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Deliverable

14.4 Case study results concerning the cost-effectiveness of the COMPARE system

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Executive summary

This is the fourth deliverable of Work Package 14, presenting the results of cost-effectiveness case studies. A total of eight case studies have been conducted (considerably more than originally envisaged) with institutions that use Whole Genome Sequencing on a routine basis for pathogen identification and surveillance. These are Animal and Plant Health Agency (APHA, UK), Friedrich-Löffler-Institut (FLI, Germany), Erasmus Medical Centre (EMC, Netherlands), Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna (IZSLER, Italy), Administración Nacional de Laboratorios e Institutos de Salud (ANLIS, Argentina), Maryland Department of Health (MDH, USA - ongoing), Public Health Agency Canada (PHAC, Canada - ongoing), and Public Health England (PHE, UK). Case study institutions were selected to ensure broad coverage of diverse surveillance contexts and applications, including sector of application (food safety, animal health, and public health), coverage of viral (influenza) and bacterial (foodborne) pathogens, routine surveillance and outbreak contexts, as well as the use of different sequencing technologies.

Methodology

The analysis focuses on the institutional perspective, i.e. the 'investment case' for implementing WGS from the perspective of the case study institutions. The costs considered therefore include equipment, consumables, staff and other costs that are directly accrued by the case study institution. The benefits are also assessed primarily from the perspective of the case study institutions, focusing on the effects of using WGS on sampling and sampling strategies, analytical results and processes, research and methods applied, and outbreak identification and response. Although the focus is on the costs and benefits accruing to the case study institutions, we also follow the recommendation of the WHO to adopt a broader societal perspective where possible and therefore report on the broader effects of the intervention for society where such effects have been concretely observed by the case study institutions. For each of the case studies, we compare the costs of using WGS to a counterfactual of processing the same number of samples during the specified reference period with the next-best conventional methods for pathogen identification. The next-best conventional methods have been defined by each individual case study institution, taking into account their own standard practice prior to the implementation of WGS. The focus of the analysis is therefore on the measurement and valuation of the marginal (incremental) costs and benefits of using WGS in the surveillance systems subject to our case studies. This has the advantage that costs and benefits that are clearly unaffected (such as costs for depreciation of laboratory buildings) do not need to be assessed, allowing the analysis to focus in detail on those costs and benefits where changes occurred. For more details on the methodology, see section 3 of this deliverable.

Costs of WGS vs conventional methods

The total per-sample cost of WGS analysis exceeds the cost of conventional methods in all case studies except one, where a non-routine method was chosen as comparator by the case study institution. Excluding this result, the use of WGS is between 1.9 and 4.3 times more expensive than the use of conventional methods, with a cost differential between EUR 55 and EUR 727. The table below summarises the costs of WGS and conventional methods according to cost type and presents the additional costs of WGS for each case study.

Table 1: Overview of costs of WGS versus conventional methods, by cost type (per sample costs)

		APHA (UK)	FLI (DE)	EMC (NL)	IZSLER (IT)	ANLIS (ARG)	MDH (USA)	PHAC (CAN)	PHE (UK)
Case study type		Outbreak	Outbreak	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance
Pathogens		Avian influenza	Avian influenza	Influenza	Foodborne*	Foodborne*	Foodborne*	Foodborne*	Foodborne*
WGS									
Equipment		€ 58.53	€ 210.71	€ 5.39	€ 163.49	€ 43.02	€ 29.53	€ 75.90	€ 35.23
Consumables		€ 830.97	€ 254.88	€ 54.88	€ 165.37	€ 104.62	€ 104.40	€ 69.75	€ 53.92
Staff costs	*Prof.*	€ 39.63	€ 42.60	€ 21.27	€ 52.35	€ 6.85	€ 20.58	€ 61.82	€ 28.30
	Tech.	€ 87.50	€ 60.19	€ 56.48	€ 13.93	€ 0.00	€ 0.00	€ 7.89	€ 7.15
Other costs		€ 0.00	€ 0.00	€ 0.00	€ 0.00	€ 0.00	€ 0.00	€ 0.00	€ 0.00
Total per-sample cost WGS		**€ 1 016.63**	**€ 568.37**	**€ 138.03**	**€ 395.14**	**€ 154.49**	**€ 154.51**	**€ 215.36**	**€ 124.59**
Conventional methods									
Equipment		€ 78.55	(€ 137.35)**	€ 3.41	€ 26.04	†	€ 5.84	€ 12.30	€ 7.11
Consumables		€ 21.91	(€ 360.88)**	€ 32.19	€ 20.17	†	€ 32.89	€ 34.95	€ 29.91
Staff costs	*Prof.*	€ 39.63	(€ 230.75)**	€ 0.00	€ 3.52	†	€ 42.43	€ 6.72	€ 2.92
	Tech.	€ 150.00	(€ 107.00)**	€ 17.79	€ 25.88	†	€ 0.00	€ 40.32	€ 23.85
Other costs		€ 0.00	(€ 0.00)**	€ 0.00	€ 16.27	†	€ 0.00	€ 0.00	€ 1.67
Total per-sample cost conventional methods		**€ 290.08**	**(€ 835.98)****	**€ 53.38**	**€ 91.87**	**€ 46.61**	**€ 81.16**	**€ 94.29**	**€ 65.46**
Cost difference between WGS and conventional methods									
Additional cost of WGS		**€ 726.54**	**(- € 267.61)****	**€ 84.63**	**€ 303.27**	**€ 107.88**	**€ 73.35**	**€ 121.07**	**€ 59.13**
Quotient of WGS over conventional methods		**3.5**	**(0.7)****	**2.6**	**4.3**	**3.3**	**1.9**	**2.3**	**1.9**

Source: Own compilation based on case study results. Note that the cost of the conventional methods is a weighted figure which accounts for the use rate of the various methods across the different pathogens. * Foodborne pathogens: Salmonella (all), Listeria (IZSLER, PHE, PHAC, MDH), E.coli and shigella (PHE, ANLIS, MDH), Campylobacter (PHE, MDH), Vibrio (MDH). ** Sequencing of a whole genome of a virus using Sanger sequencing (as indicated by FLI as comparator method) is a resource-intensive process that has generally been replaced by next-generation sequencing, and Sanger sequencing would typically be used for the (more limited and less resource-intensive) HA/NA analysis (the comparator method used by APHA). †No further cost breakdown by cost type was possible.

As can be seen in the table above, per-sample *equipment* costs are higher for WGS by a substantial margin in all but one of the case studies (APHA), when compared to the costs of conventional methods. This is particularly true for the foodborne pathogen case studies, which, as discussed in the previous section, generally rely on less costly equipment for conventional methods than the other case studies, and therefore have a greater difference between the equipment costs for WGS and for conventional methods. For the avian influenza case studies, where the alternative method (Sanger sequencing) required the purchase of a sequencer comparable in original purchase price to modern next-generation sequencers, the difference in costs is relatively smaller (FLI) or even in favour of WGS (APHA). The difference between equipment costs for WGS and conventional methods is narrowest for the case study using Nanopore sequencing (EMC).

Per-sample *consumables* costs are higher for WGS then for conventional methods in all but one case study (FLI), and sometimes considerably so, as e.g. in the case of APHA, where the consumables cost for WGS (EUR 831) is nearly 38 times the consumables cost for conventional methods (EUR 22). The determining factor, as noted before, is the higher cost of kits and reagents required for WGS. The difference in consumables costs between WGS and conventional methods can be reduced but not eliminated through higher batch sizes and higher sample volumes; this is illustrated by PHE, which has the highest batch size (40/96) and sample volume (15791), and consequently achieves one of the smallest differences in the costs of consumables between WGS and conventional methods. EMC also achieves one of the lowest cost differences, despite having a smaller batch size than PHE, due to the use of Nanopore sequencing.

With respect to per-sample *staff costs*, results depend on the staff category. Technician costs are considerably lower for WGS in four out of seven case studies, for which such data was available. In contrast, professional costs for WGS are much higher than for conventional methods in the foodborne pathogen case studies, but are either on par with or lower than for conventional methods in the avian influenza studies, which reflects the more complex procedures and analysis required for Sanger sequencing.

Overall, the variable size of the cost difference between WGS and conventional methods appears to be attributable to a combination of the type of conventional method used (as Sanger sequencing implies greater equipment and staff costs than the other methods, all else being equal) and the batch size/sample volume used in WGS analysis. As described in the detailed analysis presented in section 5.1, considerable economies of scale can be achieved with respect to equipment and consumables costs in particular. However, the results of the case study at EMC suggest that lower cost differences could also potentially be achieved at a lower batch size/sample volume through Nanopore sequencing.

Analysis of benefits

The benefits of using WGS for pathogen identification and surveillance as reported by all case study institutions are considerable. Based on the results of our exploratory interviews and literature review, the benefits associated with WGS have been divided into five different areas, which are each summarised in qualitative terms below.

Case study institutions considered that the benefits of WGS in the area of sampling and sampling strategies have so far been limited. One of the reasons for the generally low assessments in the area of sampling and sampling strategies was that sampling is mostly not within the purview of the case study institutions, as samples are independently collected by external partners and sent to the case study institutions for further analysis. An exception in this area was reported by IZSLER, whose jurisdiction employs a One Health approach, where the introduction of WGS has resulted in changes to how food safety officials conduct sampling (i.e. by moving from finished product testing to environmental sampling).

With respect to analytical results and processes, case study institutions were nearly unanimous in their assessment that WGS had a very significant impact on the quality of the results produced in terms of detail, accuracy, specificity, and sensitivity. The higher resolution data from WGS also proved to be valuable in epidemiological investigations through the production of results beyond what would be possible with conventional methods. Relative to the effects observed on the quality of results, the effects on laboratory processes and resources were considered to be not applicable or mostly negligible in the influenza case studies (APHA, FLI, and EMC), while being significant for the case study institutions that use WGS in the context of routine surveillance of foodborne pathogens. Among the specific effects and impacts of WGS related to analytical results and processes, the case study institutions reported that the effects on the time needed for the analysis were among the less significant effects, although this also depended on the type of case study. The turnaround time for conventional methods increases based on the amount of information required and the corresponding number of different tests (especially consecutive tests) that are needed, while the turnaround time for WGS analysis remains relatively consistent. Consequently, the turnaround time for WGS tends to be higher than the turnaround time for conventional methods when only basic information about the sample is needed, and lower when more a detailed characterisation of the pathogen is required. As reported by APHA, EMC, and PHAC, the difference in turnaround time for basic information can be critical in an outbreak context.

In the area of outbreak identification and response, most case study institutions considered that the use of WGS had significant or even very significant effects on the improvement of information for outbreak response. All case study institutions reported significant or very significant effects related to improved detection that outbreaks are related and improved information on outbreak epidemiology, and most considered that there had also been effects related to the earlier detection of an initial outbreak and improved information for imposing additional control/biosecurity measures. In contrast, reductions in the consequences of outbreaks, including reductions in the number of secondary outbreaks, in the duration of outbreaks, or in the overall disease burden were mostly reported by case study institutions involved in the surveillance of foodborne pathogens.

With respect to research and methods applied, almost all case study institutions considered that the use of WGS had significant or very significant effects on their understanding of disease transmission, and most considered that there had been at least moderate effects on epidemiological methods. Multiple case study institutions, including PHE, FLI, and IZSLER, have made use of WGS in order to study past outbreaks, draw new conclusions regarding outbreak epidemiology, and identify errors that had been made in the initial epidemiological investigations. PHE, PHAC and APHA also indicated that WGS had led to improved diagnostic tests, either through use as a validation tool for conventional methods (e.g. PCR assays) or by providing information to help target future conventional testing in an outbreak context.

Benefits for the wider society were observed by some of the case study institutions, but not all. APHA and IZSLER reported significant effects of WGS on trade and industry, with IZSLER citing an example of WGS being used to settle a commercial dispute regarding the source of contamination along the production chain. FLI also considered that WGS could lead to a reduction in the costs of outbreaks for the wider society through a reduction in compensation payments to operators where WGS exposed gaps in biosecurity measures. As an outlier, ANLIS indicated that no benefits had been observed in this area at all, due to the fragmented nature of the surveillance system in Argentina and a lack of communication between and within the responsible institutions.

Next steps

During the next reporting period, the two remaining case studies, which are in the process of final confirmation, will be completed. Additionally, we are in the process of

conducting a ‘breakeven’ analysis that uses the case of salmonellosis to estimate how many cases of illness would need to be prevented in each case study’s jurisdiction through the use of WGS in order to make the adoption of the technology cost-neutral from a public health perspective (results to be presented in the final deliverable, Deliverable 14.5, to be submitted in Month 60 of the project). Also, work has begun on a scientific article detailing the results of the cost-effectiveness case studies, to be published later this year.

1. Introduction

This is the fourth deliverable of Work Package 14, which aims to develop a standardised framework for estimating the cost-effectiveness of the COMPARE system and related methods and tools, including the value of safety. The activities of the work package are carried out jointly by WP partners Civic Consulting and Erasmus University Rotterdam (EUR).

In this deliverable, which was prepared by Civic Consulting, we present the results of the cost-effectiveness case studies and an assessment of costs and benefits of the COMPARE system[1] in line with Specific Objective 4 and related Task 5 of WP14. Specifically, this deliverable presents the work of Sub-task 5.3: 'Analysis of case study results and relating them to the cost-effectiveness of [the] COMPARE system.' The analysis presented here will be refined in our subsequent work and form the basis for the fifth and final deliverable of WP14, 'a report on the assessment of options for refining selected elements of COMPARE in view of improving the overall cost-effectivness of the system, with recommendations', to be completed in Month 60.

This document is structured as follows:

- Section 2 provides an overview of steps taken so far under this Work Package;
- Section 3 provides an overview of the case study methodology;
- Section 4 contains the case study reports;
- Section 5 presents the results of the case studies and provides a comparision of costs and benefits across case studies;
- Section 6 describes the next steps to be followed in this Work Package.

[1] See section 3.1.2 for a definition of the system subject to the assessment.

2. Overview of steps taken

The first deliverable of Work Package 14 corresponded to the first objective of the Work Package, namely 'to identify the important elements in calculating costs and benefits of COMPARE and related methods and tools, both regarding the system itself and from the societal perspective'. It was delivered in Month 18, and included a detailed description of the elements of COMPARE and the results of research concerning possible components of the methodological framework for the cost-effectiveness analysis. In parallel to the preparation of Deliverable 1, we also submitted a scientific article on the work conducted so far in WP14, which was published in the OIE Scientific and Technical Review in 2017.

The second deliverable of Work Package 14 corresponded to the second objective of the Work Package, i.e. to 'identify and where necessary develop state-of-the art costing methodologies for the different elements in the framework'. It was delivered in Month 30, and included a detailed description of the methodology to be used in conducting and assessing the cost effectiveness case studies, including the objectives and scope of the case studies (as well as the definition of the system and scope of activities to be assessed), criteria for case study selection, and a methodology for the assessment of costs and benefits. It also presented the approach to develop and apply a methodology to value safety in several countries.

The third deliverable of Work Package 14 consisted of a scientific paper describing the methodology and results of estimating the value of safety, with the results from consumer surveys conducted in several European countries.

Since the delivery of the second and third deliverables, we have been selecting and implementing the cost-effectiveness case studies in line with the methodology described in Deliverable 2. A total of eight case studies have been conducted with institutions that use WGS[2] on a routine basis for pathogen identification and surveillance. The cost-effectiveness case studies include both outbreak and routine surveillance scenarios, routine surveillance in a middle-income country, and routine surveillance using Nanopore sequencing. Additionally, we are in the process of conducting a 'breakeven' analysis that uses the case of salmonellosis to estimate how many cases of illness would need to be prevented in each case study's jurisdiction through the use of WGS in order to make the adoption of the technology cost-neutral from a public health perspective (results to be presented in the final deliverable). In parallel, work has begun on a scientific article detailing the results of the cost effectiveness case studies, to be published later this year in Eurosurveillance.

[2] Note that a reference to WGS in this text should be understood as refering to whole genome sequencing using Next Generation Sequencing (NGS) technologies. Whole genome sequencing with first generation sequencing methods is not covered and referred to explicitely, where relevant.

3. Methodology of the case studies

3.1. Objectives and scope

According to the description of Work Package 14, the cost-effectiveness of COMPARE and related methods and tools will be estimated using case studies. For the definition of the scope of the case studies and the methodology for the cost-effectiveness estimation it is essential to clarify the following aspects of the analytical framework (see also Deliverables 1 and 2):

- *Criteria for case-study selection*, including an overview of the case studies conducted and their main characteristics;
- *Setting and context*, including a definition of the system and activities to be assessed as well as the key research questions for the case studies; and
- *Scope of the evaluation,* including the study perspective, comparators (i.e. the counterfactual against which the system is assessed), and time horizon.

These aspects are separately discussed in the following sub-sections.

3.1.1. Criteria for case-study selection

Eight case studies were conducted with institutions that used WGS on a routine basis for pathogen identification and surveillance. The eight case studies spanned seven different countries in Europe and the Americas, and covered viral (influenza) as well as bacterial (foodborne) pathogens.

Case studies were selected to ensure broad coverage of diverse surveillance contexts and applications according to the following criteria:

- *Sector of application:* Food safety, animal health, and public health;
- *Geographical level:* Regional and national level;

Type of surveillance: Routine surveillance and outbreak contexts.

Additionally, it was determined that the case studies should include if possible a middle-income country as well as different sequencing technologies (including Nanopore sequencing, e.g. using the MinION/GridION). Eight case studies were selected and implemented on this basis, namely:

Animal and Plant Health Agency (UK)

Public Health England (UK)

Friedrich-Löffler-Institut (Germany)

Erasmus Medical Centre (Netherlands)

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna (Italy)

Administración Nacional de Laboratorios e Institutos de Salud (Argentina)

Maryland Department of Health (USA)

Public Health Agency Canada (Canada)

The table below provides an overview of the main characteristics of each case study.

Table 2: Overview of case studies

	Country	Geographical level	Sector of application	Type of surveillance	Pathogens	Remarks
Animal and Plant Health Agency (APHA)	UK	National	Animal health	Outbreak	Avian influenza virus	-
Friedrich-Löffler-Institut (FLI)	Germany	National	Animal health	Outbreak	Avian influenza virus	-
Erasmus Medical Centre (EMC)	Netherlands	National	Public health	Routine surveillance	Influenza virus	Nanopore sequencing
Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna (IZSLER)	Italy	Regional	Food safety	Routine surveillance	Foodborne bacteria*	-
Administración Nacional de Laboratorios e Institutos de Salud (ANLIS)	Argentina	National	Food safety	Routine surveillance	Foodborne bacteria*	Until recently, Argentina was classified as middle-income country**
Maryland Department of Health (MDH)	USA	Regional	Food safety	Routine surveillance	Foodborne bacteria*	-
Public Health Agency Canada (PHAC)	Canada	National	Food safety	Routine surveillance	Foodborne bacteria*	-
Public Health England (PHE)	UK	National	Food safety	Routine surveillance	Foodborne bacteria*	-

Source: Own compilation. * Foodborne pathogens: Salmonella (all), Listeria (IZSLER, PHE, PHAC, MDH), E.coli and shigella (PHE, ANLIS, MDH), Campylobacter (PHE, MDH), Vibrio (MDH). ** Since 2018, Argentina is considered a high-income country, see https://blogs.worldbank.org/opendata/new-country-classifications-income-level-2018-2019

As indicated in the table above, the eight case studies cover the criteria as follows:

- *Sector of application*: Five food safety case studies, two animal health case studies, and one public health case study;
- *Geographical level*: Six case studies at the national level, two case studies at the regional level;
- *Type of surveillance*: Six routine surveillance case studies, two outbreak case studies.

With respect to the pathogens covered by the eight case studies, five of the case studies focus on foodborne (bacterial) pathogens, including Salmonella (5 case studies), Listeria (4), E. Coli and Shigella (3), Campylobacter (2) and Vibrio (1). The remaining three case studies cover viral pathogens, including the avian influenza virus (2) and the human influenza virus (1).

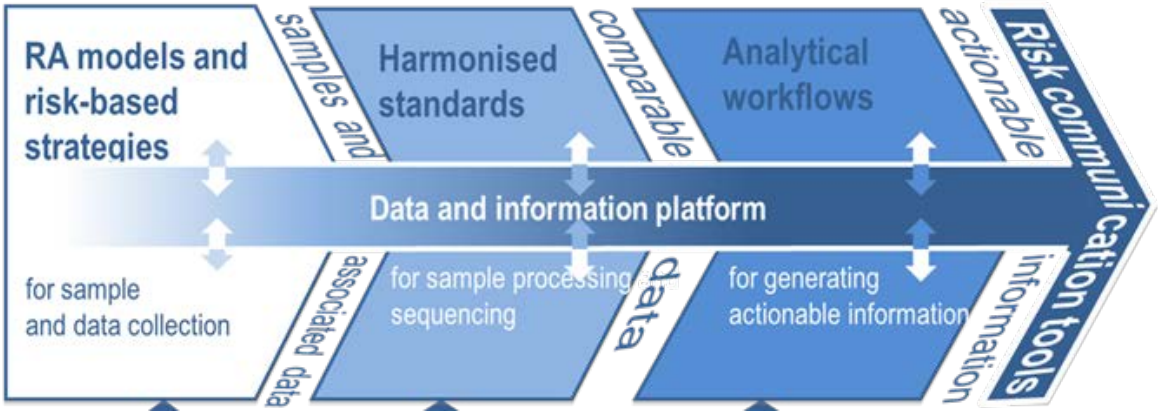
Additionally, the case studies cover routine surveillance using WGS in a country that was until recently classified as middle-income country (Argentina), as well as routine surveillance using Nanopore sequencing technology (at EMC, using the GridION).

3.1.2. Setting and context

3.1.2.1. Definition of the system subject to the cost-effectiveness estimation

The economic evaluation is carried out within the framework of the EU-funded COMPARE project. The core system targeted by the COMPARE project can be understood as *a process of information creation and analysis for pathogen identification and surveillance based on WGS*, which starts with risk-based sample and data collection strategies, continues with sample processing and sequencing based on harmonised standards, and aims at generating actionable information for pathogen and outbreak detection and related risk communication – all facilitated by a data and information sharing platform. The following figure illustrates the core system of COMPARE.

Figure 1: The core COMPARE system



Source: Adapted from COMPARE proposal.

To analyse the cost-effectiveness of this process requires its practical application in specific situations and geographical areas, which is complicated by the fact that the COMPARE project is very broad in scope (being a cross-sector and cross-pathogen framework for a globally linked data and information sharing platform), and does not only concern the practical implementation of such a system, but also intends to first develop the necessary standards and tools for sampling, processing, sequencing and data analysis and interpretation. In line with the case study approach for the cost-

effectiveness estimation it was therefore decided to focus on specific application cases with a defined sectoral and geographic scope, considering specifically the costs and benefits of a routine use of WGS for pathogen identification and surveillance. As the routine use of WGS in pathogen identification and surveillance is still relatively rare, it was also decided to widen the perspective to also include other systems that are similar to COMPARE, in that they involve harmonised methods and the use of a centralised data and information sharing platform, even if they are not directly linked to COMPARE (such as the US Genome Trakr network).

In conclusion, it was decided to define the 'system' to be assessed in the cost-effectiveness case studies as follows:

- A system for pathogen identification and surveillance using WGS on a routine basis;
- With harmonised methods (e.g. regarding data collection strategies, sample processing and sequencing, analytical tools and methods); and

Using a centralised data and information sharing platform for sequences and related metadata.

This definition of the 'system' subject to the cost-effectiveness estimation (hereafter referred to as 'WGS-based surveillance system') has been used for the identification of suitable case studies and for developing the methodological approach for the case studies.

3.1.2.2. Activities to be assessed

A system for pathogen identification and surveillance using WGS on a routine basis can be conceptualised as consisting of a process of data flow, which we divide into the following three main steps:

1. *Data acquisition*, which includes sampling and sequencing as well as all intermediate steps (sample processing, library preparation);
2. *Data analysis and storage*, which includes the bioinformatics analysis and storage of data in a reference database; and
3. *Data application,* which includes outbreak identification and response as well as other practical and research applications of genomic data.

Most of these steps are relevant for any surveillance system using WGS, independent from whether all steps and activities are conducted by one institution (as could be the case for e.g. a national food control laboratory integrated in a public food safety authority), or whether separate institutions are involved in data acquisition, data analysis and storage and data application (which would be the case for example in a network of partner institutions using a common reference database, as is the case in the COMPARE system).

With the exception of the steps that are directly related to WGS (such as sequencing and bioinformatics analysis), other key steps and the related data flow from sampling to outbreak identification and response also characterise surveillance systems that use conventional (non-WGS) laboratory methods. This model of data flow is therefore used as analytical basis for identifying the costs and benefits of WGS-based surveillance systems as well as of traditional surveillance systems.

A key question in terms of scope is to what extent response activities have to be considered when analysing the costs and benefits of a WGS-based surveillance system (i.e. the degree to which step 3 - data application should and could be included in the assessment). The COMPARE system as depicted in the figure above leads to actionable information for outbreak detection and analysis as well as risk communication, and does not necessarily include other outbreak response measures. The WHO Guide on

evaluating the costs and benefits of national surveillance and response systems concludes that an analysis of costs and benefits should consider surveillance and response systems together.[3] Nevertheless, data on costs and benefits of response activities are often very difficult to obtain ex-post, and measurement problems are significant, mostly due to the need to assess an appropriate counterfactual (such as the size of an outbreak if a specific response measure had not been taken). Therefore, while we have recorded the effects of WGS on outbreak response as concretely experienced and reported by the case study institutions, the main economic evaluation focuses rather on the first two steps concerning data acquisition and data analysis and storage, which are objectively observable and more readily quantifiable.

3.1.2.3. Research questions

In the course of the research, it became clear that in addition to better understanding the costs and benefits of the practical application of a WGS-based surveillance system, a key research interest would also be to explore potential efficiency gains due to the increased amount of information available through WGS, and the possibility that WGS provides to have surveillance systems with a cross-pathogen focus. Taking these considerations and the work package description of WP14 into account, the key research questions for the cost-effectiveness case studies for measuring and valuing elements of the system were therefore defined as follows:

1. What are the costs and benefits of a system for pathogen identification and surveillance using WGS on a routine basis, using harmonised methods and a central data hub, compared to a system that uses other ('traditional') analytical methods?
2. Does the use of WGS lead to cost savings and other benefits due to more targeted sampling and earlier/more effective outbreak response in different contexts (e.g. animal health, food safety, public health), due to the additional information obtained?
3. Are there potential efficiency gains when upgrading established surveillance systems with WGS through converting disease/target population specific surveillance systems into cross-pathogen 'catch all' surveillance systems, including in the context of low and middle income countries?
4. What main factors affect the costs and benefits of a WGS-based surveillance system, and what are possible implications in view of improving its overall cost-effectiveness?

3.1.3. Scope of the evaluation

3.1.3.1. Study perspective

The economic evaluation of costs and benefits on the basis of the case studies focuses on the institutional perspective, i.e. the 'investment case' for implementing WGS from the perspective of the case study institutions. The costs considered therefore include equipment, consumables, staff and other costs that are directly accrued by the case study institution. The benefits of the intervention are also assessed at this stage primarily from the perspective of the case study institutions, focusing on the effects of using WGS on sampling and sampling strategies, analytical results and processes, research and methods applied, and outbreak identification and response. Although the focus is on the costs and benefits accruing to the case study institutions, we also follow the recommendation of the WHO to adopt a broader societal perspective where

[3] World Health Organisation, Evaluating the Costs and Benefits of National Surveillance and Response Systems: Methodologies and Options, 2005, p. 10-1.

possible[4] and therefore report on the broader effects of the intervention for society where such effects have been concretely observed by the case study institutions.

In a second stage of the evaluation to be reported in the next deliverable, a breakeven analysis will be conducted to support the assessment of cost-effectiveness of the system, which will adopt an explicit societal perspective (see section 3.5 below).

3.1.3.2. Comparators

For each of the case studies, the economic evaluation compares the WGS-based surveillance system currently in place to a counterfactual of the same surveillance system without WGS, using the next-best conventional methods for pathogen identification and surveillance (hereafter 'conventional methods'). The next-best conventional methods have been defined by each individual case study institution on the basis of their own standard practice prior to the implementation of WGS.[5] For the purpose of the counterfactual, all other factors, such as the length of the reference period and the number of samples processed, are assumed to remain the same; only the method(s) in use are assumed to differ. The focus of the analysis is therefore on the measurement and valuation of the marginal (incremental) costs and benefits of introducing WGS in the surveillance systems subject to our case studies. This has the advantage that costs and benefits that are clearly unaffected (such as costs for depreciation of laboratory buildings) do not need to be assessed, allowing the analysis to focus in detail on those costs and benefits where changes occurred.

3.1.3.3. Time horizon and discount rate

In line with WHO recommendations for the economic evaluation of surveillance systems, the time horizon of the analysis is limited to a reference timeframe.[6] For the case studies focused on routine surveillance, the reference timeframe is typically one year, usually the last twelve month period for which data is available. The exception to this is EMC, where the reference timeframe for routine surveillance of the influenza virus was limited to a three-month period in the flu season. In the outbreak case studies, the reference timeframe is limited to the duration of the outbreak, which in practice has ranged from three to eight months. Accordingly, as the case studies covered reference periods with a maximum length of one year, no discount rate has been applied.

3.2. Evaluation of costs

3.2.1. Estimation of resources and costs

Based on a combination of the relevant WHO guidance[7] as well as previous studies concerning the evaluation of genomic sequencing technologies,[8] the costs assessed for

[4] Edejer T. Tan-Torres, R. Baltussen, T. Adam, R. Hutubessy, A. Acharya, D.B. Evans, and CJL. Murray, Making Choices in Health: WHO Guide to Cost-Effectiveness Analysis, 2003, p. 18-9.

[5] In one of the case studies (FLI), the institution preferred to use a conventional method as comparator (sequencing of a whole virus genome using Sanger sequencing), which is not a typical routine method, but is most similar in terms of results to the use of WGS.

[6] World Health Organisation, Evaluating the Costs and Benefits of National Surveillance and Response Systems: Methodologies and Options, 2005, p. 18.

[7] Edejer T. Tan-Torres, R. Baltussen, T. Adam, R. Hutubessy, A. Acharya, D.B. Evans, and CJL. Murray, Making Choice in Health: WHO Guide to Cost-Effectiveness Analysis, 2003.

each case study are broken down by both analytical step and type of cost. Following the discussion in section 3.1.2.2., the analytical steps that were considered within the scope of the economic evaluation for WGS are the following:

1. *Sample preparation and sequencing*. This step begins with receipt and opening of incoming samples. In order to provide a finer level of detail for WGS costs, this stage is divided into three sub-steps:
    - Sample preparation, including DNA/RNA extraction and purification;
    - Library preparation; and
    - Sequencing.
2. *Bioinformatics and other analyses.* This step includes data assembly, uploading, comparing sequence data with a reference dataset, result validation, and concludes with the interpretation and reporting of results. This step also considers the maintenance and curation of an internal reference dataset, where relevant.

As noted in section 3.1.2.2. above, costs related to outbreak response, e.g. concerning epidemiological analysis or the imposing of biosecurity measures, are not included. This is due to differences in the response mandate across case study institutions (e.g. while some are involved in determining response measures, others are not) as well as considerable difficulties that would emerge with respect to ex-post data collection, measurement, and construction of an appropriate counterfactual.

Within each analytical step, costs are determined on a per sample basis according to the type of resources used. The following four cost categories were selected for assessment based on the relevant WHO guidance[9] as well as past studies by Civic Consulting that analysed costs and benefits of reference laboratory networks, as well as of veterinary systems:[10]

- *Equipment costs*. This includes sequencers and ancillary equipment, IT infrastructure for bioinformatics, and other fixed assets needed for using WGS on a routine basis (including relevant software licences). Only equipment specifically used for WGS is included; basic laboratory equipment (e.g. refrigerators, centrifuges or pipettes), equipment costing less than EUR 450, standard office computers and standard office software (e.g. Word, Excel) are not included. Data collected from the case study institutions for all relevant pieces of equipment included original purchase costs, purchase year, and annual maintenance costs (if relevant). Estimated lifespans of 5 years for computers and 10 years for major laboratory equipment (e.g. sequencers) were used to ensure comparability across case studies. In order to obtain the per-sample equipment cost, we used the straightline depreciation method, i.e. the purchase cost was divided by the estimated lifespan to obtain the annualised cost. To this amount the annual maintenance costs were added to obtain the total annualised equipment

[8] Buchanan, James, Sarah Wordsworth, and Anna Schuh, "Issues Surrounding the Health Economic Evaluation of Genomic Technologies", Pharmacogenomics, Vol. 14, No. 15, 2013, Appendix 3: Costs which could be included in economic evaluations of genomic technologies. http://www.futuremedicine.com/doi/abs/10.2217/pgs.13.183.

[9] Edejer T. Tan-Torres, R. Baltussen, T. Adam, R. Hutubessy, A. Acharya, D.B. Evans, and CJL. Murray, Making Choice in Health: WHO Guide to Cost-Effectiveness Analysis, 2003.

[10] Civic Consulting (2016), Study on cost–benefit analysis of reference laboratories for human pathogens: final report, study conducted for CHAFEA of the European Commission and Civic Consulting (2009), Cost of National Prevention Systems for Animal Diseases and Zoonoses in Developing and Transition Countries, study conducted for the OIE.

cost. The annualised equipment cost was then adjusted for percentage use[11] and length of the reference period (if not 12 months) to obtain the total cost of equipment in the reference period. The resulting total equipment cost over the reference period was finally divided by the number of samples processed in the reference period to obtain the cost per sample.

- *Consumables*. Consumable materials are items that are used up in laboratory processes, such as reagents, test kits, gloves, pipette tips etc. Consumables data collected from the case study institutions included batch costs, batch sizes and average failure rates (to account for e.g. failed sequencing runs). The cost per sample was obtained by dividing the batch cost by batch size to obtain the unit cost, and then adjusting the unit cost to account for failure.
- *Staff costs.* Staff costs include wages, social contributions and non-wage income of employees, such as in-kind payment. Data was collected from case study institutions in terms of the minutes of *hands-on staff time* per sample, divided into professional and technician staff categories.[12] Hands-on staff time refers to the amount of staff time used to perform an activity, and not the duration of the activity itself: unsupervised processes (such as incubation periods or sequencing runs) are therefore not included in estimates of hands-on staff time. Where samples are processed in batches, the total amount of staff time was divided by the batch size.[13] The hands-on staff time per sample was then monetised based on country-specific standard labour costs for professional and technician staff categories,[14] plus 25% for overhead costs.

Other costs. Other cost items are those which do not fall under one of the other three categories, including subcontracting costs, shipping costs, etc. No other costs were found to be relevant for WGS. Other costs were provided by some case study institutions for conventional methods on a per-sample basis, e.g. because some tests had previously been subcontracted.

Cost data per analytical step and type of cost were collected directly from the case study institutions with the help of a dedicated questionnaire and accompanying spreadsheets; a copy of the questionnaire can be seen in Appendix I. An in-person visit was conducted with each individual case study institution in order to discuss the questionnaire and data provided in detail, clarify any questions or concerns, address

[11] For example, if an Illumina MiSeq was used during the reference period 30% of the time for the samples subject to the case study and 70% of the time for other purposes (e.g. by other users inside the institution or for external clients), then the use rate for our calculation would be 30%.

[12] For 'Professionals', occupations typically involve the performance of tasks that require complex problem-solving, decision-making and creativity based on an extensive body of theoretical and factual knowledge in a specialised field. The knowledge and skills required are typically obtained as the result of study at a higher educational institution for a period of 3-6 years following completion of secondary education leading to the award of a first degree or higher qualification. This category includes PhD candidates and Post-docs.

For 'Technicians', occupations typically involve the performance of complex technical and practical tasks that require an extensive body of factual, technical and procedural knowledge in a specialised field. The knowledge and skills required are usually obtained as the result of study at a higher educational institution for a period of 1-3 years following completion of secondary education. This category includes laboratory assistants.

[13] For example, if the simultaneous processing of 40 samples takes 160 minutes of staff time, this figure would be divided by 40 and result in a staff time estimate of 4 minutes per sample.

[14] For European Union countries, standardised labour costs for each staff category were collected from the Eurostat database 'Labour cost for LCI (compensation of employees plus taxes minus subsidies)' [lc_lci_lev]. For all other countries, average labour costs were provided directly by the case study institutions based on the definitions of professional and technician staff.

any issues unique to the case study, and ensure a consistent understanding and reporting of cost data across all case study institutions. Follow-ups were conducted by phone and email. Each case study institution was provided with a report of their case study for review and validation to ensure that the processed data was correct and complete.

Cost data was collected from each case study in the local currency, with the exception of ANLIS (Argentina), which reported costs in US dollars, due to the fluctuations in exchange rates of the national currency. Where the local currency was not the Euro, costs were converted into Euro based on the reference exchange rate of the European Central Bank for the relevant year. All costs are reported in EUR 2017.

3.3. Evaluation of benefits

The benefits of interventions such as pathogen identification and surveillance systems and/or the introduction of new genomic sequencing technologies are far-reaching and numerous; quantifying such benefits is therefore a significant challenge.

3.3.1. Choice of benefits

As indicated in above in section 3.1.3.1. on the study perspective, the focus of the economic evaluation is on the costs and benefits that accrue to individual case study institutions, although the benefits for the wider society are also considered where these have been concretely observed by the case study institutions. The main benefits considered in this evaluation are therefore the institutional cost savings and efficiency gains from using WGS, as well as other benefits that were observed by the case study institutions during the course of the study.

Based on the results of our desk research and exploratory expert interviews, we identified five key areas in which benefits might be expected to accrue, as well as an indicative list per area of potential positive effects of using WGS:

- *Sampling and sampling strategies*, including a potential reduction in the number of samples needed, simplifications in the type of samples needed (e.g. swabbing vs finished product testing), simplifications in sample storage or transport, or a reduction in the overall costs of sampling;
- *Analytical results and processes*, including improved accuracy, sensitivity, specificity and/or detail in results produced, simplified laboratory work flows, or reductions in the time, consumables, staff time, or overall costs required for the analysis;
- *Effects on research and methods applied*, including a better understanding of disease transmission, development of better diagnostic tests, improvement in epidemiological methods, or other benefits for research;
- *Outbreak identification and response*, including earlier detection of the initial outbreak, improved detection that outbreaks are related, improved information on outbreak epidemiology or for imposing additional control and biosecurity measures, reductions in the number of secondary outbreaks, in the duration of an outbreak, in overall costs for outbreak identification and response, or reductions in the disease burden for humans or livestock;

Effects on wider society, including reductions in the negative effects of outbreak(s) on consumer trust in food, on the livestock industry, on trade, on tourism, or an overall reduction in the costs of outbreak(s) for the wider society.

3.3.2. Measurement of benefits

In order to measure benefits in the five areas identified above, each case study institution was asked to assess the each of the specific effects using a Likert scale which ranged from 1 (no effect at all) to 5 (very significant positive effect). See the questionnaire in Appendix I for a complete list of the effects assessed by case study institutions. Case study institutions were also asked to indicate any other specific effects that were not included in the indicative list. During the in-person case study visits, the assessments of the case study institution for each and every specific effect were discussed in detail, with the aim of ensuring consistency across case studies and collecting in-depth contextual information to further qualify their response.

The benefit assessments of the case study institutions were further supplemented by scientific publications and other institutional reports or outbreak data that were provided to us by the case study institutions. Seven of the eight case study institutions were able to provide specific publications or further data concerning the effect of using WGS on outbreak identification and response in either retrospective studies or real-time response, including quantitative and qualitative data on the effect of using WGS for case definition and outbreak detection as well as effects of using WGS on disease control measures or the resulting disease burden.

3.4. Cost-effectiveness estimation

In this deliverable, we present the detailed results of the case studies in separate case study reports for the institutions covered (section 4). We then separately assess the costs and the benefits of using WGS on a routine basis for pathogen identification and surveillance based on a detailed analysis of the data provided by the case study institutions. The additional cost of WGS is calculated and presented across case studies, along with a mostly qualitative assessment of the main benefits as assessed by the case study institutions (section 5). This will be complemented in the following deliverable by the above mentioned break-even analysis. The break-even analysis will estimate the number of cases of illness (for the example of salmonellosis) that would need to be avoided each year through the use of WGS in order to ‘break even’ on costs, i.e. in order to make the use of WGS cost-neutral. In the final deliverable, we will also provide overall conclusions on cost-effectiveness.

4. Case study reports

This section presents the completed case study reports for the cost-effectiveness case studies.

4.1. Animal and Plant Health Agency (APHA)

Avian Influenza outbreaks – APHA, UK	
I. Institution	
Name of institution	The Animal and Plant Health Agency (APHA)
Type of institution	Public veterinary institution
Description	The Animal and Plant Health Agency (APHA) is an executive agency of the Department for Environment, Food & Rural Affairs (Defra). It also provides services to the Scottish and Welsh Governments, other government departments, and other clients. APHA is responsible for identifying and controlling endemic and exotic diseases and pests in animals, plants and bees, and for surveillance of new and emerging pests and diseases. APHA maintains essential disease investigation and response capability, as well as supporting trade in plants, animals and associated products though certification, audit and inspection, e.g. through import controls of animals, plants, seeds and products of animal origin. APHA conducts scientific research and acts as a national and international reference laboratory for the World Health Organisation (WHO), World Organisation for Animal Health (OIE), and United Nations Food and Agriculture Organisation (FAO), covering many farm animal diseases, including avian influenza. APHA was the EU reference laboratory (EU-RL) for avian influenza until the summer of 2018.
Location	Surrey, UK
II. Activities covered by case study	
Activity	Outbreak investigation[15]
Reference period	1 December 2016 – 31 July 2017
Pathogen(s) covered	Highly Pathogenic Avian Influenza (HPAI) H5N8
Outbreak summary	The outbreak of highly pathogenic avian influenza H5N8 in 2016-2017 occurred in both wild birds and poultry, infecting 13 premises across England and Wales. These included turkey and chicken producers as well as premises involved in gamebird production. The H5N8 infections in poultry are thought to have arisen independently as a result of contact with wild birds, except in the case of a cluster of three infected premises of the same commercial enterprise in Lancashire, where genomic analysis confirmed that secondary infections were likely to have occurred.[c),d)] Note that related H5N8 outbreaks also occurred in continental Europe during this period, but only samples taken in the UK are included in this case study.
Type of sample	Primarily isolates where the virus has been cultivated prior to sequencing.

[15] APHA provided data on two outbreaks: a 2016-2017 outbreak of HPAI H5N8 in wild birds and poultry and a 2017-2018 outbreak of HPAI H5N6 in wild birds only. Data on the outbreak of HPAI N5N6 is presented in Annex II for comparison purposes.

