Whole genome sequencing as a tool to strengthen foodborne disease surveillance and response

Module 3. Whole genome sequencing in foodborne disease routine surveillance

World Health Organization

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ISBN 978-92-4-002126-6 (electronic version)
ISBN 978-92-4-002127-3 (print version)

Suggested citation. Whole genome sequencing as a tool to strengthen foodborne disease surveillance and response. Module 3. Whole genome sequencing in foodborne disease routine surveillance. Geneva: World Health Organization; 2023. Licence: CC BY-NC-SA 3.0 IGO.

Cataloguing-in-Publication (CIP) data. CIP data are available at http://apps.who.int/iris.

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Design and layout. Laura Ruiz & Velvet Pineda (original design and layout); Blossom Srl (design editing)

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Abbreviations and acronyms

AMR	antimicrobial resistance
cgMLST	core genome multi-locus sequence type
DALY	disability-adjusted life year
MLVA	multiple locus variable-number tandem repeat analysis
PAHO	Pan American Health Organization
PFGE	pulsed-field gel electrophoresis
QALY	quality-adjusted life year
SNP	single nucleotide polymorphism
STEC	Shiga-toxin producing *Escherichia coli*
wgMLST	whole genome multi-locus sequence type
WGS	whole genome sequencing
WHO	World Health Organization

1. Background

1.1 Scope and purpose of this module

What this module does

- Provides guidance for countries planning to use whole genome sequencing (WGS) to enhance the routine surveillance of foodborne diseases in humans.
- Focuses on the surveillance of foodborne diseases, which includes outbreak detection and outbreak response. There is no need for a country to refer to the separate module on outbreak response, as the current module applies to the surveillance system as a whole.
- Emphasizes the need for joint work between epidemiologists and food safety professionals.
- Is intended for countries that have an existing laboratory-based surveillance system for foodborne diseases.
- Discusses decision-making aspects for building capacity for using WGS for foodborne diseases and ensuring it fits within the existing surveillance and response system.
- Provides guidance on how to develop WGS within the existing system, and how to prepare the business case to seek approval and funding from senior policy-makers. Once approval and funding have been secured, there are options for managing WGS implementation.

What this module does not do

- Include detailed technical requirements of WGS, but does provide sources of information on the matter.
- Include advice on how to use WGS for integrated food chain surveillance.
- Discuss how WGS can be applied in the food safety sector.

Before reading this module, make sure you have read the introductory module of this manual *(2)* and ensure:

- your country meets the minimum requirements for WGS implementation for enhancing routine surveillance; and
- you understand the purpose, scope, target audience, guiding principles and terminology used in this manual.

The structure of the surveillance and response system for foodborne diseases will not change significantly with the implementation of WGS. However, WGS will change the type of information reported to the surveillance system, and how that information is used for the surveillance system to meet its objectives.

This module is intended for countries that already have a foodborne diseases laboratory-based surveillance system. Specimens submitted to the laboratory as part of patient diagnosis and clinical management are also used for public health purposes. Once a foodborne pathogen has been isolated, it is possible to use WGS for further characterization, as WGS can provide additional information for surveillance, including:

- pathogen subtyping, which is used for outbreak detection, outbreak response and monitoring disease trends over time;
- identifying antimicrobial resistance (AMR) genes; and
- identifying virulence genes.

1.2 How to use this module

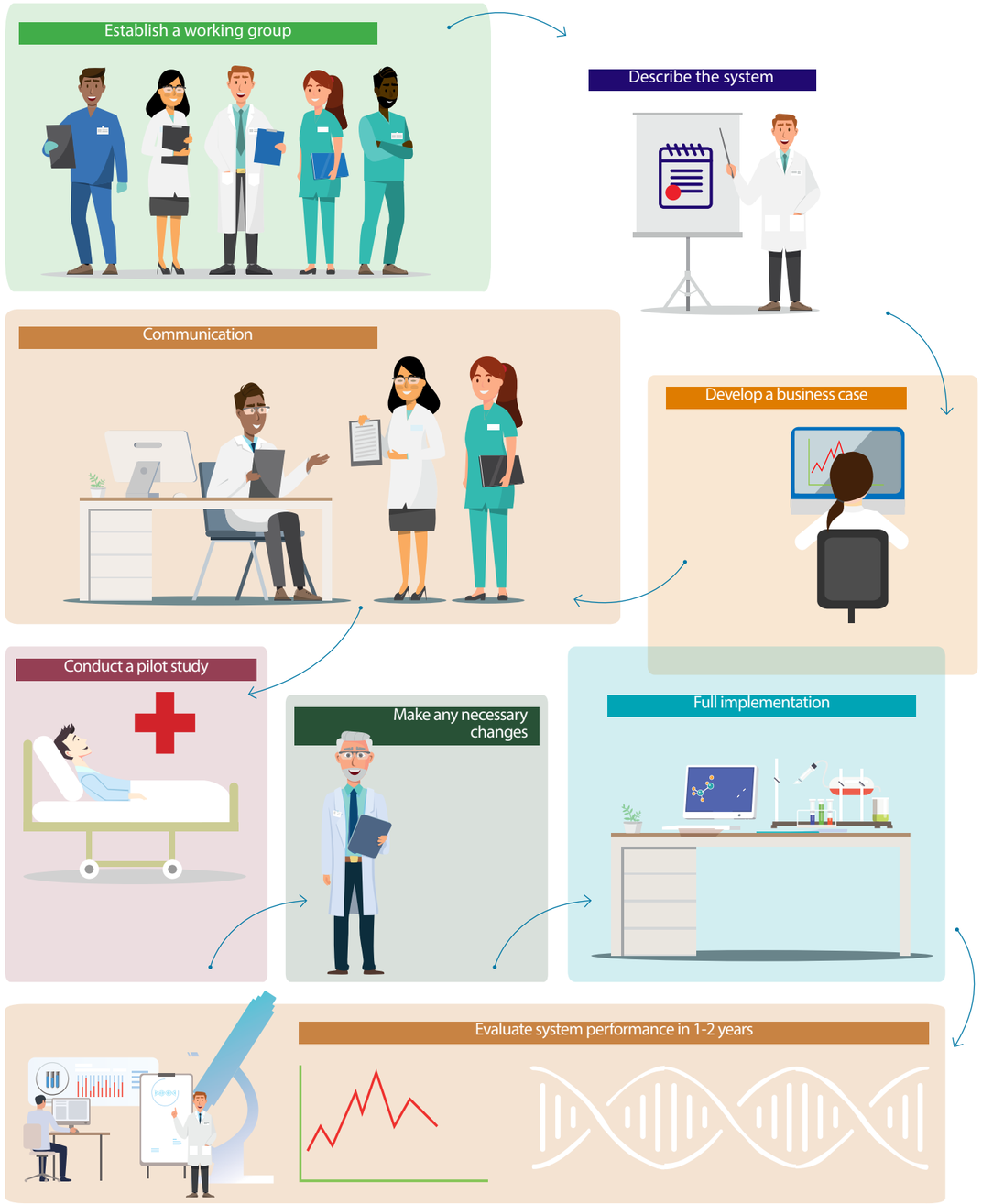
This module outlines the steps of WGS implementation. Steps can be taken in any order, and even in parallel. However, it is important to articulate the objectives of using WGS for routine surveillance in advance. The following steps are involved in foodborne pathogen WGS.

Step 1. Describe how WGS will be incorporated into the surveillance system, detailing its structure and requirements for the system to be effective.
Step 2. Develop a business case that summarizes the system's structure; cost estimates of implementation; human resources required; and system sustainability provisions.
Step 3. Conduct a pilot of the system as proposed, and make any adjustments prior to full-implementation.
Step 4. Use the managing implementation template. Based on the system's description, document all the steps necessary to make the system work, including the business case and pilot study.

Fig. 1 illustrates the steps necessary to implement WGS for routine surveillance. While working through this module, key decisions will need to be made about how sequencing will fit within the existing surveillance and response system, considering there are more than one right way to implement WGS for this purpose. Tools and case studies to help articulate national needs and decision-making are provided.

Fig. 1

Steps in implementing sequencing for outbreak investigations



2. Vision and objectives

2.1 Vision

The vision for implementing WGS to enhance the routine surveillance of foodborne diseases includes several points.

- As part of the surveillance system, sick individuals seeking health care submit specimens and culture-based methods are used to identify the pathogen responsible for the illness.
- Isolates are sequenced, and outputs are analysed and reported to public health personnel.
- Decisions are made by public health professionals, in consultation with laboratory staff, about clusters that require epidemiological investigation. A response can be quickly launched based on a small number of cases in a cluster.
- Ideally, data from the surveillance system in the human health sector are analysed together with sequencing results from the animal health and food safety sectors in real-time. Comparing human and non-human sequences of various pathogens can help identify potential sources for the pathogen and inform outbreak response and control measures.
- Sequence data can also be used for monitoring AMR and virulence factors. WGS data from the surveillance system can be used to better understand foodborne pathogen epidemiology, prioritize clusters of human infection for outbreak response and to inform clinical management policies.
- Sequence data can also be used to identify new or emerging strains of pathogens that are potentially more virulent.

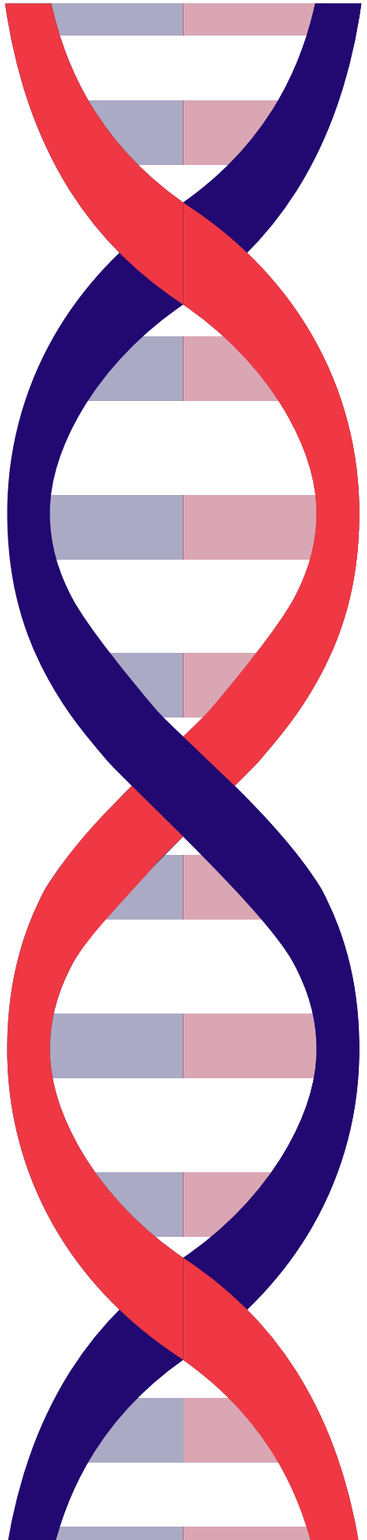
2.2 Objectives of WGS to enhance routine surveillance of foodborne diseases

The objectives of using WGS for routine surveillance of foodborne diseases are to:

- monitor trends in foodborne pathogen subtypes over time;
- detect outbreaks of foodborne diseases, including low-intensity and geographically dispersed outbreaks;
- support foodborne disease outbreak investigation;
- determine the magnitude of the problem of foodborne diseases;
- attribute food sources to specific foodborne diseases;
- inform clinical management policy where appropriate (e.g. regarding AMR in humans);
- inform antimicrobial use policy in food-producing animals and horticulture;
- contribute data from the human health sector for integration with data from other sectors at relevant points throughout the food chain, in order to guide public health action to prevent and control foodborne diseases;
- inform risk-based food safety management; and to
- monitor and evaluate interventions and measures to prevent and control foodborne diseases.

WGS for routine surveillance has the following secondary objectives:

- to determine whether the use of WGS as a tool for routine surveillance of foodborne diseases is appropriate in a country; and
- to build in-country capacity for sequencing in laboratories, bioinformatics support, as well as among epidemiologists and public health staff for results interpretation.



3. Getting started

3.1 Understanding WGS

There are resources available to help understand WGS and how it can be used for public health purposes within a surveillance and response system, including:

- the introductory module of this manual *(1)*
- this module
- the WHO whole genome sequencing for foodborne disease surveillance landscape paper *(3)*
- peer-reviewed scientific literature.

3.2 Establishing a working group

For WGS planning and implementation within the surveillance and response system, a working group of the key stakeholders should be established. The working group is likely to participate throughout the implementation process. It is important to involve key technical staff from the beginning, so that the staff participate in the entire implementation process. Key stakeholders are laboratory and bioinformatics staff, epidemiologists from the public health system and information technology (IT) support staff. It would also be beneficial to include other medical and health department staff; food safety and animal health sector personnel.

The working group should have, and document, clear terms of reference, with well-defined roles and responsibilities for each member (Web Annex A).

ACTION

- Identify key stakeholders.
- Establish a working group with key stakeholders.
- Develop terms of reference for the working group.
- Define roles and responsibilities for each member of the working group.

