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

Module 2. Whole genome sequencing in foodborne disease outbreak investigations

World Health Organization

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Whole genome sequencing as a tool to strengthen foodborne disease surveillance and response. Module 2. Whole genome sequencing in foodborne disease outbreak investigations.

ISBN 978-92-4-002124-2 (electronic version)
ISBN 978-92-4-002125-9 (print version)

Suggested citation. Whole genome sequencing as a tool to strengthen foodborne disease surveillance and response. Module 2. Whole genome sequencing in foodborne disease outbreak investigations. 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.

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

Printed in Switzerland

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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*
US FDA	United States Food and Drug Administration
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) exclusively to support outbreak investigations, following detection of an outbreak of suspected foodborne origin by the routine disease surveillance system.
- Focuses on how to use WGS information together with epidemiological outbreak investigation data.
- Emphasizes the need for joint work between epidemiologists and food safety professionals.
- Is intended for countries with limited WGS experience that would like to begin building WGS capacity within the current surveillance and response system.
- Discusses decision-making aspects of capacity-building for sequencing, and the conditions necessary for WGS to be part of the existing outbreak investigation process.
- 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

- Cover outbreak detection, a subject discussed in the surveillance module of this manual *(1)*.
- Discuss how to conduct foodborne disease outbreak investigations. For information on the latter, refer to the who manual on strengthening surveillance of and response to foodborne diseases *(2)*.
- Provide details on how to conduct food safety investigations.
- Recommend what food and environment samples to collect during outbreaks.
- Discuss detailed technical requirements, but provides relevant references.

Before reading this module, make sure you have read the introductory module of this manual *(3)* and ensure:

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

The outbreak investigation process for foodborne diseases will not change significantly with the implementation of WGS. However, WGS will change the type of information available during an outbreak, and how that information is used to identify the source of the outbreak and implement control measures.

1.2 How to use this module

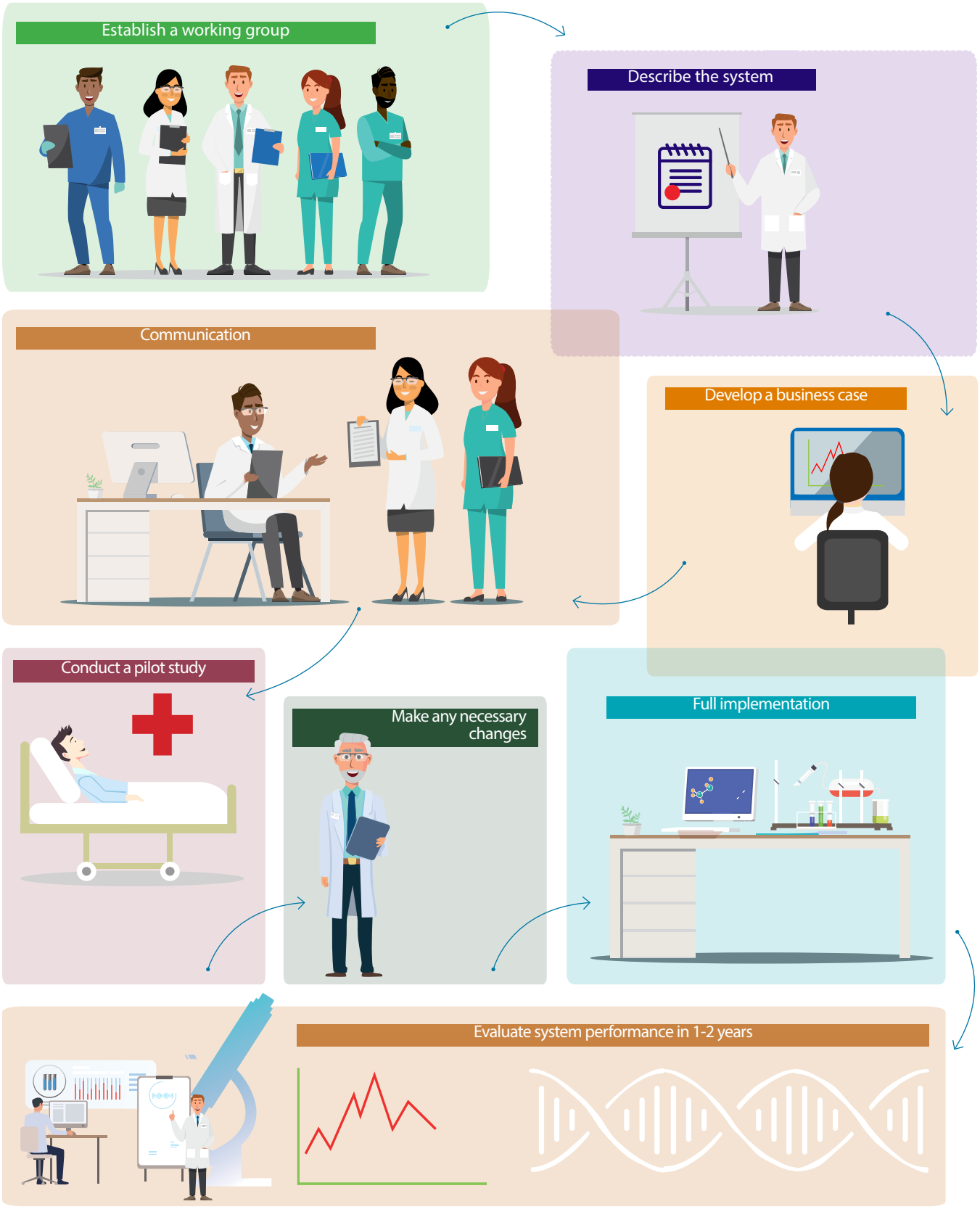
This module outlines the steps of WGS implementation. Steps can be taken in any order and even in parallel. However, it is important to articulate WGS outbreak investigation-related objectives in advance. The following steps are involved in foodborne pathogen WGS.