	However, in some time-sensitive cases the clinical sample is sequenced as-is without growing the virus first, after selecting the 'best' samples in terms of viral content based on the pre-screen PCR.		
Region covered by sampling	UK		
Number of samples analysed in reference period	*Pathogen*	*Samples analysed by conventional methods*	*Samples sequenced using WGS*
	HPAI H5N8	104 (32 HA, 72 NA)	26
Conventional methods used as reference for costing	▸ Sanger sequencing (HA and NA analyses, used on 100% of samples) ▸ Manual extraction of RNA using guanidine lysis buffer and silica column purification, generation of target double stranded DNA amplicon (150nt) by RT-PCR using specific primers according to target, BigDye method of labelling and ABI Capillary sequencing		
Sample preparation WGS	▸ Manual extraction of RNA using guanidine lysis buffer and silica column purification, 'shotgun' generation of double stranded cDNA by RT-PCR of RNA in sample with random hexamers, library generation with Nexterra kit and Illumina WGS		
Sequencer used for WGS	▸ Illumina MiSeq		
Batch size for WGS analysis	▸ During the outbreak, APHA typically sequenced batch sizes of only 1 or 2 samples due to the time-sensitivity of the results, and this number is the basis for the following cost analysis (outside outbreaks, the typical batch size of amplified isolates would be up to 10 using the MiSeq sequencer).[16]		
Reference dataset used for WGS	▸ Reference sequences are chosen from the GISAID database for initial mapping based on assumptions as to the strain identity, then the mapped reads are used in a Blast search of all GenBank sequences to determine an optimal reference sequence for each viral segment. The new reference is then used in the subsequent mapping iterations.		
Additional information	▸ WGS is not done on all incoming avian influenza samples at APHA. Sanger sequencing (HA and NA analyses) is still the standard workflow and is required as a confirmatory test. ▸ WGS is currently employed on a routine basis as an additional 'research' test, particularly in the initial stages of an outbreak, in cases that show unusual clinical characteristics (e.g. infection of an unexpected species), or in cases where an assessment of the risk to humans is needed. Once the sequence of the index case is known, decisions to sequence additional samples are also made based on epidemiological data. ▸ All incoming avian influenza samples are subject to a pre-screening using real time PCR. From the PCR results, the best samples with the highest virus content are selected for sequencing. The virus would typically be grown further before Sanger sequencing and WGS; however, depending on the time sensitivity of results, it may be sequenced directly from the clinical sample submitted.		

III. Detailed overview of costs of WGS and conventional methods

In the following, all costs are provided on a per-sample basis. Equipment costs are annualised and incorporate the annual maintenance costs as reported by the institution. They are adjusted for the

[16] APHA also has an Illumina NextSeq sequencer which can process batch sizes of up to 40 and which has been used by APHA in their capacity as the EURL for avian influenza. However, this sequencer was not used for the UK outbreaks subject to this case study.

percentage use of the equipment for the listed pathogens samples during the reference period (i.e. if a sequencer was also used for other purposes, this is taken into account). Consumables costs are adjusted for the failure rate (i.e. the percentage of consumables wasted, e.g. due to failed runs). Staff time is provided in terms of the minutes of hands-on staff time per sample, for both professionals and technicians. For the calculation of total costs, staff time is then monetised based on Eurostat data on country-specific labour costs for 2017 (by staff category), plus a 25% surcharge for overheads. For comparison purposes only, we have also provided staff costs monetised based on EU average labour costs. More detailed cost data is provided in Annex I.

a) Costs of using WGS[17]		
Sample preparation and sequencing	*Cost type*	*Cost per sample*
	Equipment costs	€ 57.33
	Consumables	€ 830.97
	Other costs	€ 0
	Staff time professionals	*0 minutes*
	Staff time technicians	*210 minutes*
	Staff costs, monetised based on labour cost data for the UK (in brackets: based on labour cost data for the EU as a whole)	€ 87.50 (85.75)
	Total	**€ 975.80**
Bioinformatics and other analyses	*Cost type*	*Cost per sample*
	Equipment costs	€ 1.20
	Other costs	€ 0.00
	Staff time professionals	*60 minutes*
	Staff time technicians	*0 minutes*
	Staff costs, based on labour cost data for the UK (for EU)	€ 39.63 (45.13)
	Total	**€ 40.83**
b) Costs of conventional methods		
Sanger Sequencing (assuming use for 100% of avian influenza samples)	*Cost type*	*Cost per sample*
	Equipment costs	€ 78.55
	Consumables	€ 21.91
	Other costs	€ 0
	Staff time professionals	*60 minutes*
	Staff time technicians	*360 minutes*
	Staff costs, based on labour cost data for the UK (for EU)	€ 189.63 (192.13)

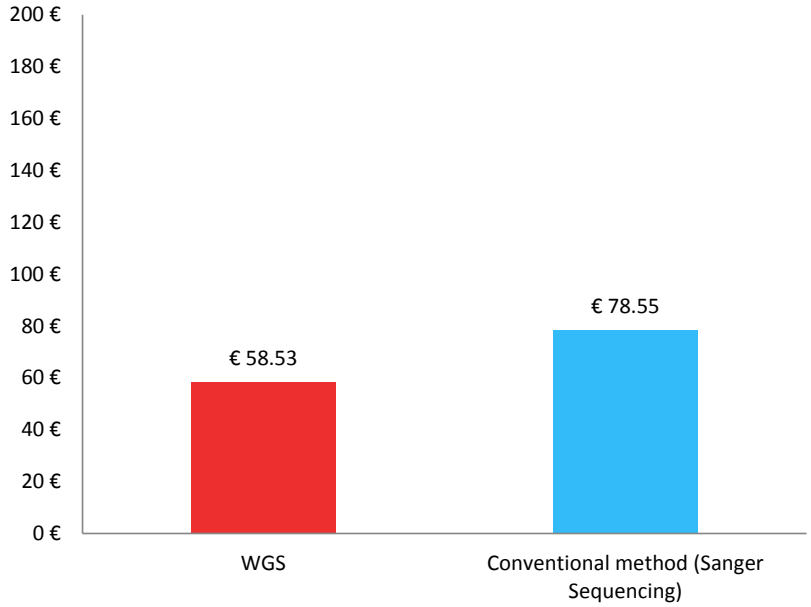
[17] APHA originally provided cost data in pounds sterling. These have been converted to Euro for comparison with the other case studies using the European Central Bank's yearly average reference exchange rate for the relevant year (i.e. the year of purchase for equipment, or 2017 otherwise).

	Total	**€ 290.08**

IV. Costs of using WGS compared to the costs of conventional methods

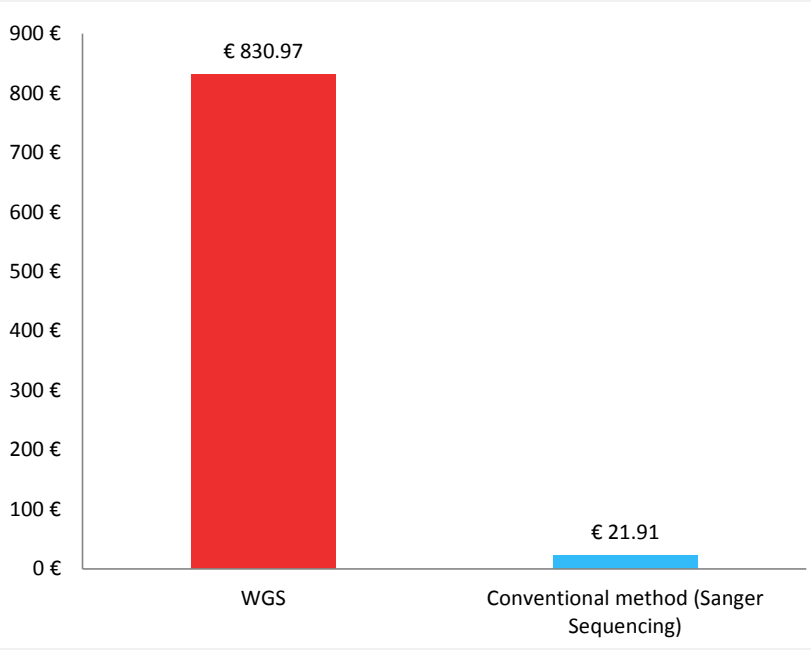
The following provides a comparison of costs per sample using WGS compared to the costs of conventional methods. See Annex I for more details.

Comparison of equipment costs



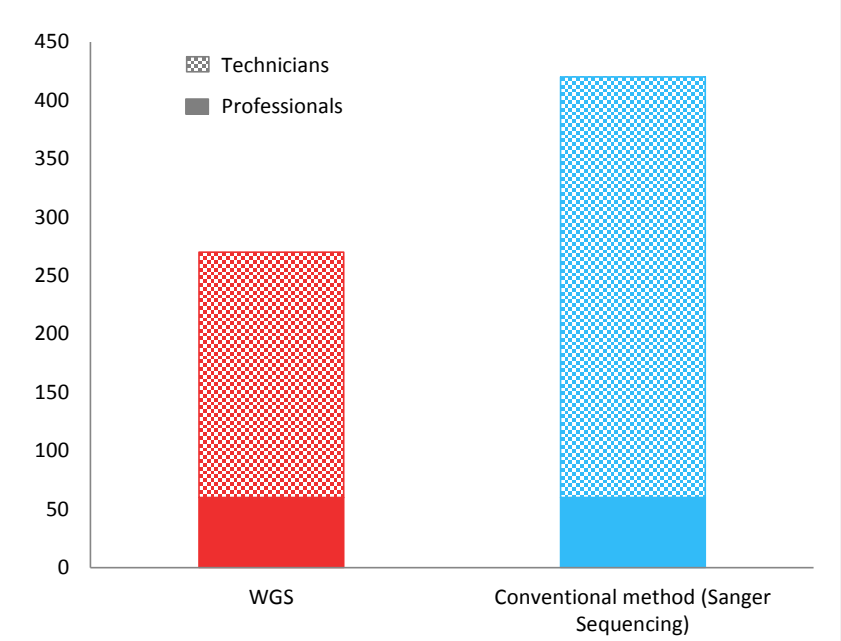
Equipment costs are lower for WGS than for Sanger sequencing (€ 58.53 vs. € 78.55 per sample). One of the factors driving the differential cost is the cost of the sequencer itself: the Illumina MiSeq used for WGS, purchased in 2012 for approximately € 105 000, is lower in price than the ABI Capillary sequencer used for Sanger sequencing, which was purchased in 2009 for approximately € 200 000. Sanger sequencing also makes use of a thermocycler and requires specialised commercial software with a licence that must be renewed annually.

Comparison of costs of consumables



The cost of consumables for WGS is considerably higher than for Sanger sequencing. The large difference in costs is attributable to the cost of the Nextera XT library preparation kit used for WGS and the reagent for the Illumina run, which costs approximately € 1200 and is used to process only one or two samples at a time in an outbreak situation.[18] In contrast, the consumables used for Sanger sequencing are both cheaper and utilisable for larger batch sizes ranging from 50 to 250.

Comparison of staff time used (in minutes)



The amount of staff time required for WGS is lower than for Sanger sequencing. Although both methods require the same amount of professional time (60 minutes), Sanger sequencing requires considerably more technician time per sample (360 minutes vs 210 minutes for WGS). All professional staff time required for WGS comes in at the bioinformatics stage; all earlier steps (sample processing, library preparation, sequencing) are handled by technician staff.

Comparison of overall costs

Cost type	*Cost per sample (WGS)*	*Cost per sample (Sanger sequencing)*
Equipment costs	€ 58.53	€ 78.55
Consumables	€ 830.97	€ 21.91
Other costs	€ 0.00	€ 0
Staff time professionals	*60 minutes*	*60 minutes*
Staff time technicians	*210 minutes*	*360 minutes*
Staff costs, based on labour cost data for the UK (for EU)	€ 127.13 (130.88)	€ 189.63 (192.13)

[18] APHA indicated that they were able to batch process samples in groups of two more than half of the time during the relevant outbreak. We have therefore assumed an average batch size of 1.6 for the Nextera XT library preparation kit.

	Total	**€ 1 016.62**	**€ 290.08**

Summary of differential costs	**A sample analysed with WGS costs considerably more than a sample analysed with Sanger sequencing, with a cost difference of € 726.54 per sample** (€ 1 016.62 vs € 290.08). The difference in total per-sample cost is entirely attributable to the large difference in the cost of consumables, which results from a combination of the cost of the Nexterra kit and the small batch size of 1-2 samples. Note that the cost data provided by APHA regarding a second (H5N6) outbreak led to very similar results, with a cost difference of € 720.10 per sample (€ 1 028.86 vs € 308.76). For details, see Annex II.

V. Effects of using WGS results

a) Turnaround time. Turnaround time is defined as the usual number of days of work from receipt and opening of an incoming sample until the reporting of results. Turnaround time does not include weekends and holidays, except in case that work has been conducted on these days, e.g. for a sequencing run or other analyses.

Turnaround time	The turnaround time for the analysis of an avian influenza sample is: ▸ Using WGS, a minimum of 3-5 days of work to sequence in a case where no virus amplification is needed. ▸ Using Sanger sequencing, a minimum of 1-2 days of work in a case where no virus amplification is needed. APHA indicated that the difference in turnaround time between Sanger sequencing and WGS arises due to machine processing time and especially the time required for analysis, as WGS results are vastly more complex and require special software to interpret. However, it indicated that the turnaround time for Sanger sequencing depends on making an accurate estimate as to the correct primers to use, and reported that the turnaround time for Sanger sequencing could be longer if the initially-selected primers are incorrect and new primers need to be designed or ordered. In cases where virus amplification (i.e. prior growth of the virus) is needed, turnaround time is higher, depending on how quickly the virus grows. The process of growing the virus adds an additional 4-6 days (on average: 4).

b) Positive effects of using WGS for pathogen identification and surveillance during the reference period

Note that or this case study, APHA provided data on two outbreaks: for the above described H5N8 outbreak (outbreak 1) and for a subsequent H5N6 outbreak (Annex II). The positive effects of using WGS described below were experienced for both outbreaks, except where indicated otherwise.

Sampling and sampling strategies	▸ APHA indicated that it saw very significant positive effects with respect to the simplification of the type of samples needed, noting that WGS was able to reduce the pre-processing required for the sample in cases where no viral amplification was necessary. This results in time savings of approximately 2 work days for generating run-ready samples. However, APHA noted that viral amplification is needed more often for WGS. ▸ APHA noted that each outbreak of HPAI was different, and that the consideration of positive effects of WGS therefore also different between cases. During the H5N8 outbreak, for example, no further effects on sampling and sampling strategies were noted, as APHA indicated that the sampling is determined by clinical findings and epidemiology, independent from whether WGS or Sanger sequencing is used. For the H5N6 outbreak, however, which was smaller and limited to isolated outbreaks in wild birds, APHA indicated that there had also been a reduction in the number of samples needed, simplification in sample storage/transport, and a

	reduction in the overall costs of sampling. It indicated that this was because WGS analysis allowed for confirmation that the separate UK isolates were all highly similar to viruses present in Continental Europe and were not direct introductions from South-East Asia.
Analytical results and processes	▸ APHA considered that using WGS had led to very significant positive effects on the accuracy, sensitivity, and specificity of results. In particular, it commented that WGS produced many reads of a sequence, resulting in higher accuracy and greater statistical confidence in the outputs, and also allowed viral genome-spanning information to be rapidly obtained regarding the genotype, pathotype, mutations, etc. ▸ APHA also noted that WGS is adaptable to high-throughput and automated pipelines. For example, APHA noted that a robot can be used for the library preparation stages (although this is not currently done at APHA). ▸ The institution indicated that another positive effect of WGS is that no prior knowledge of the target sequence is required, so no assumptions need to be made regarding the primers needed for WGS sample preparation. In contrast, if the primers available for Sanger sequencing fail to produce an amplicon, considerable time can be needed to design, order and receive new primers. ▸ During the H5N6 outbreak, APHA considered that using WGS had a significant positive effect on the simplification of laboratory work flows. This is in contrast to the situation reported in the H5N8 outbreak, where APHA considered that WGS had only a minor effect in this area. ▸ APHA considered that during the H5N6 outbreak there had been slightly more significant effects of using WGS concerning a reduction in the consumables and staff time required for the analysis than during the H5N8 outbreak, although this was not reflected in the cost data. While similar numbers of samples underwent WGS, in the case of the H5N6 outbreak, this reduced the need for additional sample analysis.
Outbreak identification and response	▸ Positive effects of using WGS were reported with respect to improved information on outbreak epidemiology, improved information for imposing additional control or biosecurity measures, and improved detection that outbreaks are related. APHA indicated that the information provided by WGS was already changing outbreak response in terms of being able to better assess the public health risk, for example by revealing the presence of mutations for mammalian host adaptation and the possible emergence of reassortant strains. It added that WGS also allowed for useful supporting information to be disseminated during outbreaks. ▸ In the H5N8 outbreak, APHA indicated that there had been a very significant positive impact of using WGS on the earlier detection of an initial outbreak, especially for the index case. APHA indicated that the information gained from WGS allows them to better assess whether the virus sampled poses a risk of transmission to humans. This effect was less pronounced for the H5N6 outbreak, once it was determined that the H5N6 outbreak strain was distinct from the H5N6 lineage associated with human infection in South-East Asia. APHA commented that WGS sometimes allows for the earlier confirmation of an outbreak and noted that WGS is still not an accredited method in the UK, but that results are given unofficially and inform the interpretation of all results. ▸ In the H5N8 outbreak, fewer positive effects were observed with respect to a reduction in the duration of the outbreak, reduction in the overall costs for outbreak identification and response, and reduction in the disease burden for livestock and humans. These effects were considered to have been comparatively larger in the H5N6 outbreak.
Research and methods applied	▸ With respect to the effects on research and methods applied, APHA reported that there had been positive effects regarding the understanding of disease transmission, an improvement in epidemiological methods, and

	the development of better diagnostic tests, although it assessed these benefits to have been higher in the case of the H5N6 outbreak than in the case of the H5N8 outbreak. Regarding the use of diagnostic tests, APHA indicated that the information gained from WGS helped determine which conventional tests to use later on in the outbreak. ▸ APHA indicated that WGS provides a lot of added value in dealing with the influenza virus, given the amount of variation observed. WGS can be used to identify novel viruses, reassortants, and mixed infections (e.g. mixed avian influenza subtypes or other pathogens) which would otherwise be missed using conventional methods. WGS also provides information on the host of origin. ▸ With respect to the H5N6 outbreak, APHA indicated that the use of WGS had allowed them to infer zoonotic risk according to mammalian adaptation signatures and to determine the likelihood or not of pre-existing immunity.
Effects on wider society	▸ APHA indicated that in the H5N8 outbreak, positive effects of using WGS could be observed with respect to a reduction in the negative effects of outbreaks for the livestock industry, for tourism, for trade, and for the wider society. Trade in particular was emphasised as an area where APHA observed positive effects from using WGS, given that HPAI had been discovered in domestic poultry. In the H5N6 outbreak, in contrast, APHA observed less significant impacts on all these domains, as the outbreak had remained confined to wild birds and did not infect poultry.
c) Negative effects of using WGS	
Negative effects of using WGS	None identified/reported other than the higher cost, although APHA indicated that from their perspective, the cost-benefit ratio of using WGS in terms of the information obtained was more favourable.
VI. Outlook	
Balance of costs and benefits achieved	▸ In general, APHA expected the balance of costs and benefits to improve. It commented that as WGS becomes more mainstream, there will be an economies of scale effect with more samples sequenced and individual run costs decreasing. Technological advances (e.g. related to the MinION) are also expected to result in further cost reductions (see below) as well as the ability to sequence clinical samples directly and to potentially sequence RNA directly.
Potential for cost reductions of using WGS for pathogen identification and surveillance in the future (through e.g. techno-logical advances)	▸ APHA expected that there will be further cost reductions in using WGS for pathogen identification and surveillance as the technology becomes more mainstream. APHA also indicated that they are currently looking at ways of optimising costs by batching samples for analysis or sequencing directly from clinical samples, thereby avoiding the virus amplification step and saving time and money. In this respect, they consider that advances in direct RNA sequencing methods and/or other technologies such as the MinION will result in considerable time and cost savings.
Future opportunities and challenges	▸ APHA considered that the cross-pathogen potential of WGS will become a reality, including across different networks and contexts. Nevertheless, APHA considered that there were unlikely to be cost reductions resulting from the cross-pathogen potential of WGS in the influenza field. However, it did see considerable future potential in the influenza field for coordination between the veterinary and public health sectors under a One Health approach. ▸ APHA commented that the bioinformatics and analysis aspect of WGS formed a sort of 'bottleneck', given that it currently relies on 'freeware'

	and the coding ability of individuals who have a rare combination of IT skills and an understanding of virology. In this respect, it considered that the COMPARE project was filling a significant gap. ▸ APHA commented that although the knowledge gained from WGS was often applied in decision-making and outbreak management, it does not easily fit into the strict quality confines of statutory testing and considered that this posed a large hurdle to making the technology 'mainstream'.

VII. Key sources/references	
Questionnaire	Questionnaire completed by APHA
Preparatory phone interview	a) Background information and description of activities
Case study visit and follow up	b) Additional data and clarifications provided
Scientific literature	c) Animal and Plant Health Agency (APHA). (2017). *National epidemiology report - Highly Pathogenic Avian Influenza H5N8 - Annex 1: Three additional infected small-holder premises - April to May 2017*. d) Animal and Plant Health Agency (APHA). (2017). *National epidemiology report - Highly Pathogenic Avian Influenza H5N8: December 2016 to March 2017*. e) Poen, M. J., Verhagen, J. H., Manvell, R. J., Brown, I., Bestebroer, T., van der Vliet, S., … Fouchier, R. A. M. (3016). Lack of virological and serological evidence for continued circulation of highly pathogenic avian influenza H5N8 virus in wild birds in the Netherlands, 14 November 2014 to 31 January 2016. *Eurosurveillance*, 21(38). h) Department for Environment Food and Rural Affairs (Defra). (2018). *Rapid Risk Assessment on the finding of H5N6 HPAI in wild birds in England and Wales.* i) Department for Environment Food and Rural Affairs (Defra). (2018). *Rapid Risk Assessment on the finding of H5N6 HPAI in wild birds in Dorset.* j) Department for Environment Food and Rural Affairs (Defra), Animal and Plant Health Agency (APHA), and Veterinary & Science Policy Advice Team - International Disease Monitoring. (2018). *Situation Assessment #4: Update on H5N6 HPAI in UK/Europe and H5N8 HPAI in Europe/Western Russia - 9 July 2018.* k) Department for Environment Food and Rural Affairs (Defra), Animal and Plant Health Agency (APHA), and Veterinary & Science Policy Advice Team - International Disease Monitoring. (2018). *Situation Assessment #3: Update on H5N6 HPAI in UK/Europe and H5N8 HPAI in Europe - 4 April 2018.* l) Department for Environment Food and Rural Affairs (Defra), Animal and Plant Health Agency (APHA), and Veterinary & Science Policy Advice Team - International Disease Monitoring. (2018). *Situation Assessment #2: Findings of H5N6 HPAI in wild birds in UK / Ireland and LPAI in poultry in France - 14 February 2018.* m) Department for Environment Food and Rural Affairs (Defra), Animal and Plant Health Agency (APHA), and Veterinary & Science Policy Advice Team - International Disease Monitoring. (2018). *Situation Assessment: Findings of H5N6 HPAI in wild birds - 30 January 2018.*
Other sources	f) APHA, Annual Report and Accounts 2016/17 g) APHA website https://www.gov.uk/government/organisations/animal-and-plant-health-agency

4.2. Friedrich-Löffler-Institut (FLI)

Avian Influenza outbreak – FLI, Germany	
I. Institution	
Name of institution	Friedrich-Loeffler-Institut (FLI)
Type of institution	Public veterinary institution
Description	The Friedrich-Loeffer-Institut (FLI) is the National Institute for Animal Health in Germany. It is a federal research institute and independent higher federal authority under the Federal Ministry for Food and Agriculture. Its work aims at the prevention of diseases, the improvement of animal welfare and the production of high quality animal-based foodstuffs. The institute performs epidemiological investigations during outbreaks of animal diseases. It also prepares risk assessments on various infectious diseases of farm animals. FLI hosts the National Reference Laboratory for Avian Influenza, which conducts application-oriented research in the field of avian influenza virus diagnostics, epidemiology and pathogenesis. It is also active within the EU-RL network for Avian Influenza. As a reference laboratory of the World Organisation for Animal Health (OIE) and of the Food and Agriculture Organization (FAO) of the United Nations, the laboratory provides advice and diagnostic assistance to countries outside Europe. FLI has a laboratory for WGS and Microarray Diagnostics. The main task of the laboratory for WGS and microarray diagnostics is full-length DNA or RNA virus genome sequencing. Beyond the sequencing activities, establishing new technical equipment, molecular biological methods, and implementing new ways for data analyses are among FLI's focus areas.[j)]
Location	Greifswald, Mecklenburg-Vorpommern, Germany
II. Activities covered by case study	
Activity	Outbreak investigation
Reference period	24/12/2016 – 28/03/2017
Pathogen(s) covered	Avian Influenza (AI)
Outbreak summary	In 2016/2017 a regional outbreak of notifiable H5 Highly Pathogenic Avian Influenza (HPAI) occurred in Lower Saxony in domestic poultry farms, principally of avian influenza subtype H5N8 with some infections of subtype H5N5. Several turkey fattening farms were affected. This was the largest outbreak in one area ever recorded in Germany, with about 30 farms affected. Culling and cleaning procedures, commercial restrictions and compensation led to high costs (estimated at EUR 500 000 per farm, depending on the number of hold poultry). Epidemiological connections were initially unknown to authorities, which therefore sought the help of FLI. Analysis using whole-genome sequencing was able to indicate that transmission occurred not only through wild birds but also through secondary infection between farms, exposing gaps in biosecurity measures in addition to other potential risk factors.[c)] The regional outbreak in Lower Saxony was part of a larger outbreak of HPAI across Germany, with more than 1 150 cases of H5Nx infection reported in wild birds and 107 outbreaks among birds kept in captivity (including both poultry and zoos) between November 8, 2016 and September 30, 2017, resulting in the death or slaughtering of approximately 1.2 million birds. Estimated direct economic losses of the total outbreak across Germany were about EUR 17 million.[f)]

Type of sample	Isolates		
Region covered by sampling	Lower Saxony, Germany		
Number of samples analysed in reference period	*Pathogen*	*Samples analysed by conventional methods*	*Samples sequenced using WGS*
	H5 Highly Pathogenic Avian Influenza Virus	The cost calculation is based on previous experiences with the listed conventional method, assuming the same number of samples as with WGS	30
Conventional method used as reference for costing	▸ Sanger sequencing of complete genomes ▸ Manual sample preparation ▸ 13 PCR products per sample, 2-fold coverage		
Sample preparation WGS	▸ Manual sample preparation		
Sequencer used for WGS	▸ Ion Torrent PGM bundle		
Batch size for WGS analysis	▸ The data provided is based on batches of 6 samples per sequencing run.		
Reference dataset used for WGS	▸ FLI maintains its own reference dataset for avian influenza, which is manually created and curated. The dataset is updated via public databases on a regular basis. Data are also shared between reference laboratories prior to publication.		
Additional information	▸ Activities covered by this case study include analyses of known avian influenza samples within the context of the relevant outbreak. ▸ Note that FLI is a research institution handling a large number of different pathogens of varying virulence. To avoid cross-contaminations, very strict laboratory procedures are applied, as was emphasised by FLI. This may lead to increased staff time and consumable costs for specific analyses. For example, when handling samples, gloves are changed after each analytical step.		

III. Detailed overview of costs of WGS and conventional methods

In the following, all costs are provided on a per-sample basis. Equipment costs are annualised and incorporate the annual maintenance costs as reported by the institution. They are adjusted for the percentage use of the equipment for the listed pathogens samples during the reference period (i.e. if a sequencer was also used for other purposes, this is taken into account). Consumables costs are adjusted for the failure rate (i.e. the percentage of consumables wasted, e.g. due to failed runs). Staff time is provided in terms of the minutes of hands-on staff time per sample, for both professionals and technicians. For the calculation of total costs, staff time is then monetised based on Eurostat data on country-specific labour costs for 2017 (by staff category), plus a 25% surcharge for overheads. For comparison purposes only, we have also provided staff costs monetised based on EU average labour costs.

More detailed cost data is provided in Annex I.

a) Costs of using WGS

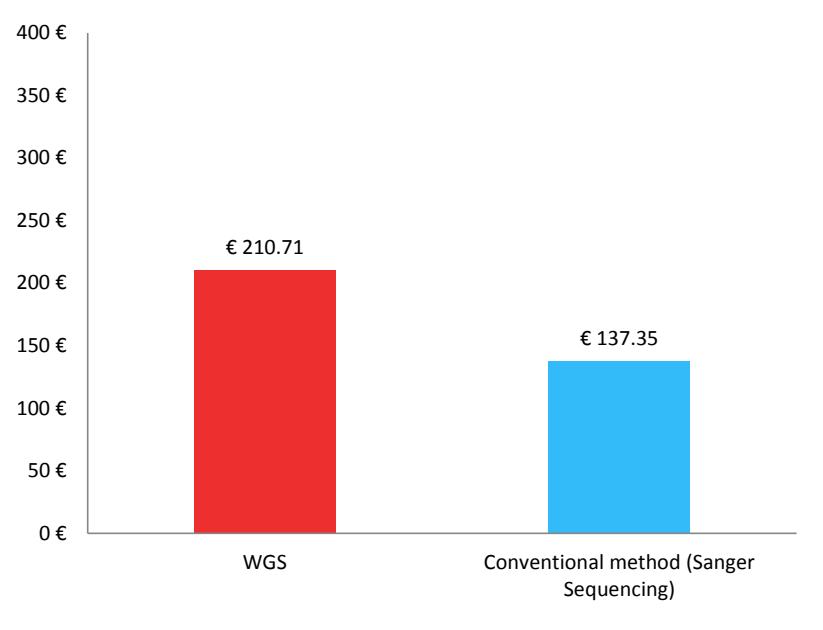
Sample preparation and sequencing	*Cost type*	*Cost per sample*
	Equipment costs	€ 198.79
	Consumables	€ 254.88
	Other costs	€ 0

	Staff time professionals	*18 minutes*
	Staff time technicians	*135 minutes*
	Staff costs, monetised based on labour cost data for Germany (in brackets: based on labour cost data for the EU as a whole)	€ 76.16 (68.66)
	Total	**€ 529.83**
Bioinformatics and other analyses	*Cost type*	*Cost per sample*
	Equipment costs	€ 11.92
	Other costs	€ 0
	Staff time professionals	*30 minutes*
	Staff time technicians	*0 minute*
	Staff costs, based on labour cost data for Germany (for EU)	€ 26.63 (22.56)
	Total	**€ 38.54**
b) Costs of conventional method (based on previous experiences with the listed method)		
Sanger Sequencing of an entire genome (assuming a use for 100% of avian influenza samples)	*Cost type*	*Cost per sample*
	Equipment costs	€ 137.35
	Consumables	€ 360.88
	Other costs	€ 0
	Staff time professionals	*260 minutes*
	Staff time technicians	*240 minutes*
	Staff costs, based on labour cost data for Germany (for EU)	€ 337.75 (293.54)
	Total	**€ 835.98**

IV. Costs of using WGS compared to the costs of conventional methods

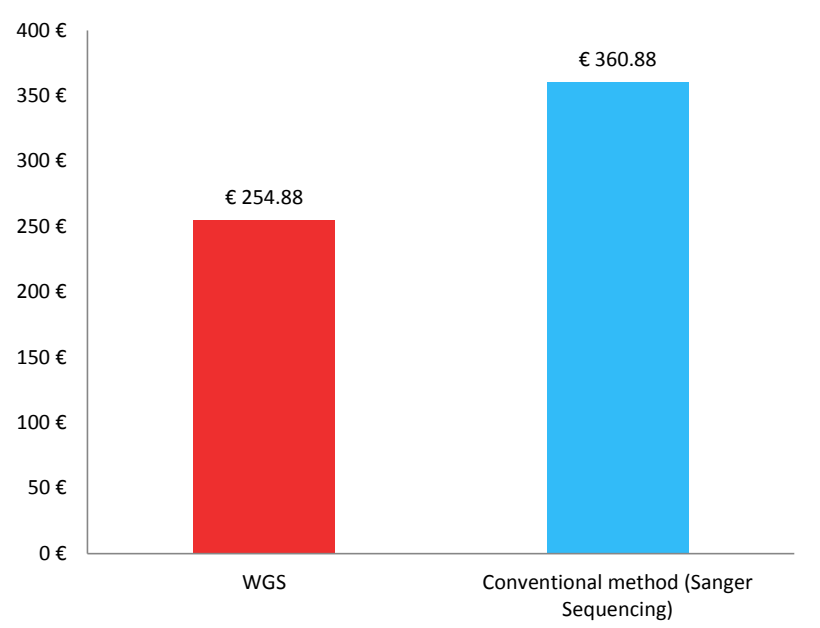
The following provides a comparison of costs per sample using WGS compared to the costs of conventional methods. See Annex I for more details.

Comparison of equipment costs



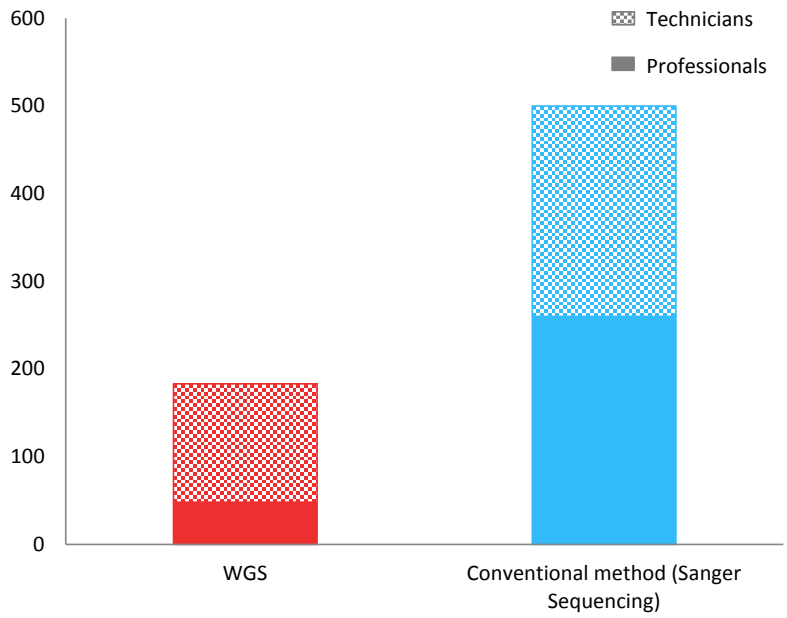
Equipment costs are significantly higher for WGS than for Sanger sequencing of an entire genome (€ 210.71 vs. € 137.35 per sample), mostly due to the purchase and maintenance costs of the IonTorrent sequencer itself.

Comparison of costs of consumables



In contrast, costs of consumables for WGS are lower than for Sanger sequencing of an entire genome (€ 254.88 vs. € 360.88 per sample). This is mostly attributable to the cost of consumables used for library preparation and sequencing, which are higher for Sanger sequencing of an entire genome.

Comparison of staff time used



The amount of staff time needed for WGS is considerably lower than for Sanger sequencing of an entire genome; however, comparatively more professional time is required for WGS, especially at the bioinformatics stage, which is exclusively conducted by professionals. Nevertheless, after monetising staff time, staff costs per sample are still more than three times higher for Sanger sequencing of an entire genome (see table below).

Comparison of overall costs	*Cost type*	*Cost per sample (WGS)*	*Cost per sample (Sanger Sequencing)*
	Equipment costs	€ 210.71	€ 137.35
	Consumables	€ 254.88	€ 360.88
	Other costs	€ 0	€ 0
	Staff time professionals	*48 minutes*	*260 minutes*
	Staff time technicians	*135 minutes*	*240 minutes*
	Staff costs, based on labour cost data for Germany (for EU)	€ 102.79 (91.23)	€ 337.75 (293.54)
	Total	**€ 568.37**	**€ 835.98**

Summary of differential costs	**A sample analysed with the use of WGS costs less than the cost of analysis with the conventional method (Sanger sequencing of an entire genome), with a cost difference of € 267.61 per sample** (€ 568.37 vs € 835.98). As indicated in the figures above, major differences in costs were found to exist in all cost categories, but especially regarding staff time.

V. Effects of using WGS results	
a) Turnaround time. Turnaround time is defined as the usual number of days of work from receipt and opening of an incoming sample until the reporting of results. Turnaround time does not include weekends and holidays, except in case that work has been conducted on these days, e.g. for a sequencing run or other analyses.	
Turnaround time	The turnaround time for the analysis of an avian influenza sample is: ▸ 4 days of work using WGS (sequencing of the full genome), compared to ▸ 8 days of work for pathogen whole genome sequencing using Sanger Sequencing. While conventional methods are therefore able to provide a fast identification of high vs. low pathogenicity of a given AI sample, WGS provides additional information on virus reassortment as well as the phylogenetic relationships. (FLI also provided the hypothetical turnaround time, if Sanger Sequencing was used to only analyse the HA segment for HPAI LPAI discrimination: This would take 2 days of work.)
b) Positive effects of using WGS for pathogen identification and surveillance during the reference period	
Sampling and sampling strategies	▸ Little or no positive effects on sampling and sampling strategies are expected from FLI's perspective despite the fact that less material is needed in terms of starting material from the extracted nucleic acids.
Analytical results and processes	▸ Overall FLI sees little evidence so far of positive effects of WGS on analytical results and processes (e.g. on the simplification of laboratory flows or consumables needed for the analysis), although it did report a clear reduction in the necessary staff time, especially when comparing WGS with Sanger sequencing of complete genomes. ▸ The institution nonetheless reported very significant positive effects of WGS on the level of detail of results produced, as well as moderately positive effects on the sensitivity of the results and reduction of overall costs for the analysis.
Outbreak identification and response	▸ Significant improvements were reported regarding the ability to detect that outbreaks are related, improved information on outbreak epidemiology (e.g. the ability to link cases to the source of infection), and a reduction in the number of secondary outbreaks. In particular, the use of WGS was able to confirm that transmission in the relevant outbreak occurred not just through wild birds but also through secondary infections between farms, highlighting potential gaps in biosecurity measures.[c),d)] Accordingly, FLI also identified positive effects regarding improved information for imposing additional control/biosecurity measures, as well as a reduction in the duration of outbreaks. ▸ FLI indicated that the genetic data provided a lot of information (on waves, clusters, and possible sources) and therefore provided hints towards certain transmission routes, allowing for some possibilities to be clearly ruled out. For example, in the present case study, FLI indicated that there were two consecutive outbreaks on one farm, raising questions regarding the effectiveness of the cleaning measures performed after the first outbreak; however, WGS analysis showed that the second outbreak on the same farm was caused by a later strain of the virus and was therefore the result of a separate introduction. ▸ Fewer benefits of WGS were reported with respect to earlier detection of the initial outbreak, given that FLI worked with samples that had already been positively identified through conventional methods. Fewer benefits were also noted with respect to a reduction in the disease burden and reduction in overall costs for outbreak identification and response.
Research and methods	▸ Regarding the positive effects on research and methods applied, FLI

applied	reported very significant improvement in the understanding of disease transmission and in epidemiological methods. FLI indicated that the same results could not be achieved with Sanger sequencing due to the level of sensitivity required.
Effects on wider society	▸ The institution considered that the use of WGS leads to positive effects for the wider society especially in relation to a reduction in the costs of outbreak(s), including through the reduction of compensation payments, and also a reduction in negative effects of the outbreak on trade (although only to a moderate extent).
c) Negative effects of using WGS	
Negative effects of using WGS	There are concerns from the industry perspective that WGS can uncover suboptimal practices e.g. in trade, biosecurity, diagnostics etc. In the present case study, for example, WGS was able to identify substantial gaps in farm biosecurity measures that contributed to the farm-to-farm transmission of avian influenza within Lower Saxony.[c),f)] Such findings could contribute e.g. to lower compensation payments or other questions of liability where secondary infections result in large economic losses. FLI indicated that to avoid a reduction in cooperation, the use of very detailed techniques and data analyses needs a proactive and careful communication strategy.
VI. Outlook	
Balance of costs and benefits achieved	▸ The efforts currently required for WGS analysis as well as the associated costs (especially equipment) are high, but it is expected that the costs of sequencing and analysis will come down, driven by the demand for sequencing. This is already the case to some extent (e.g. the cost of sequencers have already come down significantly) and the balance of costs and benefits is expected to improve in the mid- to long term.
Potential for cost reductions	▸ FLI is in the process of introducing further automation for sample preparation, which is expected to lead to a substantial reduction in hands-on staff time. ▸ In the study of the Influenza outbreak considered here, the only significant cost reduction could have been achieved by higher multiplexing in the sequencing run. This, however, would have resulted in extended turnaround times, and was therefore in this case avoided. With regard to cross-pathogen detection, FLI indicated that sample preparation was the most expensive stage and that therefore further cost reductions at the lab level could be possible with the use of different methods. This is however not feasible at the moment. ▸ Using such new methods, the costs of consumables would also be expected to decrease.
Future opportunities and challenges	▸ In the veterinary field (with a strong focus on notifiable diseases, which are well-known and for which PCR tests are available) WGS would only be used as a first step in rare cases where a diagnosis is unclear or where a novel or unknown pathogen is concerned, as WGS is much more expensive overall. Especially in case of an outbreak, under the current cost conditions, PCR would be the method of choice for initial identification of the pathogen. ▸ In the institution's perspective, the most relevant use of the cross-pathogen potential of WGS at this stage is human diagnostics in a clinical context, often through a national reference centre. For instance, FLI often receives requests regarding cases in the human field, where a hospital has an urgent case in which the pathogen could not be identified after running 30-60 PCRs (e.g. for cases of Encephalitis). These cases show most clearly the benefits of WGS and may be more economical to investigate with WGS

	rather than with multiple disease specific tests. The difficult nature of WGS for diagnostics nonetheless remains a challenge. It is expected to take at least 5-10 years before it is so simple that it can be used broadly (similarly to the past development regarding PCR diagnostics). ▸ The institution considered that metagenomics is still more of a niche topic. The analysis of an unknown pathogen for a metagenomic analysis would require more preparation, and more sequencing runs with fewer samples per run and more depth. ▸ Data accuracy is an area of concern with respect to the use of public databases, where there is a need for greater curation and validation by specialists. Data security will also be an emerging concern that will slow down the pace of analysis.