4. System description

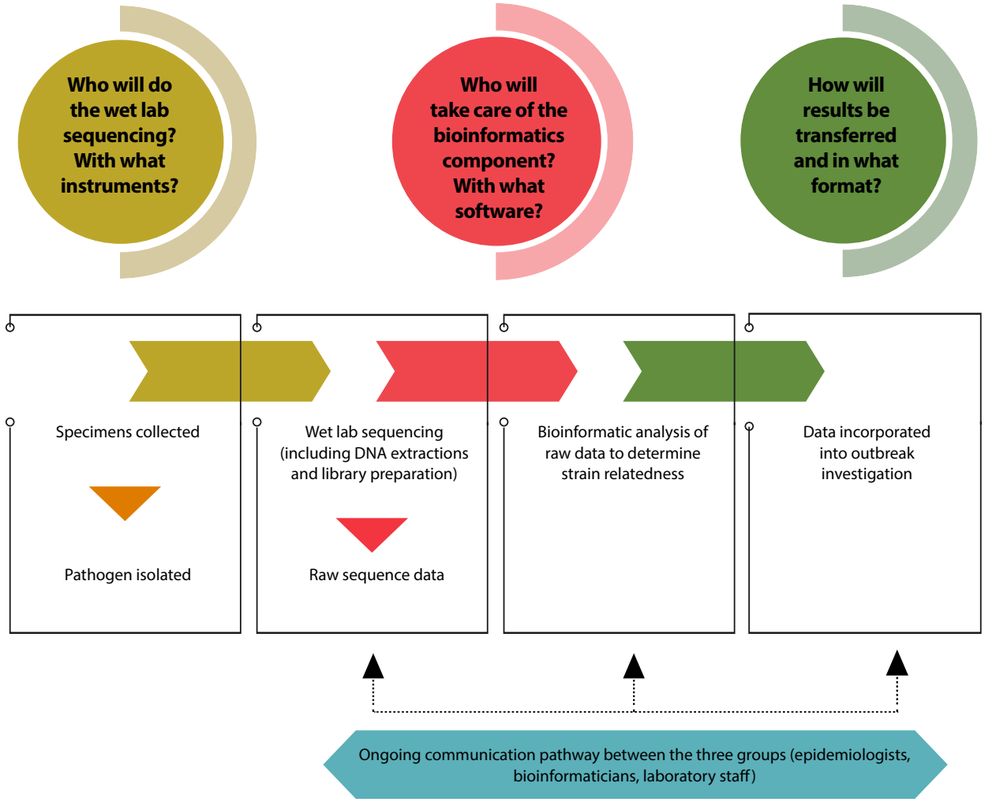
It is important to describe how WGS will be added to the existing surveillance and response system, i.e. flow of specimens and data throughout the system. The description should include:

1. goals and objectives of WGS as part of foodborne diseases routine surveillance
2. where WGS data will come from
3. who will perform bioinformatics analyses and how
4. how WGS data will be used for routine surveillance
5. human resources required within the system
6. how to measure the system's performance.

This section will analyse each step of the process, as well as related decisions that need to be made. Fig. 2 provides an overview of the system and Web Annex B will help describe how WGS will fit within the existing surveillance system.

Fig. 2

Overview of WGS to support outbreak investigations and decisions related to its implementation



4.1 Defining the goals and objectives of WGS to enhance routine surveillance of foodborne diseases

Setting goals

Short- and long-term goals can be set for WGS. WGS implementation can be used to leverage the laboratory-based surveillance system's development. For countries transitioning from syndrome-based surveillance to laboratory confirmation of disease-causing pathogens, WGS means that a single laboratory test will provide large amounts of information (e.g. subtyping, virulence factors, AMR, etc.), the cost of which would have been prohibitive in the past.

In the short-term, a country may start using WGS for a single priority foodborne pathogen, in order to establish the process and capacity for WGS in the surveillance system. Once the system is running for one pathogen, the longer-term plan would be to scale up WGS to include multiple foodborne pathogens. However, sequencing multiple pathogens could also be set as the short-term goal.

Which pathogen(s) will be sequenced?

A decision will need to be made about whether to use WGS for routine surveillance of one priority foodborne pathogen or multiple foodborne pathogens.

This decision will largely be determined by:

- financial resources available for WGS;
- human resources available in the laboratory to perform DNA extractions, library preparation and sequencing;
- access to bioinformaticians to assist with the analysis and interpretation of the results;
- human resources available within the public health authority, usually located in the Ministry of Health (i.e. epidemiologists, surveillance officers and others) to assess the clusters and carry out any outbreak response;
- the political commitment to make WGS a priority for surveillance purposes; and
- the goals of WGS within the surveillance system. If the preference is to see how WGS can be used within an existing system, choosing one priority foodborne pathogen to sequence might be better before making decisions to scale up WGS for multiple pathogens.

It is recommended that a country start with a single pathogen, and scale up once capacities are in place in the laboratory and in the public health authority. Beginning with one pathogen allows epidemiologists and microbiologists to become familiar with gene behaviour and the evolutionary history of the chosen organism, determine the best way to report output data from WGS to the surveillance system and define clusters that require follow-up. This allows for capacity-building in the public health system, as the number of clusters detected is likely to increase, creating a need to prioritize clusters for investigation.

Table 1 shows the advantages and disadvantages of choosing one pathogen versus multiple pathogens.

Table 1

Advantages and disadvantages of WGS of a single pathogen versus multiple pathogens

Options	Advantages	Disadvantages
Single pathogen	• Useful for countries where a large investment is unlikely • Can be a good starting point for piloting the WGS for surveillance and capacity development • Can provide evidence of WGS usefulness, to broaden its application • Enables staff to become more comfortable with WGS use for surveillance, as it allows time for training and building skills at a moderate pace • Does not require major changes in IT infrastructure and surveillance databases • Could facilitate WGS data sharing with the food safety and animal health sectors • Assists in the identification of needed resources and data flow gaps • WGS will be the subtyping tool of choice in the future	• Depending on the pathogen, there may not be enough specimen throughput to make sequencing financially viable • Requires investments in training existing staff and potentially hiring new staff • Ongoing subtyping of other pathogens under surveillance will need to continue while WGS is being implemented (this may stretch the laboratory and public health capacity in the early stages of WGS use) • If traditional subtyping methods are in use, it will be expensive to run it side-by-side with WGS for the chosen pathogen
Multiple pathogens	• With a high number of specimens (throughput), it will be possible to make WGS less expensive per isolate • WGS will be the subtyping tool of choice in the future	• If subtyping of foodborne pathogens is already in place, switching to WGS for multiple pathogens at once will stretch resources, which might weaken surveillance coverage • Requires large financial investments for equipment, IT infrastructure and human resources • Requires major investments to train current staff and, potentially, hiring new staff • Will not allow for staff to become more comfortable with WGS for surveillance, as there will not be time for training or acquiring new skills at a moderate pace • If traditional subtyping methods are in use, it will become expensive to run traditional typing side-by-side with WGS

Using WGS for single pathogen surveillance

The following factors need to be considered when deciding which single priority foodborne pathogen to sequence.

- Inherent characteristics of the pathogen. Does WGS provide the information required to meet the surveillance system objectives?
- Effectiveness of current subtyping methods for distinguishing isolates and identifying true outbreaks. For example, are pulsed field gel electrophoresis (PFGE) or multi locus variable-number tandem repeat analysis (MLVA) sensitive enough?
- Current burden of the pathogen in the community. Is this a pathogen regularly reported to the notifiable disease surveillance system? How many cases per year are reported?
- Resources in the public health system. Are there epidemiologists, surveillance officers and other staff available to assess and respond?
- Current scientific knowledge around implementing WGS for surveillance of the pathogen.

Case study 1 highlights the rationale for using WGS for the surveillance of listeriosis in the United States of America (USA).

Uncommon pathogen

An uncommon pathogen is one not frequently detected in the country's laboratories (e.g. several hundred annual isolates identified), and is not to be confused with a rare pathogen (e.g. fewer than ten isolates identified per year). An uncommon pathogen should be half-way between a very common pathogen (whose implementation using WGS could overwhelm the capacity of the system) and a rare pathogen (where WGS would not provide much benefit).

Countries choosing to implement WGS for an uncommon pathogen usually have a well-developed laboratory-based surveillance system, with laboratory and surveillance infrastructure already in place and used routinely. Even when this is a first attempt at incorporating sequencing data into surveillance systems, there often is laboratory capacity for isolating and further characterizing foodborne pathogens. These countries also have surveillance systems with defined processes and procedures for following up cases infected with the pathogen. In consequence, choosing an uncommon pathogen might not be useful for countries just beginning laboratory-based surveillance.

Choosing an uncommon pathogen will require a balance between gaining knowledge and building capacity for WGS, without overwhelming the system.

Choosing an uncommon pathogen may be useful for countries with:

- some sequencing experience, but unlikely to have large investments in full-scale implementation of sequencing for surveillance purposes in the short-term;
- sufficient specimen throughput in the laboratory for sequencing other pathogens; and
- a standard epidemiological response to the laboratory detection of the uncommon pathogen.

Choose an uncommon pathogen if:

- most sequencing throughput is for other non-foodborne pathogens, but it is possible that a few isolates of the uncommon pathogen can be included in each run or batched every two to three weeks;
- the selected pathogen is well understood in the country and there is the capacity to analyse and evaluate WGS outputs together with epidemiological information;
- subtyping methods are in use in the country for the selected pathogen, so there can be a short period of overlap of WGS with traditional subtyping methods to help evaluate the role of WGS in the surveillance system;
- specific funding for sequencing is limited; and
- there are uncertainties about how sequencing will work in the surveillance system. Choosing an uncommon pathogen will not overwhelm the existing system, and all key stakeholders can become comfortable with sequencing and the outputs.

Case study 1

Surveillance of listeriosis using WGS, United States of America

- Listeriosis is a serious illness with high mortality. It is foodborne and, although fairly rare, outbreaks of the disease are investigated every year in the United States of America (USA).
- For this reason, good public health and food regulatory systems are in place to combat listeriosis.
- The genome of the bacterium causing the illness, *Listeria monocytogenes*, is small and simple, making sequencing and analysis uncomplicated.
- Robust epidemiologic data on listeriosis cases was available, as public health investigators attempt to interview every patient with listeriosis in the USA to obtain the food exposure history. Having food exposure data readily available is very helpful when a cluster is detected.
- For these reasons, it was anticipated that a real life experiment could be carried out quickly (around one year) and with limited resources, to prove that real-time sequencing from farm-to-fork was superior to the current gold standard method (PFGE) for laboratory surveillance of bacterial foodborne diseases using *Listeria monocytogenes* and listeriosis as a model *(4)*.

Common pathogen

Common pathogens are those frequently detected in laboratories in the country. If a country were to choose a common foodborne pathogen, it would be necessary to ensure there was sufficient capacity in the laboratory for sequencing and results interpretation, as well as enough epidemiological capacity to assess and prioritize clusters and respond to detected outbreaks. In some circumstances, it may be necessary to choose a common pathogen to begin integration into the notifiable disease surveillance system, to make sure there are enough specimens on a sequencing run to make it financially viable and to start building capacity at both the laboratory and in the public health authority. Alternatively, a country may choose to select a common pathogen (e.g. *Salmonella*) and then sequence only specific strains that are of public health importance (e.g. *Salmonella* Enteritidis or *Salmonella* Typhimurium). This will allow for experience building with a familiar pathogen, while ensuring that the capacity is not overwhelmed by the introduction of WGS.

Choosing a common pathogen can be used in countries:

- **just beginning to use laboratory-based surveillance of foodborne pathogens**
- **with a well-developed laboratory role within the surveillance and response system.**

Choose a common foodborne pathogen if:

- many isolates are required to make a sequencing run financially viable (Box 1) and guarantee a rapid turnaround time of results for the surveillance system to detect outbreaks;
- the laboratory staff are well trained and have some experience with WGS, perhaps from a research setting or from using WGS during outbreak investigations;
- epidemiologists responsible for foodborne diseases have a basic understanding of WGS and its anticipated outputs, as well as standard processes for responding to outbreaks;
- the selected pathogen constitutes a major public health priority; and
- the aim is to build national capacity and expertise.

Case study 2 highlights the greater discrimination power in the application of WGS with an example of *Salmonella* Enteritidis surveillance in the United Kingdom of Great Britain and Northern Ireland.

BOX 1

Batching specimens to make a run financially viable

- Sequencing machines have a maximum number of specimens that can be processed in one run.
- Reaching maximum capacity will make the sequencing cost per specimen lower.
- Cost per specimen = culturing cost + DNA extraction cost + library construction cost + (sequencing run cost/number of specimens).
- It may be necessary for a laboratory to batch specimens in order to reach maximum capacity.
- Laboratories should determine if other microbes (non-foodborne) are undergoing sequencing, as it might be possible to run foodborne pathogens with those to meet the maximum number needed. This can potentially delay results after specimens are submitted for sequencing. The laboratory needs to wait until it has enough specimens to reach maximum capacity.
- If batching, epidemiologists need to know, in order to assist with the interpretation of trends (i.e. increases may be due to batching, not an outbreak) and to understand the timelines of reporting.

Case study 2

WGS for *Salmonella* Enteritidis surveillance, United Kingdom of Great Britain and Northern Ireland

- Since April 2015, WGS has been used in the United Kingdom of Great Britain and Northern Ireland for the identification and further typing of *Salmonella* strains.
- In May 2015, a cluster of 29 cases of *Salmonella* Enteritidis infection was detected *(5)*. The cases were within a five single nucleotide polymorphism (SNP) single linkage cluster, with specimen collection dates spanning two months. Of the 29 cases, 23 cases were typed as *Salmonella* Enteritidis phage type 59.
- An outbreak investigation was launched.
- The study authors concluded that the previous subtyping method of phage typing would have detected the outbreak. However, 32% of the cases (that were part of the SNP cluster but had a different phage type, i.e. not phage type 59) would not have been included in the epidemiological investigation. Consequently, their food history would not have been part of the traceback investigation.
- This case study shows the greater discrimination power of WGS when detecting genetic similarity vis-a-vis phage typing for routine surveillance of *Salmonella* Enteritidis. While phage typing would most likely have detected the outbreak, the specificity and sensitivity of WGS added precision to the epidemiological information gathered during the investigation, which facilitated traceback by the food authorities.