✓	Step 1. Describe how WGS will be incorporated into the outbreak response 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 outbreak investigation. While working through this module, key decisions will need to be made about how sequencing will fit within the existing outbreak investigation system, considering there are more than one right way to implement WGS for this purpose. This module provides tools and case studies to help articulate national needs and decision-making.

Fig. 1

Steps in implementing sequencing for outbreak investigations



2. Vision and objectives

2.1 Vision

The vision for implementing WGS to enhance foodborne disease outbreak investigations includes the following.

- Outbreaks of foodborne diseases are detected through existing surveillance systems, whether indicator-based or event-based, or both.
- An outbreak response team is set up and a case definition is developed.
- Epidemiologists, laboratory scientists and bioinformaticians discuss how WGS may support outbreak investigations, desirable timeframes and outputs for WGS results for public health use.
- Initial isolates of the relevant foodborne pathogen from human samples are sequenced. The laboratory refines the reference genome if required. An outbreak strain is identified and included in the case definition. All isolates collected as part of the outbreak investigation are characterized and classified as either an outbreak or non-outbreak strain.
- An identified microorganism that might be genetically closely related to the outbreak strain is jointly reviewed by the laboratory scientist, bioinformatician and epidemiologist, together with any epidemiological information available.

- Epidemiologists use the strain's characterization together with any epidemiological information to determine if the case meets the case definition.
- Epidemiologists and/or outbreak response team members interview cases to try to identify the suspect food item, which, depending on its nature, might need to be further investigated (traceback) for definite identification.
- Samples of the suspect food item, as well as relevant environmental samples are collected. Isolates obtained from food and/or environmental samples are sequenced and compared with those from individuals meeting the case definition of the outbreak under investigation.
- The food vehicle causing the illness is identified and control measures are implemented in the food safety sector.

2.2 Objectives of sequencing during an outbreak investigation

The objectives of sequencing foodborne pathogens during outbreaks depend on the national capacity to conduct outbreak investigations, and the routine subtyping methods already in place. One or more of the following objectives may apply.