VII. Key sources/references	
Cost questionnaire	Cost questionnaire completed by FLI
Preparatory phone interview	a) Background information and description of activities
Case study visit and follow up	b) Additional data and clarifications provided
Scientific literature	c) Conraths, F. J. (2017). Making worst case scenarios real: The introduction of highly pathogenic avian influenza of subtype H5N8 led to the largest fowl plague outbreak ever recorded in Germany. *Lohmann Information*, 51(1), 36–41. d) Conraths, F. J., et al. (2017). *Epidemiologie des aktuellen Geflügelpestgeschehens in Deutschland* [Epidemiology of the current incidence of avian influenza in Germany], presentation given at the meeting of the Gesellschaft der Förderer und Freunde für Geflügel- und Kleintierforschung e.V. at the Institut für Tierschutz und Tierhaltung in Celle on 3 May 2017. e) Friedrich-Loeffler-Institut. (2017). *Qualitative Risikobewertung zur Einschleppung sowie zum Auftreten von hochpathogenem aviären Influenzavirus H5 in Hausgeflügelbestände in Deutschland*. f) Globig, A., et al (2018). Highly Pathogenic Avian Influenza H5N8 Clade 2.3.4.4b in Germany in 2016/2017. *Frontiers in Veterinary Science*, 4(January), 2–9. http://doi.org/10.3389/fvets.2017.00240 g) Grund, C., et al. (2018). A novel European H5N8 influenza A virus has increased virulence in ducks but low zoonotic potential. *Emerging Microbes and Infections*, 7(1), 1–14. http://doi.org/10.1038/s41426-018-0130-1 h) Pohlmann, A., et al. (2018). Swarm incursions of reassortants of highly pathogenic avian influenza virus strains H5N8 and H5N5, clade 2.3.4.4b, Germany, winter 2016/17. *Scientific Reports*, 8(1), 8–13. http://doi.org/10.1038/s41598-017-16936-8 i) Pohlmann, A., et al (2017). Outbreaks among Wild Birds and Domestic Poulty Caused by Reassorted Influenza A(H5N8) Clade 2.3.4.4 Viruses, Germany, 2016. *Emerging Infectious Diseases*, 23(4), 633–636. http://doi.org/http://dx.doi.org/10.3201/eid2304.161949
Other	j) FLI website, https://www.fli.de/en

4.3. Erasmus Medical Centre (EMC)

Influenza surveillance – Erasmus MC, NL			
I. Institution			
Name of institution	Erasmus University Medical Centre (Erasmus MC)		
Type of institution	University hospital		
Description [c)]	Erasmus MC is the largest university hospital in the Netherlands. It conducts research in various fields, studying fundamental and clinical domains as well as public health and prevention. The Department of Viroscience at Erasmus MC has expertise ranging from basic virology to clinical virology, connecting medical and veterinary health, public health and ecology. The Department of Viroscience at Erasmus MC is the national reference centre for influenza and emerging infections in the Netherlands, as well as a WHO Collaborating Centre on viral infections.		
Location	Rotterdam, NL		
II. Surveillance activities covered by case study			
Activity	Routine laboratory surveillance		
Reference period	11/2018 – 01/2019		
Pathogen(s) covered	Influenza virus		
Summary of routine surveillance activities using WGS	Nanopore sequencing with the use of the GridION platform, a third generation sequencing approach, was introduced for routine surveillance of influenza at Erasmus MC at the beginning of the influenza virus season in November 2018. Nanopore sequencing replaced conventional Sanger sequencing for the 2018/2019 influenza virus season.		
Type of sample	Clinical samples		
Region covered by laboratory surveillance	The Netherlands		
Number of samples analysed in reference period	*Pathogen*	*Samples analysed by conventional methods*	*Samples sequenced using WGS*
	Influenza A (H1N1, H3N2) and B	The cost calculation is based on previous experiences with the listed conventional methods, assuming the same number of samples as with WGS	178
Conventional methods used as reference for costing	▸ Influenza: Real Time PCR (100%), Sanger Sequencing (5%)		
Sample preparation WGS	▸ Manual sample and library preparation		
Sequencer used for WGS	▸ Nanopore GridION		
Batch size for WGS analysis	▸ The typical batch size increased over the flu season from 10 to 40, with an average batch size of 20 samples		
Reference dataset used for WGS	▸ Erasmus MC does not maintain its own internal reference database, but downloads data as needed from public databases (notably GISAID). It uses the new vaccine strains as reference strains each season.		

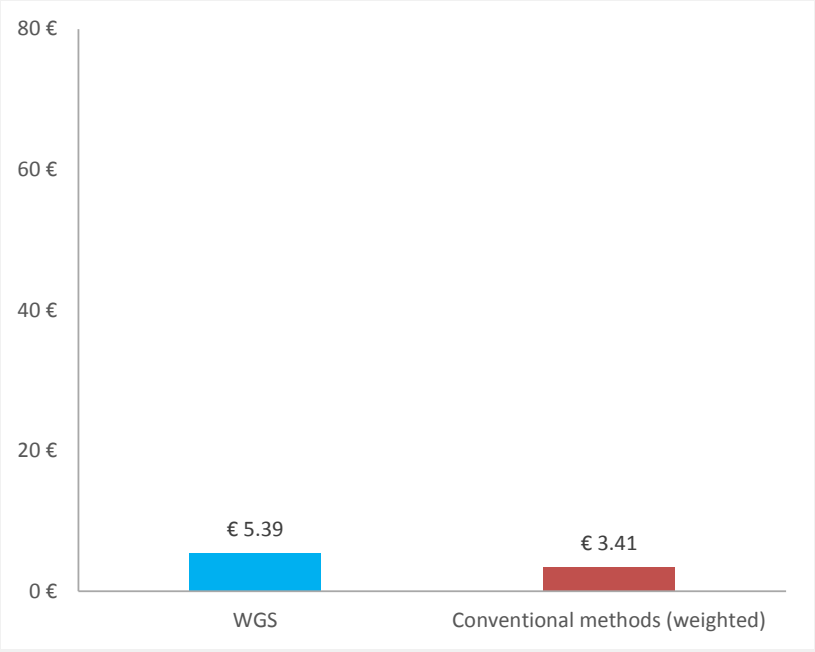
III. Detailed overview of costs of WGS and conventional methods		
In the following, all costs are provided on a per-sample basis. Equipment costs are annualised and incorporate the annual maintenance costs as reported by the institution. They are adjusted for the percentage use of the equipment for the listed pathogens samples during the reference period (i.e. if a sequencer was also used for other purposes, this is taken into account). Consumables costs are adjusted for the failure rate (i.e. the percentage of consumables wasted, e.g. due to failed runs). Staff time is provided in terms of the minutes of hands-on staff time per sample, for both professionals and technicians. For the calculation of total costs, staff time is then monetised based on Eurostat data on country-specific labour costs for 2017 (by staff category), plus a 25% surcharge for overheads. For comparison purposes only, we have also provided staff costs monetised based on EU average labour costs. More detailed cost data is provided in Annex I.		
a) Costs of using WGS		
Sample preparation and sequencing	*Cost type*	*Cost per sample*
	Equipment costs	€ 3.74
	Consumables	€ 54.88
	Other costs	€ 0
	Staff time professionals	*6 minutes*
	Staff time technicians	*72 minutes*
	Staff costs, monetised based on labour cost data for the Netherlands (in brackets: based on labour cost data for the EU as a whole)	€ 39.20 (€ 33.91)
	Total	**€ 97.82**
Bioinformatics and other analyses	*Cost type*	*Cost per sample*
	Equipment costs	€ 1.65
	Other costs	€ 0
	Staff time professionals	*18 minutes*
	Staff time technicians	*48 minutes*
	Staff costs, based on labour cost data for the Netherlands (for EU)	€ 38.54 (€ 33.14)
	Total	**€ 40.19**
b) Costs of conventional methods		
Method A: Real Time PCR	*Cost type*	*Cost per sample*
	Equipment costs	€ 2.11
	Consumables	€ 31.00
	Other costs	€ 0
	Staff time professionals	*0 minutes*
	Staff time technicians	*36 minutes*
	Staff costs, based on labour cost data for the Netherlands (for EU)	€ 16.94 (€ 14.70)
	Total	**€ 50.05**
Method B: Sanger	*Cost type*	*Cost per sample*

Sequencing	Equipment costs	€ 25.87
	Consumables	€ 23.75
	Other costs	€ 0
	Staff time professionals	*0 minutes*
	Staff time technicians	*36 minutes*
	Staff costs, based on labour cost data for the Netherlands (for EU)	€ 16.94 (€ 14.70)
	Total	**€ 66.56**

IV. Costs of using WGS compared to the costs of conventional methods

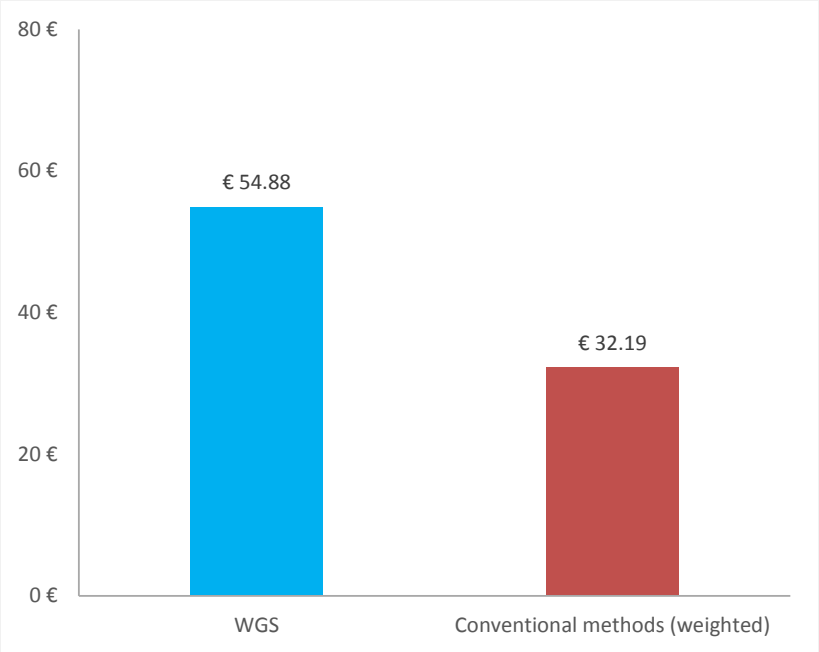
The following comparison of costs per sample using WGS compared to the costs of conventional methods considers that the number of samples processed differed for the different conventional methods. The weighted cost of the conventional methods provided here is therefore a weighted figure which accounts for the use rate of the various methods across the different pathogens. See Annex I for more details.

Comparison of equipment costs



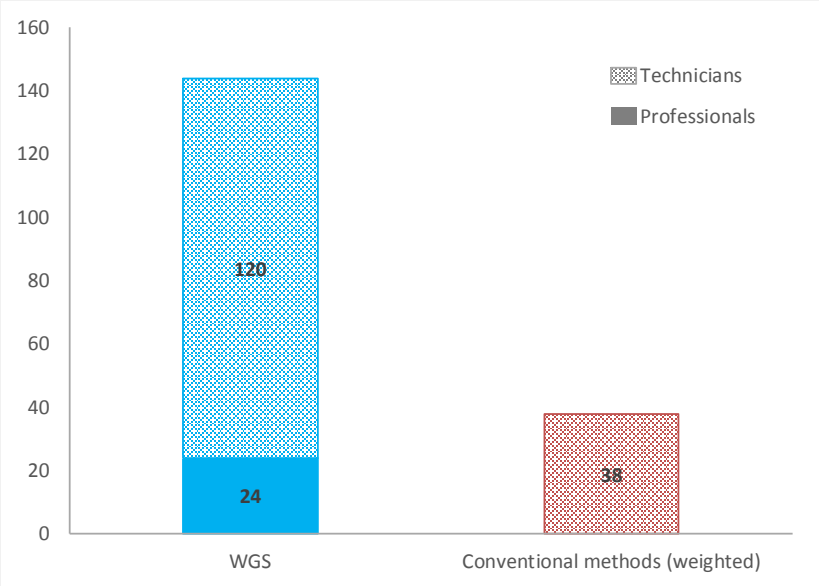
Equipment costs per sample at Erasmus MC are higher for WGS than for the weighted conventional methods (€ 5.39 vs € 3.41), although the absolute per-sample cost difference between the two methods is quite low, with an additional per-sample cost of WGS of only € 1.98. The equipment costs of the weighted conventional methods are largely determined by the costs of real time PCR, used for 100% of samples. In contrast, only 5% of samples had undergone further analysis with Sanger sequencing, reducing the relevance of this method as a cost factor.

Comparison of costs of consumables



Consumables costs for WGS clearly exceed those for conventional methods (€ 54.88 vs € 32.19). The largest cost elements for WGS are the flowcells used for Nanopore sequencing (€ 16.83 per sample, assuming an average batch size of 20) and the ligase used in library preparation (€ 15 per sample). Again, the consumables costs of the weighted conventional methods are largely determined by the costs of real time PCR, as only 5% of samples had undergone further analysis with Sanger sequencing.

Comparison of staff time used (in minutes)



WGS requires nearly three times as much technician staff time as conventional methods (120 minutes vs 38 minutes). It also requires an additional 24 minutes of professional time, mostly at the bioinformatics stage, whereas conventional methods do not require any professional staff time. Once monetised, staff costs are therefore considerably higher for WGS (€ 77.74) than for conventional methods (€ 17.79), and make up the most expensive cost item for WGS overall. As for the other costs, hands-on staff time used for the weighted conventional methods is largely determined by the real time PCR, as only 5% of samples had undergone further analysis with Sanger sequencing.

Comparison of overall costs	*Cost type*	*Cost per sample (WGS)*	*Cost per sample (conventional methods)*
	Equipment costs	€ 5.39	€ 3.41
	Consumables	€ 54.88	€ 32.19
	Other costs	€ 0	€ 0
	Staff time professionals	*24 minutes*	*0 minutes*
	Staff time technicians	*120 minutes*	*37.80 minutes*
	Staff costs, based on labour cost data for the Netherlands (for EU)	€ 77.74 (€ 67.05)	€ 17.79 (€ 15.44)
	Total	**€ 138.01**	**€ 53.38**

Differential costs	**The cost difference between WGS and conventional methods is € 84.63 per sample**. A sample analysed with WGS costs approximately 1.6 times more than analysis with conventional methods (when taking into account the use rate of the various methods). As indicated in the figures above, the largest differences are in staff and consumables costs.

V. Effects of using WGS results

a) Turnaround time. Turnaround time is defined as the usual number of days of work from receipt and opening of an incoming sample until the reporting of results. Turnaround time does not include weekends and holidays, except in case that work has been conducted on these days, e.g. for a sequencing run or other analyses.

Turnaround time	The turnaround time using the GridION is typically 2 days of work. This can be compressed to just 8-10 hours in an outbreak context, with some basic information about the sample available within the first 2-3 hours. In contrast, the turnaround time for conventional methods (PCR and Sanger sequencing) is approximately 3 days of work. In an outbreak context, this can be brought down to about 20 hours with Sanger sequencing directly on clinical material, which is performed in parallel to cultivation of the virus in eggs (which still takes 3 days). In an outbreak context, the average one day reduction in turnaround time due to WGS is reported to be very significant.

b) Positive effects of using WGS for pathogen identification and surveillance during the reference period

Sampling and sampling strategies	▸ No effects on sampling or sampling strategies were reported by Erasmus MC, as they receive clinical samples submitted by hospitals. However, Erasmus MC considered that better sampling methods could be expected in the future as a result of WGS.
Analytical results and processes	▸ Very significant positive effects were observed by Erasmus MC with respect to more detailed results produced due to NGS technology. This is due to the fact that all virus samples were now being sequenced, whereas prior to the introduction of the GridION only 5% would have undergone further analysis using Sanger sequencing. ▸ Erasmus MC reported no effects on the accuracy or specificity of results, and in fact reported negative effects on the specificity of results (see 'Negative effects of WGS' below). ▸ Moderate effects were reported with respect to a reduction in time needed for analysis. While the hands-on staff time needed increased for WGS compared to conventional methods, overall a reduction in

	turnaround time was reported for WGS (see above). This is due to the fact that more waiting periods (e.g. for viral amplification) are required for conventional methods compared to WGS. No effects were observed with respect to simplified workflows or a reduction in consumables.
Outbreak identification and response	▸ Erasmus MC reported very significant positive effects for the earlier detection of an initial outbreak and for improved detection that outbreaks are related. However, it specified that in an international context, the benefits from improved detection that outbreaks are related depended on whether partner institutions had also adopted WGS. It indicated that the benefits of WGS for detection of international outbreaks were limited if the partners still relied on conventional methods, as the results from these methods were often not comparable with results from WGS. ▸ Erasmus MC indicated that it had insufficient information with respect to possible effects on improved information through WGS for imposing additional control measures or reductions in the duration of an outbreak, in the number of secondary outbreaks, or in overall costs for outbreak identification and response. However, such effects were considered very likely to materialise in the long run (especially for other pathogens). For example, it indicated that the faster turnaround time with Nanopore sequencing could allow patients to be isolated earlier or receive more personalised medical treatment (however, this was not considered to be relevant with respect to the case study pathogen).
Research and methods applied	▸ No concrete effects on research or methods applied were reported by Erasmus MC.
Effects on wider society	▸ No concrete effects on wider society were observed by Erasmus MC during the case study period, although it was considered that such effects would likely emerge over time.
c) Negative effects of using WGS	
Negative effects of using WGS	▸ Erasmus MC reported negative effects on the sensitivity of results with WGS due to the fact that it now skips the viral cultivation step and uses a PCR approach directly on clinical samples. This is reported to save time, but results in slightly less sensitivity. Erasmus MC clarified that this is a 'problem' of internal workflow, however, not of the technology, and that the problem is not limited to Nanopore sequencing but concerns WGS in general. ▸ Erasmus MC reported limitations of Nanopore sequencing related to a failure of basecalling for homopolymeric regions in the sequences (i.e. errors in reading multiples of the same nucleotide base appearing consecutively in the DNA sequences). Erasmus MC indicated that this is a known problem specific to Nanopore sequencing and that the technology is expected to improve in the near future.
VI. Outlook	
Balance of costs and benefits achieved	▸ Erasmus MC indicated that Nanopore sequencing is a 'game changer', yet not as much as they would like due to the high prices of the required flowcells. While the costs are lower compared to e.g. Illumina sequencing, the costs are still significant. However, it was also noted that in an outbreak context 'time is more important than money', and the reduction in turnaround time was therefore considered to be very valuable.
Potential for cost reductions	▸ Erasmus MC considered that current prices (e.g. for flowcells) were relatively high, and that substantial cost reductions could be achieved through negotiation with suppliers, or increased competitive pressure.

Future opportunities and challenges	▸ Erasmus MC considered that Nanopore sequencing technology was constantly improving, with the above mentioned failure of basecalling for homopolymeric regions likely to be fixed in the very near future. ▸ The high price of the flowcells, which are only provided by one company (Oxford Nanopore), was noted as a challenge by Erasmus MC. The company also places contractual restrictions on the use of the flowcells purchased through the institutional contract between Erasmus MC and Oxford Nanopore, e.g. regarding their use outside the premises of Erasmus MC, and thereby limiting usefulness for field research and real-time analysis of outbreaks by Erasmus MC staff visiting other countries, such as China (however, the contract is in the process of being re-negotiated to remove these geographical restrictions at least partly). ▸ Erasmus MC reported that better communication was needed with hospitals to ensure that the hospitals send samples with higher viral loads in the future in order to counteract the lower sensitivity that can result from the use of metagenomic analysis without viral amplification.

VII. Key sources/references	
Cost questionnaire	Cost questionnaire completed by Erasmus MC
Preparatory phone interview	a) Background information and description of activities
Case study visit and follow up	b) Additional data and clarifications provided by the institution.
Scientific literature	As Nanopore sequencing was introduced for routine influenza surveillance at Erasmus MC for the first time during the case study period, no scientific literature related to the case study has been published yet by Erasmus MC.
Other	c) Erasmus MC Department of Viroscience website, https://www6.erasmusmc.nl/viroscience/

4.4. Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna (IZSLER)

Salmonella and Listeria surveillance – IZSLER, Italy	
I. Institution	
Name of institution	Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna (IZSLER)
Type of institution	Public veterinary institution
Description[19]	The Lombardy and Emilia-Romagna Experimental Zooprophylaxis Institute (IZSLER) is a public body entrusted with independent management, administrative and technical powers. It operates as a technical scientific institution of the state, the regions and the autonomous provinces. IZSLER's territory of jurisdiction comprises the regions of Lombardy and Emilia-Romagna in northern Italy and it is part of a network of regional institutes that covers all of Italy. The Institute's main tasks are the following: ▸ Animal diseases and zoonoses diagnostic service; ▸ Laboratory control on foodstuffs for human and animal consumption; ▸ Epidemiological monitoring in the ambit of animal health and in that of hygiene of zootechnic and foodstuff production; ▸ Analytic and advisory support to the carrying out of epidemic prevention, sanitation and eradication plans; ▸ Applied research in the field of breeding hygiene and improvement of zootechnic production and animal wellbeing; ▸ Applied and basic experimental research in the veterinary and food area. IZSLER's High Specialisation Centres carry out highly specialised activities in the field of animal health, food hygiene and zootechnic hygiene. In particular, IZSLER was appointed as the National Reference Centre for numerous diseases by the Ministry of Health, as the OIE Reference Laboratory for Foot-and-Mouth Disease, Swine Influenza, Myxomatosis, and Haemorrhagic Diseases of Lagomorphs, and as the FAO collaboration centre for Foot-and-Mouth Disease.
Location	While IZSLER's main office is located in Brescia, Italy, units are distributed on a provincial basis to cover the Lombardy Territorial Area and the Emilia-Romagna Territorial Area.
II. Surveillance activities covered by case study	
Activity	Routine laboratory surveillance
Reference period	01/2017 – 12/2017
Pathogen(s) covered	Salmonella, Listeria
Summary of routine surveillance activities using WGS	Since 2012, IZSLER routinely processes isolates of *Salmonella enterica* from human, animal and food sources as part of the One Health surveillance of foodborne infections based on PFGE, MLVA and serotyping. Isolates belonging to significant outbreaks have been sequenced and compared with SNPs and Gene-by-Gene approaches to highlight phylogenetic relationships and attribute source of infections. The same workflow is applied to isolates

[19] Source: http://www.izsler.it/izs_home_page/who_we_are_/00000047_English.html

	of *Listeria monocytogenes*. WGS is currently used as a confirmation method, and has also been used to retrospectively study past outbreaks.[c)-f)] The reference period of 2017 was a transition year, which extended to include 2018; the institute will switch to the full routine use of WGS in 2019, thereby stopping the use of conventional methods in parallel. The main reason for this is the information potential of whole genome sequencing and the potential for improving surveillance/public health. According to IZSLER, this was also requested by the industry, as major food producers, including export industries, are located in the region, e.g. in Parma.		
Type of sample	Isolates		
Region covered by sampling	Emilia-Romagna, Italy		
Number of samples analysed in reference period	*Pathogen*	*Samples analysed by conventional methods*	*Samples sequenced using WGS*
	Salmonella	1500	110 (7.3% of samples)
	Listeria	65	65 (100% of samples)
Conventional methods used	▸ Salmonella: Serotyping (100% of samples), PFGE (100%), PCR Verification for Typhimurium (50%), MLVA (60%) ▸ Listeria: PFGE (100%)		
Sample preparation WGS	▸ Manual		
Sequencer used for WGS	▸ MiSeq (Illumina)		
Batch size for WGS analysis	▸ The typical batch size for WGS analysis during the reference period was 24.		
Reference dataset used for WGS	▸ IZSLER uses its own reference dataset based on the analyses conducted, and regularly checks international databases for relevant new entries, which are then included into the database if necessary. The institution indicated that public databases have the advantage that data is available and can always be re-analysed, but noted that issues remain regarding data and metadata quality in such public databases.		
Additional information	▸ In the reference year, the institute had not used WGS to identify outbreaks but only to confirm or further analyse outbreaks that had already been identified through the use of conventional methods. Therefore, all sequenced isolates had already been typed using conventional methods. ▸ As indicated above, IZSLER has responsibilities with regard to both animal health and food safety. For the two pathogens covered by this case study, the institute routinely analyses isolates originating from animal infections, food samples, and human cases of infection, as part of a One Health approach to surveillance.		

III. Detailed overview of costs of WGS and conventional methods

In the following, all costs are provided on a per-sample basis. Equipment costs are annualised and incorporate the annual maintenance costs as reported by the institution. They are adjusted for the percentage use of the equipment for the listed pathogens samples during the reference period (i.e. if a sequencer was also used for other purposes, this is taken into account). Consumables costs are adjusted for the failure rate (i.e. the percentage of consumables wasted, e.g. due to failed runs). Staff time is provided in terms of the minutes of hands-on staff time per sample, for both professionals and technicians. For the calculation of total costs, staff time is then monetised based on Eurostat data on country-specific labour costs for 2017 (by staff category), plus a 25% surcharge for overheads. For comparison purposes only, we have also provided staff costs monetised based on EU average labour costs.

More detailed cost data is provided in Annex I.

a) Costs of using WGS

Sample preparation and sequencing	*Cost type*	*Cost per sample*
	Equipment costs	€ 123.07
	Consumables	€ 165.37
	Other costs	€ 0
	Staff time professionals	*0 minutes*
	Staff time technicians	*35 minutes*
	Staff costs, monetised based on labour cost data for Italy (in brackets: based on labour cost data for the EU as a whole)	€ 13.93 (14.29)
	Total	**€ 302.38**

Bioinformatics and other analyses	*Cost type*	*Cost per sample*
	Equipment costs	€ 40.41
	Other costs	€ 0
	Staff time professionals	*70 minutes*
	Staff time technicians	*0 minutes*
	Staff costs, based on labour cost data for Italy (for EU)	€ 52.35 (52.65)
	Total	**€ 92.77**

b) Costs of conventional methods

Serotyping (used for 100% of Salmonella samples)	*Cost type*	*Cost per sample*
	Equipment costs	€ 0
	Consumables	€ 7.76
	Other costs	€ 0
	Staff time professionals	*3 minutes*
	Staff time technicians	*38 minutes*
	Staff costs, based on labour cost data for Italy (for EU)	€ 17.36 (17.77)
	Total	**€ 25.12**

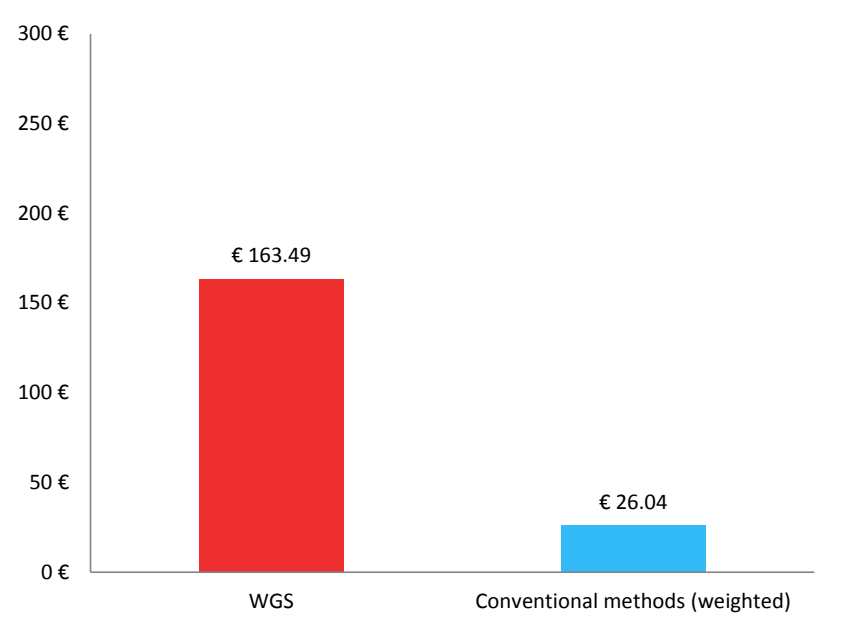
PFGE (100% of Salmonella and Listeria samples)	*Cost type*	*Cost per sample*
	Equipment costs	€ 22.84
	Consumables	€ 14.42
	Other costs	€ 0.00
	Staff time professionals	*2.5 minutes*
	Staff time technicians	*38 minutes*
	Staff costs, based on labour cost data for Italy (for EU)	€ 16.99 (17.40)
	Total	**€ 54.25**
PCR Verification for Typhimurium (50% of Salmonella samples)	*Cost type*	*Cost per sample*
	Equipment costs	€ 10.18
	Consumables	€ 2.78
	Other costs	€ 0
	Staff time professionals	*1 minute*
	Staff time technicians	*11 minutes*
	Staff costs, based on labour cost data for Italy (for EU)	€ 4.73 (4.84)
	Total	**€ 17.68**
MLVA (60% of Salmonella samples)[20]	*Cost type*	*Cost per sample*
	Equipment costs	€ 0
	Consumables	€ 0
	Other costs	€ 43.13
	Staff time professionals	*0 minute*
	Staff time technicians	*0 minute*
	Staff costs, based on labour cost data for Italy (for EU)	€ 0 (0)
	Total	**€ 43.13**

[20] Note that ISZLER has MLVA conducted externally by another lab in the network and therefore incurs no staff, consumables, or equipment costs of its own. The cost shown here is the estimated cost price.

IV. Costs of using WGS compared to the costs of conventional methods

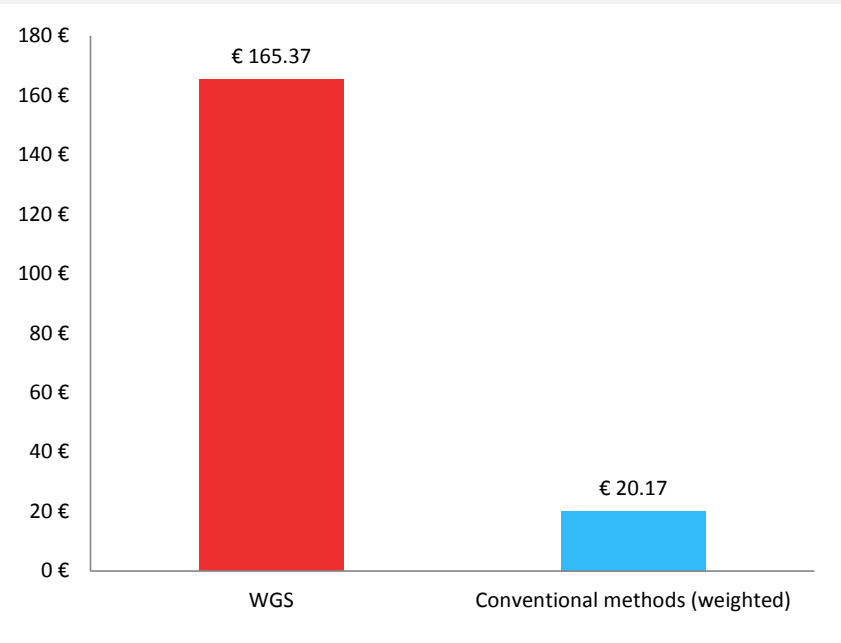
The following comparison of costs per sample using WGS compared to conventional methods takes into account the fact that the number of samples processed differed between conventional methods, e.g. serotyping is used for 100% of Salmonella samples, but MLVA is only used for 60% of Salmonella samples. The average cost of the conventional methods provided here is therefore a weighted figure which accounts for the use rate of the various methods. See Annex I for more details.

Comparison of equipment costs



Equipment costs are significantly higher for WGS (€ 163.49 vs. € 26.04 per sample), mostly due to purchase and maintenance costs of the sequencer itself. IZSLER indicated during the case study visit that larger sequencers were generally better from a cost perspective, but require a large batch size to be cost-effective. However, in a surveillance context it is not always possible to postpone analysis until a certain number of samples have accumulated.

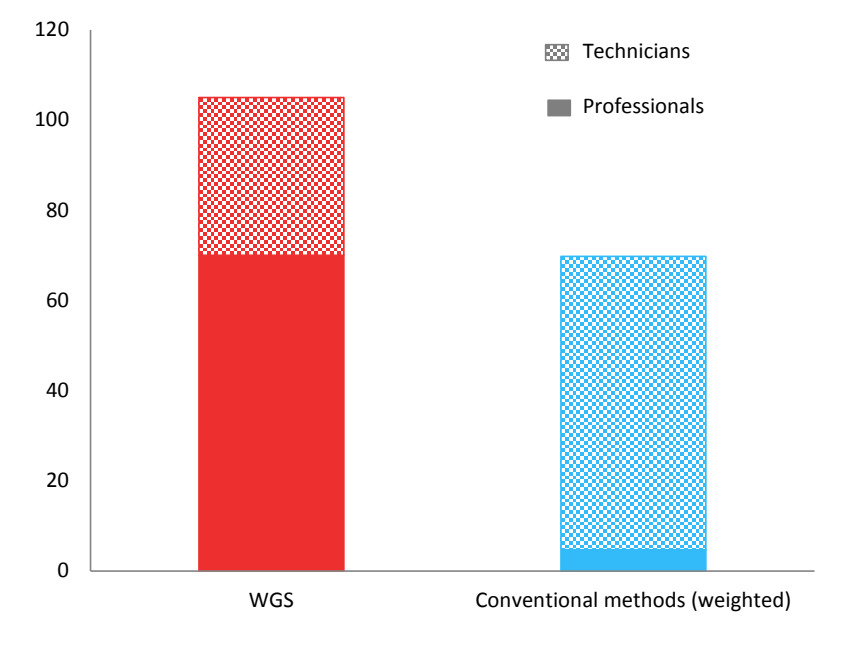
Comparison of costs of consumables



Costs of consumables for WGS are also higher than the weighted average of

conventional methods (€ 165.37 vs. € 20.17 per sample), due to the cost of consumables used for library preparation (€ 46.85 per sample using WGS) and even more importantly the cost of consumables used for sequencing (€ 114.20 per sample using WGS).

Comparison of staff time used



The amount of staff time needed for WGS is higher than for conventional methods, and the proportion of professionals' time to technicians' time is much larger for WGS. This is entirely due to the bioinformatics analysis required for WGS, as this stage is performed exclusively by professional staff, while sample preparation and sequencing are conducted exclusively by technicians. However, IZSLER indicated during the case study visit that they anticipated the bioinformatics stage to be automated for routine surveillance in the future.

Taking the different staff categories into account, monetised staff costs per sample for WGS are approximately two times the amount required for conventional methods (see table below).

Comparison of overall costs

Cost type	*Cost per sample (WGS)*	*Cost per sample (conventional methods)*
Equipment costs	€ 163.49	€ 26.04
Consumables	€ 165.37	€ 20.17
Other costs	€ 0	€ 16.27
Staff time professionals	*70 minutes*	*5 minutes*
Staff time technicians	*35 minutes*	*65 minutes*
Staff costs (monetisation based on labour cost data for Italy)	€ 66.28	€ 29.39
Staff costs (monetisation based on labour cost data for the EU)	*€ 66.94*	*€ 30.09*

	Total	**€ 395.14**	**€ 91.87**
Summary of differential costs	**The cost difference between WGS and conventional methods is € 303.27 per sample**. A sample analysed with WGS costs more than four times the amount of conventional methods (**€** 395.14 vs **€** 91.87). As indicated in the figures above, this difference is mainly due to consumables costs and equipment costs.		
V. Effects of using WGS results			
a) Turnaround time. Turnaround time is defined as the usual number of days of work from receipt and opening of an incoming sample until the reporting of results. Turnaround time does not include weekends and holidays, except in case that work has been conducted on these days, e.g. for a sequencing run or other analyses.			
Turnaround time	▸ The turnaround time for the analysis of a sample using WGS for pathogen identification is 7 days of work, compared to 10 days of work for using the specified conventional method(s) for pathogen identification.		
b) Positive effects of using WGS for pathogen identification and surveillance during the reference period			
Sampling and sampling strategies	▸ Little or no positive effects of using WGS on sampling and sampling strategies are expected from IZSLER's perspective as these are not the institution's responsibility and are independent from the institution's laboratory function. In addition, the number of samples is largely independent from the method used for analysis.		
Analytical results and processes	▸ IZSLER considered that the use of WGS for pathogen identification and surveillance has led to very significant positive effects on analytical results and processes. It reported significant improvement regarding the accuracy, sensitivity and specificity of results produced. ▸ IZSLER also indicated that WGS had led to simplified laboratory work flows, *inter alia* through the reduction of the number of hands-on steps. It also considered that WGS had led to a reduction in the amount of consumables needed for analysis and in staff time required.		
Outbreak identification and response	▸ IZSLER considered that the use of WGS for pathogen identification and surveillance has led to very significant positive effects for outbreak identification and response, and sees a reduction in the related overall costs. ▸ IZSLER reported significant improvements regarding earlier detection of initial outbreaks, detection that isolates are related, and information on outbreak epidemiology (e.g. linking cases to the source). In the institution's experience, the high resolution power of WGS is making a striking difference in pathogen typing and source attribution; this was the finding of several scientific papers published by IZSLER retrospectively examining past salmonella and listeria outbreaks using WGS.[c)-f)] In particular, a 2018 paper published by IZSLER using WGS to examine an outbreak of salmonella in 2013 concluded that PFGE and MLVA did not have the necessary resolution or accuracy, respectively, to reliably link isolates to the outbreak source, and could in fact produce misleading results.[c)-f)] ▸ Substantial advantages of WGS were therefore found to derive from the superior accuracy in the attribution of contamination responsibilities along the food chain. For example, during the above mentioned outbreak in 2013, the PFGE based surveillance system identified an outbreak of monophasic *Salmonella Typhimurium* with the potential involvement of a salami producer, a specific abattoir and a farmer. WGS and phylogenetic analyses were able to confirm the salami producer involvement in the case		

	but cleared both the farmer and the abattoir of any responsibility.[d)] ▸ IZSLER considered that WGS has also led to significant improvements regarding the information for imposing additional control/biosecurity measures. For instance, the nature (monoclonal vs polyclonal) and distribution of contamination inside food-processing facilities can be finely reconstructed by WGS. As a consequence, de-contamination of facilities can be managed and verified with high confidence. ▸ As regards the surveillance of human infections, IZSLER also considered that WGS helps identify true outbreaks, thus preventing false alerts to public health officials, and reducing the number of infections. The above quoted scientific paper concluded that, had WGS been in routine use at the time of the 2013 outbreak, the source of the outbreak could have potentially been identified up to two months earlier, possibly preventing dozens of infections if the correct mitigation measures had been taken in time. IZSLER considered that this is improving consumers' confidence in the competent authorities and in food business operators.
Research and methods applied	▸ Regarding the positive effects on research and methods applied, the institution reported very significant improvement in the understanding of disease transmission and a positive impact on epidemiological investigations.
Effects on wider society	▸ IZSLER indicated that the use of WGS has led to a significant reduction in the negative effects of food chain contamination on industry and trade relationships, and provided the example of a controversy between two operators of the Parma Ham industry following the finding of *Listeria monocytogenes* with the same PFGE type in their plants. The plants operated sequentially along the same processing chain; one was the ham producer and the second was the deboner. Considering the apparently identical contamination (based on PFGE), the operators blamed each other as the source of the contamination. WGS was able to clearly demonstrate that the isolates from the deboner and producer were unrelated despite identical having an identical PFGE type. As a result, not only were both required to improve their own hygiene procedures, but also no further commercial or legal controversy was justified. ▸ The positive impact on the food industry is also evidenced by the interest of operators in WGS and the fact that major operators have started doing their own in-house testing with WGS.
c) Negative effects of using WGS	
Negative effects of using WGS	So far, the use of WGS for pathogen identification and surveillance has not had negative effects for IZSLER, other than the currently higher costs compared to conventional methods. However, IZSLER indicates that the high resolution power of WGS might lead to the identification of a high number of smaller outbreaks which may strain existing (staff and analytical) capacities.
VI. Outlook	
Balance of costs and benefits achieved	▸ IZSLER noted that in comparison with conventional methods, using WGS is currently more expensive but should eventually reach comparable cost levels, while providing more information.
Potential for cost reductions of using WGS for pathogen identification and surveillance in the future	▸ There is a high potential for simplification of the type of samples needed for WGS with the use of metagenomics. However, IZSLER indicated that this is not expected to materialise for another 5 to 10 years. ▸ It is also expected that significant cost reduction for WGS could be achieved by scaling-up the analytical process through automation of the

(through e.g. techno-logical advances)	DNA extraction and library preparation steps. IZSLER considered that the process could eventually be almost entirely automated. ▸ Savings in the number of required staff are expected: the number of required staff for Salmonella analysis is expected to be at least halved, while maintaining the same staff categories. ▸ Technological developments might have an impact on equipment costs, although the institution noted that it is difficult to foresee how the situation will develop regarding sequencers and related equipment in the coming years.
Future opportunities and challenges	▸ The cross-pathogen potential of WGS technology is a very important advantage from IZSLER's perspective. While many conventional typing methods are pathogen-specific, using WGS can reduce the variety of methods to a single technique or to a single process. The institution noted that it is very confident that using WGS will simplify the analytical process and will improve the overall management of the laboratory. ▸ With WGS, IZSLER indicated that it will be able to satisfy a broader range of requests from public health labs, e.g. on Campylobacter, as WGS would allow them to easily switch to another pathogen in cases where there is ad hoc need to support an outbreak investigation. ▸ As indicated above, the use of WGS may lead to the identification of a high numbers of matches, i.e. potential outbreaks, which raises the question of whether they would have capacity to investigate these potential outbreaks and of the definition of an outbreak. There could also be a need for further standardisation on the approach for outbreak investigation. ▸ IZSLER noted that with the current uptake of and growing interest in WGS there is a potential for fragmentation of the system, and emphasised the importance of standards for sequencing and sharing of results. According to IZSLER quality issues with respect to public databases also indicate a need for further standards and quality assurance in this respect.

VII. Key sources/references	
Cost questionnaire	Cost questionnaire completed by IZLER
Preparatory phone interview	a) Background information and description of activities
Case study visit and follow up	b) Additional data and clarifications provided
Scientific literature	c) Comandatore, F., et al (2017). Genomic Characterization Helps Dissecting an Outbreak of Listeriosis in Northern Italy. *PLoS Currents*, 9, 1–21. http://doi.org/10.1371/currents.outbreaks.633fd8994e9f06f31b3494567c7e504c d) Morganti, M., et al. (2018). Rise and fall of outbreak-specific clone inside endemic pulsotype of salmonella 4,[5],12:i:-; insights from high resolution molecular surveillance in Emilia-Romagna, Italy, 2012 to 2015. *Eurosurveillance*, 23(13), 1–11. http://doi.org/10.2807/1560-7917.ES.2018.23.13.17-00375 e) Morganti, M., et al. (2015). Processing-dependent and clonal contamination patterns of Listeria monocytogenes in the cured ham food chain revealed by genetic analysis. *Applied and Environmental Microbiology*, 82(3), 822–831. http://doi.org/10.1128/AEM.03103-15 f) Scaltriti, E., et al. (2015). Differential single nucleotide polymorphism-based analysis of an outbreak caused by Salmonella enterica serovar Manhattan reveals epidemiological details missed by standard pulsed-field gel electrophoresis. *Journal of Clinical Microbiology*, 53(4), 1227–1238. http://doi.org/10.1128/JCM.02930-14

4.5. Administración Nacional de Laboratorios e Institutos de Salud (ANLIS)

Salmonella and E. coli surveillance – ANLIS, Argentina	
I. Institution	
Name of institution	Instituto Nacional de Enfermedades Infecciosas - Administración Nacional de Laboratorios e Institutos de Salud (INEI-ANLIS)
Type of institution	Public institution under the Ministry of Health
Description	The National Administration of Laboratories and Health Institutes is an organisation that implements the policies of the Argentinian Ministry of Health with respect to the prevention, referential diagnostics, research, and treatment of infectious, genetic, nutrition-based and non-transmissible diseases. It is also responsible for the production and quality control of immunobiological products, for the execution of health programs related to its areas of responsibility, for the coordination of laboratory networks in the country, and in the conduct of epidemiological studies. The National Institute for Infectious Diseases at ANLIS conducts and collaborates in research and methodological development concerning infectious diseases including zoonoses, foodborne infections, water infections and new microbial etiologies. It acts as the national reference laboratory for the diagnosis of viral, bacterial, fungal, and parasitic diseases.
Location	Buenos Aires, Argentina
II. Surveillance activities covered by case study	
Activity	Routine laboratory surveillance
Reference period	06/2017 – 05/2018
Pathogen(s) covered	Salmonella, E. coli
Summary of routine surveillance activities using WGS	WGS has been used at INEI-ANLIS for the routine surveillance of foodborne pathogens since 2015, having been introduced as part of a WHO Pilot Project in cooperation with the GenomeTrakr programme at the US Food and Drug Administration (US-FDA).[e-h)] Although WGS has been implemented on a routine basis for Salmonella, E. coli and Shigella, conventional methods are still being used in parallel for these pathogens due to concerns regarding the cost and availability of the relevant reagents. There are currently no plans to replace these conventional methods in the short-term. The surveillance of foodborne pathogens in Argentina is conducted through the National Diarrheal Network, in which food and clinical laboratories from the whole country participate. Depending on the pathogens, they send a number of the isolates identified to INEI-ANLIS. For Salmonella subspecies, local and provincial laboratories have the capacity to serotype the two most common serovars of Salmonella in Argentina (Salmonella enterica ser. Typhimurium and Salmonella enterica ser. Enteritidis). From these two serovars, local laboratories are required to send each month 20% of their isolates to INEI-ANLIS for further analysis. However, local laboratories must send all other serovars they isolate. To study circulating clones, INEI-ANLIS serotypes all isolates received and uses PFGE for all Salmonella enterica ser. Enteritidis and Typhimurium isolates received and for a selection of the other serovars, as well as all suspected outbreak isolates. For WGS surveillance a selection of all the isolates received at INEI-ANLIS is sequenced, including all suspected outbreak isolates.
Type of sample	Isolates (for E. Coli only: also samples)

Region covered by sampling	Argentina		
Number of samples analysed in reference period	*Pathogen*	*Samples analysed by conventional methods*	*Samples sequenced using WGS*
	Salmonella	The cost calculation is based on experiences with the listed conventional methods, assuming the same number of samples as with WGS	128
	E. Coli		192
Conventional methods used	▸ Salmonella: Biochemical testing (100% of samples), Serotyping (100%), MaldiTOF (5%), PFGE (70%) ▸ E. coli: Biochemical testing (100% of samples), PCR typing (100%), MaldiTOF (5%), PFGE (100%)		
Sample preparation WGS	▸ Manual preparation of isolates		
Sequencer used for WGS	▸ Illumina MiSeq		
Batch size for WGS analysis	▸ The typical batch size for WGS analysis during the reference period was 16 samples per run.		
Reference dataset used for WGS	INEI-ANLIS uses genomic data from publically available databases which is then complemented with genomic data from its own sequencing activities.		