Table 2 highlights the advantages and disadvantages of choosing an uncommon pathogen compared with a common foodborne pathogen.

Table 2

Advantages and disadvantages of choosing uncommon or common pathogens for WGS for surveillance purposes

	Advantages	Disadvantages
Uncommon pathogen	• Useful for countries where large investments in WGS are unlikely • Not too many cases, to not overwhelm the surveillance system • Allows time for all participants in the system to become familiar and comfortable with WGS • Provides an opportunity to determine isolate and data flow, and to establish regular communication of results between the laboratory and the public health authority	• May not have the necessary specimen throughput to make it financially viable • Isolates may be batched, which means early outbreak detection is not possible using WGS • Due to the small number of outbreaks identified, knowledge for identifying and interpreting clusters will not necessarily be generated • Might not provide the necessary information to pilot WGS as a surveillance tool, nor to detect gaps in the system or demonstrate the usefulness of WGS
Common pathogens	• Likely to have a high throughput to make rapid sequencing viable • Likely to have rapid turnaround times to assist in early outbreak detection, as little to no batching would be required • Provides enough information to allow for knowledge development and capacity to interpret WGS data, both within the laboratory and in epidemiological services	• Requires significant capacity within the laboratory for sequencing and bioinformatics analysis • Requires enough epidemiological capacity to assess outbreaks and respond to them • Requires a strong food control system to take necessary public health action

Using WGS for the surveillance of multiple foodborne pathogens

If the decision is made to use WGS for multiple foodborne pathogens, the following factors need to be considered.

- While the structure of the surveillance system will remain relatively unchanged, the types of data that will be reported and the ways data are managed through the system will undergo significant change. This will require considerable time and investments to ensure existing laboratory and public health staff are trained appropriately.
- It will be important for laboratory and epidemiological staff to communicate the changes to decision-makers, so the transition can be managed effectively.
- Laboratories may be using traditional subtyping methods for some foodborne pathogens. There will need to be a plan in place to transition from traditional typing to WGS. This will be discussed in greater detail later in this module, but transitioning subtyping methods will be a factor to consider when deciding to use WGS for multiple pathogens.
- The same analytic and reporting methods are not appropriate for all pathogens. A wider range of expertise will be necessary if WGS is used for more than one type of pathogen.

Defining the objectives of WGS for surveillance

Once short- and long-term goals are defined and a decision is made about which pathogen(s) to sequence, it will be necessary to determine if WGS will provide the information needed to meet the overall objectives of the surveillance system.

A country can use the objectives listed earlier in this module, or modify them according to the chosen pathogen(s) and the national context. It is important to document the surveillance system's objectives to evaluate performance after implementing WGS, including whether surveillance objectives are being met.

ACTION

- Define short-term and long-term sequencing goals.
- Decide which pathogen will be sequenced.
- Define the objectives of using WGS to enhance routine surveillance of foodborne diseases.

4.2 Where will WGS data come from?

Where will specimens come from?

Given that having a functional laboratory-based surveillance system is a minimum requirement for implementing WGS to enhance routine surveillance, specimens will be sent from a health facility to a laboratory for pathogen confirmation.

WGS for the chosen foodborne pathogen(s) can be implemented in a defined geographical area or nation wide. The geographical coverage of WGS in the surveillance system will depend on:

- laboratory capacities to perform WGS within the country;
- resources available (both financial and human) at the sub national and national levels;
- political commitment at the sub national and national levels; and
- structure of the health system. In some countries there is a centralized model where the public health laboratory and epidemiology capacities are at the national level. Others may have a de-centralized health system, where capacities and decision-making occurs at the sub national level.

One geographical area

Implementing WGS in one geographical area means there is a sub national area with a functional laboratory-based surveillance system that reports laboratory-confirmed pathogens to public health authorities. Decisions about the required public health follow-up take place at the sub national level.

If the selected area has the resources and political commitment, it will be possible to introduce WGS in the surveillance system as a pilot site, in preparation for scaling up to the whole country. Experiences gathered in WGS for routine surveillance from that sub national site can be shared with other similar sites and nationally.

Nation wide

If a country chooses to implement WGS across the country, all sub national sites should have a functional laboratory-based surveillance system, and should be able to contribute isolates or sequences to a national data repository. Depending on how the health system is structured, public health decisions on further action are made at the sub national level (decentralized model) or at a national level (centralized model). Regardless of where the decision about public health follow-up is made, data are collated at the national level and decisions are made about any required nation wide action.

With a decentralized surveillance system with multiple sub national laboratories contributing isolates or sequences, there needs to be national coordination to ensure that:

- sequencing and WGS analysis methods across sub national sites are comparable throughout the country;
- agreements are negotiated with each sub national site to facilitate sequence and metadata sharing, if relevant; and
- there is national level data analysis to determine whether any clusters present in more than one sub national site require investigation.

Table 3 summarizes the advantages and disadvantages of each geographical coverage option for the surveillance system.

Table 3

Advantages and disadvantages of a single geographic location or nation wide coverage of WGS for surveillance purposes

	Advantages	Disadvantages
One geographic location	• Builds on existing laboratory and public health capacities • Can be used to pilot sequencing and its interpretation before nation wide implementation • No requirement for negotiating national data sharing protocols, as data are produced and used locally	• Can direct resources to already well-established areas • Surveillance data is not representative of the whole country • Nation wide outbreaks with a few cases in region could go undetected • Might not have sufficient number of isolates for running sequences, interpreting findings or identifying outbreaks
Whole country	• Surveillance data is representative of the whole country • Can detect outbreaks throughout the country • Provides enough isolates to conduct sequencing and data interpretation • Establishes national baseline data to conduct nation wide monitoring activities (i.e. can detect national outbreaks)	• May be costly, depending on the local sequencing capacity • Depending on how the health system is structured, data sharing protocols may be needed to ensure sequences and appropriate metadata are nationally shared • If multiple sites are sequencing and running bioinformatics analyses locally (decentralized model), there will need to be harmonized testing methods, analyses and reporting (there will also need to be reporting to the national level for the analysis of WGS data to detect outbreaks across multiple sites) • If multiple sites are sequencing but bioinformatics analysis occurs nationally (centralized model), a national platform will be needed to collect all the data and perform the analyses

ACTION

- Decide the coverage required of WGS for routine surveillance.

Where will isolates be sequenced?

Upon arriving at the public health laboratory, specimens will be tested and one or more pathogens will be isolated. A decision will need to be made about where the isolate or DNA extract will be sent for the wet lab aspect of sequencing (Note: this is separate from the bioinformatics component, which will be addressed in later sections).

If the public health laboratory does not have wet lab sequencing capabilities, the options are:

- to outsource to another laboratory either in country or abroad
- to establish sequencing capacity in the public health laboratory.

Which option to choose?

To assist in this decision, Table 4 summarizes the advantages and disadvantages of outsourcing wet lab sequencing versus sequencing in the country's public health laboratory for routine surveillance purposes.

Table 4

Advantages and disadvantages of two sequencing options for wgs wet lab component used in routine surveillance

Options for sequencing	Description	Advantages	Disadvantages
Outsourcing sequencing component	Isolates obtained during routine laboratory-based surveillance are sent to a laboratory other than the national public health laboratory for sequencing.	• Reduces implementation cost in the short-term • Does not require the purchase and maintenance of sequencing nor data storage capabilities • Can be useful for low throughput (uncommon pathogen)	• May increase delays until results are available due to the need for specimen transport • Does not build national capacity in terms of infrastructure and staff • May reduce the number of isolates available for sequencing if any specimens are lost due to transportation issues • Not sustainable in the long run • May meet competing priorities at the chosen laboratory • Will increase administrative burden to ensure correct paperwork is filed and processes are followed for the international shipping of infectious materials • There are cost implications in shipping internationally
Public health laboratory	Sequencing is done by the national public health laboratory.	• Improves the public health system's capacity • Service will be available for surveillance of other pathogens, aside from foodborne diseases • Does not require international shipping of infectious materials • Working with existing staff and institutions enables familiarity with the status of foodborne disease surveillance and allows for local solutions (not dependent on third parties who may not understand surveillance) • Allows for open communication among all participants and partners in foodborne disease surveillance and response	• High implementation costs • Takes longer to establish the service and validate the outcomes • Requires trained staff

Outsourcing the wet lab component of WGS

In this case, a third party laboratory is responsible for performing the wet lab step in WGS. Mainly, it is an option for low throughput pathogens, when WGS is used for sequencing uncommon microorganisms. If there is public health follow-up after identifying the pathogen (e.g. *Listeria monocytogenes*), public health professionals will be able to detect potential links using epidemiological data, as an interim measure before WGS results are available. Outsourcing the wet lab component for an uncommon pathogen will allow a country to gather evidence on the benefits and suitability of sequencing, before investing in the required infrastructure.

Outsourcing is generally not useful for common pathogen routine surveillance, especially for foodborne diseases that: a) are outbreak prone; b) have many subtypes and require additional characterization by the laboratory, which is a necessary step to identify outbreaks; or c) require timely integration with food, animal and environmental sequences to help develop hypotheses about potential source of illness in humans.

Outsourcing the wet lab component for outbreak-prone common pathogens might work when there is a strong relationship between the public health laboratory, public health authorities and the outsourced laboratory. It may be possible to negotiate and guarantee rapid turnaround times for sequencing results to enable rapid outbreak detection.

When outsourcing the wet lab:

- develop a requirements document details the service required (Web Annex C)
- choose a laboratory to perform the sequencing (Web Annex D for mapping lab capacities)
- determine data ownership and data sharing arrangements
- develop a contract for service.

The details of each step in outsourcing the wet lab component are provided in Web Annex E.

Building wet lab WGS capacity in the public health laboratory

It is ideal to implement WGS for routine surveillance in a public health laboratory in-country, to ensure long-term sustainability as well as standardization of methods. This would also prevent data losses when switching wet lab providers.

If a steady supply of specimens to be sequenced is expected, it might be advantageous to establish WGS capacity in the public health laboratory. It is expensive to establish a sequencing laboratory, however, investigating in national capacity-building in the public health laboratory will be beneficial in the future. The system will be sustainable and reliable and will ensure data are comparable over time. In contrast, if WGS wet lab processes are outsourced, there will be ongoing costs, unsustainable in the long-term, and little local capacity-building will result.

There is an additional danger of affecting surveillance activities if wet lab service providers do not renew contracts; such a situation could lead to outbreaks not being detected.

When building in-country capacities for the wet lab component of WGS, it is important to:

- designate a laboratory to perform WGS
- plan the anticipated workflow
- choose an appropriate sequencing instrument
- ensure availability of reagents, consumables and equipment
- establish quality assurance.

The details of each step for building capacity in the public health laboratory are provided in Web Annex F.

Decision-making

When deciding whether to build capacity in the public health laboratory or outsource the wet lab component of WGS, there are a number of factors that will influence the decision, including:

- financial resources;
- objectives of WGS for routine surveillance of foodborne diseases;
- pathogen and geographic area selected for surveillance, which will determine specimen throughput;
- potential of using WGS to support non-foodborne disease surveillance and outbreak investigations; and
- political commitment (i.e. is there a long-term commitment to strengthening the capacity of the public health laboratory? Is there approval for sending specimens abroad or outside the public health system? Is there a long-term commitment to make the outsourced wet lab the permanent provider?).

Factors influencing the decision of where to conduct sequencing will vary according to national circumstances. Box 2 provides general guidance in this regard.

BOX 2

Choosing an option for the wet lab component of sequencing

1 Make sure you have read the following annexes.

A Web Annex E. Outsourcing the wet lab component of WGS

B Web Annex F. Building capacity in the public health laboratory for the wet lab component of WGS

2 Consult with colleagues from other countries where WGS has been established in support of outbreak investigations of foodborne pathogens, in order to learn about the costs, resources and data flows involved.

3 Map existing lab capacities in the public health lab (Web Annex D), other laboratories in the country and in accessible laboratories abroad. If nothing is available, new collaborations will need to be established.

4 Examine other factors that might influence the decision, including political and economic support.

5 Make sure the sequencing methods in the chosen laboratory are comparable to other regional, national or international methods.

6 If selecting outsourcing, start to think about preparing for transitioning to sequencing in the national public health laboratory within a few years.

ACTION

- Decide whether to outsource the wet lab component of WGS or build the national public health laboratory capacity:
  - if outsourcing, read Web Annex F and undertake the actions described; and
  - if choosing to build the national public health laboratory capacity, read Web Annex G and undertake the actions described.
- Determine and document the specimen referral pathways from specimen collection site to the designated laboratory.
- Make sure that specimens/isolates will be correctly transported.

Updating protocols

Once an option has been chosen, specimen referral pathways will need to be updated to reflect the change in practice. For example, if the decision is to outsource to the local university, the specimen referral pathway from the local public health laboratory to the university laboratory will need to be established. If the designated laboratory is abroad, develop protocols for international pathogen transport, including bureaucratic requirements and adequate temperature control. A protocol will also be needed to ensure that sequencing data are sent to the laboratory that will perform the bioinformatics analysis.

4.3 Who will perform the bioinformatics analyses and how?

Once sequencing in the wet lab is completed, outputs (raw sequence data) will need to be analysed with bioinformatics tools and interpreted by bioinformaticians. This process is called dry lab component. Part of the analysis will address the quality of the sequence and produce outputs that can be useful for public health authorities such as genetic relatedness, serogroup, virulence genes and AMR genes.

An entity to conduct the bioinformatics analysis needs to be selected; if the decision is to outsource the process, where and how the raw sequence data will be sent will also have to be decided. Annex 1 in the introductory module describes this process.

Steps of bioinformatic analyses

Bioinformatic analyses involves the following steps *(3)*.