- To confirm the presence of an outbreak by determining whether strains are genetically closely related, if no other subtyping already exists.
- To describe the magnitude of the outbreak, by including the suspect strain in the outbreak case definition.
- To improve specificity in epidemiology data analysis, by removing any non-outbreak cases.
- To determine if any cases detected prior to the outbreak's onset might be linked to it.
- To assist in identifying the potential source of the outbreak, by providing food history data from cases carrying the outbreak strain. This will help the food safety sector conduct trace-back activities and identify the microorganism from food or environmental samples.
- To increase specificity in analytical studies, by eliminating non-outbreak cases.
- To determine any national or international linkages to the outbreak.
- To describe any evolutionary changes in the pathogen.

As this approach will often be used where laboratory and sequencing capacities are limited, its secondary objectives are to:

- determine whether WGS is an effective tool for use during outbreak investigations
- determine whether WGS is an appropriate tool for routine surveillance in the country
- identify sequencing barriers
- assist laboratory and surveillance staff to start building sequencing capacity, with adequate technical support.

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, the "Whole genome sequencing for foodborne disease surveillance landscape paper" *(4)* and peer-reviewed scientific literature.

3.2 Establishing a working group

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

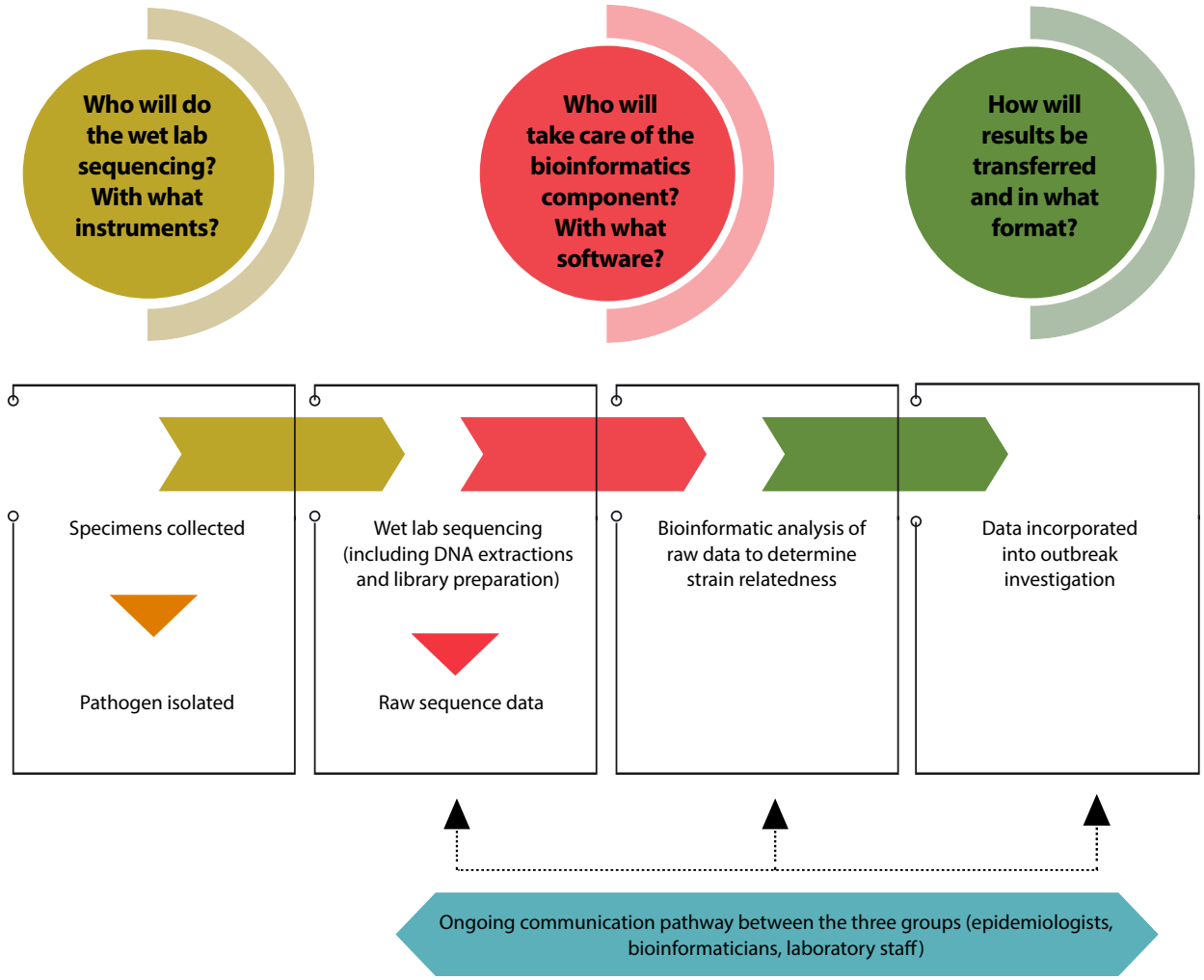
It is important to describe how WGS will be added to the current outbreak response system.
Such description should include:

1. goals and objectives of WGS as part of the outbreak response system
2. flow of specimens and data
3. where WGS data will come from
4. who will perform bioinformatics analyses and how
5. how WGS results will be applied to outbreak investigation
6. whether there are enough human resources in the system
7. 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. Web Annex B provides a template useful for this task and Fig. 2 illustrates an overview of the 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 within the current outbreak response system

Setting goals

Short- and long term goals can be set for WGS. A country may, in the long term, aspire to establish WGS for surveillance of foodborne and/or other pathogens. However, as a starting point, short-term goals may only address the use of WGS in outbreak investigations, in order to gather evidence on its usefulness for such purposes. Such evidence can later be used to lobby decision-makers for investments in WGS to support foodborne disease surveillance.

Objectives WGS for surveillance

Once short- and long term goals have been set, the overall objectives of WGS to support outbreak investigations will need to be defined. The objectives outlined in this document's introduction might be helpful, if adapted to a country-specific context. It is important to define and document the system's objectives, in order to evaluate their achievement and the system's performance.

ACTION

- Define short- and long term sequencing goals.
- Define the objectives of including WGS in the outbreak response system.

4.2 Where will WGS data come from?

Where will specimens come from?

- Collected outbreak specimens are sent to the local public health laboratory for standard testing (e.g. bacterial screening) as per normal outbreak protocols.
- All identified isolates are sequenced, and the sequence outputs are stored for immediate and future use.
- During an outbreak, consideration should be given to sequencing some historical samples of the same pathogen if available.

Regardless of how many samples are collected during the outbreak, all isolates should be sequenced and go through bioinformatics analysis. Raw sequence data can be stored for future use, for instance, to compare with data from other outbreaks or emerging epidemiological situations. This may assist in linking outbreaks in the future and determining the source of the outbreak. During the outbreak and regarding the isolate's WGS, the investigation team should determine whether:

- WGS has been previously used for outbreak investigation of this isolate, and if there was any epidemiologically relevant relatedness found between the previous and current strains;
- there is historical or current WGS data that can be used to provide context; and
- there are historical samples (human, food, animal and/or environmental) stored from previous outbreaks containing the same pathogen that could be considered for inclusion in the sequencing process.

In some outbreaks, the number of samples collected can increase dramatically in a short period of time, in which case, a decision will need to be made regarding the number of isolates to sequence during an outbreak. This may depend on the questions raised in the investigation and available resources. These will need to be discussed by epidemiologists and laboratories.

Examples of outbreaks using WGS during their investigation:

- the United States Food and Drug Administration (US FDA) investigated a multistate outbreak of listeriosis linked to Blue Bell creameries products *(5);*
- the US FDA investigation of a multistate outbreak of *Listeria* in Dole Leafy Greens Products produced in the Dole facility in Springfield, Ohio *(6);*
- whole genome and core genome multilocus sequence typing and single nucleotide polymorphism analyses of *Listeria monocytogenes* isolates associated with an outbreak linked to cheese, United States of America (USA) *(7);*
- whole genome sequencing improved case ascertainment in an outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O157 associated with raw drinking milk *(8);* and
- outbreak of *Salmonella* Bovismorbificans associated with the consumption of uncooked ham products, the Netherlands, 2016 to 2017 *(9)*.