III. Detailed overview of costs of WGS and conventional methods

In the following, all costs are provided on a per-sample basis. Equipment costs are annualised and incorporate the annual maintenance costs as reported by the institution. They are adjusted for the percentage use of the equipment for the listed pathogens samples during the reference period (i.e. if a sequencer was also used for other purposes, this is taken into account). Consumables costs are adjusted for the failure rate (i.e. the percentage of consumables wasted, e.g. due to failed runs). Staff time is provided in terms of the minutes of hands-on staff time per sample, for both professionals and technicians. For the calculation of total costs, staff time is then monetised based on estimated labour costs provided by INEI-ANLIS, plus a 25% surcharge for overheads.

More detailed cost data is provided in Annex I.

a) Costs of using WGS

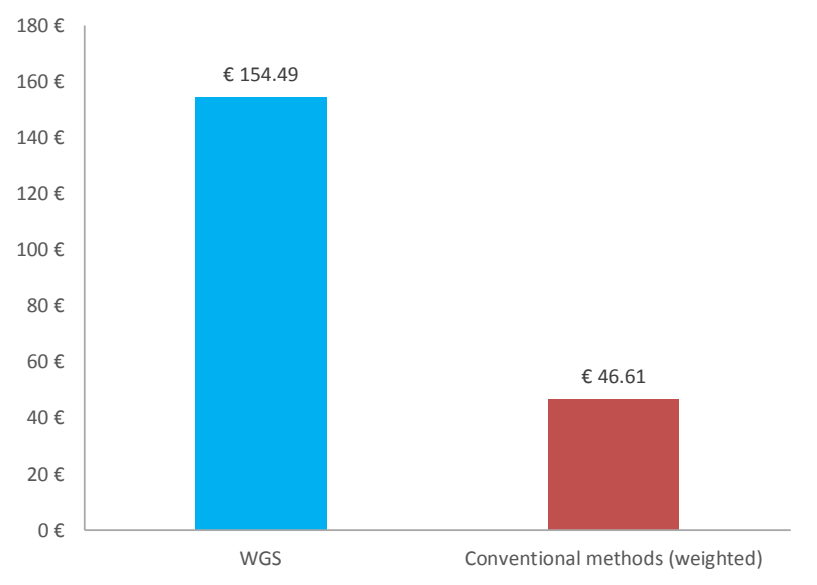
Sample preparation and sequencing	*Cost type*	*Cost per sample*
	Equipment costs	€ 35.45
	Consumables	€ 104.62
	Other costs	€ 0.00
	Staff time professionals	*31 minutes*
	Staff time technicians	*0 minutes*
	Staff costs, monetised based on labour cost data for Argentina	€ 2.33
	Total	**€ 142.40**

Bioinformatics and other analyses	*Cost type*	*Cost per sample*
	Equipment costs	€ 7.57
	Other costs	€ 0.00
	Staff time professionals	*60 minutes*
	Staff time technicians	*0 minutes*

	Staff costs, based on labour cost data for Argentina	€ 4.52
	Total	**€ 12.09**
b) Costs of conventional methods[21]		
Biochemical testing and serotyping (used for 100% of Salmonella samples)	*Cost type*	*Cost per sample*
	Total	**€ 35.41**
Biochemical testing and PCR (100% of E. Coli samples)	*Cost type*	*Cost per sample*
	Total	**€ 39.83**
MaldiTOF (5% of Salmonella and 5% of E. coli samples)	*Cost type*	*Cost per sample*
	Total	**€ 61.96**
PFGE (70% of Salmonella samples and 100% of E. coli samples)	*Cost type*	*Cost per sample*
	Total	**€ 6.64**

IV. Costs of using WGS compared to the costs of conventional methods			
The following comparison of costs per sample using WGS compared to conventional methods takes into account the fact that the number of samples processed differed between conventional methods, e.g. biochemical testing is used for 100% of Salmonella samples, but MaldiTOF is only used for 5% of Salmonella samples. The average cost of the conventional methods provided here is therefore a weighted figure which accounts for the use rate of the various methods. See Annex I for more details.			
Comparison of overall costs	*Cost type*	*Cost per sample (WGS)*	*Cost per sample (conventional methods)*
	Equipment costs	€ 43.02	-
	Consumables	€ 104.62	-
	Other costs	€ 0.00	-
	Staff time professionals	*91 minutes*	-
	Staff time technicians	*0 minutes*	-
	Staff costs (monetisation based on labour cost data for Argentina)	€ 6.85	-
	Total	**€ 154.49**	**€ 46.61**

[21] Note that costs for conventional methods were provided as lump sum figures representing the costs that were charged to external clients for the relevant tests, including equipment, consumables and staff time.



Summary of differential costs	**The cost difference between WGS and conventional methods is € 107.88 per sample**. A sample analysed with WGS costs approximately 3.3 times the amount of conventional methods (€ 154.49 vs € 46.61).

V. Effects of using WGS results

a) Turnaround time. Turnaround time is defined as the usual number of days of work from receipt and opening of an incoming sample until the reporting of results. Turnaround time does not include weekends and holidays, except in case that work has been conducted on these days, e.g. for a sequencing run or other analyses.

Turnaround time	The turnaround time for the analysis of a sample using WGS can last 5-10 days. In the case of an outbreak where the isolates are prioritised for analysis, this can be reduced to 5-6 days. The turnaround time using conventional methods lasts: ▸ 4-7 days for pathogen identification at the species level; ▸ 5-15 days for characterisation (including the serotype and toxin profile for E. coli); and ▸ 5 days must be added for identification of clonal relationship of isolates using PFGE. In a salmonella outbreak, for example, the complete turnaround time for all three steps using conventional methods can last between 7 and 15 days.

b) Positive effects of using WGS for pathogen identification and surveillance during the reference period

Sampling and sampling strategies	▸ Little or no positive effects of using WGS on sampling and sampling strategies were identified by INEI-ANLIS, although it considered that there could be a minor effect on the simplification of sample storage or transport.
Analytical results and processes	▸ INEI-ANLIS considered that the use of WGS had significant effects on improved accuracy, sensitivity, specificity and level of detail of the results produced. Results of the WHO Pilot Project to introduce WGS in Argentina also showed that WGS could obtain additional information on virulence factors.[f)] ▸ INEI-ANLIS also indicated that WGS had led to simplified laboratory work flows and could lead to a substantial reduction in required staff numbers (if it were fully implemented and used to replace conventional methods such

	as serotyping or PFGE). However, it did not see any effects on the reduction of staff time needed for the analysis (see also above the comparison of staff time used for WGS and conventional methods), due to the increased staff time needed for the bioinformatics analysis.
Outbreak identification and response	▸ INEI-ANLIS considered that the use of WGS for pathogen identification and surveillance had significant effects with respect to improved detection that outbreaks are related and improved information on outbreak epidemiology. It cited scientific publications by its staff showing the use of WGS in retrospectively distinguishing between multiple outbreaks of Shigella sonnei in Argentina.[c-d)] The study showed that even with a lack of supporting routine data WGS was an indispensable method for the tracking and surveillance of bacterial pathogens during outbreaks and was becoming a vital tool for the monitoring of antimicrobial resistant strains of *S. sonnei*.[d)] ▸ The WHO Pilot Project concluded, however, that maximising the benefit of genomic outbreak data requires long-term contextual (i.e. routine surveillance) data from local and international sources.[g)] ▸ INEI-ANLIS did not report any effects with respect to improved information for imposing additional control or biosecurity measures, nor did it indicate any effects concerning a reduction in the duration of an outbreak or a reduction in the disease burden for humans. INEI-ANLIS reported that this was due to the delay in receiving samples (see description of surveillance system above), so that typically the outbreak is already detected at the time that samples are received from local and provincial laboratories. The lack of timely availability of WGS results means that links between isolates are usually discovered too late to be of practical relevance. It was also reported that communication between the genomics team and the epidemiological team at INEI-ANLIS, as well as with the provincial public health authorities was insufficient for effective use of the additional information provided by WGS for outbreak response.
Research and methods applied	▸ The institution reported significant positive effects related to the better understanding of disease transmission and the development of better diagnostic tests. However, it did not report any effects regarding an improvement in epidemiological methods so far.
Effects on wider society	▸ INEI-ANLIS did not identify any significant effects on the wider society. It indicated that the nature of the surveillance system, gaps in communication between different units and institutions, and a lack of implementation of public health measures in response to the available data have limited the potential impact of WGS for reducing the negative effects of outbreaks for the wider society.
c) Negative effects of using WGS	
Negative effects of using WGS	INEI-ANLIS did not identify any negative effects of using WGS.
VI. Outlook	
Balance of costs and benefits achieved	▸ On balance, the benefits of using WGS outweigh the costs, given the improvements in the accuracy of results and turnaround time (for the full analysis). With the appropriate capacity-building, WGS also brings different actors of public health together.
Potential for cost reductions of using WGS for pathogen identification and	▸ Advances in sequencing technology and increasingly automated analysis of sequencing results are expected to drive further cost reductions in using WGS for pathogen identification and surveillance.

surveillance in the future (through e.g. techno-logical advances)	▸ INEI-ANLIS considered that the cross-pathogen potential of WGS was one of the most important areas of potential cost reduction. It pointed out that at the present time, INEI-ANLIS already had a genomic platform for all pathogens in their institute with equipment, reagent and personnel costs all centralised.
Future opportunities and challenges	▸ A key challenge identified affecting present and future use of WGS is the high cost of consumables, which are significantly more expensive than in other countries, such as the US or the UK. This is aggravated by exchange rate fluctuations and import duties, which make it very difficult for INEI-ANLIS to reliably purchase consumables for conducting WGS on a routine basis. It will be difficult to fully switch to WGS as long as this reliability and affordability of supplies is not ensured (either through changes in the pricing policies of producers and distributors of consumables, or through agreements with international organisations to ensure regular supply).

VII. Key sources/references	
Cost questionnaire	Cost questionnaire completed by INEI-ANLIS
Preparatory phone interview	a) Background information and description of activities
Case study visit and follow up	b) Additional data and clarifications provided
Scientific literature	c) Baker, K. S., J. Campos, M. Pichel, A. Della Gaspera, F. Duarte-Martínez, E. Campos-Chacón, H. M. Bolaños-Acuña, et al. 2017. "Whole Genome Sequencing of Shigella Sonnei through PulseNet Latin America and Caribbean: Advancing Global Surveillance of Foodborne Illnesses." Clinical Microbiology and Infection 23 (11): 845–53. doi:10.1016/j.cmi.2017.03.021. d) Chinen, Isabel, Marcelo Galas, Ezequiel Tuduri, Maria Rosa Vinas, Carolina Carbonari, Anabella Della Gaspera, Daniela Napoli, et al. 2016. "Whole Genome Sequencing Identifies Independent Outbreaks of Shigellosis in 2010 and 2011 in La Pampa Province, Argentina." *BioRxiv*. doi:10.1101/049940. e) World Health Organisation (WHO). 2018. "Implementing Whole Genome Sequencing to Support Public Health Surveillance in Argentina." f) World Health Organization (WHO). 2018. "Annex 1. Contribution/Implementation of Whole Genome Sequencing to the National Surveillance of the Shiga Toxin Producing E. Coli O157:H7 in Argentina." WHO Pilot Project. g) World Health Organization (WHO). 2018. "Annex 2. Contribution of Whole Genome Sequencing to the National Surveillance of Shigella Sonnei in Argentina Introduction." WHO Pilot Project. h) World Health Organization (WHO). 2018. "Annex 3. Contribution/ Implementation of Whole Genome Sequencing to the National and International Surveillance of Salmonella Spp." WHO Pilot Project.
Other	i) Website, ANLIS http://www.anlis.gov.ar/

4.6. Maryland Department of Health (MDH)

Under final review

4.7. Public Health Agency Canada (PHAC)

4.8. Public Health England (PHE)

Foodborne pathogen surveillance – PHE, UK			
I. Institution			
Name of institution	Public Health England (PHE)		
Type of institution	Executive agency of the Department of Health and Social Care		
Description[n)]	The Gastrointestinal Bacteria Reference Unit (GBRU) at Public Health England is the national reference laboratory for gastrointestinal bacterial pathogens for England, Wales and Northern Ireland from clinical, food and environmental samples. The GBRU also undertakes research into the genetic diversity of pathogens and the development of improved detection and characterisation techniques for food, water and environmentally borne diseases and offers expert advice, education and training on public health aspects of food microbiology and safety. In 2012, Public Health England established a central genomics service at PHE Colindale to provide sequencing capabilities for microbiology services across PHE. Whilst initially focused on a few pathogens, including Salmonella, WGS is now being used by Public Health England for routine identification, characterisation and typing of Salmonella, Listeria, E. coli & Shigella, and Campylobacter isolates from England, Wales and Northern Ireland.[f)]		
Location	Greater London, UK		
II. Surveillance activities covered by case study			
Activity	Routine laboratory surveillance		
Reference period	04/2016 – 03/2017		
Pathogen(s) covered	Salmonella, Listeria, E. coli & Shigella, Campylobacter		
Summary of routine surveillance activities using WGS	WGS has been used for routine surveillance for all referred isolates of the listed pathogens since 2015 (Campylobacter since January 2016).		
Type of sample	Bacterial isolates from clinical, food and environmental samples		
Region covered by laboratory surveillance	England, Wales and Northern Ireland		
Number of samples analysed in reference period	*Pathogen*	*Samples analysed by conventional methods*	*Samples sequenced using WGS*
	Salmonella	The cost calculation is based on previous experiences with the listed conventional methods, assuming the same number of samples as with WGS	10174
	Listeria		1000
	E. coli & Shigella		4294
	Campylo-bacter		350
Conventional methods used as reference for costing	▸ Salmonella: Taqman PCR (73% of samples), Monophasic PCR for S. Typhimurium (10%), Serotyping (98%), Phage typing (99%), D-Tartrate (3%), Glucose gas test (3%), MLVA (48%), PFGE (3%), Antimicrobial resistance (AMR) testing (68%). Use of MLVA and PFGE for Salmonella was previously based on exceedance levels for certain serotypes/phage types. ▸ Listeria: PCR (x2; 100% each), fAFLP (100%). ▸ E. coli and Shigella: Real-time PCR (100%), Serotyping (100%), Phage typing (100%), Biochemistry (100%), MLVA (100%).		

	▸ Campylobacter: Real-time PCR (100%), Serotyping (12%), Phage typing (38%), MLST (52%). PHE indicated that serotyping and phage typing would have only been done in outbreaks. ▸ Sample preparation for serotyping was partly automated through the use of a robot for the preparation of antisera plates.
Sample preparation WGS	▸ Automated laboratory processes with minimal hands-on time (for example, DNA extraction is partially automated through the use of an automated DNA extraction machine).
Sequencer used for WGS	▸ Illumina HiSeq
Batch size for WGS analysis	▸ The data provided for the reference period assumes a run of 96 samples (or batches of 40 for sample processing)
Reference dataset used for WGS	▸ PHE uses its own in-house database for SNP analysis on a routine basis as well as other public databases on an ad hoc basis as required.

III. Detailed overview of costs of WGS and conventional methods

In the following, all costs are provided on a per-sample basis. Equipment costs are annualised and incorporate the annual maintenance costs as reported by the institution. They are adjusted for the percentage use of the equipment for the listed pathogens samples during the reference period (i.e. if a sequencer was also used for other purposes, this is taken into account). Consumables costs are adjusted for the failure rate (i.e. the percentage of consumables wasted, e.g. due to failed runs). Staff time is provided in terms of the minutes of hands-on staff time per sample, for both professionals and technicians. For the calculation of total costs, staff time is then monetised based on Eurostat data on country-specific labour costs for 2017 (by staff category), plus a 25% surcharge for overheads. For comparison purposes only, we have also provided staff costs monetised based on EU average labour costs. More detailed cost data is provided in Annex I.

a) Costs of using WGS[22]

Sample preparation and sequencing	*Cost type*	*Cost per sample*
	Equipment costs	€ 30.34
	Consumables	€ 53.92
	Other costs	€ 0
	Staff time professionals	*6.85 minutes*
	Staff time technicians	*17.15 minutes*
	Staff costs, monetised based on labour cost data for the UK (in brackets: based on labour cost data for the EU as a whole)	€ 11.67 (€ 12.15)
	Total	**€ 95.93**

Bioinformatics and other analyses	*Cost type*	*Cost per sample*
	Equipment costs	€ 4.89
	Other costs	€ 0
	Staff time professionals	*36 minutes*

[22] PHE provided cost data in pounds sterling. These have been converted to Euro using the European Central Bank's yearly average reference exchange rate for the relevant year (i.e. the year of purchase for equipment, or 2017 otherwise).

	Staff time technicians	*0 minute*
	Staff costs, based on labour cost data for the UK (for EU)	€ 23.78 (€ 27.08)
	Total	**€ 28.67**

b) Costs of conventional methods

Note that detailed costing data were not available for every conventional test, as many of the conventional methods had been discontinued with the introduction of WGS. In consultation with PHE, it was decided to use similar tests for which data were available as a cost proxy. For example, as MLVA, MLST, and fAFLP are all enzyme reactions, the cost for MLVA was used as a proxy for the cost of MLST and fAFLP. Conventional tests were costed across all pathogens (e.g. the same per-sample cost calculation for Serotyping applies to Salmonella, Listeria, E. coli and Shigella, and Campylobacter).

Method A: PCR (Taqman)	*Cost type*	*Cost per sample*
	Equipment costs	€ 2.60
	Consumables	€ 2.12
	Other costs	€ 2.35
	Staff time professionals	*0 minutes*
	Staff time technicians	*5.63 minutes*
	Staff costs, based on labour cost data for the UK (for EU)	€ 2.35 (€ 2.30)
	Total	**€ 7.07**

Method B: PCR (Monophasic)	*Cost type*	*Cost per sample*
	Equipment costs	€ 2.60
	Consumables	€ 2.44
	Other costs	€ 0
	Staff time professionals	*0 minutes*
	Staff time technicians	*3.96 minutes*
	Staff costs, based on labour cost data for the UK (for EU)	€ 1.65 (€ 1.62)
	Total	**€ 6.69**

Method C: PCR (RT, other)	*Cost type*	*Cost per sample*
	Equipment costs	€ 5.12
	Consumables	€ 9.49
	Other costs	€ 0
	Staff time professionals	*2.50 minutes*
	Staff time technicians	*3.00 minutes*
	Staff costs, based on labour cost data for the UK (for EU)	€ 2.90 (€ 3.11)
	Total	**€ 17.51**

Method D: MLVA/MLST/fAFLP	*Cost type*	*Cost per sample*
	Equipment costs	€ 0
	Consumables	€ 3.87
	Other costs	€ 0
	Staff time professionals	*0 minutes*
	Staff time technicians	*7.71 minutes*
	Staff costs, based on labour cost data for the UK (for EU)	€ 3.21 (€ 3.15)
	Total	**€ 7.08**
Method E: Serotyping	*Cost type*	*Cost per sample*
	Equipment costs	€ 1.08
	Consumables	€ 13.36
	Other costs	€ 0
	Staff time professionals	*0 minutes*
	Staff time technicians	*27.25 minutes*
	Staff costs, based on labour cost data for the UK (for EU)	€ 11.35 (€ 11.13)
	Total	**€ 15.79**
Method F: Phage Typing	*Cost type*	*Cost per sample*
	Equipment costs	€ 0.08
	Consumables	€ 3.48
	Other costs	€ 0
	Staff time professionals	*2.25 minutes*
	Staff time technicians	*12.50 minutes*
	Staff costs, based on labour cost data for the UK (for EU)	€ 6.69 (€ 6.80)
	Total	**€ 10.26**
Method G: PFGE[23]	*Cost type*	*Cost per sample*
	Equipment costs	€ -
	Consumables	€ -
	Other costs	€ 97.82
	Staff time professionals	-
	Staff time technicians	-
	Staff costs, based on labour cost data for the UK (for EU)	€ - (€ -)

[23] Note that detailed cost data were not available for PFGE, so PHE's internal estimate of € 97.82 per sample was used as a unit cost.

	Total	**€ 97.82**
Method H: D-Tartrate	*Cost type*	*Cost per sample*
	Equipment costs	€ 0
	Consumables	€ 7.26
	Other costs	€ 0
	Staff time professionals	*0 minutes*
	Staff time technicians	*25.00 minutes*
	Staff costs, based on labour cost data for the UK (for EU)	€ 10.42 (€ 10.21)
	Total	**€ 17.67**
Method I: Glucose Gas	*Cost type*	*Cost per sample*
	Equipment costs	€ 0
	Consumables	€ 0.79
	Other costs	€ 0
	Staff time professionals	*0 minutes*
	Staff time technicians	*10.00 minutes*
	Staff costs, based on labour cost data for the UK (for EU)	€ 4.17 (€ 4.08)
	Total	**€ 4.96**
Method J: AMR	*Cost type*	*Cost per sample*
	Equipment costs	€ 0
	Consumables	€ 1.40
	Other costs	€ 0
	Staff time professionals	*0 minutes*
	Staff time technicians	*2.00 minutes*
	Staff costs, based on labour cost data for the UK (for EU)	€ 0.83 (€ 0.82)
	Total	**€ 2.23**
Method K: Biochemistry	*Cost type*	*Cost per sample*
	Equipment costs	€ 10.43
	Consumables	€ 25.97
	Other costs	€ 0
	Staff time professionals	*6.00 minutes*
	Staff time technicians	*36.00 minutes*
	Staff costs, based on labour cost data for the UK (for EU)	€ 18.96 (€ 19.21)
	Total	**€ 55.36**

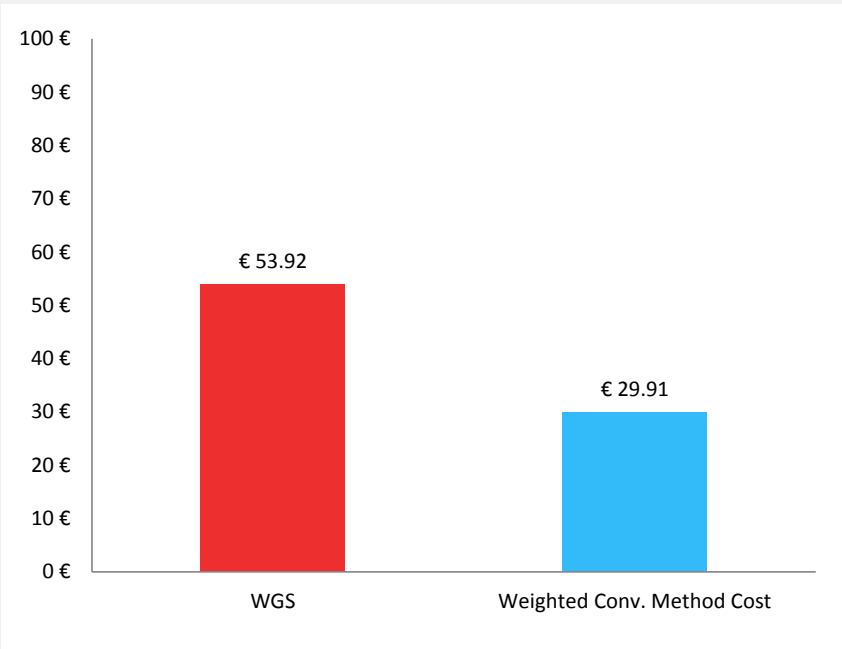
IV. Costs of using WGS compared to the costs of conventional methods

The following comparison of costs per sample using WGS compared to the costs of conventional methods considers that the number of samples processed differed for the different conventional methods. The weighted cost of the conventional methods provided here is therefore a weighted figure which accounts for the use rate of the various methods across the different pathogens. See Annex I for more details.

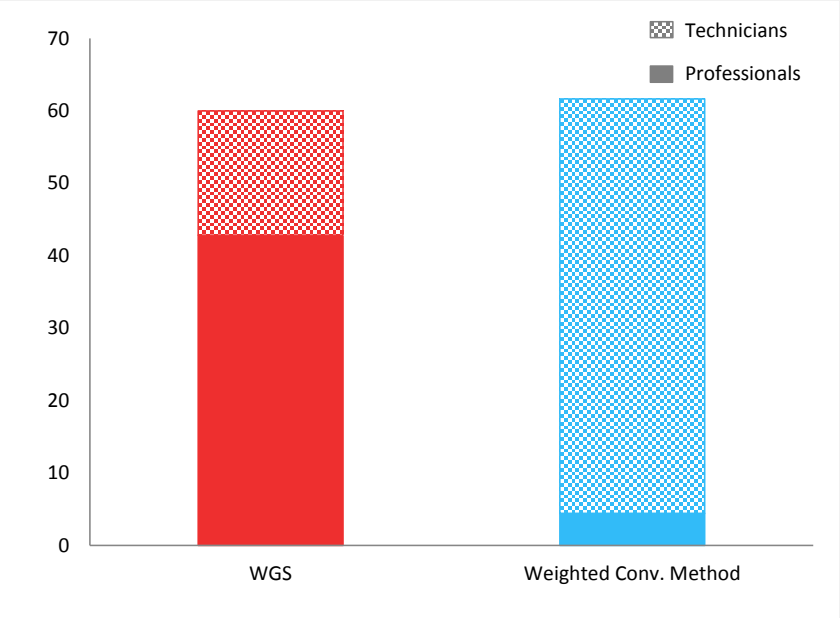
Comparison of equipment costs

Equipment costs *per sample* at PHE are higher for WGS than for the weighted conventional methods (€ 35.23 vs € 7.11), although the large volume of samples processed (15 791 during the reference period) keeps equipment costs for both WGS and conventional methods low on a per-sample basis. The large sample size and the relatively lower cost of the equipment used for the conventional methods brings the per-sample weighted cost for the conventional methods down to just € 7.11.

Comparison of costs of consumables



Consumables costs for WGS (€ 53.92) are higher than for conventional methods (€ 29.91). The higher costs for WGS result from the higher per-

	sample costs of the various kits used for library preparation, particularly the Nextera DNA Library Prep Kit for 96 samples.
Comparison of staff time used (in minutes)	 The amount of staff time needed for WGS analysis (a total of 60 minutes per sample) is slightly lower than the amount of staff time needed to carry out the various conventional methods (62 minutes). Note however that as with the other cost categories, the staff time required for conventional methods was weighted to take into account the fact that multiple tests were often performed on the same samples. The staff time required for individual conventional tests ranged from a low of 2 minutes per sample (for AMR testing) to a high of 42 minutes per sample (for the biochemistry tests). Compared to conventional methods, analysis with WGS requires a significantly larger proportion of professional staff time. As a result, once staff time has been monetised, WGS has higher staff costs (€ 35.44) than the weighted conventional methods (€ 26.77).
Comparison of overall costs	

Cost type	*Cost per sample (WGS)*	*Cost per sample (conventional methods)*
Equipment costs	€ 35.23	€ 7.11
Consumables	€ 53.92	€ 29.91
Other costs	€ 0	€ 1.67
Staff time professionals	*42.85 minutes*	*4.43 minutes*
Staff time technicians	*17.15 minutes*	*57.23 minutes*
Staff costs, based on labour cost data for the UK (for EU)	€ 35.44 (€ 39.23)	€ 26.77 (€ 26.70)
Total	**€ 124.59**	**€ 65.46**

Differential costs	**The cost difference between WGS and conventional methods is € 59.13 per sample**. A sample analysed with WGS costs approximately twice the amount of analysis with conventional methods. As indicated in the figures above, the largest differences are in equipment and consumables costs.

V. Effects of using WGS results	
a) Turnaround time. Turnaround time is defined as the usual number of days of work from receipt and opening of an incoming sample until the reporting of results. Turnaround time does not include weekends and holidays, except in case that work has been conducted on these days, e.g. for a sequencing run or other analyses.	
Turnaround time	▸ The turnaround time for the analysis of a sample using WGS for pathogen identification is 10 days of work. This figure includes weekends, as machines can be set to run over the weekend. ▸ The turnaround time using the specified conventional methods for pathogen identification is dependent on the pathogen. For example, the turnaround time would be 10-15 days of work for Salmonella (14-21 days including weekends, as machines can be set to run over the weekend), or 3 days of work for L. monocytogenes (5 days including weekends). However, these estimates do not include typing, but just confirmation of identification and serotyping. ▸ PHE considered that for most pathogens there has been an improvement in turnaround times with WGS. However, this depends on the type of analysis needed: for example, some of PHE's clients only need confirmation of identity, which takes longer with WGS than using conventional methods (i.e. PCR identification). As a result, in cases where identification is required urgently, PHE still does PCR identification tests.
b) Positive effects of using WGS for pathogen identification and surveillance during the reference period	
Sampling and sampling strategies	▸ PHE reported no effects at all on sampling and sampling strategies.
Analytical results and processes	▸ PHE indicated that WGS had very significant positive effects on analytical results and processes. It considered that WGS had significantly improved the accuracy, sensitivity, specificity, and level of detail in the results produced, citing papers in which the higher resolution data from WGS was used to produce results above and beyond what would be possible with conventional methods alone.[f)-k)] For example, PHE indicated that WGS can show how strains diversify over time, allowing strains to be identified as being phylogenetically linked, while under past methods these would have been seen to be unrelated strains. ▸ PHE indicated that WGS had led to considerable streamlining in their laboratory. It reported that WGS had simplified laboratory work flows, noting that WGS was able to replace the numerous tests that had previously been performed on each pathogen with a single, unified workflow. PHE also indicated that WGS had led to a reduction in (analytical) time, staff time, and consumables. PHE reported having reduced their lab staff considerably since introducing WGS. ▸ Another benefit noted by PHE was the ability to better monitor its own laboratory processes. PHE was able to introduce processes to report on the use of WGS (e.g. related to the number of samples processed) and indicated that it used this data to track trends in WGS usage, predict future costs, and try to reduce costs in the future.
Outbreak identification and response	▸ PHE indicated that the impact of using WGS in pathogen surveillance has been 'transformational'. It stated that WGS has dramatically changed outbreak detection, namely that more outbreaks were being detected than previously;[h)] that large multinational outbreaks are being detected that would have not been detected and confirmed with certainty before;[d)] and that 'slow burn' outbreaks with few cases over several years can also now be detected. For example, WGS was able to identify an outbreak of

	Salmonella enteritidis in reptile feeder mice that had previously been continuing undetected over a period of four years with at least 162 cases identified between 2012 and 2015.[c)] ▸ PHE also indicated that one of the benefits of WGS was the ability to monitor the effectiveness of public health interventions. As an example, it cited the case of a large EU-wide Salmonella outbreak in eggs, where action was taken to address the problem but WGS was able to identify the re-emergence of human cases, indicating an ongoing issue. With previous typing methods it would not have been possible to show it was the same strain with the level of certainty provided by WGS. ▸ PHE reported that WGS can be used for more precise case definitions in outbreak investigations. It noted that WGS provided a tool to rule cases as being in or out of the outbreak far more accurately, making subsequent epidemiological investigations more powerful by not including cases that were not actually part of the outbreak. For example, WGS was used by PHE to discriminate between three separate outbreaks of Shigella in the English Orthodox Jewish community which were circulating at the same time.[i),m)] ▸ PHE noted that WGS also allowed them to identify whether an outbreak isolate was likely to have come from outside the UK through clustering with travel-related isolates or comparisons with sequence data in external databases. It considered that WGS enabled the tracking and dissemination of emerging strains at a global scale. ▸ In sum, PHE indicated that WGS had highly significant effects on the earlier detection of an initial outbreak, improved detection that outbreaks are related, improved information on outbreak epidemiology, and improved information for imposing additional control or biosecurity measures. It also considered that WGS had contributed to a reduction in the duration of outbreaks, and had likely contributed to a reduction of the disease burden in humans (although it stated that it had not observed this directly, and that this effect might take longer to see).
Research and methods applied	▸ PHE reported very significant positive effects of using WGS regarding better understanding of disease transmission. For example, PHE described a case where an E. coli O157 isolate causing an outbreak via salad leaves was matched to isolates from UK sheep, leading it to determine that the salad leaves most likely became contaminated as a result of being grown or irrigated with river water contaminated by run-off from nearby fields where sheep had been grazing. ▸ PHE also noted other benefits for research, in particular the fact that large amounts of WGS data (sequence data) are now made publicly available and can be used freely for analysis. It also noted that WGS data made it easier to collaborate internationally, since it is now possible to send sequence data instead of isolates. ▸ Moderate effects of WGS were observed by PHE with respect to improvements in epidemiological methods. PHE indicated that the use of WGS in case definitions improves the power of analytical epidemiological studies, citing the previously-mentioned study concerning a long-undetected Salmonella outbreak linked to reptile feeder mice.[c)] ▸ Moderate effects of WGS were also observed regarding the development of better diagnostic tests. For example, PHE cited a paper co-authored by its staff which demonstrates the use of WGS as a resource for the development and evaluation of molecular diagnostic assays for Campylobacter.[l)] PHE also noted that it had recently developed and implemented a PCR assay to distinguish between typhi/paratyphi and non-typhoidal strains of Salmonella, and that it had been able to design the primers and probes and carry out extensive validation of these on a panel of over 1000 WGS results from different Salmonella samples.
Effects on wider society	▸ PHE considered that it was not able to fully assess the effects of using WGS

	on the wider society. Nevertheless, it did indicate that WGS had led to a reduction of costs of outbreaks for the wider society, citing the general principle that identifying an outbreak and putting in preventative measures should lead to the prevention of further cases going forward. ▸ PHE also considered that WGS had likely reduced the negative effects of outbreaks on consumer trust in food.
c) Negative effects of using WGS	
Negative effects of using WGS	PHE indicated that since switching to WGS, it is detecting far more outbreaks than previously (particularly with respect to Salmonella), and that this has resource implications for their epidemiological investigations.[g),h)] PHE indicated that it currently doesn't have the resources to investigate all the linked cases that they see with WGS. However, it noted that if more outbreaks are resolved, then this would lead to a reduction in the disease burden overall.
VI. Outlook	
Balance of costs and benefits achieved	▸ PHE considered that their costs had increased due to an increase in the number of outbreaks detected through WGS. However, it expected that if preventative measures are successfully implemented on the basis of better outbreak detection, improved understanding, investigation and implementation of effective control measures, the overall costs should come down from both a societal and an institutional perspective.
Potential for cost reductions	▸ PHE expected costs to come down in the long term as laboratories reorganise their operations around WGS (e.g. by replacing conventional typing methods for other gastrointestinal pathogens, through streamlining processes and needing fewer staff). It considered that there would likely be future improvements in bioinformatics, i.e. in algorithm development, which could further streamline the analysis and reduce costs. ▸ PHE also expected to see a long term reduction in the costs of outbreak detection and response through the prevention of future cases.
Future opportunities and challenges	▸ PHE considered that the full potential of WGS technology has probably not yet been fully realised, and that WGS will lead to better information on transmission of gastrointestinal pathogens and improve epidemiological investigations. It reported that some effects of WGS (e.g. on staff costs and laboratory organisation, but also on wider effects such improved epidemiological investigations and the reduction of the overall disease burden) would take longer to see. ▸ PHE considered that the MinION had a lot of potential for outbreak response in the future, and could also provide a way for laboratories to diversity their technology against price increases through supplier monopolies (e.g. from supplies who are the sole producers of necessary sequencing kits). It also considered that the MinION could be a valuable tool in developing countries, and thought there was potential for these countries to 'leapfrog' previous technology and jump right into sequencing. ▸ PHE noted that back-compatibility could be a concern going forward, as the new information provided by WGS is very different from what was collected before (e.g. through phage typing). This could cause difficulties in inter-agency communication with agencies that do not yet use WGS. ▸ Training and communications were noted as a present and future challenge, since PHE noted that WGS has a steep learning curve and re-training can require significant resources. ▸ Another future challenge noted by PHE related to the availability of bioinformatics skills, since the bioinformatics analysis requires a very

	specific set of skills in computer science, statistics, biology, and epidemiology, and people with this expertise can be difficult to recruit.
VII. Key sources/references	
Cost questionnaire	Cost questionnaire completed by PHE
Preparatory phone interview	a) Background information and description of activities
Case study visit and follow up	b) Additional data and clarifications provided
Scientific literature	c) Kanagarajah, S., Waldram, A., Dolan, G., Jenkins, C., Ashton, P. M., Martin, A. I. C., ... et al. (2018). Whole genome sequencing reveals an outbreak of Salmonella Enteritidis associated with reptile feeder mice in the United Kingdom, 2012-2015. Food microbiology, 71, 32-38. d) Inns, T., Ashton, P. M., Herrera-Leon, S., Lighthill, J., Foulkes, S., Jombart, T., et al. (2017). Prospective use of whole genome sequencing (WGS) detected a multi-country outbreak of Salmonella Enteritidis. Epidemiology & Infection, 145(2), 289-298. e) Ashton, P. M., Nair, S., Peters, T. M., Bale, J. A., Powell, D. G., Painset, A.,et al. (2016). Identification of Salmonella for public health surveillance using whole genome sequencing. PeerJ, 4, e1752. f) Ashton, P., Nair, S., Peters, T., Tewolde, R., Day, M., Doumith, M., et al. (2015). Revolutionising public health reference microbiology using whole genome sequencing: Salmonella as an exemplar. bioRxiv, 033225. g) Waldram, A., Dolan, G., Ashton, P. M., Jenkins, C., & Dallman, T. J. (2018). Epidemiological analysis of Salmonella clusters identified by whole genome sequencing, England and Wales 2014. Food microbiology, 71, 39-45. h) Mook P, Gardiner D, Verlander NQ, McCormick J, Usdin M, Crook P, Jenkins C, Dallman TJ. Operational burden of implementing Salmonella Enteritidis and Typhimurium cluster detection using whole genome sequencing surveillance data in England: a retrospective assessment. Epidemiol Infect. 2018 Jul 2:1-9. i) Vanessa Rew, Piers Mook, Suzan Trienkens, Kate S Baker, Timothy J Dallman, Claire Jenkins, Paul D Crook and Nicholas R Thomson. Whole-genome sequencing revealed concurrent outbreaks of shigellosis in the English Orthodox Jewish Community caused by multiple importations of Shigella sonnei from Israel. Microbial Genomics, 2018:4. j) Butcher H, Elson R, Chattaway MA, Featherstone CA, Willis C, Jorgensen F, Dallman TJ, Jenkins C, McLauchlin J, Beck CR, Harrison S. Whole genome sequencing improved case ascertainment in an outbreak of Shiga toxin-producing Escherichia coli O157 associated with raw drinking milk. Epidemiol Infect. 2016 Oct;144(13):2812-23. Epub 2016 Mar 10 k) Timothy J. Dallman, Marie A. Chattaway, Piers Mook, Gauri Godbole, Paul D. Crook, Claire Jenkins. Use of whole-genome sequencing for the public health surveillance of Shigella sonnei in England and Wales, 2015. 2016, Journal of Medical Microbiology 65: 882-884 l) Jansen van Rensburg MJ, Swift C, Cody AJ, Jenkins C, Maiden MC. Exploiting Bacterial Whole-Genome Sequencing Data for Evaluation of Diagnostic Assays: Campylobacter Species Identification as a Case Study. J Clin Microbiol. 2016 Dec;54(12):2882-2890. Epub 2016 Oct 12 m) J. Mcdonnell, T. Dallman, S. Atkin, D. A. Turbitt, T. R. Connor, K. A. Grant, N. R. Thomson And C. Jenkins. Retrospective analysis of whole genome sequencing compared to prospective typing data in further informing the epidemiological investigation of an outbreak of Shigella sonnei in the UK Epidemiol. Infect. (2013), 141, 2568–2575. Cambridge University Press 2013
Other	n) Website, Gastrointestinal bacteria reference unit (GBRU) https://www.gov.uk/guidance/gbru-reference-and-diagnostic-services o) Pathogen Genomics Into Practice, PHG Foundation, 2015.

5. Results

This section presents the analysis of costs, first for WGS and then for conventional methods. The costs of equipment, consumables, staff time and other costs are assessed on a per-sample basis for each analytical step. On the basis of the case study data, we derive the differential cost between WGS and conventional methods and identify major cost drivers.

5.1. Costs of WGS

The standard workflow involved in WGS for pathogen surveillance and identification is essentially pathogen-neutral and can be divided into a series of common analytical steps: sample processing, library preparation, sequencing, and bioinformatics.[24]

The following sub-sections assess the costs of WGS in detail for each major cost type (equipment, consumables, staff time, and other costs), including a breakdown by analytical step, before summarising the total costs of WGS.

5.1.1. Equipment costs

The case study institutions vary considerably with respect to the type and amount of equipment used for WGS. This is true not just for the choice of sequencer (Illumina, IonTorrent, or GridION), but also for the degree of automation in sample processing and library preparation as well as for the degree of sophistication in the bioinformatics infrastructure. The choice of equipment also influences the required consumables (see the next section for more detail).

The following table describes the type of equipment used by each of the case study institutions for WGS, including the total purchase costs, separately for each of the four analytical steps listed above (sample processing, library preparation, sequencing, and bioinformatics). Only equipment specifically used for WGS is included; basic laboratory equipment (e.g. refrigerators, centrifuges or pipettes), standard office computers and standard office software (e.g. Word, Excel) are not included.

Note that this and all following tables first present contextual information on the case studies, such as the type of case study, the number of samples analysed in the reference period, and the batch size for sample processing/sequencing, to support the analysis of the cost data presented.

[24] See section 3 on the cost assessment methodology for more detail as to what these steps comprise.