Step 1

Quality assurance

There is a quality assurance programme that analyses the quality of raw sequence data and makes sure that only sequence data meeting certain quality thresholds are used for further analyses.

Step 2

Species identification

The sequence is checked and compared against the relevant database to identify the species.

Step 3

In silico typing and phenotype prediction

The sequence is compared against the pertinent database to predict a serotype and provide information on virulence and AMR genes.

Step 4

Whole genome molecular typing

Various analyses can provide further resolution typing to assess genetic relatedness. Examples include SNP analysis, core genome multi-locus sequence type (cgMLST), whole genome multi-locus sequence type (wgMLST) and k-mer analysis.

Running the bioinformatic analyses requires appropriate data processing infrastructure, i.e. computers, data storage space and stable internet connections with adequate bandwidth for processing and sharing sequences.

Important information about bioinformatics implementation

- The dry lab component of WGS is the most complex, as it requires highly trained staff to make decisions regarding what pipelines to use and what analyses to perform.
- There is still much uncertainty regarding bioinformatic analyses for surveillance purposes; there is no internationally standardized approach, and no formal evaluations have been conducted.
- Access to a bioinformatician is necessary even in low throughput situations, or when highly automated pipelines are used. It is critical that someone involved in the implementation, with training in bioinformatics analyses, be available to review results and identify potential errors.
- The end users of bioinformatics outputs (e.g. epidemiologists and other public health professionals) need to be involved as of the planning phase, and maintain regular communication to ensure the usefulness of outputs for surveillance and outbreak response purposes.

There are multiple approaches when determining the bioinformatics approach to take. A country can:

1. purchase an off-the-shelf product containing all the necessary tools for the analyses
2. use open-source tools
3. develop their own analyses
4. use a combination of tools.

Table 5 contains a list of the advantages and disadvantages to each of the bioinformatics approaches.

Table 5

Advantages and disadvantages of various bioinformatic approaches

Approach	Advantages	Disadvantages
Off-the-shelf products	• No development is required • Does not need a full-time bioinformatician, but does require access to someone skilled in correct output interpretation • A commercial license to a software product often ensures company support and software updates • Can lead to standardization of software to be used across the country, if everyone uses the same product	• Can be originally expensive and often carry ongoing costs • Might not be able to add fields necessary for conducting further analyses of assessments • Underlying algorithms are often not publicly available
Open source products	• Free of cost • Work done in the country is replicable by the broader scientific community • Can lead to standardization of software to be used across the country, if everyone uses the same product	• Requires staff with bioinformatics skills to understand which products to use and when to use them • No guaranteed support
Open source products Develop own analytical tools	• Can customize analyses to national requirements • Can customize the type of metadata to include with each isolate's information • Epidemiologists can provide input to assist with the analysis and outputs	• Requires at least one, preferably more, highly skilled bioinformaticians • Might not be compatible with software used by other regions or countries, preventing the merging/sharing of data • Will require someone to update databases (e.g. AMR mutations are reported in multiple databases and require checking and updating own analytical tools)
Combination of approaches	• Analyses may be customized to national requirements • The purchase of widely used products may facilitate data comparability • Metadata to include with each isolate information may be customized	• Requires a bioinformatician to put all the tools together to produce the desired outputs • Might not be compatible with software used by other regions or countries, preventing the merging/sharing of data

A decision will also need to be made about where to send raw sequence data for bioinformatic analyses. The dry lab component of WGS does not take as much time to perform as the wet lab component. Once the raw sequence has been generated, outputs can be stored and re-analysed at any time. Options for outsourcing are more flexible, so that a stepwise approach may be selected whereby:

- all computing and bioinformatics analyses are outsourced;
- computing capacity is built in the public health laboratory, but bioinformatics analysis is outsourced (in this case, analysis pipelines can be installed locally by a remote expert who can also process the data and conduct the analysis remotely); and
- all computing and bioinformatics analyses are conducted in the public health laboratory.

Table 6 describes the advantages and disadvantages of outsourcing bioinformatics vis-a-vis developing national capacities.

If the public health laboratory does not have bioinformatics capabilities, the options are to:

A outsource either in-country or abroad

B establish bioinformatics capacity in the public health laboratory.

Table 6

Advantages and disadvantages of different bioinformatic options for WGS for routine surveillance

Option	Description	Advantages	Disadvantages
Outsourcing bioinformatics component	Raw sequence data are sent to a chosen organization for analysis and to provide outputs on isolate relatedness. The chosen entity might be a laboratory, a university or other.	• Reduces implementation costs • Does not require large-scale investments in computing and IT infrastructure • Does not necessarily require qualified bioinformatics staff in the public health laboratory (though still recommended that bioinformatician participate in implementation process)	• May increase delays until results are available, depending on priorities of chosen institution • Does not build national bioinformatics capacity • Will need to decide what metadata are attached to each isolate in the bioinformatics analyses • Public health laboratory staff and other public health personnel (such as epidemiologists and surveillance officers) will still need to understand bioinformatics analysis outputs • Potential lack of access for public health staff to address questions/concerns regarding bioinformatic analyses • Potential barrier to validation, due to lack of local capacity and knowledge of organisms involved in the outbreak
Computing capacity in the public health laboratory, outsource bioinformatics experts	The analysis of raw sequence data is conducted at the public health laboratory, but bioinformatics interpretation and troubleshooting are outsourced.	• Public health laboratory staff can start building capacity while using bioinformatics tools • Does not require qualified bioinformatics staff in the public health laboratory; it is still advised that bioinformatician participate in implementation process	• High implementation costs related to purchase of computing and IT infrastructure • Public health laboratory staff will need a rudimentary understanding of bioinformatics and output analyses • May increase delays until results are available, depending on priorities of chosen institution • Public health laboratory staff and other public health personnel (such as epidemiologists and surveillance officers) will still need to understand bioinformatics analysis outputs • Potential barrier to validation, due to lack of local capacity and knowledge of organisms involved in outbreak
Bioinformatics in the public health laboratory	Analysis of raw sequence data and results interpretation is conducted at the public health laboratory.	• Improves capacity within the public health system • Service will be available for other activities beyond foodborne diseases • Laboratory can ensure strict software version control, required for consistent results over time • No sensitive data are shared with external groups • Closer collaboration among epidemiologists and bioinformaticians continuously improves analysis workflows • Lab and public health staff collaborate to interpret, assess and respond to analysis results	• High implementation costs related to purchase of computing and IT infrastructure • Takes longer to establish services and validate outcomes • Requires trained bioinformatics staff in the public health laboratory • Public health staff, such as epidemiologists and surveillance officers, will need to understand the bioinformatics analyses outputs

Which option to choose?

The dry lab component is more complex than the wet lab component, given its newness in many environments. Establishing this component can be very expensive as it requires:

- access to significant IT infrastructure to conduct the analysis;
- access to data storage capability;
- access to trained bioinformaticians; and
- training of existing laboratory staff and epidemiologists to understand the general processes and bioinformatic analyses outputs.

The factors that influence the decision will be financial and political, and also determined on the availability of highly skilled bioinformatics staff. Box 3 describes the steps required to choose an option for the dry lab component of sequencing.

BOX 3

General guidance on choosing an option for the dry lab component of sequencing

1 Make sure you have read the following Web Annexes for this module.

- **A** Web Annex G. Outsourcing the dry lab component of WGS
- **B** Web Annex H. Building capacity in the public health laboratory for the dry lab component of WGS

2 Consult with colleagues from other countries where WGS has been established for routine surveillance of foodborne pathogens, in order to learn about the costs, training, data flows, types of analyses and resources involved.

3 Map existing capacities in the public health laboratory (Web Annex D), other laboratories in country and accessible laboratories abroad. If nothing is available, new collaborations will need to be established.

4 Examine factors that might influence the decision, including political and economic support.

5 If selecting outsourcing, start to think about preparing for transitioning to bioinformatic analyses within the public health laboratory within a few years.

ACTION

- Decide whether to outsource the wet lab component of WGS or build national laboratory capacity:
  - if outsourcing, read Web Annex G and undertake the actions described; and
  - if building capacity in the public health laboratory, read Web Annex H and undertake the actions described.
- Determine and document where bioinformatics analyses will be conducted.

4.4 How are the results of WGS used for routine surveillance?

There are two key stages in using WGS data for routine surveillance:

1 WGS outputs are reported to the surveillance system and stored in the surveillance database

2 the results are analysed regularly to identify when and where public health action is needed.

Reporting WGS outputs to the surveillance system

Notifiable disease surveillance databases traditionally record pathogen information in categories (e.g. each different *Salmonella* serovar is a unique category; each PFGE pattern is a different category, etc.), which are then used by epidemiologists to monitor trends over time and detect potential outbreaks.

Generating outputs that are useful for surveillance purposes is an area that is still under development internationally. The stability and accuracy of taxonomical nomenclature is not yet well established, although they are expected to be suitable for surveillance purposes (reference required!). Different countries have taken different approaches choosing different outputs from WGS to incorporate into their surveillance systems, but it is not yet possible to recommend a gold standard. Web Annex I presents a series of case studies describing how WGS outputs have been incorporated into existing surveillance and response systems.

Table 7 provides an indication of the types of outputs required if a surveillance system is to meet its objectives. It will be important for laboratory and bioinformatics staff to work with public health authorities to determine which outputs would be the most acceptable to ensure the surveillance and response system meets its objectives.

Table 7

Types of outputs required for a foodborne disease surveillance system to meet its objectives

Surveillance objective	Type of output required
Detect outbreaks of foodborne diseases, including low-intensity and geographically dispersed outbreaks	An output that enables the assessment of relatedness among all the cases in the surveillance system with sufficient discrimination to identify a likely outbreak
Monitor trends in foodborne pathogen subtypes over time	A categorical variable of sufficient discrimination to identify subtypes and track this over time (traditionally this had been at the serogroup level)
Support foodborne disease outbreak investigations	For all of the cases reported during the timeframe of the case definition, there needs to be an output that identifies an outbreak strain(s) and can be used to rule cases in or out of the outbreak (e.g. number of alleles or SNPs difference to be considered a 'confirmed' case)
Determine the magnitude of the problem of foodborne diseases	A categorical variable of sufficient discrimination to identify subtypes and track this over time (traditionally this had been at the serogroup level)
Attribute food sources to specific foodborne diseases	An output that enables the assessment of relatedness among all cases in the surveillance system, as well as comparison with outputs from food safety and animal health sectors, to assist in source attribution
Inform clinical management policy, where appropriate (e.g. regarding AMR in humans)	An output on AMR in a categorical form. This will allow to look at resistance to specific antimicrobial agents across time
Inform antimicrobial use policy in food-producing animals and horticulture	An output on AMR in a categorical form. This will allow to determine the presence of resistance to specific antimicrobial agents across time It is also important that outputs are comparable with those used for food and animals
Contribute data from the human health sector for integration with data from other sectors across relevant points in the food chain, to guide public health action to prevent and control foodborne diseases	An output that enables the assessment of relatedness among all cases in the surveillance system, as well as comparison with outputs from food safety and animal health sectors, to assist in source identification
Inform risk-based food safety management	An output that enables the assessment of relatedness among all cases in the surveillance system, as well as comparison with outputs from food safety and animal health sectors, to assist in source identification
Monitor and evaluate interventions and measures taken to prevent and control foodborne diseases	An output that enables the assessment of relatedness among all cases in the surveillance system, as well as comparison with outputs from food safety and animal health sectors, to evaluate the impact of food safety interventions on human health

To be able to interpret WGS outputs, epidemiologists and other public health professionals will need to understand the general processes required to generate those outputs as well as the differences among them. The main piece of knowledge for epidemiologists to learn during the transition is how to interpret phylogenetic trees or nomenclature data provided by the laboratory. Epidemiologists and laboratory staff will also need to be in constant communication to define clusters based on the WGS outputs and basic epidemiological data (i.e. travel history and other data in terms of person, time and place).

The main challenge in combining WGS outputs with epidemiological data is how to record assessments of genetic relatedness in existing surveillance databases. The issues are listed below.

- Traditionally, further typing provided a serogroup, which could be recorded as a categorical variable in the surveillance database. A serogroup can be inferred from WGS for some pathogens. However, phylogenetic trees, which are useful for identifying clusters, may be difficult to incorporate into surveillance databases. The analyses associated with phylogenetic trees cannot be ignored and it is important that this information be analysed and stored in a larger genomics database.
- When data are displayed in phylogenetic trees, there needs to be a common identifier so that information in the trees can be linked to epidemiological data. There will need to be agreement amongst the laboratory staff and epidemiologists on what common identifier is appropriate to link the data. An example is to link WGS data with epidemiological data using the unique laboratory identification number of the isolate.
- If the laboratory makes an assessment and identifies a new group of genetically related sequences amongst a group of cases, each case with that sequence can be coded in the surveillance database (e.g. by a cluster code). Those cases with unique sequences may be recorded as unique, but there is no easy way to record an assessment of relatedness between each unique case, in the absence of internationally standardized nomenclature. There may be local ways to record this information depending on the bioinformatic analyses used.
- A case may not remain unique forever, which means that at a certain point in time that unique case may become genetically related to another. The surveillance database must allow changes to the field(s) recording whether a case is unique or related. Also, methods for identifying clusters must take this into account.

It is not necessary to redevelop the surveillance system database to accommodate WGS outputs. The main data required for surveillance purposes that should be captured in the surveillance database are as follows.

Nomenclature code
This code identifies the pathogen of interest at a level of sufficient discrimination to monitor trends and detect outbreaks. It may be stored as a categorical variable in the surveillance database.

Cluster code
Once an outbreak has been detected, all cases included in the cluster are assigned a code unique to the cluster. This may be stored as a free text or numerical field in the surveillance database.