ACTION

- Ensure that outbreak response protocols are in place and up to date and that they include appropriate specimen collection and transport.
- All specimens collected should be sent for processing and isolates should be sent for sequencing.

Where will isolates be sequenced?

Upon arriving in 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 its DNA extract will be sent for wet lab sequencing. Note: this is separate from the bioinformatics component which will be addressed in later sections.

The use of sequencing as part of this option is characterized by a limited and infrequent isolate throughput.

Which option to choose?

Key options

If wet lab sequencing capabilities do not already exist at the public health laboratory, the options are:

- **to outsource to another national laboratory or internationally**
- **to establish sequencing capacity in the public health laboratory.**

Table 1 shows the advantages and disadvantages of outsourcing wet lab sequencing processes, compared with sequencing in the country's public health laboratory.

Table 1

Advantages and disadvantages of two options for WGS during outbreak investigations

Options for sequencing	Task description	Advantages	Disadvantages
Outsourcing	Samples/specimens obtained during an outbreak are sent to a laboratory other than the national public health laboratory for sequencing	• Reduces implementation costs in the short-term • Does not require the purchase and maintenance of sequencing equipment nor data storage capabilities • Facilitates outbreak investigation in countries with no capacity or few resources	• 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 that correct paperwork is filed and that processes are followed for 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 other projects or outbreaks, aside from foodborne illnesses • Does not require international shipping of infectious material • Working with existing staff and institutions enables familiarity with the status of foodborne disease outbreak investigations, and allows for local solutions (not dependent on third parties who may not understand outbreak investigations) • Allows for open communication among all participants and partners in foodborne disease outbreak investigations	• High implementation costs • Takes longer to establish the service and validate the outcomes • Requires trained staff

Outsourcing wet lab component of WGS

Outsourcing will allow a country to gather evidence on the benefits and suitability of sequencing before investing in the required infrastructure. Outsourcing the wet lab component means that a third-party laboratory will be responsible for performing the wet lab step in WGS. Time is important during outbreaks and outsourcing the wet lab component will generate delays after microorganism isolation. It may be possible to negotiate and guarantee rapid turnaround of sequencing results, so that the information obtained may be useful in outbreak investigations in real-time or close to real-time.

Important steps to follow when outsourcing the wet lab component include:

- developing a document to state the service required in detail (Web Annex C)
- choosing a sequencing laboratory (Web Annex D)
- determining data ownership and data-sharing arrangements
- preparing service contracts.

Web Annex E provides the details on each step.

Building wet lab WGS capacity within the public health laboratory

Countries planning to progressively move from sequencing for outbreak investigations to sequencing for surveillance, may prefer to build national capacity within the public health system. If a steady number of isolates is expected to be sequenced, it might be preferable to set up WGS capacity in the public health laboratory. In spite of the expense of establishing a sequencing laboratory, the investment will prove to be beneficial in the future. The system will be sustainable and reliable. On the contrary, if WGS wet lab processes are outsourced, ongoing costs could become unsustainable in the long run and will not strengthen the local capacity.

The following steps will contribute to capacity-building in the public health laboratory in terms of the WGS wet lab component:

- designating a laboratory to perform WGS
- planning the anticipated workflow
- choosing an appropriate sequencing instrument
- making reagents, consumables and equipment available
- implementing quality assurance.

For details on each step, please refer to 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 related to outbreak investigations of foodborne diseases;
- 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 service provider?).

Factors influencing the decision of where to conduct sequencing will vary according to national circumstances. Box 1 provides general guidance in this regard.

BOX 1

Choosing an option for the wet lab component of sequencing

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

   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, outbreak response protocols and/or 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 antimicrobial resistance (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.

Bioinformatic analyses involves the following steps *(4)*.

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 single nucleotide polymorphism (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 aspects of 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.

Each of these have advantages and disadvantages as discussed in Table 2.

Table 2

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. 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); or
- all computing and bioinformatics analyses are conducted in the public health laboratory.