Table 3: WGS – Type and total purchase costs of equipment used by case study institutions, by analytical step

		APHA (UK)	FLI (DE)	EMC (NL)	IZSLER (IT)	ANLIS (ARG)	MDH (USA)	PHAC (CAN)	PHE (UK)
Case study type		Outbreak	Outbreak	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance
Pathogens		Avian influenza	Avian influenza	Influenza	Foodborne*	Foodborne*	Foodborne*	Foodborne*	Foodborne*
Batch size for sample processing/sequencing		1-2	6	20	24	12	24	32	Processing: 40 Sequencing: 96
No. of samples analysed in reference period		26 *(in 8 months)*	30 *(in 3 months)*	178 *(in 3 months)*	175 *(in 12 months)*	320 *(in 12 months)*	1 767 *(in 12 months)*	8 630 *(in 12 months)*	15 791 *(in 12 months)*
Analytical steps	Sample processing	Basic lab equipment only (€ 0)**	- *Covaris sonicator* - *Agilent bioanalyser* (€ 49 300)	- *Gel electrophoresis system* (€ 4 000)	Basic lab equipment only**	- *Qiacube DNA* (€ 13 724)	- *MagNA Pure 24* (€ 44 260)	Basic lab equipment only**	- *2 Qiasymphony* - *Roche Magna Pure 96* (€ 218 582)
	Library preparation	Basic lab equipment only (€ 0)**	Basic lab equipment only (€ 0)**	- *PCR machine* - *Qubit* - *Magnate 96 wells* (€ 8 800)	- *Biorad-T100 thermal cycler* - *Biorad-CFX96 RT-system* - *Microplate Genie-Shake* (€ 29 100)	- *Qubit 3.0* - *Bioshake iQ Thermomixer* (€ 2 943)	- *Multichannel & Single Channel Pipette* (€ 3 203)	- *Tapestation* - *Blue Pippin* - *QUBIT* (€ 51 641)	- *2 cBot Cluster Generation System* - *2 LabChip GX* - *3 Assy-Sciclone G3* - *LabChip-DS Spectrophotometer 96* - *2 Glomax: 96 well plate Fluorometer* - *Biomek NXP Span-8 with integrated sealer and chilled storage* - *Biomek NXP Span-8* - *3 Biomek NXP Multichannel* (€ 1 033 590)
	Sequencing	- *Illumina MiSeq* (€ 104 826)	- *IonTorrent PGM* (€ 93 000)	- *GridION* (€ 45 000)	- *Illumina MiSeq* (€ 100 000)	- *Illumina MiSeq* (€ 75 273)	- *2 Illumina MiSeq* (€ 155 624)	- 3 Illumina MiSeq (€ 264 345)	- *2 Illumina HiSeq* (€ 1 212 821)
	Bioinformatics	- *Workstation*(€ 2 355)	- *Server* (€ 34 700)	- *Server* - *Storage* - *CLC* (€ 16 560)	- *3 Workstations* - *Storage* - *BioNumerics* (€ 44 220)	- *Server* - *2 Computers* (€ 26 702)	- *CLC* - *BaseSpace subscription* - *PC* (€ 5 665)	- *Storage* - *Networking* - *Servers* - *BioNumerics* (€ 2 892 662)	- *Computing system* - *Network* - *Storage* (No purchase cost provided)
Total purchase costs		**€ 107 181**	**€ 177 000**	**€ 74 360**	**€ 173 320**	**€ 118 641**	**€ 208 751**	**€ 3 208 648**	**€ 2 464 922†**

Source: Own compilation based on case study results. * Foodborne pathogens: Salmonella (all), Listeria (IZSLER, PHE, PHAC, MDH), E.coli/shigella (PHE, ANLIS, MDH), Campylobacter (PHE, MDH), Vibrio (MDH). **Costs for basic laboratory equipment are not included in the assessment. Purchase costs of € 0 therefore imply that no other equipment than basic laboratory equipment was used by the institution. † Not including bioinformatics costs.

As shown in the table above, the specific equipment used for WGS varies considerably between case study institutions. The total purchase cost in the year of purchase ranges from a low of EUR 74 360 (for third-generation sequencing using the GridION) to a high of EUR 3 208 648 (for three MiSeqs and a top-of-the-line custom bioinformatics infrastructure). Overall, higher purchase costs tend to reflect higher throughputs (i.e. multiple sequencers, or higher-capacity sequencers such as the Illumina HiSeq) as well as greater investment in automation and/or bioinformatics capacity.

Large variations in equipment costs emerge already in the sample processing and library preparation steps, depending on the type of equipment used and in particular on the degree of automation. At one extreme, one of the institutions (APHA) relies only on basic laboratory equipment during the first two analytical steps (sample processing and library preparation) and therefore reports for these steps no additional purchase costs for WGS-related equipment. Other institutions (FLI, IZSLER and PHAC) also report relying on basic laboratory equipment for at least one of the first two analytical steps. At the other extreme, however, another institution (PHE) reports having a highly automated process for sample and library preparation, with total purchase costs of the equipment used in these steps totalling more than EUR 1.2 million.

For institutions with a comparatively low throughput of samples that do not require an extensive bioinformatics infrastructure, the most expensive single piece of equipment by purchase cost is the sequencer itself. For third-generation sequencing, this is the GridION, at a cost of EUR 45 000 for this institution. The cost of second-generation sequencers ranges from EUR 75 273 (for an Illumina MiSeq purchased in 2014) to EUR 606 410 (for an Illumina HiSeq purchased in 2013). In at least one case (PHAC), the purchase cost of equipment related to the high-performance computing (HPC) systems exceeds the purchase cost of the sequencer(s) by a considerable amount. At PHAC, the purchase costs of storage and servers for the HPC system amount to approximately EUR 1.4 million and EUR 1.3 million respectively (both purchased in 2017), compared to a cost of EUR 264 345 for three MiSeq sequencers.

In order to assess costs in a comparable way across case studies, we have calculated the cost of equipment, consumables, staff, and other costs on a per-sample basis. The per-sample equipment costs were calculated by dividing the original purchase cost of the equipment over the estimated lifespan[25] to obtain an annualised cost, to which annual maintenance costs were added.[26] As described in detail in section 3 above, the annualised cost was then adjusted for percentage use[27] and length of the reference period (if not 12 months) to obtain the total cost of equipment in the reference period, which was then divided by the number of samples in the reference period to reach the per-sample cost. These per-sample equipment costs are shown per analytical step in the table below.

[25] Estimated lifespans of 5 years for computers and 10 years for major laboratory equipment (e.g. sequencers) were used to ensure comparability across case studies.

[26] Some case study institutions indicated that multiyear service contracts were included in the original purchase cost of certain equipment. In these cases, annual maintenance costs have not been separately assessed, but have been kept as part of the original purchase cost.

[27] For example, if an Illumina MiSeq was used during the reference period 30% of the time for the samples subject to the case study and 70% of the time for other purposes (e.g. by other users inside the institution or for external clients), then the use rate for our calculation would be 30%.

Table 4: WGS – Equipment costs per sample, by analytical step

Institution		APHA (UK)	FLI (DE)	EMC (NL)	IZSLER (IT)	ANLIS (ARG)	MDH (USA)	PHAC (CAN)	PHE (UK)
Case study type		Outbreak	Outbreak	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance
Pathogens		Avian influenza	Avian influenza	Influenza	Foodborne*	Foodborne*	Foodborne*	Foodborne*	Foodborne*
Batch size for sample processing/sequencing		1-2	6	20	24	12	24	32	Processing: 40 Sequencing: 96
No. of samples analysed in reference period		26 *(in 8 months)*	30 *(in 3 months)*	178 *(in 3 months)*	175 *(in 12 months)*	320 *(in 12 months)*	1 767 *(in 12 months)*	8 630 *(in 12 months)*	15 791 *(in 12 months)*
Analytical steps	Sample processing	€ 0.00 **	€ 38.09	€ 0.03	€ 0.00 **	€ 5.13	€ 6.24	€ 0.00	€ 2.17
	Library preparation	€ 0.00 **	€ 0.00 **	€ 0.52	€ 3.65	€ 0.57	€ 0.32	€ 1.05	€ 14.01
	Sequencing	€ 57.33	€ 160.70	€ 3.19	€ 119.43	€ 29.75	€ 21.45	€ 6.27	€ 15.14
	Bioinformatics	€ 1.20	€ 11.92	€ 1.65	€ 40.41	€ 7.57	€ 1.52	€ 68.59	€ 4.89
Total per-sample cost		**€ 58.53**	**€ 210.71**	**€ 5.39**	**€ 163.49**	**€ 43.02**	**€ 29.53**	**€ 75.90**	**€ 35.23**

Source: Own compilation based on case study results. Note: Equipment costs per sample are calculated on basis of the purchase price and predicted lifespan of the equipment, also taking into account maintenance costs and the percentage use of the equipment during the reference period. * Foodborne pathogens: Salmonella (all), Listeria (IZSLER, PHE, PHAC, MDH), E.coli and shigella (PHE, ANLIS, MDH), Campylobacter (PHE, MDH), Vibrio (MDH). **Costs for basic laboratory equipment are not included in the assessment. Costs of € 0.00 for a specific analytical step therefore imply that no other equipment than basic laboratory equipment was used by the institution (see previous table).

As indicated in the table above, the total per-sample equipment costs of WGS analysis show substantial variation across case study institutions. The cost of third-generation sequencing using the GridION lies well below the other case studies at about EUR 5 per sample. Among the second-generation sequencing case studies, the costs range from a low of EUR 30 to a high of EUR 211. Comparing the second-generation figures to the total purchase costs illustrates the influence of increasing returns to scale in WGS analysis with respect to the total sample volume: While PHE reports the second-highest total purchase costs of equipment by a wide margin over the other case studies, at nearly EUR 2.5 million, the high sample volume (more than 15 000 during the reference period of 12 months) also leads it to achieve the second-lowest per-sample equipment cost, at just EUR 35 per isolate sequenced.

A largely similar picture can be observed if the four analytical steps are considered separately, with a notable exception being the bioinformatics step:

- As discussed above, per sample costs vary in the sample processing and library preparation steps based on how much specialised equipment is used (as opposed to basic laboratory equipment) as well as the degree of automation. However, when considering the costs on a per-sample basis, economies of scale play a much larger role. For example, the total purchase cost of equipment used by FLI in the first two steps amounts to EUR 49 300, compared to the cost of more than EUR 1.2 million for PHE's highly automated system; however, because PHE processed far more samples than FLI during the relevant reference period (15 791 compared to 30), the per-sample cost of equipment in the pre-sequencing steps comes down to just EUR 16 at PHE, while FLI's per-sample equipment cost in these steps is more than twice as high, at EUR 38.
- In the sequencing step, the most relevant factor affecting per-sample cost, other than sequencing generation (second vs third), is again economies of scale resulting from the volume of samples. For the most part, the per-sample costs in the sequencing stage are inversely related to the number of samples. However, sample volume is not the only determinant of costs, as illustrated by a comparison of the per-sample costs between APHA and FLI. Although both are outbreak case studies with comparable sample volumes during their reference periods (respectively 26 and 30), the per-sample sequencing equipment cost at FLI (EUR 161) is almost three times higher than at APHA (EUR 57). This is not due to significant differences in the purchase costs of their respective sequencers (as shown in Table 5, the purchase costs are comparable), but rather to differences in the use rate of the equipment. While FLI indicated that their sequencer was used 90% during the reference period for the 30 samples connected to the outbreak, APHA indicated that their sequencer was used only 10% during the reference period for the 26 outbreak samples. As APHA's sequencer was mostly used for other purposes during this period, the per-sample equipment cost once the use rate is accounted for is lower than would otherwise be expected from the sample volume alone.

In the bioinformatics step, economies of scale are once again a factor, although the sophistication of the equipment purchased appears to play an even larger role here than in the previous steps. A highly sophisticated bioinformatics infrastructure is expensive, more so than the sequencer(s), and can cost a large institution millions of EUR, as illustrated by the purchase cost data from PHAC, as well as the fact that bioinformatics equipment makes up approximately 90% of its total per-sample equipment cost. Smaller institutions, in contrast, appear able to build a less extensive but still adequate (for the lower volume of samples) bioinformatics infrastructure for a fraction of the cost, i.e. for EUR 50 000 or less.

5.1.2. Consumables

Consumables costs cover all items that are used up in laboratory processes, ranging from standard items such as gloves and pipette tips to specialised reagents and kits. Failure rates are also incorporated into the analysis to account for e.g. failed sequencing runs. The table below lists the types of consumables used by each institution to conduct WGS.

Table 6: WGS – Types of consumables, by analytical step

		APHA (UK)	FLI (DE)	EMC (NL)	IZSLER (IT)	ANLIS (ARG)	MDH (USA)	PHAC (CAN)	PHE (UK)
Case study type		Outbreak	Outbreak	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance
Pathogens		Avian influenza	Avian influenza	Influenza	Foodborne*	Foodborne*	Foodborne*	Foodborne*	Foodborne*
Batch size for sample processing/sequencing		1-2	6	20	24	12	24	32	Processing: 40 Sequencing: 96
Analytical steps	Sample processing	*- Qiagen viral RNA extraction kit*	*- Covaris-Vials* *- RNA-Purification* *- RT-PCR* *- Agilent Bioanalyzer RNA Pico Kit* *- Lab gloves* *- DNA/RNA-Extraction* *- Gelextraction/ DNA-Purification* *- PCR* *- Pipette tips* *- 96-Well PCR-plates* *- Reaction tubes*	*- RNA isolation kit* *- RT-PCR kit* *- Other consumables*	*- Qiagen DNAeasy Kit* *- Tips* *- Eppendorf vials* *- Gloves* *- General Reagents*	*- Qiacube box* *- Eppendorf DNA LoBindMicrocentifuge tubes* *- Filter tips for Qiacube*	*- MagNA pure 24 processing cartridge* *- MagNA pure 24 total NA isolation kit* *- MagNA pure filter tips* *- MagNA pure tube* *- Sealing foil* *- MagNA pure tip park & piercing tools*	*- EZ1 kit* *- BioRad plates*	*- Various reagents and consumables*
	Library preparation	*- Roche cDNA synthesis kit*	*- GeneRead Library Prep Kit* *- KAPA Library Quant IonTorrent* *- Adapter* *- Agilent Bioanalyzer DNA HS Kit*	*- Ligase* *- Sequencing kit* *- Other consumables*	*- Nextera XT DNA SAMP Prep* *- Nextera Xt index* *- Agencourt Ampure XP* *- Microseal A* *- Micro-Plate* *- Deepwell plate* *- Microseal B* *- Tips* *- PCR-tube* *- Gloves*	*- 96 samples* *- 96 indices, 384 samples* *- Agencourt AMPure XP Beads* *- Qubit reagent BR* *- Qubit reagent HS* *- Filter tips*	*- Nextera XT library prep (v2 kit)* *- Nextera XT library prep (v3 kit)* *- Index Set A* *- Index Set C* *- Ampure XP* *- Other disposables*	*- Plates* *- PCR CleanDX* *- TapeStation Tape + Reagent* *- TapeStation Tips* *- TapeStation 8-strip tubes* *- TapeStation plate* *- Reservoirs* *- Nextera XT Library Kit* *- 2X KAPA HiFi HotStart ReadyMix*	*- Nextera 96* *- PE Rapid cluster kit 2x96* *- 96 indices* *- 384 samples* *- cBot loading kit (rapid only) 2x 96*

								- *Pippin casette* - *Qubit rgt & tubes* - *Micrcon column*	
	Sequencing	- *Nextera XT kit*	- *Chips (316v2)* - *Sequencing Reagents* - *Onetouch Reagents* - *W2-Bottles* - *Enrichment Beads* - *Nitrogen*	- *Flowcell*	- *MiSeq Reagent Kit V2 (2x250)*	- *MiSeq Reagent Kit v2 500 cycles*	- *500 cycle v2 kit* - *500 cycle v3 kit*	- *Cartridge + flow cell (600 v3)*	- *200 cycle rapid v2 2x96* - *Other various costs*
	Bioinformatics	*None*	*None*	*None*	*None*	*None*	*None*	*None*	*None*

Source: Own compilation based on case study results. Notes: * Foodborne pathogens: Salmonella (all), Listeria (IZSLER, PHE, PHAC, MDH), E.coli and shigella (PHE, ANLIS, MDH), Campylobacter (PHE, MDH), Vibrio (MDH).

The table above shows the variation in consumables used for WGS across case studies. It is notable that the required consumables are dependent on the type of equipment that a particular institution has purchased, as this determines the types of reagents or kits that are required for sequencing. Case study institutions using Illumina sequencers, for example, also use Illumina consumables such as Nextera or MiSeq kits (see APHA, IZSLER, ANLIS, MDH, PHAC, PHE), while the IonTorrent requires a different set of consumables (see FLI), and the GridION by Oxford Nanopore requires flowcells that are uniquely produced by Oxford Nanopore (see EMC).

The dependency on specific consumables for sequencing was noted by some of the case study institutions as a significant factor driving costs, making WGS less affordable. Particularly in our interviews with institutions in Kenya[28] and Argentina, this was highlighted as a serious problem. The costs of kits and reagents were reported to be significantly higher in these countries, making it cheaper to purchase kits through partner organisations abroad or even to outsource the sequencing itself to institutions in Europe or the US. Possible reasons mentioned for the higher costs included pricing policies of the producers or local distributors of consumables, import duties, and variations in exchange rates. Similarly, it was noted by another case study institution (EMC) that substantial discounts on consumables (as well as equipment) could be obtained through negotiation with suppliers to buy in bulk. For example, EMC reported that it was able to obtain discounts of up to 60% over the list price for key consumables and WGS equipment by joining together with other university hospitals and collectively negotiating with the suppliers.

The table below shows the consumables costs per sample for each case study institution by analytical step.

[28] In addition to the interviews with and visits to the case study institutions, the results of which are presented in this report, several exploratory interviews have also informed the analysis, including with relevant experts in Kenya.

Table 7: WGS – Costs of consumables per sample, by analytical step

		APHA (UK)	FLI (DE)	EMC (NL)	IZSLER (IT)	ANLIS (ARG)	MDH (USA)	PHAC (CAN)	PHE (UK)
Case study type		Outbreak	Outbreak	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance
Pathogens		Avian influenza	Avian influenza	Influenza	Foodborne*	Foodborne*	Foodborne*	Foodborne*	Foodborne*
Batch size for sample processing/sequencing		1-2	6	20	24	12	24	32	Processing: 40 Sequencing: 96
Analytical step	Sample processing	€4.63	€ 45.44	€ 16.80	€ 4.32	€ 3.45	€ 11.47	€ 12.07	€ 6.84
	Library preparation	€ 70.28	€ 77.95	€ 21.25	€ 46.85	€ 35.24	€ 44.16	€ 24.08	€ 36.45
	Sequencing	€ 756.06	€ 131.49	€ 16.83	€ 114.20	€ 65.93	€ 48.76	€ 33.60	€ 10.62
	Bioinformatics	-	-	-	-	-	-	-	-
Total		**€ 830.97**	**€ 254.88**	**€ 54.88**	**€ 165.37**	**€ 104.62**	**€ 104.40**	**€ 69.75**	**€ 53.92**

Source: Own compilation based on case study results. Notes: Costs per sample are adjusted for failure rate. * Foodborne pathogens: Salmonella (all), Listeria (IZSLER, PHE, PHAC, MDH), E.coli and shigella (PHE, ANLIS, MDH), Campylobacter (PHE, MDH), Vibrio (MDH).

As can be seen in the table above, large variations were observed in the costs of consumables reported by the case study institutions, ranging from EUR 54 to EUR 831 per sample. Most variation in costs occurs in the sequencing step, which ranges from a per-sample cost of EUR 11 to EUR 756.

One of the most significant cost drivers for consumables appears to be the batch size. Excluding the EMC case study with Nanopore sequencing for the moment, the case studies can be roughly divided into three groups based on batch size:

- Low-throughput case studies with batch sizes below 12 (FLI, APHA). These two case studies are also the two case studies that concern outbreak situations, in which batch size is often low due to the time-sensitivity of the information on the pathogen required;
- Medium-throughput case studies with batch sizes of between 12 and 24 in routine surveillance situations (IZSLER, ANLIS, MDH); and
- High-throughput case studies with batch sizes of 32 or higher in routine surveillance situations (PHAC, PHE).

Among these three groups, the total per-sample consumables costs decreases as the average throughput increases. Accordingly, low-throughput (outbreak) case studies have the highest per-sample consumables costs, ranging from EUR 255 to EUR 831; medium-throughput (routine surveillance) case studies have lower consumables costs, ranging from EUR 104 to EUR 165; and the high-throughput (routine surveillance) case studies have the lowest per-sample consumables costs of EUR 54 to EUR 70. Notably, while the EMC case study with Nanopore sequencing would belong to the medium-throughput case study by batch size (20), its per-sample costs (EUR 55) are more similar to the high-throughput case studies.

The impact of batch size on the per-sample consumables costs is particularly well-illustrated by a comparison between APHA and PHE, as both institutions make use of different Nextera kits by Illumina for library preparation and sequencing. For both institutions, these kits constitute the most expensive consumable cost. As APHA uses an Illumina Nextera kit for batches of 1-2 samples at a time[29] due to the time sensitivity of the information on the pathogen required in the context of an avian influenza outbreak, the costs of the kit come out to EUR 756 per sample. In contrast, at PHE, which handles a much larger volume of samples in the context of routine surveillance of foodborne diseases, batch size is much larger, reducing the per-sample cost of its Nextera kits to approximately EUR 24.

5.1.3. Staff costs

Staff time for WGS analysis is provided in terms of the minutes of *hands-on staff time* per sample and divided into professional and technician staff categories.[30] It refers to

[29] An average batch size of 1.6 was assumed for this consumable.

[30] For 'Professionals', occupations typically involve the performance of tasks that require complex problem-solving, decision-making and creativity based on an extensive body of theoretical and factual knowledge in a specialised field. The knowledge and skills required are typically obtained as the result of study at a higher educational institution for a period of 3-6 years following completion of secondary education leading to the award of a first degree or higher qualification. This category includes PhD candidates and Post-docs. For 'Technicians', occupations typically involve the performance of complex technical and practical tasks that require an extensive body of factual, technical and procedural knowledge in a specialised field. The knowledge and skills required are usually obtained as the result of study at a higher educational institution for a period of 1-3 years following completion of secondary education. This category includes laboratory assistants.

the amount of staff time used to perform an activity, and not the duration of the activity itself: unsupervised processes (such as incubation periods or sequencing runs) are therefore not included in estimates of hands-on staff time. Where samples are processed in batches, the total amount of staff time is divided by the batch size.[31] The hands-on staff time per sample is then monetised based on country-specific standard labour costs for professional and technician staff categories, plus 25% for overhead costs.

Hands-on staff time estimates per sample differ considerably between case study institutions. Estimates of professional staff time per sample range from 24 to 90 minutes (with the costs ranging from EUR 7 to EUR 62), while estimates of technician staff time per sample range from 0 to 210 minutes (with associated costs between EUR 0 and EUR 88). The following table provides a breakdown of hands-on staff time per sample by analytical step and staff category.

[31] For example, if the simultaneous processing of 40 samples takes 160 minutes of staff time, this figure would be divided by 40 and result in a staff time estimate of 4 minutes per sample.

Table 8: WGS – Staff time per sample, by analytical step (in minutes, and Euro)

			APHA (UK)	FLI (DE)	EMC (NL)	IZSLER (IT)	ANLIS (ARG)	MDH (USA)	PHAC (CAN)	PHE (UK)
Case study type			Outbreak	Outbreak	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance
Pathogens			Avian influenza	Avian influenza	Influenza	Foodborne*	Foodborne*	Foodborne*	Foodborne*	Foodborne*
Batch size for sample processing/sequencing			1-2	6	20	24	12	24	32	Processing: 40 Sequencing: 96
Analytical step	Sample processing	Prof.	0	8	0	0	11	2	‡	2.6
		Tech.	*60*	*40*	*48*	*20*	*0*	*0*	‡	*16.8*
	Library preparation	Prof.	0	3	0	0	18	21†	‡	1.6
		Tech.	*60*	*60*	*18*	*10*	*0*	*0*†	‡	*0.0*
	Sequencing	Prof.	0	7	6	0	2	†	0	2.6
		Tech.	*90*	*35*	*6*	*5*	*0*	†	*19*‡	*0.3*
	Bioinformatics	Prof.	60	30	18	70	60	15	90**	36.0
		Tech.	*0*	*0*	*48*	*0*	*0*	*0*	*19***	*0.0*
Total minutes per sample		Prof.	**60**	**48**	**24**	**70**	**91**	**29**	**90**	**42.9**
		Tech.	***210***	***135***	***120***	***35***	***0***	***0***	***19***	***17.2***
Total cost per sample, based on country-specific labour costs		Prof.	**€ 39.63**	**€ 42.60**	**€ 21.27**	**€ 52.35**	**€ 6.85**	**€ 20.58**	**€ 61.82**	**€ 28.30**
		Tech.	***€ 87.50***	***€ 60.19***	***€ 56.48***	***€ 13.93***	***€ 0.00***	***€ 0.00***	***€ 7.89***	***€ 7.15***

Source: Own compilation based on case study results. Note: Not included are costs for training or other one-off staff costs. Total cost per sample is based on country-specific labour costs for professional and technician staff categories sourced from Eurostat (lc_lci_lev, 'Professional, scientific and technical activities' and 'Administrative and support service activities', extracted 27 June 2018) for EU countries; labour cost data provided by case study institutions for Canada, USA and Argentina. In all cases 25% has been added to cover overhead costs. * Foodborne pathogens: Salmonella (all), Listeria (IZSLER, PHE, PHAC, MDH), E.coli and shigella (PHE, ANLIS, MDH), Campylobacter (PHE, MDH), Vibrio (MDH). ** Bioinformatics staff time for PHAC includes IT support for the reference database as well as staff time for genomic epidemiology. † Sequencing staff time included in the estimate for library preparation. ‡ All staff time for 'wet-lab' steps from sample processing to sequencing are reported under sequencing.

As the table above shows, for all but two case study institutions, the first three stages of the analysis (sample processing and library preparation, to some extent also sequencing) are mostly carried out by technician-level staff, with some institutions indicating a limited amount of professional time for supervisory or quality control purposes. This is not the case at ANLIS or MDH, where the relevant departments do not employ technician staff and where therefore all staff time is professional. The bioinformatics stage is exclusively performed by professional staff in all case studies except the Nanopore case study with EMC.

The ratio of professional to technician staff time and the total hands-on staff time per sample varies across case studies, and particularly across types of case studies. The relevant institutions emphasised that in an outbreak situation, it is not always possible to wait for more samples to arrive so that they can be batched together for more efficient sample processing, library preparation or sequencing. In the routine surveillance case studies, in contrast, the institutions indicated that their standard procedure (outside an outbreak context) was to accumulate samples and run their sequencer(s) with higher batch sizes.

In addition to the batch size, the level of automation emerges as another key cost driver with respect to staff time. As indicated in the overview table above, the lowest total staff time requirements in the pre-bioinformatics stages are consistently achieved by PHE, which also has the highest level of automation in these stages (see the discussion in section 5.1.1 on equipment costs). Based on the data provided in the table above, the high level of automation at PHE appears to have the effect of generating considerable savings with respect to technician time in particular.

5.1.4. Other costs

None of the case study institutions indicated any additional costs for WGS analysis.

5.1.5. Total costs of WGS

The following table summarises the total per-sample costs of WGS by cost type.

Table 9: WGS – Total per sample costs, by cost type

		APHA (UK)	FLI (DE)	EMC (NL)	IZSLER (IT)	ANLIS (ARG)	MDH (USA)	PHAC (CAN)	PHE (UK)
Case study type		Outbreak	Outbreak	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance
Pathogens		Avian influenza	Avian influenza	Influenza	Foodborne*	Foodborne*	Foodborne*	Foodborne*	Foodborne*
Batch size for sample processing/sequencing		1-2	6	20	24	12	24	32	Processing: 40 Sequencing: 96
No. of samples analysed in reference period		26 *(in 8 months)*	30 *(in 3 months)*	178 *(in 3 months)*	175 *(in 12 months)*	320 *(in 12 months)*	1 767 *(in 12 months)*	8 630 *(in 12 months)*	15 791 *(in 12 months)*
Equipment		€ 58.53	€ 210.71	€ 5.39	€ 163.49	€ 43.02	€ 29.53	€ 75.90	€ 35.23
Consumables		€ 830.97	€ 254.71	€ 54.88	€ 165.37	€ 104.62	€ 104.40	€ 69.75	€ 53.92
Staff costs	*Prof.*	€ 39.63	€ 42.60	€ 21.27	€ 52.35	€ 6.85	€ 20.58	€ 61.82	€ 28.30
	Tech.	€ 87.50	€ 60.19	€ 56.48	€ 13.93	€ 0.00	€ 0.00	€ 7.89	€ 7.15
Other costs		€ 0.00	€ 0.00	€ 0.00	€ 0.00	€ 0.00	€ 0.00	€ 0.00	€ 0.00
Total per-sample cost WGS		**€ 1 016.63**	**€ 568.37**	**€ 138.01**	**€ 395.14**	**€ 154.49**	**€ 154.51**	**€ 215.36**	**€ 124.59**

Source: Own compilation based on case study results. * Foodborne pathogens: Salmonella (all), Listeria (IZSLER, PHE, PHAC, MDH), E.coli and shigella (PHE, ANLIS, MDH), Campylobacter (PHE, MDH), Vibrio (MDH).

As shown in the table above, the total per-sample cost of WGS ranges from a low of EUR 125 to a high of EUR 1 017. An inverse relationship can be observed between sample volume/batch size and total per-sample costs. Excluding the EMC Nanopore case study, the total per-sample costs decrease almost uniformly across the table from left to right as the total sample volume increases. The exception to this trend is PHAC, where the more extensive bioinformatics infrastructure (an outlier among the case studies) contributes to a significantly higher equipment and staff time cost. In general, low-throughput (outbreak) case studies (APHA, FLI) have the highest per-sample costs, ranging between EUR 568 and EUR 1 017; medium-throughput (routine surveillance) case studies have moderate per-sample costs, ranging between EUR 154 to EUR 395; and the high-throughput (routine surveillance) case studies have among the lowest per-sample costs at EUR 125 to EUR 215. As with the consumables costs (see 5.1.2.), the total per-sample costs in the Nanopore case study (EUR 138) are closer to the high-throughput costs than the medium-throughput costs.

Notably, the increasing returns to scale described above are visible to at least some extent in all major cost types, although the effect is most clear with respect to consumables and technician staff time.

5.2. Costs of conventional methods

In this analysis, the costs of WGS are compared to the costs of using relevant conventional methods for pathogen identification. Unlike WGS, conventional methods are mostly pathogen-dependent and can follow complex workflows depending on the method and pathogen. Different methods are often applied to the same isolate based on previous results (e.g. certain serovars are selected to undergo further testing) or pre-established workflows (e.g. 10% of all isolates are selected to undergo further testing). Determining the costs of conventional methods and comparing these across institutions and pathogens is therefore less straightforward than for WGS.

The following table provides an overview of the range of conventional methods that are or were in use by each of the case study institutions for each relevant pathogen, along with the percentage of samples per pathogen that would undergo each test.

Table 10: Conventional methods – Overview of methods per institution and pathogen (with percentage of samples typically processed using the method)

	APHA (UK)	FLI (DE)	EMC (NL)	IZSLER (IT)	ANLIS (ARG)	MDH (USA)	PHAC (CAN)	PHE (UK)
Avian influenza	Sanger sequencing – HA/NA analysis (100%)	Sanger sequencing – whole genome (100%)	-	-	-	-	-	-
Influenza	-	-	PCR (100%) Sanger sequencing (5%)	-	-	-	-	-
Salmonella	-	-	-	Serotyping (100%) PFGE (100%) PCR (50%) MLVA (60%)	Biochemical testing (100%) Serotyping (100%) MaldiTOF (5%) PFGE (100%)	PFGE (100%)	Biochemical analysis (100%) Serotyping (100%) PFGE (65%)	PCR x2 (73%, 10%) MLVA (48%) Serotyping (98%) Phage typing (99%) PFGE (3%) D-Tartrate (3%) Glucose gas (8%) AMR (68%)
Listeria	-	-	-	PFGE (100%)	-	PFGE (100%)	Biochemical analysis (100%) PFGE (100%)	PCR x2 (100%) fAFLP (100%)
E. Coli & Shigella	-	-	-	-	Biochemical testing (100%) PCR typing (100%) MaldiTOF (5%) PFGE (100%)	PFGE (100%) PCR (100%)	-	PCR (100%) MLVA (100%) Serotyping (100%) Phage typing (100%) Biochemistry (100%)
Campylobacter	-	-	-	-	-	PFGE (100%) MaldiTOF (100%)	-	PCR (100%) MLST (52%) Serotyping (12%) Phage typing (38%)
Vibrio	-	-	-	-	-	PFGE (100%) PCR (100%)	-	-

Source: Own compilation based on case study results. Figures in parentheses are the share of samples typically processed using the method.

The table above illustrates the diversity and complexity of the workflows involving conventional methods that were previously in place for each pathogen. Some of the case study institutions indicated that these conventional workflows were still either partially or fully in place at the time of the case study for various reasons, e.g. due to the need to fully validate the new technology or to meet statutory testing or reporting requirements.

In order to make the cost assessment of conventional methods comparable between institutions and comparable with the cost assessment for WGS despite the use of different methods and different workflows, we have calculated the *weighted average cost per sample* of the conventional methods in use at each institution. The weighted average cost takes into account the different use rates of each method per pathogen in order to arrive at a single estimate for the cost of an 'average' sample.[32] For detailed costs and other information (e.g. equipment and consumable types) on the different conventional methods in use at each institution, see the case study reports in section 4.

The analysis of conventional costs encompasses the same cost types as the analysis of WGS costs (equipment, consumables, staff time, other costs). However, unlike the cost assessment of WGS, the costs of the conventional methods are not further broken down by analytical step, as the analytical steps are not consistent between the different methods. The table below therefore presents an overview of the weighted average cost per sample according to cost type.

[32] For example, if an institution indicated that 100% of Salmonella samples were subject to serotyping (at a cost of e.g. EUR 20) and 50% subject to PFGE (at a cost of e.g. EUR 50), then the weighted average cost per sample would be EUR 20 *1 + EUR 50 *0.5 = EUR 45. Where an institution analysed multiple pathogens with different methods, the average costs would be calculated per pathogen and then further weighted by the proportion of each pathogen in the total samples analysed. To further increase comparability of the costs calculated, we have also assumed as part of the cost assessment that the same number of samples that were processed with WGS would have instead been processed using conventional methods, following the workflows indicated in the table above.

Table 11: Conventional methods – Weighted average cost per sample, by cost type

		APHA (UK)	FLI (DE)	EMC (NL)	IZSLER (IT)	ANLIS (ARG)	MDH (USA)	PHAC (CAN)	PHE (UK)
Case study type		Outbreak	Outbreak	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance
Pathogens		Avian influenza	Avian influenza	Influenza	Foodborne*	Foodborne*	Foodborne*	Foodborne*	Foodborne*
No. of samples analysed in ref. period (WGS)†		26 *(in 8 months)*	30 *(in 3 months)*	178 *(in 3 months)*	175 *(in 12 months)*	320 *(in 12 months)*	1 767 *(in 12 months)*	8 630 *(in 12 months)*	15 791 *(in 12 months)*
Conventional method(s)		Sanger sequencing (HA/NA analysis)	Sanger sequencing (whole genome**)	PCR; Sanger sequencing (HA/NA)	Serotyping; PFGE; PCR; MLVA	Biochemical analysis; Serotyping; PCR typing; MaldiTOF; PFGE	PFGE; PCR; MaldiTOF	PFGE; Biochemical testing; Serotyping	PCR; MLVA; MLST; fAFLP; Serotyping; Phage typing; PFGE; D-Tartrate; Glucose gas; AMR; Biochemistry
Equipment		€ 78.55	(€ 137.35)**	€ 3.41	€ 26.04	‡	€ 5.84	€ 12.30	€ 7.11
Consumables		€ 21.91	(€ 360.88)**	€ 32.19	€ 20.17	‡	€ 32.89	€ 34.95	€ 29.91
Staff time (minutes)	*Prof.*	*60*	*(260)***	*0*	*5*	‡	*61*	*10*	*4*
	Tech.	*360*	*(240)***	*38*	*65*	‡	*0*	*98*	*57*
Staff costs	*Prof.*	€ 39.63	(€ 230.75)**	€ 0.00	€ 3.52	‡	€ 42.43	€ 6.72	€ 2.92
	Tech.	€ 150.00	(€ 107.00)**	€ 17.79	€ 25.88	‡	€ 0.00	€ 40.32	€ 23.85
Other costs		€ 0.00	(€ 0.00)**	€ 0.00	€ 16.27	‡	€ 0.00	€ 0.00	€ 1.67
Total per-sample cost		**€ 290.08**	**(€ 835.98)****	**€ 53.38**	**€ 91.87**	**€ 46.61**	**€ 81.16**	**€ 94.29**	**€ 65.46**

Source: Own compilation based on case study results. Notes: The weighted average cost of the conventional methods accounts for the use rate of the various methods across the different pathogens. * Foodborne pathogens: Salmonella (all), Listeria (IZSLER, PHE, PHAC, MDH), E.coli and shigella (PHE, ANLIS, MDH), Campylobacter (PHE, MDH), Vibrio (MDH). ** Note that the sequencing of a whole genome of a virus using Sanger sequencing (as indicated by FLI as comparator method) is a resource-intensive process that has generally been replaced by next-generation sequencing (i.e. what we refer to in this study as WGS), and Sanger sequencing would typically be used for the (more limited and less resource-intensive) HA/NA analysis. The figures from the FLI case study are therefore placed in brackets and are provided for comparison purposes only. † Note that the total number of samples in the reference period refers to the total number of samples processed by WGS, which was also used as a basis for determining the costs of conventional methods (as it considers the hypothetical situation of using these methods under similar circumstances). It does not refer to the actual number of samples processed using conventional methods in this period. ‡No further cost breakdown by cost type was possible.

As indicated in the table above, the case study institutions are highly diverse with respect to the cost of conventional methods in total and per cost type. The per-sample cost is considerably higher for the two outbreak case studies than for the routine surveillance case studies. Of the two outbreak case studies, the FLI case study stands out with a relatively high per-sample cost of EUR 836. However, the conventional method assessed in this case study is the sequencing of a whole virus genome using Sanger sequencing, which is a resource-intensive process that has generally been replaced by next-generation sequencing (i.e. what we refer to in this study as WGS), and is not a typical routine method. In contrast, Sanger sequencing is also used for (more limited and less resource-intensive) HA/NA analysis (as at APHA and EMC), which is a more suitable point of reference. Excluding the case of whole genome sequencing with Sanger sequencing, the total weighted average cost of conventional methods across the case studies ranges from approximately EUR 58 to EUR 290.

For reasons discussed with respect to each cost type below, the cost difference between these two types of case studies appears to be due more to the difference in the choice of methods (Sanger sequencing vs. other conventional methods) than to the difference in sample volume, as had been the case with WGS analysis.

- Equipment costs are on average higher in the avian influenza case studies compared to the foodborne pathogen case studies. The higher equipment costs in these case studies result from a combination of lower sample volumes in the context of an outbreak (which raise the per-sample cost, all else being equal) and the high purchase cost of the ABI sequencers used to conduct Sanger sequencing, which range from EUR 120 000 to EUR 199 999. In contrast, the conventional methods used for foodborne pathogens rely on less expensive equipment (e.g. thermal cyclers, PCR machines), and some methods (e.g. MLVA) are conducted using standard laboratory equipment only.
- In terms of consumables, the costs are generally comparable between case studies; the weighted average consumables costs for conventional methods are relatively low, ranging from about EUR 22 to EUR 30, except in the case of ANLIS (EUR 90).
- Staff time and therefore staff costs are much higher in both of the avian influenza case studies than in the foodborne pathogen case studies. This is most likely a consequence of the lower batch sizes due to the outbreak context, the more complex and time-intensive steps involved in Sanger sequencing (e.g. RNA extraction, PCR amplification, purification, sequencing, bioinformatics) as well as the greater involvement of professional staff in supervision, quality control and bioinformatics.[33] In contrast, the conventional methods used in the foodborne pathogen case studies tend to be more straightforward (e.g. PCR or MLVA analysis, which each require less than 10 minutes of staff time) and rely often entirely on technician staff rather than professional staff.
- Other costs listed in the table above refer to the cost price of certain conventional methods where the test had either been outsourced by the institution (e.g. MLVA at IZSLER) or where no detailed costing data was available to provide a breakdown by cost type (e.g. PFGE at PHE). No costs other than these were noted by the case study institutions.

[33] Sanger sequencing was also used in the EMC influenza virus case study. However, at EMC, Sanger sequencing was only performed on 5% of samples (and therefore factors less into the weighted average costs of conventional methods), while at APHA and FLI it was performed on 100% of samples.

5.3. Costs of WGS vs conventional methods

The total per-sample cost of WGS analysis exceeds the cost of conventional methods in all case studies except one, where a non-routine method was chosen as comparator by the case study institution (see above). Excluding this result, the use of WGS is between 1.9 and 4.3 times more expensive than the use of conventional methods, with a cost differential between EUR 55 and EUR 727. The table below summarises the costs of WGS and conventional methods according to cost type and presents the additional costs of WGS for each case study.

Table 12: Overview of costs of WGS versus conventional methods, by cost type (per sample costs)

		APHA (UK)	FLI (DE)	EMC (NL)	IZSLER (IT)	ANLIS (ARG)	MDH (USA)	PHAC (CAN)	PHE (UK)
Case study type		Outbreak	Outbreak	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance
Pathogens		Avian influenza	Avian influenza	Influenza	Foodborne*	Foodborne*	Foodborne*	Foodborne*	Foodborne*
WGS									
Equipment		€ 58.53	€ 210.71	€ 5.39	€ 163.49	€ 43.02	€ 29.53	€ 75.90	€ 35.23
Consumables		€ 830.97	€ 254.88	€ 54.88	€ 165.37	€ 104.62	€ 104.40	€ 69.75	€ 53.92
Staff costs	*Prof.*	€ 39.63	€ 42.60	€ 21.27	€ 52.35	€ 6.85	€ 20.58	€ 61.82	€ 28.30
	Tech.	€ 87.50	€ 60.19	€ 56.48	€ 13.93	€ 0.00	€ 0.00	€ 7.89	€ 7.15
Other costs		€ 0.00	€ 0.00	€ 0.00	€ 0.00	€ 0.00	€ 0.00	€ 0.00	€ 0.00
Total per-sample cost WGS		**€ 1 016.63**	**€ 568.37**	**€ 138.03**	**€ 395.14**	**€ 154.49**	**€ 154.51**	**€ 215.36**	**€ 124.59**
Conventional methods									
Equipment		€ 78.55	(€ 137.35)**	€ 3.41	€ 26.04	†	€ 5.84	€ 12.30	€ 7.11
Consumables		€ 21.91	(€ 360.88)**	€ 32.19	€ 20.17	†	€ 32.89	€ 34.95	€ 29.91
Staff costs	*Prof.*	€ 39.63	(€ 230.75)**	€ 0.00	€ 3.52	†	€ 42.43	€ 6.72	€ 2.92
	Tech.	€ 150.00	(€ 107.00)**	€ 17.79	€ 25.88	†	€ 0.00	€ 40.32	€ 23.85
Other costs		€ 0.00	(€ 0.00)**	€ 0.00	€ 16.27	†	€ 0.00	€ 0.00	€ 1.67
Total per-sample cost conventional methods		**€ 290.08**	**(€ 835.98)****	**€ 53.38**	**€ 91.87**	**€ 46.61**	**€ 81.16**	**€ 94.29**	**€ 65.46**
Cost difference between WGS and conventional methods									
Additional cost of WGS		**€ 726.54**	**(- € 267.61)****	**€ 84.63**	**€ 303.27**	**€ 107.88**	**€ 73.35**	**€ 121.07**	**€ 59.13**
Quotient of WGS over conventional methods		**3.5**	**(0.7)****	**2.6**	**4.3**	**3.3**	**1.9**	**2.3**	**1.9**

Source: Own compilation based on case study results. Note that the cost of the conventional methods is a weighted figure which accounts for the use rate of the various methods across the different pathogens. * Foodborne pathogens: Salmonella (all), Listeria (IZSLER, PHE, PHAC, MDH), E.coli and shigella (PHE, ANLIS, MDH), Campylobacter (PHE, MDH), Vibrio (MDH). ** Sequencing of a whole genome of a virus using Sanger sequencing (as indicated by FLI as comparator method) is a resource-intensive process that has generally been replaced by next-generation sequencing, and Sanger sequencing would typically be used for the (more limited and less resource-intensive) HA/NA analysis (the comparator method used by APHA). †No further cost breakdown by cost type was possible.