AMR information
Describes the recognized AMR genes that may be present in an isolate. It may be stored as a free text field or coded as Yes/No for each key AMR gene of interest in the surveillance system.

Virulence genes
For some pathogens, it will be important to systematically collect information about virulence (e.g. Shiga-toxin producing *Escherichia coli* (STEC)). These data may be stored as a free text field or coded as Yes/No for each key virulence gene of interest in the surveillance system.

Depending on the stability and flexibility of the surveillance database, it may be possible to add fields to it to accommodate a range of outputs from sequencing. Ideally, there would be a category assigned to a pathogen at a level of sufficient discrimination for cluster detection. Once a clustering of a particular type has been observed, there is flexibility to generate a phylogenetic tree or other representation of relatedness (e.g. SNP matrix) of cases within the cluster.

ACTION

- Laboratory and public health staff together decide:
  - how WGS results from in the laboratory will be reported/shared with public health authorities;
  - on an agreeable frequency for reporting WGS results to public health authorities, depending on the objectives of the surveillance system; and
  - how to interpret the results and report trends over time.
- Public health authorities modify the surveillance database to capture agreed WGS outputs.
- Make sure the surveillance database data dictionary is updated to reflect the changes to the database.

Linking to public health action

As with any surveillance system, data needs to be collected and analysed to inform public health action. The four main areas where sequencing data can link to public health action are discussed below. The link between the surveillance data and the types of public health action that can occur are summarized in Table 8.

Outbreak detection

Using traditional typing methods, epidemiologists would receive results from the laboratory and analyse the data for: a) clustering in terms of time, place and person; and b) detecting increases when the observed number of notifications for a pathogen exceeds the 'normal' level.

With WGS it will require the epidemiologist to continuously work with the bioinformatician to understand WGS results, add important epidemiological data to WGS data and to make decisions about public health action. This will require a change in practice for most epidemiologists.

One key challenge that the laboratories and public health authorities face is how to define clusters that require public health follow-up. It may be possible to establish criteria for defining clusters for each pathogen in the surveillance system being sequenced. Some elements that may be included in the criteria for defining a cluster are:

- the minimum number of isolates to include in a cluster
- the number of SNP differences in isolates included in the cluster
- the date range (i.e. case onset date, specimen collection date, etc.).

Given that WGS is better at discriminating than other subtyping methods, it is likely that more clusters will be identified. It will be important to prioritize clusters for investigation within the existing resources available. Clusters that may be given higher priority are those with a higher number of cases, a tighter clustering in place and time (e.g. three cases occurring in a small geographic area within one week may be given a higher priority than three cases in a larger geographic area with months between specimen collection dates), or an unusual demographic feature in the cases (e.g. all the cases are aged <5 years; all of the cases are male).

Each pathogen will have its own criteria for clustering and for assigning a priority for public health action. The information to assign criteria for defining clusters and priority for public health action is built over time during WGS implementation. Defining thresholds at the beginning is helpful, but expect changes during implementation.

Outbreak investigation

Once a cluster has been identified and a decision is made to follow up, cases in the cluster are interviewed and their food histories are analysed to determine if there is a common food or event that may link the cases. Sequencing information is used when it is incorporated in the case definition for the outbreak. Using WGS in outbreak case definitions will ensure greater specificity in assigning 'case' status for analytical studies and reduce misclassification bias, which may exist for some pathogens using traditional subtyping methods.

Once a food item or premise has been identified, food safety staff can collect food or environmental samples. If these samples are positive, an isolate can be sequenced and compared with those of human cases. Food safety staff who are part of the outbreak response team will also conduct tracebacks. Epidemiological, traceback and microbiological evidence are analysed to inform food safety authorities to intervene to stop the spread of the infection.

Evaluating the impact of control measures

Whether control measures are taken during an outbreak or as part of handling a pathogen's endemic strain, evaluating the success of control measures is important to determine their usefulness in future cases. Intervention success can be assessed by determining whether the strain continues to be detected in human cases, or in food and/or environmental samples from the areas where control measures were implemented.

Integrated food chain surveillance

Using the One Health approach, data from the animal health, food safety and human health sectors are regularly shared and jointly interpreted to better understand the epidemiology of foodborne diseases and the occurrence and spread of foodborne hazards along the food chain. WGS approaches should be harmonized across sectors to ensure outputs are comparable. In this context, comparing sequences from clinical specimens from humans with non-human isolates can lead to early identification and control of potential food contamination events before a full-scale outbreak even occurs. When using WGS across sectors, it will be important that all partners agree on the metadata details to be shared.

Table 8

Linking WGS for foodborne disease surveillance and outbreak response to public health action

	Sectors involved	How sequencing is linked to public health action	How to establish links
Outbreak detection	Laboratory, public health authorities	The presence of two or more genetically related isolates will trigger an assessment of existing epidemiological information, and a decision about whether further case information is required (e.g. interviewing cases for a food and exposure history)	• Assign focal points in the laboratory and in the public health authority (epidemiologist) • Focal points will work together regularly to incorporate sequencing into the surveillance system
Outbreak investigation	Laboratory, public health authorities, food safety personnel	• Sequencing evidence is considered along with epidemiological and traceback evidence to suggest a contamination source • Sequencing information becomes part of the case definition's exclusion/inclusion criteria • Presence of genetically related food or animal source can guide investigation, sampling and hypothesis generation • Food safety authorities take necessary action (e.g. food withdrawal, food recall, improvement notices issued on a food premise)	• Staff from different sectors have defined roles within the outbreak response team
Evaluating interventions	Laboratory, public health authorities, food safety and animal health personnel	• Once an intervention is put in place, either during an outbreak or as part of controlling an endemic strain, sequencing is important to understand if contamination of the source has been controlled and if the intervention(s) were successful	• In outbreak situations, staff from different sectors have defined roles within the outbreak response team • If the strain persists, the information is communicated to food safety and animal health staff for further action, as public health authorities continue to monitor
Integrated food chain surveillance	Laboratory, public health authorities, food safety and animal health personnel	• Sequencing data are contributed from key points across the food chain to understand the risk, transmission pathways and evolutionary changes • Results are considered in a risk analysis context	• Establish a working group with representation from all sectors • Reference to World Health Organization (6) for detailed guidance

Source: reproduced from World Health Organization *(6)*.

ACTION

- Designate one or more persons from the laboratory who will work regularly with public health authorities to assess clusters and participate in the outbreak response team in outbreak investigations.
- Laboratory and public health authority jointly develop criteria for detecting clusters.
- Document the methods for cluster detection in standard operating procedures.
- Ensure multi-sectoral collaboration, including all relevant stakeholders, when using WGS for outbreak investigation.
- Work with key stakeholders should begin early in the process. They should start at the point of drafting the surveillance system's description, to define roles and responsibilities and the type of information to be shared, in order for the system to be effective.

4.5 Are there sufficient human resources in the system?

The availability of human resources is key to WGS for surveillance and response. Successful implementation of WGS in foodborne disease surveillance and response requires an understanding of molecular epidemiology, WGS-specific microbiology and molecular laboratory methods, in addition to an understanding of bioinformatics.

Some countries may be using subtyping methods, such as PFGE and antigen testing. The transition from traditional microbiological methods to WGS involves a drastic change in methodology and processing, and the current workforce will need to be retrained in the required skills. Knowledge and experience gaps will need to be identified, and followed up with appropriate training. For countries that do not have staff working with traditional typing methods, it may be necessary to recruit new staff, or outsource certain aspects of the workflow *(3)*. As a minimum, sequencing requires the following staff (please refer also to Web Annex J).

Molecular microbiologist
This professional should be able to culture pathogens, prepare isolates for sequencing and run sequencing equipment. The molecular microbiologist and/or the bioinformatician will need to work closely with the epidemiologist to continuously review sequencing outputs.

Bioinformatician
This person will be responsible for bioinformatics data analysis, some results interpretation and providing the results to public health authorities. The bioinformatician will need to work closely with the epidemiologist to continuously review sequencing outputs.

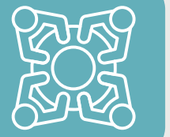
Epidemiologist
This health professional will need to work closely with the molecular microbiologist and/or bioinformatician on a regular basis to interpret WGS outputs, and to incorporate WGS outputs into the outbreak investigation process. Epidemiologists will need to work closely with food safety and/or animal health colleagues to interpret results and make decisions concerning public health.

The above three roles are described in more detail in Web Annex J and additional information may be found in the World Health Organization (WHO) landscape paper on the subject *(3)*.

Once a decision has been made regarding how to incorporate WGS into routine surveillance (estimated specimen throughput, where the sequencing will be conducted, etc.), an adequate number of trained personnel will need to be available to assume the required role in the surveillance system.

Training epidemiologists will be especially important. Using WGS for the routine surveillance of foodborne pathogens will require a cultural shift for many epidemiologists, as they are likely not to have training in molecular epidemiology. They may not require as detailed training as laboratory staff, but they must be able to understand WGS principles, capacities and limitations, in addition to WGS outputs and results interpretation. The latter is vital to ensure sequencing information is turned into public health action.

There are several options for training staff to build their capacity in WGS methods and interpretation of sequencing results.



Training programs
There are training courses for wet lab and dry lab processes. There are many online courses that are available, and it may also be possible to leverage regional, national or international networks to access training and education materials.

Partnering with collaborators in other countries
It may be possible to establish collaborations with countries that already have WGS experience. Case study 3 on the collaboration between United Republic of Tanzania and Denmark describes training conducted as part of a broader collaboration on WGS. Collaboration should be established prior to finalizing the description of how WGS will be incorporated into the surveillance system, in order to guarantee that staff will receive appropriate training as part of the implementation. This case study is an example of multilateral collaboration for training in WGS. To establish these networks, conduct a literature review to see what has been done in other countries. This will also help to identify key collaborators in various countries, who can be contacted regarding WGS.

Internships
Internships or mentoring programmes are useful for pairing students with experienced mentors from institutions that use WGS. Case Study 4 is an internship example.

Self-directed learning using online resources
Resources are available online for staff who have some sequencing knowledge and skills and wish to expand that knowledge. This is especially useful for end users of sequencing results in the public health and food safety sectors.

Case study 3

Collaboration between United Republic of Tanzania and Denmark for WGS implementation

- In a project supported by the Danish International Development Agency of the Ministry of Foreign Affairs (DANIDA), the Technical University of Denmark (DTU) collaborated with the Kilimanjaro Christian Medical Centre (KCMC) in Moshi, United Republic of Tanzania, to test the feasibility of setting up WGS in a resource limited setting.
- Two doctoral students were enrolled at the KCMC medical college to set up sequencing and data analysis. The students were trained in the principles and practical aspects of DNA sequencing at DTU, and implemented this technology at the KCMC.
- Due to the absence of bioinformaticians in the country, DTU provided short training on bioinformatics to both students, including how to use and interact with web-based tools developed at DTU for data analysis, and enrolling in bioinformatics courses at DTU.
- To date, more than 350 bacterial genomes have been sequenced as part of this project.

Case study 4

Genomics and Epidemiological Surveillance of Bacterial Pathogens course and internship

The Genomics and Epidemiological Surveillance of Bacterial Pathogens course is funded and organized by the Wellcome Genome Campus Advanced Courses and Scientific Conferences. This is an annual training programme for microbiologists and public health scientists in WGS laboratory techniques, computational analysis and interpretation.

The course was first held in Costa Rica in 2013, originally designed through a collaboration between regional public health scientists from the Pan American Health Organization (PAHO), PulseNet Latin America and the Caribbean, and research scientists at the Wellcome Trust Sanger Institute. The course originated in and was inspired by the obvious synergy between academic research and efforts to implement WGS in public health.

The course has a detailed online application process; all related costs including travel are covered to enable participation from across the Region of the Americas.

In six years, in Latin America alone, more than 100 individuals have been trained. Courses last for six consecutive days (Sunday to Friday) and are meant for around 20 participants. To maximize hands-on time, there is one computer per participant. The modules are detailed in an illustrated manual that participants work through with instructor support. Also included are discussion sessions and team exercises that simulate real world public health events such as outbreaks.

Hands-on training is emphasized. The course focus has been slightly modified every year to accommodate for increased exposure to new technologies in the Region, technological developments and shifting regional healthcare priorities. The yearly appraisal of the content is seen as central to the success of the course.

A concerted effort to train the trainer has helped to ensure knowledge dissemination beyond the course. Course programmes and files are provided to participants in a portable computing environment at the end of the course. This allows everyone to continue to practice, recreate, review or teach themselves elements of the material. All programmes used on the course are open access and can be freely downloaded; there are also detailed online manuals and an active user support network.

To improve research capacity to understand regional public health issues, a year-long pilot programme begun with seed-funding and mentorship for course participants, aimed at designing a genomics project to sequence and analyse regional *Klebsiella pneumoniae* and *Salmonella enterica* isolates.

Through these courses it was possible to identify leaders in the field, and to support participants while conducting research at the instructors' base institutions. This allowed for more detailed training built around shared interests in infectious diseases and ongoing work. Setting up links among scientists in public health and academic research is, at this stage, a key strategy to ensure that cutting-edge techniques, approaches, ideas and experience can be accessed and transferred, as WGS develops, in a public health setting.

ACTION

- Define the staffing requirements for incorporating WGS in the surveillance system.
- Determine whether new staff needs to be recruited.
- Identify training options for upskilling existing staff, including laboratory and public health staff.

Measuring system performance

Once the decisions have been made about how to structure the surveillance system using WGS, it will need to run for 1 to 2 years. Following this, a formal evaluation of the role of WGS in the surveillance system should be conducted. The evaluation should examine the following.

Usefulness
How useful was WGS for early outbreak detection, for supporting outbreak investigations, and to inform public health interventions?

Timeliness
How long did it take for public health authorities to get results from the laboratory? Could this time be reduced?

Flexibility
How well did the existing system adapt to the sequencing outputs?

Cost
Was sequencing done within the allocated budget? Were there unexpected costs?

Given that this is a new technology, the evaluation should also include qualitative data. Interviews with the following key individuals involved in WGS will be essential to determine acceptability:

- wet lab professionals who perform the sequencing;
- staff running bioinformatics analyses and the interpretation of results;
- epidemiologists who combine WGS outputs with epidemiological data and decide on public health action; and
- other members of the food safety system, such as regulatory officials or environmental health specialists who use WGS results to decide which public health interventions to conduct.

The updated guidelines for evaluating public health surveillance systems of the United States Centers of Disease Control and Prevention (US CDC) *(7)* are a good source on evaluation of surveillance and outbreak response systems. Further along this module, there is a discussion on how to pilot outbreak response systems and how to monitor and evaluate system performance.

ACTION

- Define when to evaluate the role of WGS in surveillance and outbreak response system.
- Decide on the surveillance system's attributes that will be most important to evaluate.

Transition from traditional typing methods

This section is only applicable to countries with laboratories that are already doing further typing of foodborne pathogens.

Performing WGS in parallel with traditional tests for long periods of time is very expensive and should be avoided. New methods must be sufficiently validated in order to support public health and/or regulatory actions and all stakeholders must have the capacity to understand and apply the new results. There may be resistance to discontinuing the old method, due to the comfort level it provides, as well as the fear around new genomics and bioinformatics technology. Consider the following in managing this transition period.

1 Address parallel testing costs from the outset in the description of the surveillance system, and include the expense in the cost estimates in the business case. Define a specific end date for legacy methods. This will help demonstrate that high costs are temporary and that ongoing costs are more reasonable and sustainable.

2 Communicate the projected date for discontinuing legacy tests to all stakeholders early and remind them often. This gives partners advanced notice and allows them to prepare for the change. Ensuring that affected parties do not feel blindsided by WGS implementation is a key component of a successful transition that is supported by everyone involved.

3 Avoid unnecessary duplication of validation work. Spending extensive time and resources on validation will extend the parallel testing period. Leverage the vast amount of validation that has been completed for foodborne disease WGS. For example, use the PulseNet International network's standardized methods, which have undergone international validation.

4 The same transition period need not be repeated for each organism if implementing WGS in a staged manner, one organism at a time, the period of parallel testing required for the first organism will not be the same for subsequent rollouts. Implementing WGS for the first foodborne pathogen is likely to have the longest transition period. Most operational issues can be optimized during this first transition, which will make transitioning subsequent organisms faster and less expensive.

5 Verify that a validated method works for your program. A method that already has validation data will only need to be verified in your laboratory. In this case, select a well-characterized panel of samples representing the typical isolate diversity in your local area, perform the test and evaluate the findings, in order to ensure that the test is being performed and analysed correctly. The same principles as for designing a pilot study apply; consult published resources *(8)* for more details.

It will also be necessary to think about how the transition period can be used to evaluate the effectiveness of WGS in the surveillance and response system. For example, it will be possible to compare how successful traditional methods were at detecting clusters and the timeliness of cluster detection with the success and timeliness of WGS results. This information can be used later as a justification for discontinuing traditional typing, if WGS proves to be successful.

ACTION

- Define the transition period when traditional typing methods will run in parallel with WGS.
- Provide a statement about how the transition will be managed.
- Define how traditional typing results will be compared with WGS results in order to help evaluate the success of WGS.

Summary

The following are key decisions that will need to be made when using WGS in support of outbreak investigations of food pathogen(s).

- **What are the goals and objectives of using WGS in the surveillance system?**
- **What pathogen(s) will be sequenced?**
- **What is the geographic coverage of the surveillance system?**
- **Who will perform the wet lab component of WGS?**
- **How will the wet lab methods be performed?**
- **Who will perform the bioinformatics analyses?**
- **What tools will be used in the bioinformatics analyses?**
- **How will WGS outputs be reported to the surveillance system?**
- **How will the epidemiologists link WGS data to surveillance data?**
- **How will WGS outputs be used to guide public health action (considering other sectors may need to be involved, such as food safety)?**
- **What are the human resource requirements?**
- **How will existing staff be reskilled?**
- **When and how will the role of WGS in the surveillance system be evaluated?**
- **If applicable, how will the transition from traditional typing to WGS methods happen?**

Table 9 summarizes the key features of the different approaches to using WGS for surveillance purposes.

Table 9

Features of various approaches to using WGS to enhance routine surveillance of foodborne pathogen(s)

Type of pathogen under surveillance	Geographic coverage	
	Sub national coverage	**National coverage**
Uncommon foodborne pathogen	Too few isolates for this approach to be viable or useful for routine surveillance.	• Low throughput of specimens for WGS • Wet lab WGS could be outsourced to one laboratory with sequencing capacity, or developed in public health laboratory • Needs access to bioinformatics expertise, but not necessary to hire a bioinformatician • Requires a national database to collate and analyse data nationally • Can detect outbreaks and monitor trends on a national scale • May require national data sharing agreements • Relatively low amount of resources required
Common foodborne pathogen	• Low-to-medium throughput of specimens for WGS • Wet lab WGS could be outsourced to one laboratory with sequencing capacity or developed in public health laboratory • Needs access to bioinformatics expertise, but not necessary to hire a bioinformatician • Processes for managing WGS data are assigned to laboratory and local public health staff • Can detect outbreaks and monitor trends at sub national level • Moderate amount of resources required. This model is likely to be implemented in sub national areas that can afford to invest in WGS and staff training	• Wet lab WGS capacity should be built in the public health lab and across multiple sites if there are sub national laboratories • If only one lab in country performs WGS, throughput will be high • If multiple sub national sites in country perform WGS, throughput will likely be low-to-medium at each site • Needs access to bioinformatics expertise, but not necessary to hire a bioinformatician • Requires national agreement on WGS methods and analyses to allow comparison across the country • Data sharing agreements between sub national and national sites possibly required • Requires a national database for the collation and analysis of WGS outputs • Can detect outbreaks and monitor trends on a national scale • Moderate to high amount of resources required to ensure national consistency, depending on how many sub national sites, will contribute to surveillance

Multiple foodborne pathogens	• Wet lab WGS capacity should be built in the public health laboratory
	• Consider hiring a bioinformatician
	• Processes for managing WGS data are assigned to laboratory and local public health staff for each pathogen
	• Can detect outbreaks and monitor trends at the sub national level
	• High amount of resources required
		• Wet lab WGS capacity should be built in the public health laboratory and across multiple sites if there are sub national laboratories
		• If only one lab in the county performs WGS:
		  ◦ throughput will be very high
		  ◦ a bioinformatician will be required
		• If multiple sub national sites in the country perform WGS:
		  ◦ throughput is likely to be medium-to-high at each site; and
		  ◦ ensure at least one site has a bioinformatician who might be a resource for other sites
		• Requires national agreement on WGS methods and the analyses to be performed to allow comparisons across the country
		• Data sharing agreements between sub national and national sites possibly required
		• Requires a national database for the collation and analysis of WGS outputs
		• Can detect outbreaks and monitor trends of multiple pathogens on a national scale.
		• Large amount of resources required, with investments at sub national and the national level likely

5. Business case

To implement WGS for outbreak investigations, a country will need to develop a business case. This is a document that describes why a change in practice is required, what the proposed change is, the resources required for implementing the new practice and any risks that may be associated with said change.

Writing a business case that lays out the vision and some basic planning is the first step toward accessing additional funding, re-allocating existing resources to support WGS implementation and establishing partnerships with collaborators and stakeholders. The drafting process itself helps to refine and clarify the vision, to elucidate the potential paths to achieving it and to identify specific needs. Creating this document also ensures a clear and concise description of what is intended from the early stages, which will become useful, for instance, when briefing senior officials, building stakeholder buy-in and applying for funding.

The business case should be written in non-technical language, suitable to a general audience. It will be the overview of how the system will work, based on the details contained in the description of the outbreak response system. The business case must be easily understood by non-technical personnel, such as policy-makers in governmental and/or donor institutions, who will be deciding whether to fund the proposal.

The business case should include sections on the following:

- a rationale for sequencing foodborne pathogens for outbreak investigation purposes;
- the current status of WGS in your local jurisdiction, the region and/or internationally;
- current subtyping methods (if they exist) other than WGS, including an emphasis on why WGS will improve outbreak investigations;
- the approach to WGS in the surveillance and response system;
- stakeholder details;
- specific requirements for WGS in the surveillance and response system;
- results of any local pilot studies (if applicable);
- transition to phase-out tests (if applicable);
- budget estimate for WGS in the surveillance and response system;
- timelines for implementation including key milestones;
- communication plan;
- potential risks;
- sustainability plan of the system; and
- evaluation of the role of sequencing within the surveillance and response system at the end of a trial period.

The business case will help to ensure efficient implementation and to engage decision-makers and other stakeholders. Web Annex K provides a template that may be used to collate all the relevant information required to develop a business case. Some sections of the business case template are discussed below in greater detail. The content for other sections can be summaries of the description of how WGS will become part of the surveillance system.

5.1 Rationale for WGS

The rationale for WGS should include a brief background on the benefits of WGS for foodborne outbreak investigations. This section can discuss the technical benefits of sequencing, such as greater discriminatory power for case definitions. If possible, local, national or regional examples should be used. If this is not possible, a literature search may provide examples of the benefits of WGS, such as those listed in the Bibliography.

ACTION

- Find examples of the benefits of WGS in foodborne disease surveillance and response. Ideally, they should be local or regional examples, but if it is not possible, search scientific literature for examples.

5.2 Estimating the cost of implementation

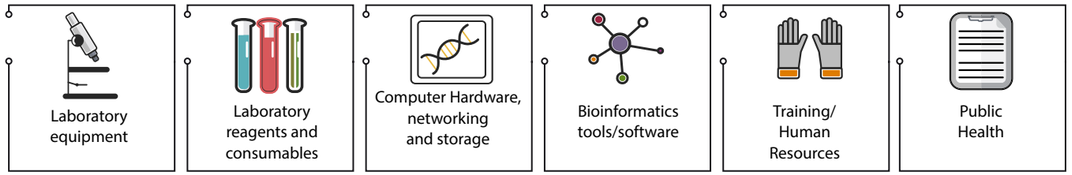
The costs associated with implementing WGS are high, due to the cost of equipment and reagents; training of laboratory staff to perform sequencing and analyse the results and of epidemiologists to interpret the results; IT infrastructure; and bioinformatics. These costs are significant even for laboratories that routinely perform traditional and molecular testing of foodborne pathogens.

Once the decision has been made on how WGS will be incorporated into the surveillance system, it will be important to define gaps and estimate the associated costs of sequencing across the surveillance and response system. If establishing the sequencing laboratory, it will be essential to separate the costs of purchasing the necessary equipment from maintenance, consumables and personnel cost See a template for estimating the costs in Web Annex L. The areas to be considered in the cost estimates are shown below and in Fig. 3.

- Laboratory equipment: sequencing platform and ancillary equipment (centrifuges, incubators, fluorometer, thermocyclers, spectrophotometer, etc). This includes personnel for equipment operation and maintenance.
- Laboratory reagents and consumables: reliable access to reagents and kits, ability to routinely procure glassware and plasticware (pipette tips, tubes, 96 well plates, film, etc.).
- Computer hardware, networking and storage: computers for sequencing data analyses, reliable internet connection, data storage facility, maintenance and support contracts, etc.
- Bioinformatics tools/software: these are the analysis tools and pipelines to generate WGS outputs.
- Training: once training requirements have been estimated, it will be necessary to determine how training will occur.
- Human resources: additional staff or different skillsets may be required.
- The cost estimate of each item will require research and obtaining quotes from local, regional or international suppliers. Knowledge of any existing operational or laboratory costs based on the current national situation as well as from research, should be used when describing technical requirements related to the outbreak response system.

Fig. 3

Areas to consider in estimating costs for WGS



Initial costs

Laboratory equipment	Laboratory reagents and consumables	Computer Hardware, networking and storage	Bioinformatics tools/software	Training/ Human Resources	Public Health
• Sequencing platform equipment • Ancillary equipment • Personnel	• Proprietary reagent procurement or generic reagent procurement production • Consumables	• Desktop hardware • High performance computing/ cloud access • Data storage facility and/or cloud access • Internet connections- speed and bandwidth • Personnel	• License fees (commercial) • Personnel	• Training for microbiologists, epidemiologists, managers, policy-makers, stakeholders, courses, workshops, visiting scientists, online training	• Adapting the existing surveillance database to incorporate WGS outputs • Personnel

Ongoing costs

Laboratory equipment	Laboratory reagents and consumables	Computer Hardware, networking and storage	Bioinformatics tools/software	Training/ Human Resources	Public Health
• Service contracts • Maintenance & repair • Expected lifespan before replacement • Personnel	• Need to know: volume of isolates expected to be sequenced • Proprietary reagent procurement or generic reagent procurement production • Consumables	• Expected lifespan before replacement • High performance computing and storage and/or cloud access • Internet connections, speed and bandwidth • Personnel • Maintenance/ service contracts	• License fees (commercial) • Open source: maintenance and upgrades, troubleshooting • Personnel	• Staff turnover • Expansion of WGS utilization requires more personnel with suitable knowledge in all related sectors	• May require additional staff • Personnel

ACTION

- Estimate the costs of WGS based on how WGS will be incorporated into the surveillance system.