As can be seen in the table above, per-sample *equipment* costs are higher for WGS by a substantial margin in all but one of the case studies (APHA), when compared to the costs of conventional methods. This is particularly true for the foodborne pathogen case studies, which, as discussed in the previous section, generally rely on less costly equipment for conventional methods than the other case studies, and therefore have a greater difference between the equipment costs for WGS and for conventional methods. For the avian influenza case studies, where the alternative method (Sanger sequencing) required the purchase of a sequencer comparable in original purchase price to modern next-generation sequencers, the difference in costs is relatively smaller (FLI) or even in favour of WGS (APHA). The difference between equipment costs for WGS and conventional methods is narrowest for the case study using Nanopore sequencing (EMC).

Per-sample *consumables* costs are higher for WGS then for conventional methods in all but one case study (FLI), and sometimes considerably so, as e.g. in the case of APHA, where the consumables cost for WGS (EUR 831) is nearly 38 times the consumables cost for conventional methods (EUR 22). The determining factor, as noted before, is the higher cost of kits and reagents required for WGS. The difference in consumables costs between WGS and conventional methods can be reduced but not eliminated through higher batch sizes and higher sample volumes; this is illustrated by PHE, which has the highest batch size (40/96) and sample volume (15 791), and consequently achieves one of the smallest differences in the costs of consumables between WGS and conventional methods. EMC also achieves one of the lowest cost differences, despite having a smaller batch size than PHE, due to the use of Nanopore sequencing.

With respect to per-sample *staff costs*, results depend on the staff category. Technician costs are considerably lower for WGS in four out of seven case studies. In contrast, professional costs for WGS are much higher than for conventional methods in the foodborne pathogen case studies, but are either on par with or lower than for conventional methods in the avian influenza studies, which reflects the more complex procedures and analysis required for Sanger sequencing.

Overall, the variable size of the cost difference between WGS and conventional methods appears to be attributable to a combination of the type of conventional method used (as Sanger sequencing implies greater equipment and staff costs than the other methods, all else being equal) and the batch size/sample volume used in WGS analysis. As described previously, considerable economies of scale can be achieved with respect to equipment and consumables costs in particular. However, the results of the case study at EMC suggest that lower cost differences could also potentially be achieved at a lower batch size/sample volume through Nanopore sequencing.

5.4. Analysis of benefits

This section presents the analysis of the benefits of using WGS for pathogen identification and surveillance as compared to conventional methods, based on the experiences of the case study institutions.

According to the results of our desk research and exploratory expert interviews, benefits of using WGS for pathogen identification and surveillance may accrue in different areas, including:

- Sampling and sampling strategies;
- Analytical results and processes;
- Effects on research and methods applied;
- Outbreak identification and response; and

Effects on wider society.

To understand better the relevance and significance of potential benefits, we provided case study institutions a list of specific positive effects in each of the five areas listed above. We asked the institutions to assess whether they had experienced these effects in practice on a scale from 1 (no effect at all) to 5 (very significant positive effect).

The following subsections describe the effects observed by the case study institutions in detail for each benefit area and presents related evidence.

5.4.1. Effects on sampling and sampling strategies

The use of WGS could result in simplifications in the type of samples needed or in the storage/transport of samples, as well as a potential reduction in the number of samples needed. For example, effects on the sampling process could be expected to result from the ability to more easily conduct environmental sampling using WGS (e.g. through the use of swabs in lieu of finished product testing), as well as from the ability to adapt sampling strategies in response to better epidemiological data in real time. As a result, these effects could reduce the overall costs of sampling. However, six of the eight case study institutions reported that they observe little to no effects in the area of sampling and sampling strategies, or in the related costs. One of the reasons for the generally low assessments in the area of sampling and sampling strategies was that sampling is mostly not within the purview of the case study institutions, as samples are independently collected by external partners and sent to the case study institutions for further analysis. Any potential effects of WGS on sampling or sampling strategies would therefore be limited.

The following table presents the assessments of the case study institutions for specific effects related to sampling and sampling strategies, based on their practical experience.

Table 13: Effects of WGS on sampling and sampling strategies experienced by case study institutions

	APHA (UK)	FLI (DE)	EMC (NL)	IZSLER (IT)	ANLIS (ARG)	MDH (US)	PHAC (CAN)	PHE (UK)
Simplifications in the type of samples needed	5	1	1	3	1	–	1	1
Simplifications in sample storage or transport	–	1	1	–	2	–	1	1
Reductions in the number of samples needed	1	2	1	–	1	–	1	1
Reductions in the overall costs of sampling	–	1	1	–	1	–	1	1

Note: Case study institutions were asked to assess specific positive effects or impacts of using WGS on a scale from 1 (no effect at all) to 5 (very significant positive effect). '–' indicates not applicable or don't know/no answer. Assessments of moderate to very significant positive effects (3 to 5) are highlighted in colour.

Only one of the specific positive effects listed – simplification of the type of samples needed – was considered by two of the case study instutions (APHA and IZSLER) to be moderate to very significant. APHA indicated that WGS had been able to reduce the pre-processing required for samples in cases where no viral amplification was necessary, resulting in time savings of approximately two work days per sample. IZSLER indicated that while it is not responsible for sampling, its partner organisations in food safety that do conduct sampling have changed their processes in response to IZSLER's adoption of WGS by replacing finished product testing with more environmental samples from surfaces in food facilities.

5.4.2. Effects on analytical results and processes

Effects of WGS on analytical results and processes could include effects related to the quality of results (e.g. improved accuracy or specificity) as well as effects on laboratory processes, costs and organisation (e.g. simplified workflows or a reduction in time needed for the analysis). WGS is expected to have effects in this area due to the higher resolution of the data produced as well as the potential to replace multiple conventional tests with more streamlined, pathogen-neutral workflows using WGS.

The following table presents the assessments of the case study institutions for specific effects experienced by them with respect to analytical results and processes.

Table 14: Effects of WGS on analytical results and processes experienced by case study institutions

	APHA (UK)	FLI (DE)	EMC (NL)	IZSLER (IT)	ANLIS (ARG)	MDH (US)	PHAC (CAN)	PHE (UK)
More detailed results produced	5	5	5	5	4	5	5	5
Improved accuracy of results produced	5	–	1	5	4	4	5	5
Improved specificity of results produced	5	–	1	5	4	5	5	5
Improved sensitivity of results produced	5	3	1	5	4	5	5	5
Simplified laboratory work flows	2	1	1	5	4	4	4	5
Reduction of staff time	2	1	3	5	4	2	1	5
Reduction of overall costs for analysis	2	3	–	–	–	1	4	5
Reduction of consumables needed for analysis	2	1	1	5	4	1	3	5
Reduction of time needed for analysis	3	1	1	4	1	1	*	4

Note: Case study institutions were asked to assess specific positive effects or impacts of using WGS on a scale from 1 (no effect at all) to 5 (very significant positive effect). '–' indicates not applicable or don't know/no answer. Assessments of moderate to very significant positive effects (3 to 5) are highlighted in colour. * PHAC indicated a very significant negative effect in this category, of temporary nature, for reasons described in the text.

Case study institutions were nearly unanimous in their assessment that WGS had a very significant positive effects on the quality of the results produced in terms of detail, accuracy, specificity, and sensitivity. For example, in the context of avian influenza identification and surveillance, the use of WGS was reported to provide many reads of a sequence, resulting in higher accuracy and greater statistical confidence in the outputs, and allowed viral genome-spanning information to be rapidly obtained regarding the genotype, pathotype, mutations, etc (reported by APHA).

For foodborne pathogens, it was indicated that WGS shows how bacterial strains diversify over time, allowing strains to be identified as linked when under previous methods they would have been considered unrelated (indicated by PHE). The higher resolution data from WGS also proved to be valuable in epidemiological investigations through the production of results beyond what would be possible with conventional methods (documented in detail by both PHE and ISZLER).[34] For a more detailed

[34] See Ashton, P., et al. (2015). Revolutionising public health reference microbiology using whole genome sequencing: Salmonella as an exemplar. *bioRxiv*, 033225; Butcher, H., et al. (2016). Whole genome sequencing improved case ascertainment in an outbreak of Shiga toxin-producing E. coli O157 associated with raw drinking milk. *Epidemiology and Infection*, 144(13), 2812–2823; Rew, V., et al. (2018). Whole-genome sequencing revealed concurrent outbreaks of shigellosis in the English Orthodox Jewish Community caused by multiple importations of Shigella sonnei from Israel. *Microbial Genomics*, 4(3), 1–7. Comandatore, F., et al (2017). Genomic Characterization Helps Dissecting an Outbreak of Listeriosis in Northern Italy. *PLoS Currents*, 9, 1–21.; Morganti, M., et al. (2015). Processing-dependent and clonal contamination patterns of Listeria monocytogenes in the cured ham food chain revealed by genetic analysis.

description of the applications of WGS data in epidemiological investigations, see the discussion in the next sub-section.

Relative to the effects observed on the quality of results, the effects on laboratory processes and resources were considered to be not applicable or mostly negligible in the influenza case studies (APHA, FLI, and EMC), while being significant for the case study institutions that use WGS in the context of routine surveillance of foodborne pathogens. IZSLER, ANLIS, MDH, PHAC and PHE all assessed the positive effects on laboratory workflow to be significant or very significant, while IZSLER, ANLIS and PHE also considered that there had been very significant positive effects on laboratory resources (e.g. a reduction in necessary consumables). IZSLER and PHE both considered that WGS had already reduced the number of hands-on steps for analysis. In a report published in August 2018,[35] PHE outlined the following specific effects on the streamlining of laboratory processes that it had observed since adopting WGS:

- At least 10 different validated processes for different bacteria had been replaced with WGS;
- Pathogens were being processed in fewer rooms (e.g. Salmonella samples are now being processed in one room rather than travelling through nine different laboratories); and
- Samples containing live organisms now required fewer hands-on interactions (from being handled 7-9 times before WGS to 2 times after WGS), reducing accident risks for laboratory staff.

PHE also indicated that WGS had improved laboratory management, reporting that replacing conventional methods with WGS made it easier to monitor its own laboratory processes, predict costs, and identify ways to reduce costs in the future.

Among the specific effects and impacts of WGS related to analytical results and processes, the case study institutions reported that the effects on the time needed for the analysis were among the less significant positive effects, although this also depends on the type of case study. Data on turnaround time of WGS compared to conventional methods provided by the case study institutions provides more detailed information in this respect. The following table presents the usual number of days of work[36] from receipt and opening of an incoming sample to the reporting of the results, as reported by the case study institutions.

Applied and Environmental Microbiology, 82(3), 822–831; Scaltriti, E., et al. (2015). Differential single nucleotide polymorphism-based analysis of an outbreak caused by Salmonella enterica serovar Manhattan reveals epidemiological details missed by standard pulsed-field gel electrophoresis. *Journal of Clinical Microbiology*, 53(4), 1227–1238.

[35] Grant, K., Jenkins, C., Arnold, C., Green, J., & Zambon, M. (2018). *Implementing pathogen genomics. A case study.* Public Health England. p. 22.

[36] Note that days of work do not include weekends and holidays, except where work has been conducted on these days, e.g. for a sequencing run or other analyses.

Table 15: Turnaround time of WGS compared to conventional methods, in days of work

Institution	APHA (UK)	FLI (DE)	EMC (NL)	IZSLER (IT)	ANLIS (ARG)	MDH (US)	PHAC (CAN)	PHE (UK)
Case study type	Outbreak	Outbreak	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance
Pathogens	Avian influenza	Avian influenza	Influenza	Foodborne*	Foodborne*	Foodborne*	Foodborne*	Foodborne*
WGS	3-5 days†	4 days	2 days; can be reduced to 8-10 hours if needed	7 days	5-10 days; can be reduced to 5-6 days when given priority	7 days	10-14 (ideal); 30-35 days (observed)‡	10 days
Conventional methods	1-2 days† (HA/NA analysis)	8 days (whole genome) 2 days (HA/NA analysis)	3 days; can be reduced to 20 hours if needed	10 days	7-15 days, depending on the analysis required	4 days in most cases; can be 4-10 days for Salmonella and Campylobacter	10-15 days	10-15 days (Salmonella); 3 days (Listeria)

Source: Own compilation based on case study results. Note: Turnaround time does not include weekends and holidays * Foodborne pathogens: Salmonella (all), Listeria (IZSLER, PHE, MDH), E.coli and shigella (PHE, ANLIS, MDH), Campylobacter (PHE, MDH), Vibrio (MDH). † Assumes no viral amplification needed; otherwise, this estimate would increase by an average of 4 days. ‡The estimate of 30-35 days of observed turnaround time for WGS reported by PHAC is primarily due to the batching required (i.e. waiting to accumulate enough samples to run the sequencers cost-efficiently) as well as the time to ship isolates from provincial labs across Canada to the central laboratory in Winnipeg for sequencing. The shipping time is unique to the WGS transition period and was not relevant for conventional methods, as conventional methods were previously done entirely at the provincial level. PHAC indicated that the turnaround time for WGS would likely be faster than for conventional methods once the transition to a more decentralised model (i.e. with sequencing done in individual provinces) was complete.

As shown in the table above, the influenza virus case studies generally report lower turnaround times both for WGS and for conventional methods than the foodborne pathogen studies.

- For the influenza virus, the reported turnaround time ranged between 3 and 5 days of work for WGS analysis (and as low as 10 hours using Nanopore sequencing), compared to 1-2 days for HA/NA analysis or 8 days for analysis of a whole genome using Sanger sequencing;
- For foodborne pathogens, the usual turnaround time was 5-10 days for WGS analysis,[37] versus a turnaround time of 4-15 days for the full analysis of a foodborne pathogen using conventional methods.

The results of the case studies indicate that the differential effect of WGS on turnaround time depends on the level of information required about the pathogen and thus the complexity of the conventional analysis that would be required. The turnaround time for conventional methods increases based on the amount of information required and the corresponding number of different tests (especially consecutive tests) that are needed, while the turnaround time for WGS analysis remains relatively consistent. Consequently, the turnaround time for WGS tends to be higher than the turnaround time for conventional methods when only basic information about the sample is needed, and lower when more a detailed characterisation of the pathogen is required.

For the avian influenza case studies, for example, the turnaround time using WGS (3-5 days with second-generation sequencing) is longer than a simple HA/NA analysis that would be conducted to determine the pathogenicity of the sample (1-2 days), but shorter compared to a whole genome analysis using Sanger sequencing (8 days). In the foodborne pathogen case studies, the turnaround time for WGS tends to be less than or at least on the lower end of the turnaround time required for a full analysis using conventional methods, depending on the pathogen. While individual conventional methods used to gain more basic information about the sample (e.g. confirmation of identify at the species level) have lower turnaround times than an analysis with second-generation WGS technology (such as the Illumina MiSeq), EMC indicated that with Nanopore sequencing, basic information about the sample can already be obtained within 2-3 hours.

In an outbreak context, the difference in turnaround time for basic information about a sample can be non-trivial. APHA, for example, performs a cleavage site and full HA analysis using Sanger sequencing on every incoming avian influenza sample in order to determine the subtype and pathogenicity before subjecting the sample to WGS analysis. While APHA considered that the additional information provided by WGS in terms of more detailed and accurate results is invaluable in terms of informing epidemiological investigation and outbreak response (see below), it also reported that timing is a critical aspect in the avian influenza context, and that the priority is often to get basic information about the sample as quickly and reliably as possible in order to notify other responsible agencies and avoid delays in the decision-making process. APHA therefore indicated that it could not envision dropping the initial conventional tests as long as the turnaround time for receiving basic information using WGS exceeded that for conventional methods. The critical importance of turnaround time

[37] A significant outlier in this respect was PHAC. The reference period for the case study took place during a transitional period in which all sequencing for routine foodborne surveillance was centralised at PHAC's National Microbiology Laboratory (NML) in Winnipeg, Manitoba. The turnaround time of 30-35 days therefore included time needed for laboratories in Canada's ten provinces to ship samples to the central laboratory. PHAC indicated that it expected the turnaround time for WGS to come down significantly after the transition to a decentralised model (in which provinces conduct their own sequencing) is complete. In this respect, it estimated the actual time of work for molecular characterisation using WGS at 10-14 days, which is an estimate more in line with the observations of other case studies.

was also confirmed by PHAC, which reported that the move to WGS (with the associated temporary increase in turnaround time) was 'extremely relevant' and highly disruptive, requiring provincial and federal laboratories in Canada to adjust their workflows as a result. In the foodborne surveillance context, PHAC expressed concerns that the delay in turnaround time currently observed may compromise the recall of patients when questioned on their food histories, and may lead to the slower detection of outbreaks. However, PHAC indicated that the turnaround time for WGS would likely be faster than for conventional methods once the transition to a more decentralised model (i.e. with sequencing done in individual provinces) was complete.

5.4.3. Effects on research and methods applied

In addition to changing the character of the analytical results and processes in the case study institutions, the use of WGS is expected to lead to further benefits for research and epidemiological methods applied. The large amount of information that can be obtained through WGS provides data beyond what could have been obtained through conventional methods, allowing for better insights into e.g. how pathogens evolve over time or how diseases spread. WGS can also be used as a tool to develop and validate conventional diagnostic tests.

The following table presents the assessments by the case study institutions of specific positive effects on research and methods applied due to the use of WGS, as experienced by these institutions in practice.

Table 16: Effects on research and methods applied as experienced by case study institutions

	APHA (UK)	FLI (DE)	EMC (NL)	IZSLER (IT)	ANLIS (ARG)	MDH (US)	PHAC (CAN)	EUR
Better understanding of disease transmission	4	5	1	5	4	4	5	5
Other benefits for research	–	4	–	–	–	1	5	5
Improvement in epidemiological methods	4	5	1	5	1	1	–	3
Development of better diagnostic tests	4	2	1	–	4	–	3	2

Note: Case study institutions were asked to assess specific positive effects or impacts of using WGS on a scale from 1 (no effect at all) to 5 (very significant positive effect). '–' indicates not applicable or don't know/no answer. ssessments of moderate to very significant positive effects (3 to 5) are highlighted in colour.

All case study institutions except one considered that the use of WGS had significant or very significant positive effects on their understanding of disease transmission, and most considered that there had been at least moderate positive effects on epidemiological methods. Multiple case study institutions, including PHE, FLI, and IZSLER, have made use of WGS in order to study past outbreaks, draw new conclusions regarding outbreak epidemiology, and identify errors that had been made in the initial epidemiological investigations; see also the following sub-section for more detailed examples related to the use of WGS in an outbreak context.

With respect to the analysis of viruses in general and HPAI in particular, APHA and FLI both experienced the benefits of using WGS in terms of obtaining more detailed genetic information regarding virus strains and how these evolve. As viruses mutate particularly quickly, APHA indicated that WGS can be used to identify novel viruses, reassortants, and mixed infections that would be missed by other methods. It also

indicated that WGS analysis of viral RNA provides data on the host of origin, which can be used to understand more about the hosts' response to viral RNA. With respect to the WGS analysis of viruses, FLI also reported that the same results could not be achieved with Sanger sequencing due to the level of sensitivity required.

In the foodborne disease context, PHE and IZSLER both reported that WGS had improved epidemiological methods. PHE indicated in a recent report that the use of WGS had improved its microbiological understanding as well as its understanding of the transmission pathways of enteric pathogens.[38] For example, PHE staff indicated that cluster analysis of Shigella sonnei infections using WGS had uncovered novel transmission routes.[39] A concrete example of WGS research applications in a non-outbreak context was also demonstrated in a 2015 paper by IZSLER staff which presents the results of intensive environmental sampling for Listeria along the production chain for Parma ham using WGS.[40] The analysis indicated at which stages in the production chain and on which types of environmental surfaces contamination was most likely to occur. Clonal contamination patterns were also examined to draw insights on transmission within and between plants, as well as assess the efficacy of hygiene measures through repeated sampling more than six months later.

Three of the case study institutions reported significant positive effects of WGS on the development of better diagnostic tests. Specific examples were provided by PHE, which cited a study in which its staff had used publicly-available WGS data to evaluate the robustness of real-time PCR assays for Campylobacter in the face of high levels of genetic diversity and introgression, and to determine the underlying reasons for high or low levels of specificity in the assay.[41] More recently, PHE reported that it had also used WGS in the development of a PCR assay to distinguish between typhi/paratyphi and non-typhoidal strains of Salmonella. Another example was provided by APHA, which reported that the detailed information provided by WGS helped them to better target conventional testing at later stages of an avian influenza outbreak.

Other benefits of WGS for research were reported by PHE, FLI and PHAC. PHE reported that one of the key benefits of WGS was the public availability of sequence data, indicating that there is currently a large amount of sequence data available for analysis, with considerable benefits for the broader research community (e.g. for validating molecular diagnostic assays, as PHE had done for Campylobacter). Similarly, PHAC reported that the large amounts of data generated by WGS are used for scheme development, genome-wide association studies, machine learning, anti-microbial resistance prediction, and pathogenomics. Another benefit noted by PHE was that WGS makes it easier to collaborate internationally, as whole genome sequences could be sent more quickly and easily between laboratories than physical samples.

[38] Grant, K., Jenkins, C., Arnold, C., Green, J., & Zambon, M. (2018). *Implementing pathogen genomics. A case study.* Public Health England. p. 23-4.

[39] Dallman, Timothy J, Marie A Chattaway, Piers Mook, Gauri Godbole, Paul D Crook, and Claire Jenkins, 'Use of Whole Genome Sequencing for the Public Health Surveillance of Shigella Sonnei in England and Wales, 2015', *Journal of Medical Microbiology*, 2016, 882–84.

[40] Morganti, Marina, Erika Scaltriti, Paolo Cozzolino, Luca Bolzoni, Gabriele Casadei, Marco Pierantoni, and others, 'Processing-Dependent and Clonal Contamination Patterns of Listeria Monocytogenes in the Cured Ham Food Chain Revealed by Genetic Analysis', *Applied and Environmental Microbiology*, 82 (2015), 822–31.

[41] Jansen van Rensburg MJ, Swift C, Cody AJ, Jenkins C, Maiden MC. Exploiting Bacterial Whole-Genome Sequencing Data for Evaluation of Diagnostic Assays: Campylobacter Species Identification as a Case Study. *J Clin Microbiol.* 2016 Dec;54(12):2882-2890.

5.4.4. Effects on outbreak identification and response

Ultimately, the use of WGS in pathogen identification and surveillance is expected to lead to improvements in outbreak detection and response by providing high resolution data which can be used to more precisely track outbreaks in real time, contribute to more precise case definitions, and assist in tracing cases back to the source(s) of contamination, allowing outbreaks to be resolved more quickly. Potential specific effects of WGS in this area could therefore include improved information for outbreak response (e.g. improved detection that outbreaks are related, improved information for imposing biosecurity measures) and reductions in the consequences of outbreaks (e.g. a reduction in the disease burden for humans and/or livestock).

The following table presents the assessments by the case study institutions of specific effects of using WGS which were experienced by them in practice with respect to outbreak identification and response.

Table 17: Effects of WGS on outbreak identification and response experienced by case study institutions

	APHA (UK)	FLI (DE)	EMC (NL)	IZSLER (IT)	ANLIS (ARG)	MDH (US)	PHAC (CAN)	PHE (UK)
Improved detection that outbreaks are related	4	5	5	5	4	5	5	5
Improved information on outbreak epidemiology	4	5	5	5	4	4	5	5
Earlier detection of an initial outbreak	5	2	5	5	–	–	*	5
Improved information for imposing additional control/biosecurity measures	4	4	–	5	1	–	5	5
Reduced number of secondary outbreaks	3	5	–	–	1	–	–	3
Reduction of overall costs for outbreak identification and response	2	2	–	5	–	1	–	–
Reduction of the duration of an outbreak	2	3	–	–	1	4	–	5
Reduction of the disease burden (humans)	2	–	–	–	1	2	–	5
Reduction of the disease burden (livestock)	2	2	–	–	1	–	–	–

Note: Case study institutions were asked to assess specific positive effects or impacts of using WGS on a scale from 1 (no effect at all) to 5 (very significant positive effect). '–' indicates not applicable or don't know/no answer. ssessments of moderate to very significant positive effects (3 to 5) are highlighted in colour. ** PHAC reported a (temporary) negative effect, see the discussion in section 5.4.2 above.

As shown in the table above, most case study institutions considered that the use of WGS had significant or even very significant effects on the improvement of information for outbreak response. All case study institutions reported significant or very significant effects related to improved detection that outbreaks are related and improved information on outbreak epidemiology, and most considered that there had

also been effects related to the earlier detection of an initial outbreak and improved information for imposing additional control/biosecurity measures.[42]

In contrast, reductions in the consequences of outbreaks, including reductions in the number of secondary outbreaks, in the duration of outbreaks, or in the overall disease burden were mostly reported by case study institutions involved in the surveillance of foodborne pathogens. One of them noted very significant effects on overall costs for outbreak identification and response (IZLER), and two reported significant reductions in the duration of an outbreak (MDH, PHE) as well as at least somewhat significant effects on a reduction in the human disease burden (MDH, PHE). The other case study institutions saw more limited effects regarding reductions in the consequences of outbreaks. This was especially the case for ANLIS, which mostly did not observe significant effects of WGS in this area, other than effects on outbreak detection and epidemiology. ANLIS reported that this was due to the nature of the surveillance system in place in Argentina, which limited the timely availability of WGS results and feedback between the laboratory analysis and outbreak control. In Argentina, samples are initially collected by local and provincial labs, which send samples on to the national laboratory at ANLIS for further analysis. This delay in receiving samples meant that links between samples were usually discovered too late to be of practical relevance. It also reported that communication between the genomics team, the epidemiological team, and provincial public health authorities was insufficient for effective use of the additional information provided by WGS for outbreak response.

The practical benefits of using WGS in outbreak identification and response were documented by several of the case study institutions with respect to specific outbreaks. Four of the case study institutions (IZLER, ANLIS, MDH and PHE) re-examined samples collected during a past outbreak, and identified the potential benefits that the use of WGS would have had in this particular situation. The following table presents the results of these retrospective analyses. The table provides details on the respective outbreak that was re-examined, the study approach and the results in terms of the effects of WGS on case definition/detection and disease control/burden, as well as other conclusions. Note that due to the ex-post character of the analysis, the benefits listed below were by definition not observed in practice but represent a possible counterfactual if WGS had been in use at the time.

[42] A significant (temporary) outlier with respect to the earlier detection of an outbreak was PHAC, see the discussion in section 5.4.2 above.

Table 18: Results of retrospective analyses of past outbreaks with WGS, as reported by case study institutions

Insti-tution	Patho-gen	Region / year	Description of outbreak	Type of study	WGS effect on case definition / detection	Effect on disease control / burden	Other conclusions
IZSLER (IT) [a)]	Salmonella 4,[5],12:i:-	Emilia-Romagna (2013)	The outbreak was detected by routine surveillance. Epidemiological investigation rapidly identified the food involved (fermented dry-cured salami made from pork) and the facility implicated, but was not able to attribute several individuals infected with the implicated strain to the outbreak and could not confirm or exclude the role of suspect sources at abattoir and farm level. Overall, 137 human isolates with with the outbreak pulsotype were recovered from the outbreak territory	98 Salmonella isolates from human, animal and food sources were re-examined using WGS over a 3-year period (2012–15)	With WGS, first isolates unambiguously linking human cases to the salami facility which had been the source of the outbreak were available more than a month in advance of the outbreak onset (as identified based on incidence) and more than 2 months before the source had been identified using PFGE and MLVA	Had WGS been in routine use at the time of the 2013 outbreak, the source of the outbreak could have potentially been identified up to two months earlier, possibly preventing dozens of infections if the correct mitigation measures had been taken in time	MLVA and PFGE were not only unable to reliably link isolates to the outbreak source, but had in fact produced misleading results by incorrectly classifying some cases as being part of the outbreak when they were not
ANLIS (ARG) [b)]	Shigella sonnei	La Pampa (2010-2011)	Two suspected outbreaks in 2010 and 2011 in La Pampa province. In 2010, 26 reported cases were spread throughout the city of General Pico; 9 isolates were recovered. The second outbreak occurred in 2011 in the city of Castex. No supporting epidemiological data is available for the second outbreak, but 7 isolates were recovered. At the time, it was uncertain whether the two outbreaks were related.	17 Shigella isolates from the two outbreaks were re-examined using PFGE and WGS to test for relatedness.	Both PFGE and WGS confirmed that the two outbreaks were independent. However, PFGE over-predicted variability within the genomic structures. WGS confirmed conventional results and provided a more detailed view of the relationships between and within outbreaks.	WGS detected the presence of the ESBL gene OXA-1, which is suspected to be associated with resistence to 3rd generation cephalosporin, a standard treatment for Shigellosis in Argentina.	The retrospective study shows that even with a lack of supporting routine data, WGS becomes an indispensible method for the tracking and surveillance of bacterial pathogens during outbreaks.
ANLIS (ARG) [c)]	Shigella sonnei	Country-wide (2010, 2011, 2016)	Three outbreaks of Shigellosis occurred in various locations around the country in 2010 (5 isolates recovered), 2011 (3 isolates recovered) and 2016 (8 isolates recovered).	16 isolates from 3 outbreaks in Argentina were re-examined with WGS to supplement a pan-Latin American study on Shigella sonnei	WGS confirmed the results of conventional tests which suggested that the outbreaks were phylogenetically distinct. WGS also uncovered that the 2011 and 2016 isolates fell into multiple sublineages, indicating that the outbreaks may have multiple epidemiologic origins.	WGS was used to characterise the anti-microbial resistance (AMR) profiles of Shigella sonnei recovered from the three outbreaks at a more granular level, showing increasing levels of AMR in Shigella sonnei across Latin America.	WGS detected the presence of closely related isolates from different countries within an individual sublineage, indicating that international transmission of S. sonnei occurs across Latin America.

MDH (USA) [d]	Vibrio parahaem-olyticus	MD (2010)	Two individuals became ill after eating raw oysters in two different restaurants in Baltimore, MD. Isolates were collected from the two individuals as well as nine outbreak-implicated oysters.	Retrospective WGS analysis to determine the identity, genetic makeup, relatedness, and potential pathogenicity of the 2010 MD samples	Strains isolated from stool and oyster samples were indistinguishable with PFGE. WGS analysis was able to clarify the phylogenetic relationships of the clinical and food samples and identify the clinical strains as belonging to a clonal complex described only in Asia, confirming their local vector and their likely path from Asia to MD	Not discussed	The wgMLST method employed using WGS was found to be easy, robust, and scalable to multiple strains to be used in future *Vibrio parahaemolyticus* outbreak investigations
MDH (USA) [e]	Vibrio parahaem-olyticus	MD (2012-2013)	In the summers of 2012 and 2013, spikes in cases of *Vibrio parahaemolyticus* were reported in the US state of MD. Overall, 46 cases of *Vibrio parahaemolyticus* gastroenteritis associated illnesses were reported over this period, out of which 34 strains could be isolated.	Retrospective analysis of the 34 MD samples as well as other national and international samples for comparison	WGS analysis provided far more precise case definitions than those that had been achieved with PFGE and MLST. Five distinct clusters of sequence types (STs) were detected through WGS. WGS was also able to provide detailed information on sublineages within each cluster, e.g. by differentiating between West Coast and East Coast strains of ST36, which had not been possible with MLST.	Not discussed	In addition to the phylogenomic analysis, WGS was also used to determine the pathogenicity of particular strains
PHE (UK) [f]	Shigella sonnei	UK (2011)	Outbreak in the Orthodox Jewish (OJ) community in the UK. Gastrointestinal illness was reported in 86 people, of whom 82 met the case definition for possible, probable or confirmed outbreak case of *S. sonnei*, across 18 family clusters and six further individuals. Of these, 27 cases were laboratory confirmed at the local laboratory	Retrospective assessment of the value of WGS compared to conventional typing methods. Twenty-four isolates were selected for WGS	WGS and SNP analysis facilitated a more precise case definition. WGS analysis showed that the strains implicated in the outbreak formed three phylogenetically distinct clusters. One cluster represented cases associated with recent exposure to a single strain, whereas the other two clusters represented related but distinct strains of *S. sonnei* circulating in the OJ community across the UK	The lack of clarity in conclusions drawn from MLVA prevented (at the time of the outbreak) broadcasting of specific risks associated with the outbreak. Greater confidence that an outbreak was occurring would have facilitated a more pro-active approach to spread public health messages on infection control more effectively	WGS data challenged the conclusions drawn during the initial outbreak investigation and allowed cases of dysentery to be implicated or ruled out of the outbreak that were previously misclassified

Sources: Case study reports, and the following publications: a) Morganti, M., et al. (2018). Rise and fall of outbreak-specific clone inside endemic pulsotype of salmonella 4,[5],12:i:-; insights from high resolution molecular surveillance in Emilia-Romagna, Italy, 2012 to 2015. Eurosurveillance, 23(13), 1–11; b) Chinen, I. et al. (2016) 'Whole genome sequencing identifies independent outbreaks of Shigellosis in 2010 and 2011 in La Pampa Province, Argentina', bioRxiv, (April). doi: 10.1101/049940; c) Baker, K. S. et al. (2017) 'Whole genome sequencing of Shigella sonnei through PulseNet Latin America and Caribbean: advancing global surveillance of foodborne illnesses', Clinical Microbiology and Infection, 23(11), pp. 845–853. doi: 10.1016/j.cmi.2017.03.021; d) Haendiges, J. et al. (2016) 'A Nonautochthonous U.S. Strain of Vibrio parahaemolyticus Isolated from Chesapeake Bay Oysters

Caused the Outbreak in Maryland in 2010', Applied and Environmental Microbiology, 82(11), pp. 3208–3216. doi: 10.1128/aem.00096-16; e) Haendiges, J. et al. (2015) 'Characterization of Vibrio parahaemolyticus clinical strains from Maryland (2012-2013) and comparisons to a locally and globally diverse V. parahaemolyticus strains by whole-genome sequence analysis', Frontiers in Microbiology, 6(FEB), pp. 1–11. doi: 10.3389/fmicb.2015.00125; f) McDonnell, J., et al. (2013). Retrospective analysis of whole genome sequencing compared to prospective typing data in further informing the epidemiological investigation of an outbreak of Shigella sonnei in the UK. Epidemiology and Infection, 141(12), 2568–75.

The results of the restrospective analyses in the table above confirm the potential for significant positive effects of using WGS with respect to outbreak identification and response. Had WGS been used at the time of the listed outbreaks, it would have likely contributed to more precise case definitions, with implications for the effectiveness of public health actions and communications during an outbreak.

In particular, the retrospective analysis of the 2013 outbreak in Italy (Salmonella in Emilia-Romagna) provides evidence that the use of WGS would have allowed for human cases to be linked to the source of the outbreak much earlier than had been possible at the time using conventional methods. This outbreak also illustrates the importance of employing a One Health approach in the surveillance of foodborne infections: identifying linkages between human cases and sources in the food system through WGS in real-time critically depends on the routine laboratory surveillance of isolates from human, animal and food sources being conducted through the same institution; or, if several institutions are involved, timely identification of these linkages depends on a continuous exchange of sequencing data between the relevant institutions.

The Argentinian outbreaks listed in the table demonstrate the potential of WGS to detect and characterise anti-microbial resistance (AMR), with possible implications for patient treatment and public health actions if the results could have been available and communicated to the relevant parties in a timely manner. The Argentinian outbreaks and the US and UK outbreaks also demonstrate the benefits of having a better case definition concerning the outbreak with WGS data, compared to the more limited information available through conventional methods. In particular, in the 2011 UK outbreak of *Shigella sonnei*, the retrospective study suggests that if WGS data been available during the outbreak, public health authorities could have provided clear messaging to the affected community regarding the nature of the outbreak and the risks involved, which was not possible at the time due to a lack of clarity regarding the case definition.

As an important caveat for all the retrospective analysis listed above, it is not possible to determine which mitigation and control measures would have been taken in practice had the additional information provided through WGS been available. It is therefore not possible to determine the actual or potential reduction in disease burden that could have occurred as a result of using WGS. In principle, however, it is possible that more timely and effective control measures in the cases listed above could have made a difference in the disease burden associated with each outbreak.

The evaluation of more recent outbreaks, in which many of the case study institutions were able to apply WGS in a real-time setting for outbreak detection and response, presents a second approach for documenting the practical benefits of WGS in an outbreak context. The following table presents the results of several real-time analyses carried out by the case study institutions. The table provides details on the respective outbreaks in which WGS was used, the study approach, and the results in terms of the effects of WGS on case definition/detection and disease control/burden, in addition to other conclusions.

Table 19: Effects of applying WGS for outbreaks in real-time (i.e. not retrospective), as reported by case study institutions

Insti-tution	Patho-gen	Regio n / year	Description of outbreak	Type of study	WGS effect on case definition / detection	Effect on disease control / burden	Other conclusions
APHA (UK)	HPAI (H5N8)	UK (2016-2017)	The outbreak occurred in both wild birds and poultry, infecting 13 premises across England and Wales. These included turkey and chicken producers as well as gamebird production. The infections in poultry are thought to have arisen independently as a result of contact with wild birds, except in the case of a cluster of three infected premises of the same commercial enterprise in Lancashire, where secondary infections were likely to have occurred.	Real-time use of WGS during outbreak	There had been a very significant positive impact of using WGS on the earlier detection of an initial outbreak, especially for the index case.	Information provided by WGS allowed for a better assessment of the public health risk, for example by revealing the presence of mutations for mammalian host adaptation and the possible emergence of reassortant strains. WGS also allowed for useful supporting information to be disseminated during outbreaks.	Using WGS led to a reduction in the negative effects of outbreaks for the livestock industry, for tourism, for trade, and for the wider society. Trade in particular was emphasised as an area where positive effects from using WGS were observed, given that HPAI had been discovered in domestic poultry.
FLI (DE)	HPAI (principally H5N8 with some infections of subtype H5N5)	Germany (2016-2017)	The outbreak occurred in Lower Saxony in domestic poultry farms. About 30 farms were affected, including several turkey fattening farms. The outbreak was part of a larger outbreak across Germany, with more than 1 150 cases of H5Nx infection reported in wild birds and 107 outbreaks among birds kept in captivity (including both poultry and zoos), resulting in the death or slaughtering of approximately 1.2 million birds. Estimated direct economic losses of the total outbreak across Germany were about EUR 17 million.	Real-time use of WGS during outbreak	Benefits of WGS with respect to earlier detection of the initial outbreak were not experienced, as samples had already been positively identified through conventional methods before reaching the case study institution.	Analysis using WGS was able to indicate that transmission occurred not only through wild birds but also through secondary infection between farms, exposing gaps in biosecurity measures in addition to other potential risk factors. Also, the additional information through WGS allowed for some possibilities to be clearly ruled out. For example, two consecutive outbreaks on one farm raised questions regarding the effectiveness of the cleaning measures performed after the first outbreak; however, WGS analysis showed that the second outbreak on the same farm was caused by a later strain of the virus and was therefore the result of a separate introduction.	WGS led to a reduction in the costs of outbreak(s), including through the reduction of compensation payments, and also a reduction in negative effects of the outbreak on trade (although only to a moderate extent), according to the case study institution.

ANLIS (ARG) [a)]	Shigella sonnei	Buenos Aires (2016)	An outbreak of dysentry was detected in Buenos Aires in April 2016 with more than 900 associated cases, including two fatalities. Ten samples were collected for further analysis, of which eight tested positive for *Shigella sonnei*.	Real-time use of WGS during outbreak	WGS had no effect on the detection of the outbreak, as the outbreak was already apparent before the samples were collected for analysis. Real-time analysis of the 2016 analysis indicated that this outbreak was distinct from past outbreaks of *S. sonnei* in 2010 and 2011 (see previous table), except for one sample in 2016 which was found to be linked to the 2011 outbreak. WGS analysis allowed for better differentiation between isolates than PFGE analysis, which had been carried out at the same time.	None reported	The investigation concluded that maximising the benefit of genomic outbreak data in specific settings requires long term contextual isolate data from organisms isolated locally and internationally
MDH (US) [b)]	Salmonella (multiple strains)	23 US states	A multistate outbreak of Salmonella infections was detected in 2017, and ultimately linked to imported Maradol papayas from the Carica de Campeche farm in Mexico. A total of 220 people infected with the outbreak strains of *Salmonella* Thompson (144), *Salmonella* Kiambu (54), *Salmonella* Agona (12), *Salmonella* Gaminara (7), or *Salmonella* Senftenberg (3) were reported in 23 states. 68 people were hospitalised and one death was reported in New York.	Real-time use of WGS during outbreak	WGS was used to show that the infections across more than 20 states were genetically linked. WGS was also able to distinguish between four independent outbreaks of various *Salmonella* strains linked to Mexican papayas that were ongoing during the same period.	WGS was used by the US state lab to link isolates of *Salmonella* Kiambu and *Salmonella* Thompson collected from grocery store papayas to clinical isolates from affected persons using the CDC PulseNet database. The outbreak was linked back to the Carica de Campeche papaya farm in Mexico as a likely source. On the basis of the WGS results, Maradol papayas imported from Mexico were recalled by several producers. The investigation of this outbreak with WGS also allowed three other unrelated Salmonella outbreaks linked with Mexican papayas to be identified and the products removed from the market.	WGS was used to test for anti-microbial resistance. WGS did not identify anti-microbial resistance genes among isolates from 139 ill people. However, one ill person's isolate, a *Salmonella* Senftenberg, contained a gene known to decrease susceptibility to ciprofloxacin.
PHAC (CAN)[e)]	Salmonella Enteritidis	Canada	Following the adoption of WGS for routine surveillance in 2017, 17 individual outbreaks of *Salmonella* Enteritidis linked to raw chicken products, particularly frozen breaded raw chicken products, have been identified across Canada. As of May 2019, a total of 573 laboratory-confirmed cases have	Real-time use of WGS during outbreak	WGS led to the detection of the 17 *Salmonella* outbreaks linked to raw chicken. 14 of these outbreaks were detected in the first 6 months of using WGS for routine surveillance in 2017.	Based on WGS data, 14 food products were linked to the outbreaks. 13 products were recalled by food inpection authorities and one product was voluntarily removed by the retailer. In 2018, the Government of Canada adopted new, stricter requirements for producers of raw frozen breaded chicken products, which were	The use of WGS allowed food safety authorities to identify an entire product category (raw frozen breaded chicken) that was responsible for a disproportionate amount of the *Salmonella* Enteritidis

			been investigated in association with outbreaks related to raw chicken. 96 individuals were hospitalised and 3 deaths were reported, although it was unclear to what extent Salmonella had contributed to those three deaths.			estimated by PHAC to comprise approximately 40% of the disease burden attributable to *Salmonella* Enteritidis.	disease burden.
PHE (UK) [c)]	Salmonella Enteritidis	UK and Spain (2015)	The outbreak was detected [in the UK] using WGS data and investigated. UK cases were interviewed to obtain a food history and links between suppliers were mapped to produce a food chain network for chicken eggs. Food and environmental samples were taken from premises linked to cases and tested for Salmonella. Within the outbreak single nucleotide polymorphism (SNP) defined cluster, 136 cases were identified in the UK and 18 in Spain. One isolate from a food containing chicken eggs [omlette] was within the outbreak cluster.	Detection with and real-time use of WGS during outbreak	The investigation concluded that that UK and Spanish cases were exposed to a common source of Salmonella-contaminated chicken eggs. Using WGS provided additional sensitivity over phage-typing. Of the UK cases, 68% corresponded to PT59. Had WGS not been used, it is likely that the outbreak would still have been recognized. [However,] the loss of 32% of cases would have likely slowed the recognition of the outbreak, and made made it harder to pull together a food chain network, although whether the same source would have been identified is a matter of speculation.	Routine WGS changed the way the outbreak was managed; it was previously accepted practice in infectious intestinal disease outbreaks to exclude cases with travel history to focus on possible in-country exposures. With the greater specificity of WGS information, travel histories and other geographical metadata can now provide information on which other countries may have cases from the same source (with respect to this outbreak, additional cases were found in Spain (18) and the US (3)).	One of the limitations of the investigation was that it did not isolate Salmonella from any of the eggs sampled during the outbreak investigation. Food chain information was difficult to obtain. This situation arose both from the challenges in contacting cases and poor recall, but also the very resource-intensive nature of traceback investigations.
PHE (UK) [d)]	Salmonella Enteritidis	UK (detected 2015)	Analysis of WGS data uncovered the previously undetected outbreak that had been on-going for four years. Between April 2014 and September 2015, 714 isolates of S. Enteritidis PT8 were reported and 147 fell within the five SNP outbreak cluster.	Retrospective and real-time use of WGS during outbreak	Following the implementation of SNP typing at PHE, analysis of WGS data revealed a large sub-cluster o fisolates of S. Enteritidis PT8 associated with an outbreak of salmonellosis. A coordinated investigation generated microbiological, analytical and descriptive evidence to show that the outbreak was linked to handling of feeder mice or snakes infected by the mice. The outbreak had been occurring undetected by traditional surveillance procedures since at least January 2012. The continuous mode of transmission meant that the number of cases each	Following the investigation, a series of recommendations were made to control infections. At the farm level, these included a recommendation to depopulate mice rooms in a stepwise fashion, disinfect and repopulate with mice from a Salmonella free source. In the UK, controls were focused on improved labelling and provision of hygiene advice at the point of sale. A press release was issued which provided public health advice. The Reptile Trade Association produced their own advice which was sent to all major suppliers of	The investigation highlighted the potential of WGS to have a significant impact on decreasing the incidence of Salmonella by identifying low level, continuous transmission ("slow burn") outbreaks. SNP typing of the core genome provided evolutionary context making it possible to

					month was relatively low compared to other cases belonging to S. Enteritidis PT8, and had not triggered the traditional exceedance algorithm based on serotyping and phage typing data.	reptile feed.	confidently link cases from four years earlier to the contemporary cluster.