5.3 Managing the cost of transition

The transition period between old and new tests is most expensive during the implementation of any new technology. Based on the description of the surveillance system, describe how the transition period will be managed and set a date when traditional typing methods will be discontinued.

Clearly state the rationale for having a transition period and provide an estimate of the cost of traditional typing methods during that time. Indicate that this is a temporary situation required prior to the introduction of WGS.

5.4 Cost–benefit analysis

A clear demonstration of the specific and local benefits of implementing WGS for foodborne disease surveillance is critical for:

A building buy-in from management and stakeholders;

B increasing the chances of securing new funding; and

C convincing decision-makers to re-allocate existing funds from other priorities to WGS.

There may be insufficient data to conduct a local cost–benefit analysis. Evidence of the benefits of implementing WGS originating, exclusively, in other countries makes it difficult to accept that those benefits could be locally realized as well. It is important to review the technical literature and determine what the experience in other countries has been, and determine the potential benefits in current local conditions.

To conduct a cost–benefit analysis, examine global and local evidence. Consider all elements of cost, benefits and value-added indicators. Assess the net value (benefits minus costs) and describe the results.

Examine global and local evidence

Global. Use any and all available evidence to wholly describe the cost–benefit of WGS for public health, foodborne disease and food safety (see case studies for examples). Repeated demonstrations of WGS implementation success in other jurisdictions/sectors will provide substantial rationale for your program. Using a wide variety of sources (e.g. from different countries, sectors, diseases) also demonstrates that the benefits of WGS are not isolated occurrences, which adds confidence to the expectation of successful outcomes in your situation.

Local. Highlight any benefits derived from WGS use in countries, sectors, jurisdictions that are most like yours. Conduct your own analysis using cost and benefit elements that are specific to your program. It is critical to be able to demonstrate that your program is likely to realize benefits that outweigh costs using locally relevant data. It also allows you to place your cost–benefit analysis in the context of all public health priorities in your local area.

Consider all elements of cost, benefits and indicators of added value. While qualitative evidence is valuable, avoid using it exclusively. Quantitative data should be included, as it may be perceived as stronger evidence; easier to understand and communicate; and useful for solving other problems as well (e.g. obtain support from policy-makers).

References related to economic benefits and added value indicators are found in the Bibliography and examples of cost and benefit elements are listed in Table 10. An example of developing laboratory cost estimates to demonstrate the cost–benefit of WGS is described in Case study 5.

Case study 5

Demonstrating cost–benefit: calculating laboratory operational costs

For countries already conducting laboratory-based surveillance for foodborne diseases, WGS is a replacement test, i.e. the introduction of WGS will allow for multiple laboratory tests to be discontinued. Calculating whole operational costs of tests to be replaced and comparing those to the cost of WGS, is critical to the cost–benefit analyses and to demonstrate long-term sustainability. For countries without extensive laboratory-based surveillance, WGS may provide a sustainable solution for strengthening surveillance. Prior to WGS, a vast array of equipment, reagents and expertise were required for all the tests needed to identify and characterize each organism, whose maintenance is very difficult for a programme with limited resources. WGS, on the other hand, is a universal test that handles all organisms (i.e. not limited on species, or the same type of organism) and a single lab assay provides data to address multiple public health needs.

EXAMPLE

The European Centre for Disease Prevention and Control (ECDC) developed laboratory cost estimates as part of their Expert Opinion on the introduction of next-generation typing methods for food- and waterborne diseases in the European Union (EU) and European Economic Area (EEA) *(9)*. In preparing those estimates, it was found that WGS was a cost-effective replacement for *Escherichia coli* and *Campylobacter* tests, and was approaching cost-effectiveness for *Listeria monocytogenes* and *Salmonella* (depending on volume/throughput). The cost estimate model takes into account: local reagent pricing; median and range of expenses for all traditional tests and WGS from up to 24 different countries; the proportion of organisms that get characterized by each traditional test; sample throughput; operator hands-on time; and total turnaround time. The model is applicable to cost estimates in any location. However, to have an accurate estimate of human resources costs, the total operator time should be multiplied by local laboratory worker wages.

Table 10

Examples of cost and benefit elements

Cost element (units)	Benefit element (units)[1]
Laboratory expenses: initial (unit of money)	Impact on disability-adjusted life years (DALY), i.e. change in DALY
Laboratory expenses: ongoing (unit of money)	Impact on quality-adjusted life years (QALY), i.e. change in QALY
Epidemiology expenses: initial and ongoing (unit of money)	Impact on the proportion of successful outbreak investigations
Training (time, money): include initial and ongoing training needs	Impact on incidence of disease (number of cases per population)
Human resources (number of full-time equivalents, money)	Impact on size of outbreaks before intervention (number of cases)
Dependence on external partners (qualitative and financial or in-kind support)	Impact on amount of food waste due to incorrect food recalls (weight in kg or t)
Uncertainty during transition change and resistance to change (qualitative)	Impact on laboratory operational costs (money): see case study[2]
Disruption of established procedures (qualitative)	Knowledge of transmission, attribution of burden of illness(qualitative)

1 Benefits can be predicted or measured/estimated.

2 For countries without existing laboratory-based surveillance, compare the cost of implementing WGS with those of implementing traditional methods.

Assess the net value (benefits minus costs) and describe the results

It will be necessary to compare the costs and benefits of using WGS in the surveillance system. Provide a rationale for a qualitative assessment of costs and benefits, and for cases when measurement units are not directly comparable. Conclude the cost–benefit analysis with a statement describing the results.

ACTION

- Conduct a cost–benefit analysis to be included in the business case.

5.5 Sustainability

A common and ongoing challenge is to design a long-term WGS programme. Smaller, time-limited research projects and pilot studies may be easier to be funded than a permanent programme. However, in order to truly reap the benefits of WGS for strengthening surveillance, laboratory and surveillance, data should be collected and acted upon consistently over time.

There is no single path to successful long-term funding, but there are some elements that will likely contribute to sustainable funding for a WGS programme such as: considering cost-saving alternatives, estimating the cost-effectiveness of WGS replacing multiple laboratory tests, and accounting for the long-term benefits of increased knowledge overall and broader mitigation to the burden of foodborne diseases. The following approaches may help with sustainable funding.

- Conduct a careful evaluation (and periodic re-evaluation) of your programme needs and associated costs with particular attention to any cost-savings alternatives. For example, accessing centralized computing and data storage resources may provide significant cost savings in both the short- and long-term, when compared with maintaining your own high-performance computing facility.
- Long-term costs may be perceived as higher than they actually are, especially for laboratories where WGS is a replacement technology. WGS may largely replace many tests, including speciation, serotyping, AMR typing, virulence factor identification and characterization and molecular subtyping. Thus, for laboratories currently offering a similar suite of tests, WGS implementation may be cost-neutral or even result in a decrease in financial needs. Ensuring that sectors/centres responsible for foodborne disease surveillance and those responsible for monitoring AMR are collaborating is one important way to obtain the most efficient use of ongoing resources.
- Make sure that your successes are continually documented and championed. Keep an active tally of achievements including successful outbreak investigations, surveillance stories, collaboration on international events and cost savings, and have manuscripts, abstracts, presentations, briefings, memos and other such products up-to-date and championed frequently to (and by) your decision-makers.
- One benefit of WGS technology is that once the isolate is sequenced, data can be stored electronically and become a rich source for scientific study. Thus, while WGS is highly effective at delivering accurate data for surveillance and outbreak investigations, the data may also be used to answer other important questions. Enquiries comprising transmission pathways throughout the food safety continuum, microbial population biology and evolution, virulence and pathogenesis are likely to determine future treatments and interventions, and may contribute to significant decreases in the burden of illness overall. This is a longer-term benefit whose tangible outcomes are difficult to see in the short-term, but it is important enough that it should be part of the ongoing rationale supporting WGS use.

5.6 Risks

It is important to state the risks associated with WGS implementation and those of not establishing WGS for foodborne disease surveillance, so policy makers can understand the ramifications of both alternatives.

Implementation-related risks

- WGS implementation can be costly and require long-term financial support.
- Too many changes too fast may compromise the stability of the surveillance system.
- There is no gold standard for WGS analysis for most pathogens. There is a risk in choosing a method that may need to be replaced in the future. Also, at the present time, it may not be comparable with neighbouring jurisdictions.

Risks of not implementing

- A falling behind perception by other countries regarding the ability to detect and respond to outbreaks early.
- Potential reduction in trade opportunities.
- Inability to link public health or outbreak data across borders.

6. Communication

With the introduction of a new technology, the process of successfully managing change involves communication. In particular, it will be important to communicate with key stakeholders, but more so it will be necessary to communicate regularly with senior decision-makers.

6.1 Decision-makers

When communicating with decision-makers it is important to use non-technical language, but still be able to convey the concepts behind using WGS for foodborne disease surveillance and response purposes. The following aspects should be covered:

- the burden of foodborne disease, both in terms of human illness and the costs to the food system;
- a description of WGS;
- how WGS will benefit foodborne disease surveillance above and beyond what is the norm in the country;
- the benefits of using WGS for foodborne surveillance to other programmes, for example AMR and emerging and re-emerging diseases programmes; and
- local examples of the use of WGS and its benefits.

Case study 6 is an example of communication with decision-makers and Web Annex M contains some general talking points for communicating key aspects of WGS to senior management.

Developing a business case ensures you have a clear and concise description of your intent from the early stages. This will be useful for briefing senior officials, building stakeholder buy-in and applying for funding. In addition, this will also be for regular feedback by way of verbal briefings, memos and other means.

Case study 6

How the US FDA engaged decision-makers in the WGS process

The process for engaging decision-makers includes the following actions.

1. Selecting a case study to retrospectively sequence, in order to demonstrate what might be achieved through WGS. It might be an outbreak that was solved through epidemiology and food safety investigation, but still had a few un-answered questions.
2. Conducting the WGS study.
3. Presenting the results and discussing the value of WGS in the case study in question and its potential broader applications.

There was a large outbreak of *Salmonella* Montevideo in 2009-2010 in the USA. The epidemiological investigation and food tracing identified a food manufacturer as the source and control measures were applied. However, traditional typing methods were not able to distinguish clinical specimens from multiple potential food items at the time of the outbreak. The United States Food and Drug Administration (US FDA) decided to use WGS retrospectively, in order to determine if WGS would have provided additional information. In 2010, 35 isolates where sequenced from:

- ingredient suppliers
- patients who ingested the suspected food products
- temporally and geographically diverse food sources.

By means of WGS, it was determined that clinical specimens were genetically related to a drain swab from a manufacturing facility of Italian-style meats and pepper used in meat production.

These results were presented to all stakeholders, with presentations to different areas of the US FDA every month for a whole year and the case study was written up for publication *(10)*. Decision-makers at the US FDA requested additional evidence regarding the degree of variation, and a second study was conducted to evaluate variability *(11)* by determining the level of genetic diversity when different isolates were sequenced; changes related to thaw and refreezing; and changes related to sequencing by different technicians. This work documented the reproducibility of the methods. Results were presented to various groups of stakeholders throughout the FDA, with varying degrees of WGS knowledge, and were discussed for nearly a year prior to publication. After several case studies highlighting different aspects of the power of WGS with different foodborne pathogens *(12)*, different sections of the US FDA found additional applications for WGS.

ACTION

- Ensure that there is a mechanism for updating senior decision-makers.

6.2 Translating the technology to decision-makers

It is important to engage decision-makers early in the development process. Where possible, presentations on WGS for foodborne disease surveillance should demonstrate the new technology and what it can achieve. Local examples should be developed and included in the rationale section of the business plan.

6.3 Highlighting the priority of foodborne diseases

There are many competing priorities for funding. When communicating with decision-makers, the importance of foodborne diseases and the potential for implementing public health interventions should be emphasized. There are many international documents highlighting the burden of foodborne disease, but local data are preferred if available. In the absence of local data, the WHO's reports *(13)* or data from countries with similar population dynamics may be used.

Developing collaborations with colleagues from food safety and animal health sectors can also help strengthen submissions and assist in highlighting the importance of integrated food chain surveillance for foodborne diseases (case study 7). Data from the whole food chain will provide a better understanding of foodborne disease epidemiology, inform risk assessment and risk management, including the identification of the most efficient and effective control measures. This will benefit public health as well as the economy, including food industry and trade.

Case study 7

Real-time sequencing of *Salmonella* spp. isolates, 2014

Public Health England began real-time sequencing of all presumptive *Salmonella* spp. isolates from human specimens starting in April 2014 *(14)*. In June 2014, a large multi-national outbreak of *Salmonella* Enteritidis was linked to egg consumption. Over 350 cases were reported in several European countries. A clear statistical correlation between the egg distribution network of the United Kingdom of Great Britain and Northern Ireland and individuals affected by the outbreak was revealed by WGS, which pointed to the eggs as the source of the outbreak of *Salmonella*.

WGS showed that five-point source outbreaks were distinct but linked. Clinical, food and environmental samples in several European countries showed that separate introductions of contamination had occurred from at least two premises owned by a single European egg producer with broad product distribution.

This case showed the power of WGS in revealing the epidemiology behind an outbreak, and allowed the definitive source of the outbreak — a single egg producer — to be identified and targeted for intervention, rather than just the point source locations where the contamination reached the population. Targeted interventions farther up the food production chain can be additionally effective in reducing further risks. This case also highlighted the importance of genome sequencing data from multiple countries, demonstrating how global sharing of WGS data could enhance the response to a foodborne outbreak, to further protect public health and identify a particular source of contamination.