Sources: Case study reports, and the following publications: a) World Health Organization (WHO) (2018) Annex 2. Contribution of Whole Genome Sequencing to the National Surveillance of Shigella sonnei in Argentina.; b) Centers for Disease Control and Prevention (2017) Multistate Outbreak of Salmonella Infections Linked to Imported Maradol Papayas (Final Update). https://www.cdc.gov/salmonella/kiambu-07-17/index.html; c) Inns, T., et al. (2017). Prospective use of whole genome sequencing (WGS) detected a multi-country outbreak of Salmonella Enteritidis. Epidemiology & Infection, 145(2), 289-298; d) Kanagarajah, S., et al. (2018). Whole genome sequencing reveals an outbreak of Salmonella Enteritidis associated with reptile feeder mice in the United Kingdom, 2012-2015. Food Microbiology, 71, 32-38. g) Public Health Agency of Canada (2019), Public Health Notice - Outbreaks of Salmonella infections linked to raw chicken, including frozen raw breaded chicken products. https://www.canada.ca/en/public-health/services/public-health-notices/2018/outbreaks-salmonella-infections-linked-raw-chicken-including-frozen-raw-breaded-chicken-products.html.

As shown in the table above, the effects of applying WGS in real-time during outbreaks of disease confirm the results of the restrospective studies in that typically better case definitions with respect to the outbreaks were possible. An earlier detection of the outbreak through WGS was reported for four of the listed outbreaks. At least two of these three outbreaks (an avian influenza outbreak in the UK and Salmonella outbreak in the UK and Spain) would likely have also been detected with conventional methods (albeit with delays). In the case reported by PHAC, the use of WGS allowed for the identification of 17 separate outbreaks associated with the same food (raw chicken), and more specifically with the same product category (raw frozen breaded chicken products), which had not been picked up with conventional methods. Of special interest is the case of Salmonella in feeder mice in the UK, which might not have been detected at all without WGS due to its character as 'slow-burn' outbreak with low case numbers. This case provides clear evidence that the use of WGS has led in practice to a reduction in the disease burden in humans, as also indicated by PHE, because measures could be taken to end this (previously undetected) outbreak.

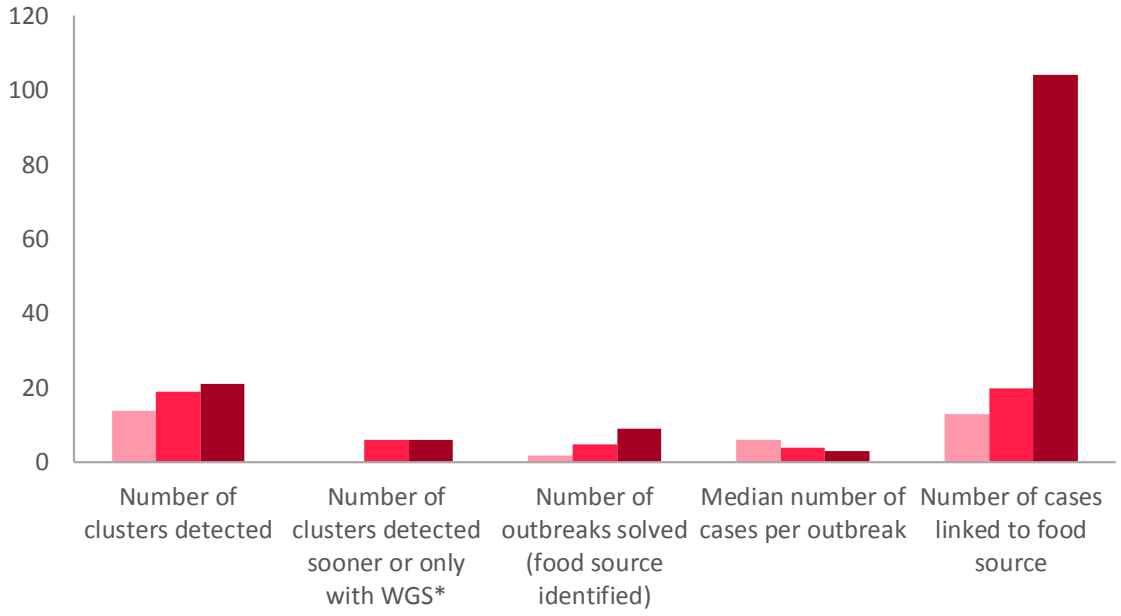
For the 2016-17 HPAI outbreak in Germany and the 2016 *Shigella sonnei* outbreak in Buenos Aires, the use of WGS did not lead to an earlier detection, as sequencing was only conducted after the pathogen had been detected through conventional methods. This confirms that certain benefits of using WGS for pathogen identification and surveillance depend on the functioning of the surveillance system – the later in the chain WGS is used, the more limited are the potential benefits in terms of earlier detection.

The outbreak cases listed in the table above also confirm that WGS allows for a better linkage to the sources of the outbreak, as the analysis of single nucleotide polymorphism (SNP) defined clusters creates valuable information that allows to identify or exclude specific sources. This is observed both in the avian influenza cases (e.g. the 2016-17 HPAI outbreak in Germany) as well as in the foodborne pathogen cases (e.g. the 2017 Mexican papaya outbreak in the US, the raw chicken cases in Canada, the two Salmonella outbreaks in the UK).

This better identification of the source of infection is also evidenced by data provided by the Centers for Disease Control in the United States with respect to cluster size and outbreak resolution for listeriosis infections from the periods before, during, and after the full introduction of WGS in routine surveillance, which replaced the previous network based on PFGE (see the following figure).[43]

[43] Jackson, B. R. et al. (2016) 'Implementation of Nationwide Real-time Whole-genome Sequencing to Enhance Listeriosis Outbreak Detection and Investigation', Clinical Infectious Diseases. Edited by P. M. Griffin, 63(3), pp. 380–386.

Figure 2: Listeriosis clusters detected and outbreaks solved before and after implementation of real-time WGS in the United States, 2012-2015



Source: Jackson, B. R. et al. (2016) 'Implementation of Nationwide Real-time Whole-Genome Sequencing to Enhance Listeriosis Outbreak Detection and Investigation', Clinical Infectious Diseases. Edited by P. M. Griffin, 63(3), p. 382. * Cluster detection sooner or only by WGS, as compared with PFGE.

As indicated in the figure above, the number of solved listeriosis outbreaks (food source identified) as well as the number of individual cases linked to the food source were shown to increase considerably in the period after implementing WGS. Nearly a third of the clusters were also identified either sooner or only by WGS, as compared to PFGE.

In this context, it is notable that the number of clusters detected increased (from 14 to 21), while the median number of cases per Listeria cluster or outbreak decreased (from 6 to 3) in the second year of implementing WGS (see figure above). Similar outcomes related to the use of WGS were reported by several case study institutions, which experienced a clear effect on the number and size of clusters detected, with a larger number of smaller outbreaks being identified. In a 2018 publication aiming to quantify the operational burden associated with the use of WGS for cluster analysis of two Salmonella serovars, PHE determined that during a one year period between 2014 and 2015, WGS had identified a significantly larger number of both *Salmonella* enteritidis and *Salmonella* typhimurium clusters than conventional methods.[44] While PHE reported that it currently did not have the resources to investigate all the clusters uncovered with WGS, it expected that resolving more outbreaks through the use of WGS would eventually lead to an overall reduction in the disease burden. Additional data in this respect was provided by PHAC, which reported that the number of

[44] 147 clusters at the 0-SNP level, 40 at 5-SNP, and 16 at 10-SNP for Salmonella enteritidis, and 64 at 0-SNP, 11 at 5-SNP and 4 at 10-SNP clusters for Salmonella typhimurium. For 0-SNP clusters, the median number of cases for both serovars was 2, while for 5-SNP clusters, the median number of cases ranged from 5 to 7 depending on the serovar; the median number of cases was considerably higher for 10-SNP clusters, at 14.5 (for Salmonella enteritidis) or 26.5 (for Salmonella typhimurium). In contrast, over the same period, conventional methods had only identified a total of 13 clusters for both serovars with a median number of 17 cases (for Salmonella enteritidis) or 23 cases (for Salmonella typhimurium). See: Mook, P., et al. (2018). Operational burden of implementing Salmonella Enteritidis and Typhimurium cluster detection using whole genome sequencing surveillance data in England: a retrospective assessment. *Epidemiology and Infection*, 1-9.

Salmonella Enteritidis outbreaks detected increased substantially from less than 20 each year between 2012 and 2016 to more than 100 in 2017, the first year with routine use of WGS. PHAC also reported, however, that the number of Listeria outbreaks detected had actually decreased in the first year of WGS implementation, as PFGE had previously been detecting outbreaks that did not exist, leading to an inefficient use of resources investigating non-existent outbreaks.

While better linkage of the identified clusters or outbreaks with the sources is therefore experienced in practice by case study institutions, the practical hurdles for source identification remain considerable. This was reported in particular with respect to the Salmonella outbreak in the UK and Spain, where comprehensive tracing activities allowed for the identification of the affected food (omelettes), but where it was not possible to isolate Salmonella from any of the eggs sampled during the outbreak investigation. One possible reason is that the time between the egg consumption and egg sampling is typically greater than the shelf life of chicken eggs, meaning that the affected batch is often no longer available when the sampling is conducted. Food chain information was also difficult to obtain due to the highly resource-intensive nature of traceback investigations, as well as the challenges in contacting cases and poor recollection among the affected persons (e.g. of foods eaten prior to the onset of illness). In spite of these challenges, it was possible to link the infections in the UK to cases in Spain using WGS. Furthermore, facilitated by the GenomeTrakr database hosted at the National Center for Biotechnology Information (NCBI), WGS data allowed for the outbreak to be linked to three additional Salmonella cases in New York State.[45]

Finally, the outbreak cases listed in the previous table confirm that WGS provides better information for imposing control measures and for assessing the effectiveness of the measures taken. For example, with respect to the initial implementation of control measures, APHA reported that the data provided by WGS enabled them to better assess the public health risk of an avian influenza outbreak by revealing whether particular avian influenza strains included mutations that could pose a risk of transmission to humans, and indicating whether the samples received are related to outbreak strains with known cases of human infection. Also in the avian influenza context, the use of WGS allowed for confirmation that cases of avian influenza in domestic poulty occurred not just through introduction by wild birds, but also through secondary infections between farms, indicating the presence of gaps in farm biosecurity measures.[46] WGS data provided additional information (on waves, clusters, and possible sources) that helped to determine possible transmission routes, concerning e.g. whether a subsequent infection on the same farm was the result of inadequate cleaning measures or a separate introduction. Similar experiences were reported by PHE and IZSLER, which used WGS to monitor the effectiveness of public health interventions. For example, PHE cited the case of a large EU-wide Salmonella outbreak in eggs, where action was taken to address the problem but WGS was able to identify the re-emergence of human cases, indicating an ongoing issue. With previous typing methods it would not have been possible to show that it was the same strain with the level of certainty provided by WGS. Concrete benefits related to WGS-supported control measures were also reported by PHAC, as data provided by WGS

[45] The two cases for which additional information was available had had a history of travel to Europe during their incubation period. See: Inns, T., et al. (2017). Prospective use of whole genome sequencing (WGS) detected a multi-country outbreak of Salmonella Enteritidis. Epidemiology & Infection, 145(2), 289-298.

[46] Conraths, F. J. (2017). Making worst case scenarios real: The introduction of highly pathogenic avian influenza of subtype H5N8 led to the largest fowl plague outbreak ever recorded in Germany. *Lohmann Information*, 51(1), 36–41; Conraths, F. J., et al. (2017). Epidemiologie des aktuellen Geflügelpestgeschehens in Deutschland [Epidemiology of the current incidence of avian influenza in Germany], presentation given at the meeting of the Gesellschaft der Förderer und Freunde für Geflügel- und Kleintierforschung e.V. at the Institut für Tierschutz und Tierhaltung in Celle on 3 May 2017.

allowed for stricter, Canada-wide regulations to be adopted for a product category that was estimated to be responsible for up to 40% of the disease burden attributable to Salmonella Enteritidis.

5.4.5. Effects on wider society

Improved pathogen surveillance and better outbreak response through the use of WGS can generate benefits for the wider society through reducing the negative effects of outbreaks on trade, livestock industry, and consumer trust, as well as reducing the overall costs of the outbreak for society.

The following table presents the assessments by the case study institutions of specific effects on the wider society from the use of WGS, which have been experienced by the case study institutions in practice.

Table 20: Wider effects of WGS on society as experienced by case study institutions

	APHA (UK)	FLI (DE)	EMC (NL)	IZSLER (IT)	ANLIS (ARG)	MDH (US)	PHAC (CAN)	PHE (UK)
Reduction of costs of outbreak(s) for the wider society	4	5	–	–	1	–	–	–
Reduction of negative effects of outbreak(s) on trade	4	3	–	5	1	–	–	–
Reduction of negative effects of outbreak(s) on livestock industry	4	2	–	5	1	–	–	–
Reduction of negative effects of outbreak(s) on consumer trust in food	3	2	–	–	1	–	3	4
Reduction of negative effects of outbreak(s) on tourism	4	–	–	–	1	–	–	–

Note: Case study institutions were asked to assess specific positive effects or impacts of using WGS on a scale from 1 (no effect at all) to 5 (very significant positive effect). '–' indicates not applicable or don't know/no answer. Assessments of moderate to very significant positive effects (3 to 5) are highlighted in colour.

As the high number of not applicable or don't know/no answer items in the table above indicates, case study institutions were often unable or hesitant to assess effects on the wider society due to a lack of concrete data, although most considered that these effects were likely to exist (or would be likely to exist in the future).

Three case study institutions considered that there had been significant effects of WGS with respect to reducing the negative effects of outbreaks on trade. In particular, APHA considered that there had been significant positive effects on wider society related to the identification of highly-pathogenic avian influenza in domestic poultry during a recent H5N8 outbreak, which had affected international trade. It indicated that the use of WGS had significantly reduced negative effects on livestock industry and trade as well as improved the overall biosecurity of the country, as it was able to confirm and validate the results obtained by conventional methods while providing additional information regarding the public health risk. IZSLER also considered that the use of WGS had a very significant positive impact on industry and trade, as evidenced by the fact that major operators in the region were starting to adopt WGS to do their own in-house testing. IZSLER also provided an example where WGS was able to provide clarity in a case where two strains of Listeria with the same PFGE type were discovered in two plants operating sequentially along the same processing chain

in the Parma ham industry. Through the use of WGS, IZSLER was able to show that the two isolates were unrelated, despite having an identical PFGE type, with the result that both plants were required to improve their own hygiene procedures and further commercial or legal controversy between the two plants could be avoided.

Another perspective on the benefits of WGS in this area was provided by FLI, which reported a highly significant positive effect concerning the reduction of the costs of outbreaks for the wider society. It indicated that WGS analysis could have the effect of reducing compensation payments to operators in the livestock or poultry industry where WGS indicated that biosecurity measures had been insufficient. However, it reported that the use of WGS had also provoked concerns from industry as a better identification of transmission routes could raise questions of liability for livestock producers which were found to have been the source of an outbreak, or which had employed insufficient biosecurity measures. It therefore indicated that a proactive and careful communication strategy would be needed to maintain industry cooperation.

Another effect of WGS for wider society which was considered to be 'very significant' by PHAC was the ability to leverage the WGS infrastructure and protocols that have been developed for foodborne disease surveillance for other disease areas as well.

APHA, PHAC and PHE all indicated that WGS had significant impacts on consumer trust in food, with FLI also considering that there had been at least some effect in this area. PHE indicated that WGS 'probably' did improve consumers' general confidence in food, or at least food from certain places of origin (e.g. consumer trust in the safety of eggs from the UK). The least significant effects on wider society were considered to be a reduction in the negative effects of outbreaks on tourism, with most case study institutions indicating that this was not applicable or that they did not have enough information to make this assessment.

With respect to benefits for the wider society, ANLIS was again an outlier among the case study institutions, indicating that none of the specific effects related to the wider society had been observed in Argentina.[47] The reasoning provided by ANLIS was similar to that discussed previously in the section on outbreak identification and response, namely that the characteristics of the surveillance system in Argentina as well as communication issues have limited the potential impact of WGS on outbreak response and wider impacts (see previous sub-section).

[47] MDH and EMC indicated that they were unable to assess any specific effects on the wider society based on their own concrete experiences during the case study period, but both institutions considered that such effects did exist in principle.

6. Next steps

This section discusses the next steps to be carried out as part of Work Package 14.

During the next (and final) period of the COMPARE project, WP partners Civic Consulting and Erasmus University Rotterdam will prepare the final deliverable to be produced by Work Package 14, namely Deliverable 14.5: A report on the assessment of options for refining selected elements of COMPARE in view of improving the overall cost-effectiveness of the system, with recommendations (to be submitted in Month 60 of the project).

Appendix I. Questionnaire

Cost-effectiveness studies (WP 14)

Case study questionnaire – [###Institution name]

[###Unit within institution]

1. Contact information

1.1. Name: …
1.2. Institution: …
1.3. Telephone: …
1.4. Email: …

2. Reference period

Please indicate the last period for which financial data on expenditure for routine surveillance activities is available, preferably the last calendar year.

2.1. Start: MM / YY End month: MM / YY

Please take this period as the reference period when responding to the questionnaire, and provide data accordingly. If data does not refer to this period, please indicate this in your answer.

3. Summary of routine surveillance activities using WGS

Please provide a brief background of your routine surveillance activities using WGS[1] (e.g. how long WGS has been in routine use for each pathogen) and refer to any relevant reports/documents describing these activities and related impacts: …

4. Pathogens sequenced using WGS

Please indicate in the table below the type and number of pathogen samples sequenced using WGS during the reference period.

Pathogen	Nr. of samples sequenced
…	…
…	…
…	…
…	…
…	…

[1] Note that where this questionnaire refers to WGS, it should be understood that this refers to WGS using Next Generation Sequencing (NGS).

Part I: COSTS OF USING WGS FOR PATHOGEN IDENTIFICATION

This section of the questionnaire refers to the cost of using WGS for pathogen identification, which encompasses the following key stages:

1. **Sample preparation and sequencing**, including receipt and opening of incoming samples, DNA extraction/purification, library preparation, sequencing;
2. **Bioinformatics and other analyses**, including data assembly, uploading, comparing sequence data with reference dataset, serotyping and antimicrobial testing (where relevant), and result validation, and interpretation and reporting of results. This step also considers the maintenance and curation of your (own) reference dataset.

The following subsections are divided according to the two key stages listed above and differentiate between the *equipment costs, consumables, staff time* and *other costs* directly related to implementing the step.[2]

The following cost tables are separately provided in the Excel workbook "WGS" – please complete all tables in Excel where indicated below, not in this Word questionnaire, and add additional rows where needed.

1. Sample preparation and sequencing

Please briefly describe the **process of sample preparation** for WGS analysis and indicate whether parts of the process are automated (e.g. through the use of a laboratory robot) or conducted manually. Where parts of the process are automated, please provide details: …

Please also indicate the **average batch size** that would be used in a typical sequencing run during the reference period:[3] …

The following questions refer to costs incurred in all steps involved in sample preparation and sequencing, including receipt and opening of incoming samples, DNA extraction/purification, library preparation, sequencing.

1.1. Equipment costs

In the following table, please indicate the equipment used for sample preparation and sequencing. For each piece of equipment, please provide the **total price at the time of purchase** (including VAT),[4]

[2] This questionnaire therefore focuses on direct costs of pathogen identification and does not consider indirect costs related to e.g. maintenance of the laboratory building, costs of personnel management etc.

[3] Note that this number should reflect the *average* batch size during the reference period, not the ideal batch size. For example, if your sequencer has the capacity to sequence 10 samples at a time but you would typically sequence only 1 sample at a time, this number should be 1.

year of purchase, annual maintenance costs, predicted lifespan, and the proportion of the equipment's capacity that is used specifically for WGS-related analysis of the pathogen samples listed in question 4 above.

Type of equipment	Total purchase price (Euro)	Year of purchase	Annual maintenance costs (Euro)	Predicted lifespan (years)*	% use during reference period**
Sample processing					
…	…	…	…	…	…
…	…	…	…	…	…
Library preparation					
…	…	…	…	…	…
…	…	…	…	…	…
Sequencing					
…	…	…	…	…	…
…	…	…	…	…	…

PLEASE COMPLETE IN EXCEL – WGS TABLE 1

Note: Indicate currency, if not Euro. Please only include items of equipment costing EUR 400 (approx. GBP 350) or more that qualify as capital expenditure relevant for sample preparation and sequencing, such as sequencing machines and durable lab equipment as well as specific software purchasing or licensing fees. Please do not include *basic laboratory equipment (e.g. refrigerators, centrifuges or pipettes), standard office computers or standard office software (e.g. Word, Excel)*. Please complete one row per piece of equipment (i.e. if two pieces are required of the same type of equipment, use two different rows). * See a list of typical lifespans used in previous studies in the Excel sheet. ** % use during reference period refers to the use of the equipment for the pathogen samples listed in question 4 above. For example, if an Illumina DNA sequencing system was used during the reference period for 30% of the time for the pathogen samples listed in question 4, and 70% for other purposes, indicate 30%.

Comments: …

1.2. Consumables

In the table below, please list the consumables used during all steps involved in sample preparation and sequencing, and provide the related **costs per batch**. Consumables include items that are used up in laboratory processes, such as chemicals, petri dishes, etc.

[4] In case that you have no data on the purchasing price, please provide an estimate, e.g. based on current costs of purchasing a similar piece of equipment.

Type of consumable	Batch size	Cost per batch (Euro)	% failure*
Sample processing			
…	…	…	
…	…	…	
Library preparation			
…	…	…	
…	…	…	
Sequencing			
…	…	…	
…	…	…	

PLEASE COMPLETE IN EXCEL – WGS TABLE 2

Note: Indicate currency, if not Euro. Please indicate for each consumable the batch size, i.e. the number of samples that can be processed using one batch of the consumable in question, and provide costs per batch. If you provide costs per sample, indicate batch size = 1.

* % failure refers to the percentage of consumables that are wasted, e.g. due to failed runs, etc. If not relevant, indicate 0%.

Comments: …

1.3. Staff time

In the table below, please estimate the amount of **hands-on staff time**[5] spent on all steps involved in sample preparation and sequencing. This should include any time spent on quality control.

Please differentiate between the categories "Professionals" and "Technicians" (see further details on these categories in Annex I) and provide **staff time per sample in minutes**.

Staff type	Staff time per sample in minutes
Sample processing	
Professionals	…
Technicians	…
Library preparation	
Professionals	…
Technicians	
Sequencing	
Professionals	…
Technicians	…

PLEASE COMPLETE IN EXCEL – WGS TABLE 3

Note: If several samples are treated at the same time, please divide staff time per category by the number of samples to obtain the per-sample staff time. For example, if sample processing for 40 samples takes 2 hours and 40 minutes for a laboratory technician, convert this figure to minutes (160 minutes), and divide by 40 (resulting in a technician staff time of 4 minutes per sample). Please base your estimate on all hands-on staff time used for sample preparation and sequencing related to the pathogen samples listed in question 4, including for maintenance of equipment and staff time used for failed runs, etc.

Comments: …

[5] 'Hands-on staff time' refers to the amount of staff time actually used to perform an activity, not the duration of the activity. Unsupervised incubation periods are not included in hands-on staff time.

1.4. Other costs

If you have incurred any other costs for sample preparation and sequencing (e.g. for subcontracting or external services), please indicate these in the table below and provide **costs per sample**.

Type of other cost	Cost per sample (Euro)
Sample processing	
…	…
…	…
Library preparation	
…	…
…	
Sequencing	
…	…
…	…

PLEASE COMPLETE IN EXCEL – WGS TABLE 4

Note: Indicate currency, if not Euro.

Comments: …

2. Bioinformatics and other analyses

Please indicate whether you maintain your own in-house reference dataset for the bioinformatics analysis and if so, whether this uses information from public databases (e.g. from the NCBI database). Please also indicate whether any part of your bioinformatics analysis is outsourced: …

The following questions refer to costs related to the **bioinformatics and other analyses**, which includes data assembly, uploading, comparing sequence data, and result validation, and interpretation and reporting of results. This step also considers the costs for maintenance and curation of **your (own) reference dataset** (such as costs for downloading sequence data from other, e.g. international databases, cleaning database entries, etc.).

2.1. Equipment costs

In the following table, please indicate the equipment used for bioinformatics and other analyses, as well as using and maintaining your (own) reference dataset. For each piece of equipment, please provide the **total price at the time of purchase** (including VAT), year of purchase, annual maintenance costs, predicted lifespan, and the proportion of the equipment's capacity that is used specifically for WGS-related analysis of the pathogen samples listed in question 4 above.

Type of equipment	Total purchase price (Euro)	Year of purchase	Annual maintenance costs (Euro)	Predicted lifespan (years)*	% use during reference period**
Bioinformatics & other analyses					
…	…	…	…	…	…
…	…	…	…	…	…
Reference dataset					
…	…	…	…	…	
…	…	…	…	…	…

PLEASE COMPLETE IN EXCEL – WGS TABLE 5

Note: Indicate currency, if not Euro. Please only include items of equipment costing EUR 400 (approx. GBP 350) or more that qualify as capital expenditure relevant for bioinformatics and other analyses, such as specific server computers, storage devices and equipment as well as specific software purchasing or licensing fees, *not including standard office computers or software (e.g. Word, Excel)*. Please complete one row per piece of equipment (i.e. if two pieces are required of the same type of equipment, use two different rows). * See a list of typical lifespans used in previous studies in the Excel sheet. ** % use during reference period refers to the use of the equipment for the pathogen samples listed in question 4 above. For example, if a specific server system was used during the reference period for 50% of the time for the pathogen samples listed in question 4, and 50% for other purposes, indicate 50%.

Comments: …

2.2. Staff time

In the table below, please estimate the amount of hands-on staff time spent on all steps involved in bioinformatics and other analyses, as well as using and maintaining your (own) reference dataset.

Please differentiate between the categories "Professionals" and "Technicians" (see further details on these categories in Annex I) and provide **staff time per sample in minutes**.

Staff type	Staff time per sample in minutes
Bioinformatics & other analyses	
Professionals	…
Technicians	
Reference dataset	
Professionals	…
Technicians	…

PLEASE COMPLETE IN EXCEL – WGS TABLE 6

Note: If several samples are treated at the same time, please divide staff time per category by the number of samples to obtain the per-sample staff time. See note under Table in section 1.3.
Please base your estimate on all hands-on staff time used for bioinformatics analysis and use of reference dataset related to the pathogen samples listed in question 4, including for maintenance of equipment and staff time used for failed runs, etc.

Comments: …

2.3. Other costs

If you have incurred any other costs for bioinformatics and other analyses, as well as using and maintaining your (own) reference dataset (e.g. for consumables, subcontracting or external services), please indicate these in the table below and provide **costs per sample**.

Type of other cost	Cost per sample (Euro)
Bioinformatics & other analyses	
…	…
…	
Reference dataset	
…	…
…	…

PLEASE COMPLETE IN EXCEL – WGS TABLE 7

Note: Indicate currency, if not Euro.

Comments: …

3. Cost price of WGS calculated/used by you

If you have calculated internally the cost price for using WGS for pathogen identification per sample (from sample preparation and sequencing to bioinformatics and further analysis), e.g. for offering this as a service to third parties, please indicate this cost price per sample, and specify how it was calculated: …

4. Use of WGS for confirmation of results obtained with conventional method(s)

Do you use WGS for confirmation of results obtained with the conventional method(s) described in the next section of the questionnaire? If yes, please specify the share of samples analysed with the conventional method(s) that are additionally analysed with WGS for confirmation purposes: …

PART II: COSTS OF RELEVANT CONVENTIONAL METHOD(S) FOR PATHOGEN IDENTIFICATION

This section of the questionnaire refers to the cost of using relevant conventional method(s) for pathogen identification - typically this would be the method(s) you previously used for identification of the pathogens listed in question 4, or would use in case WGS would not be available ('second-best' methods). This method/these methods are hereafter referred to as "**Conventional method(s)**".

In this section, please consider the "normal use" of these conventional method(s) in terms of equipment and staff time needed, i.e. the way that you previously used the method (if you no longer use it), or continue to use the method (if this is the case). Please cover the same steps as in section I of the questionnaire, i.e. from receipt and opening of incoming samples until reporting of results.

The following tables are separately provided in the Excel workbook "CONVENTIONAL" – please complete all tables in Excel, not in this Word questionnaire, and add additional rows, where needed.

5. Costs of conventional methods

Please list the conventional method(s) used by you for identification of each of the pathogens listed in question 4. Please also indicate the share of samples that would typically have been processed using these methods.

Pathogen	Method	Conventional method(s)	Share of samples processed using the methods
Pathogen 1	A.		… %
	B.	…	
Pathogen 2	…	…	…%

PLEASE COMPLETE IN EXCEL – CONVENTIONAL TABLE 1

Note: If you indicate a share of 100% for method A and 50% for method B, this would indicate that all samples are analysed with method A, while every second sample is in addition analysed with method B.

Comments: …

Are any of these methods **still in use** in parallel to the analysis using WGS? If so, please explain: …

Please briefly describe the **process of sample preparation** for the methods listed above and indicate whether parts of the process are automated (e.g. through the use of a laboratory robot) or conducted manually. Where parts of the process are automated, please provide details: …

In the following, please provide all relevant costs related to the listed methods, covering the key steps from sampling preparation to further analysis, equivalent to all steps related to WGS listed above. We again differentiate between *equipment costs, consumables, staff time* and *other costs* directly related to implementing the methods.

5.1. Equipment costs

In the following table, please indicate the equipment used for each method. For each piece of equipment, please provide the **total price at the time of purchase** (including VAT), year of purchase, annual maintenance costs, predicted lifespan, and the proportion of the equipment's capacity used for applying the method.

Pathogen	Type of equipment	Total purchase price (Euro)	Year of purchase	Annual maintenance costs (Euro)	Predicted lifespan (years)*	% use for method**
Pathogen 1	*Metho*					
…	…	…				…
…	…	…	…	…	…	…

PLEASE COMPLETE IN EXCEL – CONVENTIONAL TABLE 2

Note: Indicate currency, if not Euro. Please only include items of equipment costing EUR 400 (approx. GBP 350) or more that qualify as capital expenditure relevant for using the conventional method(s), such as machines and durable lab equipment as well as specific software purchasing or licensing fees. Please do not include *basic laboratory equipment (e.g. refrigerators, centrifuges or pipettes), standard office computers or standard office software (e.g. Word, Excel)*. Please complete one row per piece of equipment (i.e. if two pieces are required of the same type of equipment, use two different rows). * See a list of typical lifespans used in previous studies in the Excel sheet. ** % use for methods refers to the (hypothetical) use of the equipment for processing the pathogen samples listed in question 4 above. For example, if a specific piece of equipment would have been used for 30% of the time during a period similar to the reference period to process the same number of pathogen samples as listed in question 4, indicate 30%.

Comments: …

5.2. Consumables

In the table below, please list the consumables used for each method, and provide the related **costs per batch**. Consumables include items that are used up in laboratory processes, such as chemicals, petri dishes, etc.

Pathogen	Type of consumable	Batch size	Cost per batch (Euro)	% failure*
Pathogen 1				
…	…	…		
…	…	…	…	

PLEASE COMPLETE IN EXCEL – CONVENTIONAL TABLE 3

Note: Indicate currency, if not Euro. Please indicate for each consumable the batch size, i.e. the number of samples that can be processed using one batch of the consumable in question, and provide costs per batch. If you provide costs per sample, indicate batch size = 1.

* % failure refers to the percentage of consumables that are wasted, e.g. due to failed runs, etc. If not relevant, indicate 0%.

Comments: …

5.3. Staff time

In the table below, please estimate the amount of hands-on staff time used for each method. Please differentiate between the categories "Professionals" and "Technicians" (see further details on these categories in Annex I) and provide **staff time per sample in minutes**.

Pathogen	Staff type	Staff time per sample in minutes
Pathogen 1	*Method A*	
…	Professionals	…
…	Technicians	…

PLEASE COMPLETE IN EXCEL – CONVENTIONAL TABLE 4

Note: Please base your estimate on all hands-on staff time that would be used for processing the pathogen samples listed in question 4 above, including for maintenance of equipment and staff time used for failed runs, etc.

Comments: …

5.4. Other costs

If you have incurred any other costs (e.g. for subcontracting or external services), please indicate these in the table below and provide **costs per sample**.

Pathogen	Type of other cost	Cost per sample (Euro)
Pathogen 1	*Met*	
…	…	…
…	…	…

PLEASE COMPLETE IN EXCEL – CONVENTIONAL TABLE 5

Note: Indicate currency, if not Euro.

Comments: …

6. Cost price of conventional method(s) calculated/used by you

If you have calculated internally the cost price for using the above listed method(s) for pathogen identification per sample (from sample preparation to further analysis), e.g. for offering this as a service to third parties, please indicate this cost price per sample, and specify how this was calculated: …

7. Use of conventional method(s) for confirmation of WGS results

Do you use conventional method(s) for confirmation of WGS results? If yes, please specify the share of WGS samples that are additionally analysed with conventional method(s) for confirmation purposes: …

PART III: EFFECTS OF USING WGS RESULTS

This section of the questionnaire refers to the effects of the application of the results of using WGS for pathogen identification and surveillance during the reference period, compared to a situation in which conventional methods would have been applied.

8. Turnaround time

In this question we define the *turnaround time* as the usual number of **days of work** from receipt and opening of an incoming sample until the reporting of results. Turnaround time does not include weekends and holidays, except in case that work has been conducted on these days, e.g. for a sequencing run or other analyses. Please indicate:

- the turnaround time for the analysis of a sample using WGS for pathogen identification, (in days of work): …
- the turnaround time for the analysis of a sample using the specified conventional method(s) for pathogen identification (in days of work): …

If turnaround time is different: How relevant has the difference in turnaround time been for outbreak identification and response? …

9. Positive effects of using WGS for pathogen identification and surveillance during the reference period

a) Please summarise how, in your experience, the use of WGS for pathogen identification and surveillance has led to positive effects/impacts: …

b) Please assess the extent to which the use of WGS for pathogen identification and surveillance has led to the following specific positive effects/impacts. Please only assess effects that you have concretely experienced or noticed. If you don't know, or an item is not applicable, please indicate this.

	1 (No effect at all)	2	3	4	5 (Very significant positive effect)	Not appli-cable	Don't know	Please quantify effects, if possible	Comments
Sampling and sampling strategies									
Reduction in number of samples needed	☐	☐	☐	☐	☐	☐	☐	…	…
Simplification of type of samples needed (e.g. use of swabbing instead of other types of sample)	☐	☐	☐	☐	☐	☐	☐	…	…
Simplification of sample storage or transport	☐	☐	☐	☐	☐	☐	☐	…	…
Reduction of overall costs for sampling	☐	☐	☐	☐	☐	☐	☐	…	…
Other effects: …	☐	☐	☐	☐	☐	☐	☐	…	…
Analytical results/processes									
Improved accuracy of results produced	☐	☐	☐	☐	☐	☐	☐	…	…
Improved sensitivity of results produced	☐	☐	☐	☐	☐	☐	☐	…	…
Improved specificity of results produced	☐	☐	☐	☐	☐	☐	☐	…	…
More detailed results produced	☐	☐	☐	☐	☐	☐	☐	…	…
Simplified laboratory work flows	☐	☐	☐	☐	☐	☐	☐	…	…
Reduction of time needed for analysis	☐	☐	☐	☐	☐	☐	☐	…	…
Reduction of consumables needed for analysis	☐	☐	☐	☐	☐	☐	☐	…	…
Reduction of staff time	☐	☐	☐	☐	☐	☐	☐	…	…
Reduction of overall costs for analysis	☐	☐	☐	☐	☐	☐	☐	…	…
Other effects: …	☐	☐	☐	☐	☐	☐	☐	…	…
Outbreak identification and response									
Earlier detection of an initial outbreak	☐	☐	☐	☐	☐	☐	☐	…	…

	1 (No effect at all)	2	3	4	5 (Very significant positive effect)	Not appli-cable	Don't know	Please quantify effects, if possible	Comments
Improved detection that outbreaks are related	☐	☐	☐	☐	☐	☐	☐	…	…
Reduced number of secondary outbreaks	☐	☐	☐	☐	☐	☐	☐	…	…
Improved information on outbreak epidemiology (e.g. linking cases to source)	☐	☐	☐	☐	☐	☐	☐	…	…
Improved information for imposing additional control/biosecurity measures	☐	☐	☐	☐	☐	☐	☐	…	…
Reduction of the duration of an outbreak	☐	☐	☐	☐	☐	☐	☐	…	…
Reduction of the disease burden (livestock)	☐	☐	☐	☐	☐	☐	☐	…	…
Reduction of the disease burden (humans)	☐	☐	☐	☐	☐	☐	☐	…	…
Reduction of overall costs for outbreak identification and response	☐	☐	☐	☐	☐	☐	☐	…	…
Other effects: …	☐	☐	☐	☐	☐	☐	☐	…	…
Effects on research and methods applied									
Better understanding of disease transmission	☐	☐	☐	☐	☐	☐	☐	…	…
Improvement in epidemiological methods	☐	☐	☐	☐	☐	☐	☐	…	…
Development of better diagnostic tests	☐	☐	☐	☐	☐	☐	☐	…	…
Other benefits for research: …	☐	☐	☐	☐	☐	☐	☐	…	…
Effects on wider society									
Reduction of negative effects of outbreak(s) on consumer trust in food	☐	☐	☐	☐	☐	☐	☐	…	…
Reduction of negative effects of outbreak(s) on livestock industry	☐	☐	☐	☐	☐	☐	☐	…	…
Reduction of negative effects of outbreak(s) on tourism	☐	☐	☐	☐	☐	☐	☐	…	…

	1 (No effect at all)	2	3	4	5 (Very significant positive effect)	Not appli-cable	Don't know	Please quantify effects, if possible	Comments
Reduction of negative effects of outbreak(s) on trade	☐	☐	☐	☐	☐	☐	☐	…	…
Reduction of costs of outbreak(s) for the wider society (i.e. reduced consequential costs/losses)	☐	☐	☐	☐	☐	☐	☐	…	…
Other effects: …	☐	☐	☐	☐	☐	☐	☐	…	…

Comments: …

10. Negative effects of using WGS

Has the use of WGS for pathogen identification and surveillance led to any negative effects/impacts, both for routine surveillance and during outbreaks? If so, please explain: …

11. Closing questions regarding use of WGS in pathogen identification and surveillance

a) Do you see any potential for significant cost reductions for using WGS in pathogen identification and surveillance in the future (through e.g. technological advances)? For which steps are they most relevant? …
b) To what extent are there current or potential cost reductions as a result of the cross-pathogen potential of WGS? For which steps are they most relevant? …
c) Please provide any additional comments you think we should take into account for the assessments of costs and benefits of using WGS for pathogen surveillance and identification, including any additional data sources you consider relevant. …

Annex I: Definition of staff categories "Professionals" and "Technicians"

For "Professionals", occupations typically involve the performance of tasks that require complex problem-solving, decision-making and creativity based on an extensive body of theoretical and factual knowledge in a specialised field. The knowledge and skills required are typically obtained as the result of study at a higher educational institution for a period of 3-6 years following completion of secondary education leading to the award of a first degree or higher qualification. This category includes PhD candidates and Post-docs.

For "Technicians", occupations typically involve the performance of complex technical and practical tasks that require an extensive body of factual, technical and procedural knowledge in a specialised field. The knowledge and skills required are usually obtained as the result of study at a higher educational institution for a period of 1-3 years following completion of secondary education. This category includes laboratory assistants.

Appendix II. Cost data collected from case study institutions

ANNEX : Data collected for cost calculation - APHA

I. WGS

Equipment

In the following, the equipment used for sample preparation, sequencing, bioinformatics and other analyses considered for the cost calculation is listed. For each piece of equipment, the table provides the total unit price at the time of purchase (including VAT), annual maintenance costs, and predicted lifespan. Only equipment was considered that costed EUR 400 or more that qualify as capital expenditure relevant for WGS, such as sequencing machines and durable lab equipment as well as specific software purchasing or licensing fees. Not included were basic laboratory equipment (e.g. refrigerators, centrifuges or pipettes), standard office computers and standard office software.

This approach was similarly applied for all methods listed below.

	Total purchase price (Euro)	*Annual maintenance costs (Euro)*	*Predicted lifespan (years)*
Illumina MiSeq	€ 104 826	€ 12 000	10
Computer	€ 2 355	€ 0	5

Consumables

In the following, the consumables used for sample preparation and sequencing considered for the cost calculation are listed. Consumables include items that are used up in laboratory processes, such as chemicals, petri dishes, etc. For each item, the table provides the cost per sample, the step of analysis it is used for and the failure rate. The failure rate refers to the percentage of consumables that are wasted, e.g. due to failed runs, and is taken into account in the cost calculation.

This approach was similarly applied for all methods listed below.

	Cost per sample (Euro)	*Step of analysis*	*% failure*
Qiagen viral RNA extraction kit	€ 4.59	Sample processing	1
Roche cDNA synthesis kit	€ 69.58	Library preparation	1
Nextera XT kit	€ 748.57	Sequencing	1

Staff time per sample in minutes

The following provides the estimated staff time per sample spent on each step, separately for professionals and for technicians. The amount of 'hands-on staff time' is indicated, i.e. the amount of staff time actually used to perform an activity, and not the duration of the activity, including for maintenance of equipment and staff time used for failed runs. Where several samples are treated at the same time, total staff time is divided to obtain the per-sample staff time. For example, if sample processing for 40 samples takes 2 hours and 40 minutes for a laboratory technician, this figure is converted to minutes (160 minutes), and divided by 40, resulting in a technician staff time of 4 minutes per sample.