7. Conducting a pilot study

A pilot study should be conducted to test how WGS will be incorporated into the outbreak response system, based on the system's description developed prior to full implementation. The system to be piloted should be exactly the same as the one described in the business case. Piloting the role of WGS in the outbreak response system will:

- demonstrate the feasibility of WGS implementation
- demonstrate the benefits of using WGS for foodborne disease surveillance
- produce accurate estimates of the cost of full-scale implementation
- establish collaborative partnerships
- build local capacity for WGS data generation, analysis and interpretation
- build local capacity for combining WGS outputs with epidemiological data to guide public health action
- identify barriers to widespread adoption of WGS
- generate preliminary results to seek additional funding or re-allocate existing funds.

There are several steps in developing a pilot study to test how WGS has been incorporated into the surveillance system (Fig. 4). Box 4 highlights some key differences between the description of the system and the pilot study plan. A template for developing a pilot study plan is provided in Web Annex N.

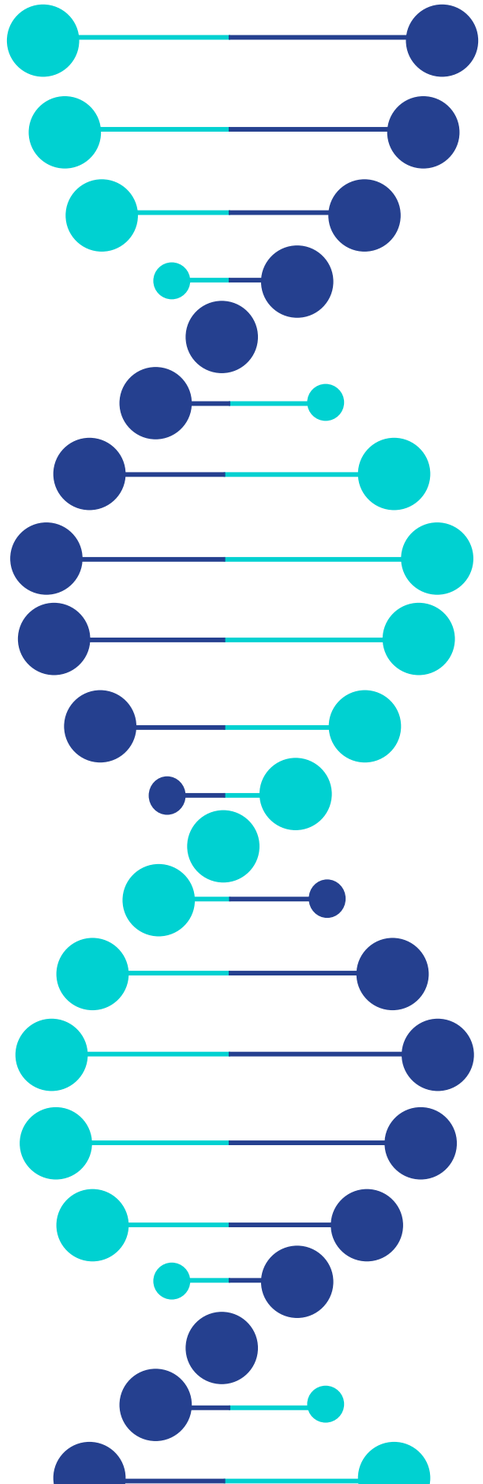


Fig. 4

Steps of a pilot study to test WGS for an outbreak system

BOX 4

Differences between a pilot study plan and a description of the surveillance system

The pilot study plan:

- should use elements from the description of the surveillance system that you wish to test before going to full-scale implementation;
- has objectives are about assessing the feasibility of implementing WGS within the surveillance and response system;
- is time-limited;
- may focus on a smaller geographic area or on a single pathogen; and
- has evaluation criteria specific to WGS outputs within the surveillance and response system.

The description of the surveillance system:

- defines how the entire system will function, from specimen collection to public health action;
- has objectives about the functioning of the entire surveillance and response system;
- is intended to reflect practice into the future with no time limitations; and
- evaluation is considered a continuous quality improvement process within the surveillance and response system.

7.1 Step 1. Set out governance arrangements

An advisory group should be established to guide the planning and execution of the pilot study and to facilitate communication and collaboration between stakeholder organizations. The group should be removed from the day to day running of the pilot and should include decision-makers, i.e. a level above those involved in the day to day pilot study activities. This is a good way to involve other agencies and sectors, such as food safety, in the study.

A pilot study team should be designated. The team of technical people who developed the description of how WGS will be incorporated into the surveillance system should be utilized during the pilot. The team should meet regularly in the early stages to ensure there are opportunities to reflect on the processes and the outputs.

ACTION

- Set up an advisory group or equivalent.
- Outline who will participate in the pilot team.

7.2 Step 2. Define pilot study objectives

In general, the objectives focus on assessing the feasibility of implementing WGS in the surveillance system based on the system's description. Objectives can be defined:

- to assess the criteria for determining relatedness of cases in outbreak investigations
- to determine best practices for incorporating WGS data into existing streams of evidence
- to elucidate the diversity of foodborne pathogens
- to evaluate the performance and suitability of computer analysis and data storage and transfer
- to set guidelines for interpreting WGS data.

It might also be useful to document specific questions the pilot study needs to address, such as the ones below, but there may be others.

Validity

1. Can sequencing be compared with that of other partners? For example, to compare pathogen outputs with those of other countries)?
2. What are the minimum quality standards for sequencing results, and how can they be monitored?

Public health outcomes

3. Does WGS correctly identify cases in an outbreak (if conducting a retrospective pilot study)?
4. Do clusters identified by WGS differ from those identified by current methods in terms of:
   - A. size (number of cases)
   - B. duration
   - C. agreement with epidemiological information
   - D. identification of source/transmission routes?
5. Is the discriminating power of WGS higher than that of other typing methods?
6. What will be the impact on investigation resources?
7. Does WGS produce data timely enough to inform a public health response in outbreak management?

Suitability and acceptability

8. How fast are WGS results available in comparison to current typing methods?
9. Are results compatible with current public health databases?
10. Can the WGS process accommodate changing demands?
11. Are public health staff able to use WGS to guide action?
12. Do public health staff have a good understanding of WGS and the information it can provide?

ACTION

- Define objectives of the pilot study.
- Define the pilot study questions.

7.3 Step 3. Design a pilot study

Scope

A pilot study will allow the testing of WGS and how it will be incorporated into the surveillance system on a smaller scale, and/or over a defined time period. For example, if implementing WGS throughout the country, the pilot could take place in a single geographical area, or if the plan is to implement WGS for all diseases, the pilot could be conducted on just one pathogen.

The pilot must be a trial of the integration of WGS into surveillance, and not just a laboratory-based project. Delaying the involvement of epidemiologists and other personnel who will ultimately be responsible for acting on laboratory results may weaken the impact of the pilot study in garnering support for more widespread WGS use. The expertise and input from laboratory and epidemiology colleagues, in addition to that of non-public health sectors, will ensure the strongest possible pilot study. All partners in the pilot study should participate from the very beginning.

Approach

The pilot study should test the plan to incorporate WGS into the surveillance system. This can be done prospectively or retrospectively. At first, a retrospective study may be important to demonstrate the use of WGS locally. A well-designed retrospective study can provide strong rationale for continuing staged implementation for surveillance. However, a prospective pilot study design will yield the greatest benefits, as it will involve real-time sequencing which can help to determine realistic turnaround times and will allow laboratory staff and epidemiologists to meet regularly and find out how to use the outputs from WGS. Table 11 lists the advantages and disadvantages of prospective and retrospective study designs.

Table 11

Advantages and disadvantages of a prospective and retrospective study design

Study type	Definition	Advantages	Disadvantages
Prospective	Samples are analysed as they become available over the duration of the study period; often referred to as in real-time.	• Conditions of the study represent the reality of implementing WGS • Accurate identification of barriers, bottlenecks and strengths that can be leveraged at a small scale to ensure the success of (and smooth transition to) more widespread adoption	• The number and type of cases may not be predictable • Ensuring a sufficient sample set (e.g. size, genetic content diversity, epidemiological follow-up) may be challenging • Stakeholders might not be able or willing to take public health action based on a test that may be viewed as experimental or not validated • If the laboratory or public health workload is high, it may be difficult to find time to make necessary improvements in the system
Retrospective	Samples from a past surveillance period are analysed.	• Well-defined and optimal sample sets can be selected from existing surveillance data for maximum efficiency and effectiveness • Stakeholders not yet able or willing to take public health actions based on WGS data have the opportunity to participate with lower actual or perceived risks • Samples can be sequenced in large batches, lowering the cost of data generation	• Retrospective pilot studies cannot replicate the conditions of real-time surveillance • Epidemiological follow up may not be possible to confirm the WGS findings • Elements, such as the impact on surveillance data life cycles and timeliness, potential problems with data processing, transfer and storage, and the pressures for rapid public health interventions, cannot easily be simulated or assessed • There is a risk of the pilot study being designed or perceived as a laboratory validation exercise

ACTION

- Define the scope of the pilot study, in line with the description of the outbreak response system.
- Select a prospective or retrospective design or a combination of both.
- Outline the methodology (based on the description of the outbreak response system).
- If determining or validating interpretation criteria is a study objective, specify how it will be done.

7.4 Step 4. Outline milestones and deliverables

Determine the milestones and deliverables during and at the end of the pilot study period. Milestones are critical points, such as the development of the pilot study plan, the sequencing start and end dates and the date the evaluation, will be completed.

Deliverables are any tangible outputs from the pilot study, such as the plan, sequencing results and the final evaluation report. It is also important to include here mechanisms for refining/optimizing activities during the pilot study.

ACTION

- List key milestones with dates and responsible person or group.
- List any deliverables, such as reports and dates of completion.

7.5 Step 5. Communicate

One of the main differences between a pilot study and a research project is the real-life aspect, i.e. pilot studies are conducted under the operational conditions (or replicate those conditions) and pace of surveillance and outbreak response. Whereas research projects can typically produce a single final report or manuscript at the end of the project, a prospective pilot study should include means to assess and communicate findings as they occur. Providing a single final report or manuscript to be reviewed by stakeholders and partners should be avoided, as maintaining a high degree of engagement throughout the pilot project is key to building capacity, knowledge and support for more widespread adoption of WGS. Table 12 outlines examples of elements of a communication plan.

Table 12

Elements of a pilot study communication plan

Content	To/from	Frequency (method)
Phylogenetic trees and spreadsheets containing WGS results and epidemiological data	Joint laboratory and epidemiological analysis and interpretation of results	Depends on the nature of outbreak. Daily, weekly, fortnightly (in person, by teleconference or video conference)
Summary of results and public health actions	From study leads to all stakeholders and partners	At conclusion of outbreak (by email and teleconference to allow for discussion)
Project plan and progress, issues encountered successes and challenges	Advisory group	Depending on the length of the pilot, this may just occur at the midpoint of the pilot. If the pilot is longer than one year, consider producing a report every six months
Report and/or manuscript	All partners and stakeholders	

ACTION

- Include communications in the pilot study plan.

7.6 Step 6. Develop evaluation criteria

Some barriers may be easily overcome, but others may be difficult to be removed and may even change the potential description of the surveillance system. It is important to document solutions or potential solutions during the pilot study.

The main criteria to assess the usefulness of WGS during the pilot study are listed below.

Timeliness
Recording dates as results and information are produced. It is possible to track the dates of specimen collection, pathogen identification and sequencing results in a spreadsheet.

Outbreak detection
Describe how outbreaks were detected and which outputs from WGS were the most useful.

Usability of WGS output
Could the output be integrated into surveillance system?

If existing typing methods are already in place, it would be ideal to run it in parallel with WGS. This would make it possible to evaluate and compare the outcomes of both methods. This may include determining:

- how early outbreaks were detected
- the method providing the greatest discrimination for detecting similarities between isolates
- the size of the clusters when they were first recognized
- the resulting public health action and if it was faster than traditional typing methods.

ACTION

- Define the evaluation criteria and how to measure them.
- Create a log to systematically document barriers/constraints during the pilot study.

7.7 Step 7. Finalize the pilot study plan

Document all decisions in the pilot study planning template in Web Annex N. Case study 8 is an example of approaches to pilot studies.

Case study 8

Canada's pilot studies for WGS implementation

The Public Health Agency of Canada launched multiple pilot studies prior to its staged implementation of WGS for foodborne disease surveillance and outbreak response *(15)*.

First, a retrospective pilot study was conducted, using well-defined historical outbreaks as the sample set. This provided preliminary guidelines for interpreting WGS results in all future work. Also, in the early stages, not all stakeholders in Canada were comfortable using WGS in real-time for public health and regulatory decision-making, that is, it was a non-validated method that many did not understand yet. Performing a retrospective pilot study as a first step was successful in providing early evidence of the benefits while building the support of stakeholders.

Following the retrospective pilot study, another pilot test was started using WGS to support outbreak response in parallel with molecular methods, PFGE and multiple locus variable-number tandem repeat analysis (MLVA). Both pilots were conducted in collaboration with provincial public health partners as well as Canada's national food regulatory agencies (Health Canada and the Canadian Food Inspection Agency). Following these successful pilot projects, the implementation of WGS for surveillance is being rolled out for one organism at a time.

8. Managing implementation

Implementing WGS in the surveillance and response system for foodborne diseases is likely to take some time. The first step, once approval has been granted and funds are provided, is to procure the necessary equipment and reagents, based on the needs defined in the system's description. It will also be necessary to recruit new staff and begin training existing staff if necessary.

Given the several steps in planning for WGS to support outbreak investigations, the tool provided in Web Annex O might be useful. This tool helps document every step of the process, from the initial concept through full-scale implementation. Each action described in the boxes throughout this module is included in the implementation tool, to assist countries in overall planning.

Using the template requires having read this module and convened a working group to see through each step in implementation. The working group can then follow the guidance document, plan accordingly and take the required actions, which can be systematically recorded in the template.

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