This approach was similarly applied for all methods listed below.

Staff category / *Step*	*Professionals* (staff time in minutes)*	*Technicians** (staff time in minutes)*
Sample processing	0	60
Library preparation	0	60
Sequencing	0	90
Bioinformatics & other	60	0

analyses		
Reference dataset	0	0

The definition of these catgories is based on the International Standard Classification of Occupations of the International Labour Office (ILO).

**For "Professionals", occupations typically involve the performance of tasks that require complex problem-solving, decision-making and creativity based on an extensive body of theoretical and factual knowledge in a specialised field. The knowledge and skills required are typically obtained as the result of study at a higher educational institution for a period of 3-6 years following completion of secondary education leading to the award of a first degree or higher qualification. This category includes PhD candidates and Post-docs.*

***For "Technicians", occupations typically involve the performance of complex technical and practical tasks that require an extensive body of factual, technical and procedural knowledge in a specialised field. The knowledge and skills required are usually obtained as the result of study at a higher educational institution for a period of 1-3 years following completion of secondary education. This category includes laboratory assistants.*

II. Conventional method A: Sanger Sequencing

Equipment

	Total purchase price (Euro)	*Annual maintenance costs (Euro)*	*Predicted lifespan (years)*
ABI Capillary sequencer 37/30	€ 198 667	€ 8 000	10
G storm thermocycler	€ 2 355	€ 388	5
LazerGene software licence	€ 16 474	0	1

Consumables

	Cost per sample (Euro)	*% failure*
Viral RNA extraction kit	€ 4.59	1
PCR kit	€ 4.75	1
Gel extraction kit	€ 1.68	0
Labelling kit	€ 10.28	5

Staff time per sample in minutes

	Professionals	*Technicians*
Staff time in minutes	60	360

III. Key variables

Labour costs

The following table provides the hourly labour cost data (in Euro) used for monetisation of staff time. Figures below refer to Eurostat data on labour costs for 2017 (by staff category), plus a 25% surcharge for overheads.

	Professionals	*Technicians*
UK	€ 39.63	€ 25.00
EU	€ 45.13	€ 24.50

Source: Eurostat, Labour cost levels by NACE Rev. 2 activity [lc_lci_lev]. Construct: Labour cost for LCI (compensation of employees plus taxes minus subsidies). NACE categories: Professional, scientific and technical activities; Administrative and support service activities. Extracted in June 2018.
Other

…	

ANNEX : Data collected for cost calculation - FLI

I. WGS

Equipment

In the following, the equipment used for sample preparation, sequencing, bioinformatics and other analyses considered for the cost calculation is listed. For each piece of equipment, the table provides the total unit price at the time of purchase (including VAT), annual maintenance costs, and predicted lifespan. Only equipment was considered that costed EUR 400 or more that qualify as capital expenditure relevant for WGS, such as sequencing machines and durable lab equipment as well as specific software purchasing or licensing fees. Not included were basic laboratory equipment (e.g. refrigerators, centrifuges or pipettes), standard office computers and standard office software. Note that the predicted lifespan of equipment is based on standard values and applied uniformly across case studies. Lifespans used for accounting purposes by each case institution may differ.

This approach was similarly applied for all methods listed below.

	Total purchase price (Euro)	*Annual maintenance costs (Euro)*	*Predicted lifespan (years)*
Covaris sonicator	€ 27 300	€ 0	10
Agilent bioanalyzer	€ 22 000	€ 0	10
Ion Torrent PGM bundle	€ 93 000	€ 11 500	10
Server for assembly computation	€ 34 700	€ 0	5

Consumables

In the following, the consumables used for sample preparation and sequencing considered for the cost calculation are listed. Consumables include items that are used up in laboratory processes, such as chemicals, petri dishes, etc. For each item, the table provides the cost per sample, the step of analysis it is used for and the failure rate. The failure rate refers to the percentage of consumables that are wasted, e.g. due to failed runs, and is taken into account in the cost calculation.

This approach was similarly applied for all methods listed below.

	Cost per sample (Euro)	*Step of analysis*	*% failure*
96-Well PCR-plates qPCR	€ 0.86	Sample processing	10
96-Well PCR-plates PCR	€ 0.69		
Reaction tubes 1.5 ml	€ 0.40		
Reaction tubes 2 ml	€ 0.54		
Pipette tips 1000 µl	€ 1.12		
Pipette tips 200 µl	€ 1.05		
Pipette tips 100 µl	€ 1.05		
Pipette tips 10 µl	€ 1.05		
Pipette tips 2 µl	€ 1.05		
RNA-Purification	€ 5.59		
Gelextraction/DNA-Purification	€ 2.04		
DNA/RNA-Extraction	€ 3.71		
RT-PCR	€ 5.15		
PCR	€ 1.33		
Lab gloves	€ 3.76		

Covaris-Vials	€ 6.91		
Agilent Bioanalyzer RNA Pico Kit	€ 5.00		
GeneRead Library Prep Kit	€ 29.35	Library preparation	10
Adapter	€ 12.01		
Agilent Bioanalyzer DNA HS Kit	€ 5.86		
KAPA Library Quant IonTorrent	€ 23.64		
Onetouch Reagents	€ 21.31	Sequencing	10
Enrichment Beads	€ 0.86		
Chips (316v2)	€ 50.52		
Sequencing Reagents	€ 45.16		
Nitrogen	€ 0.48		
W2-Bottles	€ 1.21		

Staff time per sample in minutes

The following provides the estimated staff time per sample spent on each step, separately for professionals and for technicians. The amount of 'hands-on staff time' is indicated, i.e. the amount of staff time actually used to perform an activity, including maintenance of equipment and staff time used for failed runs, but excluding unsupervised processes (e.g. time that the sequencer is running unsupervised). Where several samples are treated at the same time, total staff time is divided to obtain the per-sample staff time. For example, if sample processing for 40 samples takes 2 hours and 40 minutes for a laboratory technician, this figure is converted to minutes (160 minutes), and divided by 40, resulting in a technician staff time of 4 minutes per sample.

This approach was similarly applied for all methods listed below.

Staff category *Step*	*Professionals** *(staff time in minutes)*	*Technicians*** *(staff time in minutes)*
Sample processing	8	40
Library preparation	3	60
Sequencing	7	35
Bioinformatics & other analyses	20	0
Reference dataset	10	0

The definition of these categories is based on the International Standard Classification of Occupations of the International Labour Office (ILO).

**For "Professionals", occupations typically involve the performance of tasks that require complex problem-solving, decision-making and creativity based on an extensive body of theoretical and factual knowledge in a specialised field. The knowledge and skills required are typically obtained as the result of study at a higher educational institution for a period of 3-6 years following completion of secondary education leading to the award of a first degree or higher qualification. This category includes PhD candidates and Post-docs.*

***For "Technicians", occupations typically involve the performance of complex technical and practical tasks that require an extensive body of factual, technical and procedural knowledge in a specialised field. The knowledge and skills required are usually obtained as the result of study at a higher educational institution for a period of 1-3 years following completion of secondary education. This category includes laboratory assistants.*

II. Conventional method A: Sanger Sequencing			
Equipment			
	Total purchase price (Euro)	*Annual maintenance costs (Euro)*	*Predicted lifespan (years)*
ABI Sequencer	€ 120 000	€ 8 000	10

Consumables		
	Cost per sample (Euro)	*% failure**
96-Well PCR-plates qPCR	€ 0.86	10
96-Well PCR-plates PCR	€ 0.69	
Reaction tubes 1.5 ml	€ 0.40	
Reaction tubes 2 ml	€ 0.54	
Pipette tips 1000 µl	€ 1.12	
Pipette tips 200 µl	€ 1.05	
Pipette tips 100 µl	€ 1.05	
Pipette tips 10 µl	€ 1.05	
Pipette tips 2 µl	€ 1.05	
RNA-Purification	€ 10.00	
Gelextraction/DNA-Purification	€ 24.25	
RT-PCR	€ 34.87	
Lab gloves	€ 3.76	
EtOH	€ 0.05	0
2-Mercaptoethanol	€ 0.02	
Agarose	€ 1.95	
TBE-Buffer (0.5X)	€ 0.52	
Ethidiumbromid-Lsg.	€ 0.26	
52/4000 Seq.-Kit	€ 120.00	
Nucleoseq Columns	€ 72.28	
Formamide	€ 0.50	
Capillary array	€ 39.80	
Sequencing buffer	€ 0.13	
Polymer POP7	€ 36.60	

Staff time per sample in minutes		
	Professionals	*Technicians*
Staff time in minutes	260	240

III. Key variables

Labour costs		
The following table provides the hourly labour cost data (in Euro) used for monetisation of staff time. Figures below refer to Eurostat data on labour costs for 2017 (by staff category), plus a 25% surcharge for overheads.		
	Professionals	*Technicians*
Germany	€ 53.3	€ 26.8
EU	€ 45.1	€ 24.5
Source: Eurostat, Labour cost levels by NACE Rev. 2 activity [lc_lci_lev]. Construct: Labour cost for LCI (compensation of employees plus taxes minus subsidies). NACE categories: Professional, scientific and technical activities; Administrative and support service activities. Extracted in June 2018.		

ANNEX : Data collected for cost calculation - EMC

I. WGS

Equipment

In the following, the equipment used for sample preparation, sequencing, bioinformatics and other analyses considered for the cost calculation is listed. For each piece of equipment, the table provides the total unit price at the time of purchase (including VAT), annual maintenance costs, and predicted lifespan. Only equipment was considered that costed EUR 400 or more that qualify as capital expenditure relevant for WGS, such as sequencing machines and durable lab equipment as well as specific software purchasing or licensing fees. Not included were basic laboratory equipment (e.g. refrigerators, centrifuges or pipettes), standard office computers and standard office software. Note that the predicted lifespan of equipment is based on standard values and applied uniformly across case studies. Lifespans used for accounting purposes by each case institution may differ.

This approach was similarly applied for all methods listed below.

	Total purchase price (Euro)	*Annual maintenance costs (Euro)*	*Predicted lifespan (years)*
Gel electrophoreses system	€ 4 000	€ 0	10
PCR machine	€ 5 000	€ 0	10
Qubit	€ 3 000	€ 0	10
Magnate 96 wells	€ 800	€ 0	10
GridION	€ 45 000	€ 4 500	10
Computer (server)	€ 15 060	€ 0	5
Computer (back-up)	€ 0	€ 700	1
Computer (CLC)	€ 1 000	€ 0	5
CLC Software	€ 500	€ 0	1

Consumables

In the following, the consumables used for sample preparation and sequencing considered for the cost calculation are listed. Consumables include items that are used up in laboratory processes, such as chemicals, petri dishes, etc. For each item, the table provides the cost per sample, the step of analysis it is used for and the failure rate. The failure rate refers to the percentage of consumables that are wasted, e.g. due to failed runs, and is taken into account in the cost calculation.

This approach was similarly applied for all methods listed below.

	Cost per sample (Euro)	*Step of analysis*	*% failure*
RNA isolation kit	€ 6.00	Sample processing	20
RT-PCR kit	€ 5.00		
Consumables	€ 3.00		
Ligase	€ 15.00	Library Preparation	0
Sequencing kit	€ 3.75		
Consumables	€ 2.50		
Flowcell	€ 16.50	Sequencing	2

Staff time per sample in minutes

The following provides the estimated staff time per sample spent on each step, separately for professionals and for technicians. The amount of 'hands-on staff time' is indicated, i.e. the amount of staff

time actually used to perform an activity, including maintenance of equipment and staff time used for failed runs, but excluding unsupervised processes (e.g. time that the sequencer is running unsupervised). Where several samples are treated at the same time, total staff time is divided to obtain the per-sample staff time. For example, if sample processing for 40 samples takes 2 hours and 40 minutes for a laboratory technician, this figure is converted to minutes (160 minutes), and divided by 40, resulting in a technician staff time of 4 minutes per sample.

This approach was similarly applied for all methods listed below.

Staff category *Step*	*Professionals** *(staff time in minutes)*	*Technicians*** *(staff time in minutes)*
Sample processing	0	48
Library preparation	0	18
Sequencing	6	6
Bioinformatics & other analyses	18	48
Reference dataset	0	0

The definition of these catgories is based on the International Standard Classification of Occupations of the International Labour Office (ILO).

**For "Professionals", occupations typically involve the performance of tasks that require complex problem-solving, decision-making and creativity based on an extensive body of theoretical and factual knowledge in a specialised field. The knowledge and skills required are typically obtained as the result of study at a higher educational institution for a period of 3-6 years following completion of secondary education leading to the award of a first degree or higher qualification. This category includes PhD candidates and Post-docs.*

***For "Technicians", occupations typically involve the performance of complex technical and practical tasks that require an extensive body of factual, technical and procedural knowledge in a specialised field. The knowledge and skills required are usually obtained as the result of study at a higher educational institution for a period of 1-3 years following completion of secondary education. This category includes laboratory assistants.*

II. Conventional method A: Real Time PCR

Equipment

	Total purchase price (Euro)	*Annual maintenance costs (Euro)*	*Predicted lifespan (years)*
Lightcycler	€ 40 200	€ 3 931	10
Magnapure 96	€ 125 619	€ 9 309	10

Consumables

	Cost per sample (Euro)	*% failure**
RNA isolation kit	€ 6.00	0
Real Time PCR kit (5x per sample)	€ 25.00	

Staff time per sample in minutes

	Professionals	*Technicians*
Staff time in minutes	0	36

III. Conventional method B: Sanger Sequencing

Equipment			
	Total purchase price (Euro)	*Annual maintenance costs (Euro)*	*Predicted lifespan (years)*
3130XL sequencer	€ 44 118	€ 13 759	10
Computer + DNAstar	€ 500	€ 0	5

Consumables		
	Cost per sample (Euro)	*% failure**
RT-PCR Kit (2x per sample HA NA)	€ 20.00	0
Big Dye Terminator	€ 0.75	
Consumables	€ 3.00	

Staff time per sample in minutes		
	Professionals	*Technicians*
Staff time in minutes	0	36

XIII. Key variables

Labour costs

The following table provides the hourly labour cost data (in Euro) used for monetisation of staff time. Figures below refer to Eurostat data on labour costs for 2017 (by staff category), plus a 25% surcharge for overheads.

	Professionals	*Technicians*
Netherlands	€ 53.20	€ 28.20
EU	€ 45.10	€ 24.50

Source: Eurostat, Labour cost levels by NACE Rev. 2 activity [lc_lci_lev]. Construct: Labour cost for LCI (compensation of employees plus taxes minus subsidies). NACE categories: Professional, scientific and technical activities; Administrative and support service activities. Extracted in June 2018.

Other	
…	
…	

ANNEX: Data collected for cost calculation - IZLER

I. WGS

Equipment

In the following, the equipment used for sample preparation, sequencing, bioinformatics and other analyses considered for the cost calculation is listed. For each piece of equipment, the table provides the total unit price at the time of purchase (including VAT), annual maintenance costs, and predicted lifespan. Only equipment was considered that costed EUR 400 or more that qualify as capital expenditure relevant for WGS, such as sequencing machines and durable lab equipment as well as specific software purchasing or licensing fees. Not included were basic laboratory equipment (e.g. refrigerators, centrifuges or pipettes), standard office computers and standard office software. Note that the predicted lifespan of equipment is based on standard values and applied uniformly across case studies. Lifespans used for accounting purposes by each case institution may differ.

This approach was similarly applied for all methods listed below.

	Total purchase price (Euro)	*Annual maintenance costs (Euro)*	*Predicted lifespan (years)*
Biorad-T100 thermal cycler	€ 4 000	€ 0	10
Biorad-CFX96 RT-System	€ 24 400	€ 0	10
Microplate-Genie-Shaker	€ 700	€ 0	10
MiSeq (Illumina, USA)	€ 100 000	€ 12 000	10
Workstations (3 pieces)	€ 5 000	€ 0	5
Storage unit	€ 18 500	€ 0	5
Bionumerics License	€ 10 720	€ 0	10

Consumables

In the following, the consumables used for sample preparation and sequencing considered for the cost calculation are listed. Consumables include items that are used up in laboratory processes, such as chemicals, petri dishes, etc. For each item, the table provides the cost per sample, the step of analysis it is used for and the failure rate. The failure rate refers to the percentage of consumables that are wasted, e.g. due to failed runs, and is taken into account in the cost calculation.

This approach was similarly applied for all methods listed below.

	Cost per sample (Euro)	*Step of analysis*	*% failure*
Qiagen DNAeasy Kit	€ 4.00	Sample processing	1
Tips	€ 0.25		
Eppendeorfs vials	€ 0.01		
Gloves	€ 0.01		
General Reagents	€ 0.01		
Tips 200ul	€ 0.37	Library preparation	5
Tips 100 ul	€ 0.36		
Tips 1000 ul	€ 0.01		
Nextera Xt index	€ 2.49		
Agencourt Ampure XP	€ 1.77		
Tips 20 ul	€ 0.37		
PCR-tube	€ 0.02		

Micro-Plate	€ 0.29		
Gloves	€ 0.01		
Deepwell plate	€ 0.25		
Microseal A	€ 0.48		
Microseal B	€ 0.08		
Nextera XT DNA SAMP Prep	€ 38.12		
MiSeq Reagent Kit V2 (2x250)	€ 113.07	Sequencing	1

Staff time per sample in minutes

The following provides the estimated staff time per sample spent on each step, separately for professionals and for technicians. The amount of 'hands-on staff time' is indicated, i.e. the amount of staff time actually used to perform an activity, including maintenance of equipment and staff time used for failed runs, but excluding unsupervised processes (e.g. time that the sequencer is running unsupervised). Where several samples are treated at the same time, total staff time is divided to obtain the per-sample staff time. For example, if sample processing for 40 samples takes 2 hours and 40 minutes for a laboratory technician, this figure is converted to minutes (160 minutes), and divided by 40, resulting in a technician staff time of 4 minutes per sample.

This approach was similarly applied for all methods listed below.

Staff category *Step*	*Professionals** *(staff time in minutes)*	*Technicians*** *(staff time in minutes)*
Sample processing	0	20
Library preparation	0	10
Sequencing	0	5
Bioinformatics & other analyses	60	0
Reference dataset	10	0

The definition of these categories is based on the International Standard Classification of Occupations of the International Labour Office (ILO).

**For "Professionals", occupations typically involve the performance of tasks that require complex problem-solving, decision-making and creativity based on an extensive body of theoretical and factual knowledge in a specialised field. The knowledge and skills required are typically obtained as the result of study at a higher educational institution for a period of 3-6 years following completion of secondary education leading to the award of a first degree or higher qualification. This category includes PhD candidates and Post-docs.*

***For "Technicians", occupations typically involve the performance of complex technical and practical tasks that require an extensive body of factual, technical and procedural knowledge in a specialised field. The knowledge and skills required are usually obtained as the result of study at a higher educational institution for a period of 1-3 years following completion of secondary education. This category includes laboratory assistants.*

II. Conventional method A: Serotyping

Equipment

No equipment other than basic laboratory equipment is used for serotyping, therefore there are no associated costs.

Consumables

	Cost per sample (Euro)	*% failure**
Media	€ 2.29	0.1
Antisera	€ 4.84	
Plasticware and gloves	€ 0.62	

Staff time per sample in minutes

	Professionals	*Technicians*
Staff time in minutes	3	38

III. Conventional method B: PFGE

Equipment

	Total purchase price (Euro)	*Annual maintenance costs (Euro)*	*Predicted lifespan (years)*
Shacking waterbath	€ 3 000	€ 0	10
Biorad Mapper Apparatus	€ 21 000	€ 0	10
Image Acquisition apparatus	€ 12 000	€ 0	10
Bionumerics License	€ 11 170	€ 0	10

Consumables

	Cost per sample (Euro)	*% failure**
Media	€ 0.17	3
Buffers	€ 12.31	
Restriction Enzymes	€ 1.06	
Plasticware and gloves	€ 0.46	

Staff time per sample in minutes

	Professionals	*Technicians*
Staff time in minutes	2.5	38

IV. Conventional method C: PCR Verification

Equipment

	Total purchase price (Euro)	*Annual maintenance costs (Euro)*	*Predicted lifespan (years)*
Biorad-T100 thermal cycler	€ 4 000	0	10
Image Acquisistioin apparatus	€ 12 000	0	10

Consumables

	Cost per sample (Euro)	*% failure**

Media	€ 0.02	5
Baffers and reagents	€ 2.03	
Oligos and Taq	€ 0.36	
Plasticware and gloves	€ 0.24	
Staff time per sample in minutes		
	Professionals	*Technicians*
Staff time in minutes	1	10
V. Conventional method D: MLVA		
MLVA is outsourced to another lab in the institute's network, for a cost of € 43.13 per sample.		
VI. Key variables		
Labour costs		
The following table provides the hourly labour cost data (in Euro) used for monetisation of staff time. Figures below refer to Eurostat data on labour costs for 2017 (by staff category), plus a 25% surcharge for overheads.		
	Professionals	*Technicians*
Italy	€ 44.9	€ 23.9
EU	€ 45.1	€ 24.5
Source: Eurostat, Labour cost levels by NACE Rev. 2 activity [lc_lci_lev]. Construct: Labour cost for LCI (compensation of employees plus taxes minus subsidies). NACE categories: Professional, scientific and technical activities; Administrative and support service activities. Extracted in June 2018.		
Exchange rate (if relevant)		
…		
…		
Other		
…		
…		

ANNEX: Data collected for cost calculation - ANLIS

I. WGS

Equipment

In the following, the equipment used for sample preparation, sequencing, bioinformatics and other analyses considered for the cost calculation is listed. For each piece of equipment, the table provides the total unit price at the time of purchase (including VAT), annual maintenance costs, and predicted lifespan. Only equipment was considered that costed EUR 400 or more that qualify as capital expenditure relevant for WGS, such as sequencing machines and durable lab equipment as well as specific software purchasing or licensing fees. Not included were basic laboratory equipment (e.g. refrigerators, centrifuges or pipettes), standard office computers and standard office software. Note that the predicted lifespan of equipment is based on standard values and applied uniformly across case studies. Lifespans used for accounting purposes by each case institution may differ.

This approach was similarly applied for all methods listed below.

	Total purchase price (Euro)	*Annual maintenance costs (Euro)*	*Predicted lifespan (years)*
Qiacube DNA	€ 13 724	€ 974	10
Qubit 3.0	€ 1 743	€ 0	10
Bioshake iQ Thermomixer	€ 1 201	€ 0	10
MiSeq Illumina	€ 75 273	€ 6 072	10
Server	€ 19 474	€ 0	5
Computer	€ 3 614	€ 452	5
Computer	€ 3 614	€ 452	5

Consumables

In the following, the consumables used for sample preparation and sequencing considered for the cost calculation are listed. Consumables include items that are used up in laboratory processes, such as chemicals, petri dishes, etc. For each item, the table provides the cost per sample, the step of analysis it is used for and the failure rate. The failure rate refers to the percentage of consumables that are wasted, e.g. due to failed runs, and is taken into account in the cost calculation.

This approach was similarly applied for all methods listed below.

	Cost per sample (Euro)	*Step of analysis*	*% failure*
Qiacube box	€ 1.70	Sample processing	0
2mL Eppendorf DNA LoBindMicrocentifuge Tubes	€ 0.00		
Filter tips 200ul (1024) for Qiacube	€ 1.04		
Filter tips 1000ul (1024) for Qiacube	€ 0.70		
96 samples (Illumina, Cat # FC-131-1096)	€ 27.66	Library preparation	5
96 indices, 384 samples (Illumina, Cat # FC-131-1002)	€ 2.24		
Agencourt AMPure XP Beads, 60 ml (Beckman	€ 0.69		

Coulter, Cat # A63881)			
Qubit reagent BR	€ 0.46		
Qubit reagent HS	€ 0.46		
100 ul Filter tips	€ 0.11		
10 ul Filter tips	€ 0.11		
1000 ul filter tips	€ 0.07		
General consumables	€ 1.77		
MiSeq Reagent Kit v2 500 cycles	€ 62.79	Sequencing	5

Staff time per sample in minutes

The following provides the estimated staff time per sample spent on each step, separately for professionals and for technicians. The amount of 'hands-on staff time' is indicated, i.e. the amount of staff time actually used to perform an activity, including maintenance of equipment and staff time used for failed runs, but excluding unsupervised processes (e.g. time that the sequencer is running unsupervised). Where several samples are treated at the same time, total staff time is divided to obtain the per-sample staff time. For example, if sample processing for 40 samples takes 2 hours and 40 minutes for a laboratory technician, this figure is converted to minutes (160 minutes), and divided by 40, resulting in a technician staff time of 4 minutes per sample.

This approach was similarly applied for all methods listed below.

Staff category *Step*	*Professionals** *(staff time in minutes)*	*Technicians*** *(staff time in minutes)*
Sample processing	11	0
Library preparation	18	0
Sequencing	2	0
Bioinformatics & other analyses	60	0
Reference dataset	0	0

The definition of these categories is based on the International Standard Classification of Occupations of the International Labour Office (ILO).

**For "Professionals", occupations typically involve the performance of tasks that require complex problem-solving, decision-making and creativity based on an extensive body of theoretical and factual knowledge in a specialised field. The knowledge and skills required are typically obtained as the result of study at a higher educational institution for a period of 3-6 years following completion of secondary education leading to the award of a first degree or higher qualification. This category includes PhD candidates and Post-docs.*

***For "Technicians", occupations typically involve the performance of complex technical and practical tasks that require an extensive body of factual, technical and procedural knowledge in a specialised field. The knowledge and skills required are usually obtained as the result of study at a higher educational institution for a period of 1-3 years following completion of secondary education. This category includes laboratory assistants.*

II. Conventional method A: Biochemical testing

Equipment

No equipment other than basic laboratory equipment is used for biochemical testing, therefore there are no associated costs.

Consumables		
	Cost per sample (Euro)	*% failure**
General consumables	*Not available*	*Not available*

Staff time per sample in minutes		
	Professionals	*Technicians*
Staff time in minutes	2	13.8

III. Conventional method B: Serotyping

Equipment

No equipment other than basic laboratory equipment is used for biochemical testing, therefore there are no associated costs.

Consumables		
	Cost per sample (Euro)	*% failure**
General consumables	*Not available*	*Not available*

Staff time per sample in minutes		
	Professionals	*Technicians*
Staff time in minutes	10	35

IV. Conventional method C: PCR typing

Equipment

	Total purchase price (Euro)	*Annual maintenance costs (Euro)*	*Predicted lifespan (years)*
Biorad Mycycler thermal cycler	€ 2 466	0	10

Consumables		
	Cost per sample (Euro)	*% failure**
General consumables	*Not available*	*Not available*

Staff time per sample in minutes		
	Professionals	*Technicians*
Staff time in minutes	20	0

V. Conventional method D: MaldiTOF

Equipment

	Total purchase price (Euro)	*Annual maintenance costs (Euro)*	*Predicted lifespan (years)*

MaldiTOF	€ 188 239	0	10

Consumables		
	Cost per sample (Euro)	*% failure**
General consumables	*Not available*	*Not available*

Staff time per sample in minutes		
	Professionals	*Technicians*
Staff time in minutes	10	0

VI. Conventional method E: PFGE

Equipment			
	Total purchase price (Euro)	*Annual maintenance costs (Euro)*	*Predicted lifespan (years)*
PFGE Biorad	€ 32 157	0	10

Consumables		
	Cost per sample (Euro)	*% failure**
General consumables	*Not available*	*Not available*

Staff time per sample in minutes		
	Professionals	*Technicians*
Staff time in minutes	25	0

VII. Key variables

Labour costs

The following table provides the hourly labour cost data (in Euro) used for monetisation of staff time. Figures below refer to data provided by ANLIS on labour costs for professional staff for 2017, plus a 25% surcharge for overheads. Labour costs for technician staff were imputed from professional staff costs.

	Professionals	*Technicians*
Argentina	€ 4.52	€ 2.67

ANNEX : Data collected for cost calculation - PHE

I. WGS

Equipment

In the following, the equipment used for sample preparation, sequencing, bioinformatics and other analyses considered for the cost calculation is listed. For each piece of equipment, the table provides the total unit price at the time of purchase (including VAT), annual maintenance costs, and predicted lifespan. Only equipment was considered that costed EUR 400 or more that qualify as capital expenditure relevant for WGS, such as sequencing machines and durable lab equipment as well as specific software purchasing or licensing fees. Not included were basic laboratory equipment (e.g. refrigerators, centrifuges or pipettes), standard office computers and standard office software. Note that the predicted lifespan of equipment is based on standard values and applied uniformly across case studies. Lifespans used for accounting purposes by each case institution may differ.

This approach was similarly applied for all methods listed below.

	Total purchase price (Euro)	*Annual maintenance costs (Euro)*	*Predicted lifespan (years)*
QIASYMPHONY	€ 59 693	€ 17 681	10
QIASYMPHONY	€ 59 693	€ 17 681	10
ROCHE MAGNA PURE 96	€ 99 195	€ 6 844	10
cBot Cluster Generation System	€ 49 174	€ 4 563	10
cBot Cluster Generation System	€ 49 174	€ 4 563	10
LABCHIP GX	€ 52 950	€ 6 844	10
LABCHIP GX	€ 52 950	€ 6 844	10
ASSY-SCICLONE, G3 WGS, HV HEAD, L GRIP	€ 91 635	€ 10 266	10
ASSY-SCICLONE, G3 WGS, HV HEAD, L GRIP	€ 91 635	€ 10 266	10
ASSY-SCICLONE, G3 WGS, HV HEAD, L GRIP	€ 91 635	€ 10 266	10
LABCHIP-DS SPECTROPHOTOMETER 96	€ 48 584	€ 5 703	10
Glomax: 96 well plate Fluorometer	€ 14 749	€ 2 281	10
Glomax: 96 well plate Fluorometer	€ 14 749	€ 2 281	10
Biomek NXp Span-8 with integrated sealer and chilled storage	€ 160 770	€ 9 125	10
Biomex NXP Multichannel	€ 78 896	€ 8 745	10
Biomex NXP Multichannel	€ 78 896	€ 8 745	10
Biomex NXP Multichannel	€ 78 896	€ 8 745	10
Biomek NXP Span-8	€ 63 600	€ 9 125	10

Illumina HI-SEQ	€ 606 410	€ 57 034	10
Illumina HI-SEQ	€ 606 410	€ 57 034	10
Bioinformatics	Per-sample cost provided by PHE: € 4.89		

Consumables

In the following, the consumables used for sample preparation and sequencing considered for the cost calculation are listed. Consumables include items that are used up in laboratory processes, such as chemicals, petri dishes, etc. For each item, the table provides the cost per sample, the step of analysis it is used for and the failure rate. The failure rate refers to the percentage of consumables that are wasted, e.g. due to failed runs, and is taken into account in the cost calculation.

This approach was similarly applied for all methods listed below.

	Cost per sample (Euro)	*Step of analysis*	*% failure*
Various reagents and consumables	€ 6.84	Sample processing	0
96 indices, 384 samples	€ 1.94	Library preparation	0.1
nextera 96	€ 23.71		
PE Rapid cluster kit 2x96	€ 5.64		
cBot loading kit (rapid only) 2x 96	€ 1.84		
200 cycle rapid v2 2x96	€ 7.77	Sequencing	0.1
Other various costs	€ 1.88		

Staff time per sample in minutes

The following provides the estimated staff time per sample spent on each step, separately for professionals and for technicians. The amount of 'hands-on staff time' is indicated, i.e. the amount of staff time actually used to perform an activity, including maintenance of equipment and staff time used for failed runs, but excluding unsupervised processes (e.g. time that the sequencer is running unsupervised). Where several samples are treated at the same time, total staff time is divided to obtain the per-sample staff time. For example, if sample processing for 40 samples takes 2 hours and 40 minutes for a laboratory technician, this figure is converted to minutes (160 minutes), and divided by 40, resulting in a technician staff time of 4 minutes per sample.

This approach was similarly applied for all methods listed below.

Staff category *Step*	*Professionals** *(staff time in minutes)*	*Technicians*** *(staff time in minutes)*
Sample processing	2.65	16.85
Library preparation	1.60	0
Sequencing	2.60	0.30
Bioinformatics & other analyses	36.00	0
Reference dataset	0	0

The definition of these catgories is based on the International Standard Classification of Occupations of the International Labour Office (ILO).

**For "Professionals", occupations typically involve the performance of tasks that require complex problem-solving, decision-making and creativity based on an extensive body of theoretical and factual knowledge in a specialised field. The knowledge and skills required are typically obtained as the result of study at a higher educational institution for a period of 3-6 years following completion of secondary education leading to the award of a first degree or higher qualification. This category includes PhD candidates and Post-docs.*

***For "Technicians", occupations typically involve the performance of complex technical and practical tasks that require an extensive body of factual, technical and procedural knowledge in a specialised field. The knowledge and skills required are usually obtained as the result of study at a higher educational institution for a period of 1-3 years following completion of secondary education. This category includes laboratory assistants.*

II. Conventional method A: PCR (Taqman)			
Equipment			
	Total purchase price (Euro)	*Annual maintenance costs (Euro)*	*Predicted lifespan (years)*
TaqMan 7500	€ 43 000	€ 1 141	10
TaqMan 7500	€ 43 000	€ 1 141	10
TaqMan 7500	€ 43 000	€ 1 141	10
TaqMan 7500	€ 43 000	€ 1 141	10
TaqMan 7500	€ 43 000	€ 1 141	10

Consumables		
	Cost per sample (Euro)	*% failure**
Cupule	€ 0.08	*Costed into per-sample price*
Molecular water	€ 0.05	
Pipette tips	€ 0.07	
Plastic loops	€ 0.02	
Pre-aliquoted PCR strip HilA	€ 1.78	
Pre-aliquoted PCR strip lacZ+ttR	€ 0.14	

Staff time per sample in minutes		
	Professionals	*Technicians*
Staff time in minutes	0	5.63

III. Conventional method B: PCR (Monophasic)			
Equipment			
	Total purchase price (Euro)	*Annual maintenance costs (Euro)*	*Predicted lifespan (years)*
TaqMan 7500	€ 43 000	€ 1 141	10
TaqMan 7500	€ 43 000	€ 1 141	10
TaqMan 7500	€ 43 000	€ 1 141	10
TaqMan 7500	€ 43 000	€ 1 141	10
TaqMan 7500	€ 43 000	€ 1 141	10

Consumables		
	Cost per sample (Euro)	*% failure**

Cupule	€ 0.08	*Costed into per-sample price*
Molecular water	€ 0.05	
Takyon PCR mastermix	€ 1.07	
fliC probe	€ 0.18	
fljB probe	€ 0.15	
fljB/IS200 probe	€ 0.14	
fliC_fw primer	€ 0.04	
fliC_rev primer	€ 0.05	
fljB_fw primer	€ 0.07	
fljB_rev primer	€ 0.05	
fliB/IS200_fw primer	€ 0.22	
fliB/IS200_rev primer	€ 0.05	
Fast 96 well PCR plate	€ 0.22	
Pipette tips	€ 0.05	
Plastic loops	€ 0.02	
Eppendorf tubes	€ 0.00	

Staff time per sample in minutes		
	Professionals	*Technicians*
Staff time in minutes	0	3.96

IV. Conventional method C: PCR (Real-Time)

Equipment

	Total purchase price (Euro)	*Annual maintenance costs (Euro)*	*Predicted lifespan (years)*
Thermal cyclers	€ 2 446	€ 570	10
Thermal cyclers	€ 2 446	€ 570	10
Thermal cyclers	€ 2 446	€ 570	10
Thermal cyclers	€ 2 446	€ 570	10
Rotor gene	€ 30 831	€ 1 528	10
Rotor gene	€ 30 831	€ 1 528	10
Robot (Beckman etc.)	€ 61 662	€ 8 003	10

Consumables

	Cost per sample (Euro)	*% failure**
Pipette tips filter	€ 0.77	*Costed into per-sample price*
Pastette fine tip	€ 0.06	
Pastette graduated	€ 0.03	
Universal Plastic 25ml	€ 0.56	
1.5ml skirted Microtube	€ 0.05	

gloves nitrile	€ 0.06	
Dispojar	€ 4.12	
Rotagene PCR strips	€ 0.09	
Probes	€ 1.39	
Primers	€ 1.39	
Water	€ 0.24	
Takyon	€ 0.74	

Staff time per sample in minutes		
	Professionals	*Technicians*
Staff time in minutes	2.50	3.00

V. Conventional method D: MLVA/MLST/fAFLP

Equipment

No equipment other than basic laboratory equipment is used for serotyping, therefore there are no associated costs.

Consumables		
	Cost per sample (Euro)	*% failure**
Cupule	€ 0.08	*Costed into per-sample price*
Molecular water	€ 0.05	
Difco NA plates	€ 0.71	
MOLIS labels	€ 0.06	
Primers	€ 0.41	
Qiagen taq mix	€ 0.27	
2 ml tube	€ 0.04	
Nuclease free water (Severn)	€ 0.01	
filtered tips	€ 0.14	
microamp PCR plate	€ 0.36	
microamp PCR caps	€ 0.03	
Hi-Di	€ 0.04	
PCR plate Foil	€ 0.00	
Liz 1200	€ 0.62	
DBHT Frag. Analysis	€ 1.01	
Tips	€ 0.04	

Staff time per sample in minutes		
	Professionals	*Technicians*
Staff time in minutes	0	7.71

VI. Conventional method E: Serotyping			
Equipment			
	Total purchase price (Euro)	*Annual maintenance costs (Euro)*	*Predicted lifespan (years)*
Thermal cyclers	€ 2 466	€ 570	10
Thermal cyclers	€ 2 466	€ 570	10
Thermal cyclers	€ 2 466	€ 570	10
Thermal cyclers	€ 2 466	€ 570	10
Robot (Beckman etc.)	€ 61 662	€ 8,003	10
Consumables			
	Cost per sample (Euro)	*% failure**	
MaConkey plates	€ 0.25	*Costed into per-sample price*	
GIA	€ 0.75		
BHI (5ml,UV)	€ 1.86		
BHI (5ml,Tube)	€ 1.42		
Craigies	€ 1.19		
NA slopes (Tubes)	€ 0.71		
DE slopes	€ 0.68		
MOLIS labels	€ 0.02		
Microtitre plates	€ 0.43		
Serum (for'O' microtitre plates, 1:8)-2.7ml/plate	€ 2.89		
Serum (for'H' microtitre plates, 1:32)-2.7ml/plate	€ 0.88		
Serum (for craigies, 1:4)	€ 1.14		
Serum(for slide agglutination)	€ 0.19		
Serum (for titrations)	€ 0.06		
Formal saline	€ 0.41		
Phenol saline	€ 0.02		
Plastic loops	€ 0.11		
Plastic needles	€ 0.02		
Pastettes (short)	€ 0.17		
Gilson tips	€ 0.16		
Staff time per sample in minutes			
	Professionals	*Technicians*	
Staff time in minutes	0	27.25	
VII. Conventional method F: Phage Typing			
Equipment			

	Total purchase price (Euro)	*Annual maintenance costs (Euro)*	*Predicted lifespan (years)*
Thermal cyclers	€ 2 466	€ 570	10
Thermal cyclers	€ 2 466	€ 570	10
Thermal cyclers	€ 2 466	€ 570	10
Thermal cyclers	€ 2 466	€ 570	10

Consumables		
	Cost per sample (Euro)	*% failure**
Difco NA plates	€ 1.42	*Costed into per-sample price*
Dorsets egg slopes	€ 0.68	
Difco nutrient broth (double strength-4ml in tubes)	€ 0.59	
Pastettes	€ 0.07	
Plastic tips (for Pipetmax)	€ 0.14	
Phage suspension (0.16ml/NA plate)	€ 0.08	
Pipette tips	€ 0.05	
MOLIS labels (small)	€ 0.26	
MOLIS labels (V.small)	€ 0.19	

Staff time per sample in minutes		
	Professionals	*Technicians*
Staff time in minutes	2.25	12.5

VIII. Conventional method G: PFGE

No detailed cost data was available for PFGE. PHE's internal calculation of € 97.82 per sample was used instead as a unit cost.

IX. Conventional method H: D-Tartrate

Equipment

No equipment other than basic laboratory equipment is used for serotyping, therefore there are no associated costs.

Consumables		
	Cost per sample (Euro)	*% failure**
D-Tartrate tubes	€ 3.84	*Costed into per-sample price*
Plastic loops	€ 0.05	
Pastettes (short)	€ 0.03	
Lead acetate - saturated solution	€ 3.20	

MOLIS labels	€ 0.13	

Staff time per sample in minutes		
	Professionals	*Technicians*
Staff time in minutes	0	25.00

X. Conventional method I: Glucose gas

Equipment

No equipment other than basic laboratory equipment is used for serotyping, therefore there are no associated costs.

Consumables

	Cost per sample (Euro)	*% failure**
Glucose tube	€ 0.71	*Costed into per-sample price*
Plastic loop	€ 0.02	
MOLIS label	€ 0.06	

Staff time per sample in minutes		
	Professionals	*Technicians*
Staff time in minutes	0	10.00

XI. Conventional method J: AMR

Equipment

No equipment other than basic laboratory equipment is used for serotyping, therefore there are no associated costs.

Consumables

	Cost per sample (Euro)	*% failure**
Mackoney plates	€ 0.07	*Costed into per-sample price*
Saline in tubes	€ 0.07	
Microtitre plate	€ 0.01	
Plates	€ 0.58	
ISO agar + antibiotic	€ 0.33	
Muller hinton agar + antibiotic	€ 0.08	
Chromagenic agar	€ 0.01	
Loops	€ 0.04	
Tips	€ 0.04	
Labels	€ 0.14	
Eppendorf tubes	€ 0.04	

Staff time per sample in minutes		
	Professionals	*Technicians*
Staff time in minutes	0	2.00

XII. Conventional method K: Biochemistry

Equipment			
	Total purchase price (Euro)	*Annual maintenance costs (Euro)*	*Predicted lifespan (years)*
Thermal cyclers	€ 2 466	€ 570	10
Thermal cyclers	€ 2 466	€ 570	10
Thermal cyclers	€ 2 466	€ 570	10
Thermal cyclers	€ 2 466	€ 570	10
Biolog	€ 110 930	€ 11 115	10
Biolog	€ 110 930	€ 11 115	10

Consumables		
	Cost per sample (Euro)	*% failure**
Pipette tips filter	€ 1.54	*Costed into per-sample price*
Pastette fine tip	€ 0.13	
Pastette graduated	€ 0.07	
Universal Plastic 25ml	€ 0.56	
Gloves nitrile	€ 0.11	
Dispojar	€ 0.41	
Microgen plate	€ 9.73	
Inoculators	€ 1.16	
Reservoirs	€ 0.53	
Inoculating fluid	€ 0.44	
Other biochemistry media	€ 11.30	

Staff time per sample in minutes		
	Professionals	*Technicians*
Staff time in minutes	6.00	36.00

XIII. Key variables

Labour costs		
The following table provides the hourly labour cost data (in Euro) used for monetisation of staff time. Figures below refer to Eurostat data on labour costs for 2017 (by staff category), plus a 25% surcharge for overheads.		
	Professionals	*Technicians*
UK	€ 39.6	€ 25.0

EU	€ 45.1	€ 24.5
Source: Eurostat, Labour cost levels by NACE Rev. 2 activity [lc_lci_lev]. Construct: Labour cost for LCI (compensation of employees plus taxes minus subsidies). NACE categories: Professional, scientific and technical activities; Administrative and support service activities. Extracted in June 2018.		
Other		
…		
…		