Table 3 describes the advantages and disadvantages of outsourcing bioinformatics for developing vis-a-vis developing national capacities. If planning a stepwise progression from sequencing for outbreak investigations to sequencing for surveillance, building the national capacity may be preferable.

Table 3

Advantages and disadvantages of different bioinformatic options for WGS during outbreak investigations

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

Key options

If the public health laboratory does not have bioinformatics capabilities, the options are either to establish bioinformatics capacity in the public health lab, or to outsource either within the country or internationally.

Which option to choose?

The dry lab is more complex than the wet lab component (although it is less time-consuming than the latter), 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 skilled human resources. Box 2 describes the steps required to choose an option for the dry lab component of sequencing.

BOX 2

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 in support of outbreak investigations 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 the 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 dry lab component of WGS or build the national public health laboratory capacity:
  - if outsourcing, read Web Annex G and undertake the actions described; and
  - if choosing to build the national public health laboratory capacity, 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 in outbreak investigation?

Incorporation into outbreak response process

Every outbreak is different, as different microorganisms might be involved, different food sources might be identified and different at-risk populations might be affected. In some outbreaks, the source may be somewhat easily and quickly identified through epidemiological and traceback information, but others may be more difficult. Web Annex I provides an example of how WGS would be used during an outbreak investigation.

During outbreak investigations, epidemiologists need to determine what cases could be related to a common source, versus those cases that are likely not related to that source. Therefore, sequencing outputs should:

- classify persons who have the outbreak strain and are linked to the suspect sample, so they can be recorded in the outbreak line list/database;
- detect suspected cases; and
- identify which suspected cases carry a strain very similar to the outbreak strain, so that epidemiological data may be reviewed to determine whether this is a case.

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).

WGS data by itself does not identify nor confirm the source of an outbreak, but it provides a stronger laboratory link and can assist in narrowing the focus of the investigation and thus, improve epidemiologic and traceback findings. However, the latter findings are still critical in determining an infection's vehicle, especially in polyclonal outbreak situations, where food and/ or food facilities may contain multiple strains/serotypes.

The *Salmonella* Agona example in Web Annex I highlights the difficulties of including or excluding a suspected case in an outbreak. There needs to be excellent communication and data-sharing among epidemiologists, laboratory staff and bioinformaticians. When dealing with food and environmental samples, the communication should also include food safety officers.

Outbreak response protocol

The outbreak response protocol should be updated to include a section on the appropriateness of sequencing in any given outbreak. Consider the following questions.

- Are we likely to collect specimens?
- Has a pathogen been isolated yet by the public health laboratory?
- Is the sequencing facility set up to process the identified pathogen, e.g. reference genomes?
- What additional information will sequencing provide that is not already available through existing practices?

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.

4.5 Key points

- **A variety of outputs can be generated.**
- **Sequencing output alone cannot confirm an outbreak or classify a case. Epidemiological data are also required.**
- **Epidemiologists, laboratory staff and bioinformaticians must continually work together during the outbreak investigation to refine what constitutes a closely related strain.**

ACTION

- Laboratory and public health staff jointly decide:
  - how to report the results from WGS in the laboratory to, or share them with, public health authorities;
  - the frequency of reporting WGS results to public health authorities, based on the aims of the surveillance system; and
  - how to interpret the results and report trends over time.
- Update relevant outbreak response protocols to reflect sequencing considerations during an outbreak response.

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 pulsed field gel electrophoresis (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 *(4)*. 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.

Once other technical aspects of the outbreak response system have been defined (e.g. estimated number of samples and isolates or to be analysed, laboratory in charge of sequencing), an adequate number of trained personnel will need to be available.

Training epidemiologists will be especially important. Using WGS for outbreak investigation will require a cultural shift for many epidemiologists, as they are likely not to have training in molecular epidemiology. They may not require detailed training in laboratory science, but they must be able to understand WGS principles, capacities and limitations, in addition to WGS outputs and results interpretation. The latter is crucial to ensure that data are 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 programmes
There are training courses for wet lab and dry lab processes, both onsite and online.

Partnering with collaborators from other countries

It may be possible to establish collaborations with countries that already have WGS experience. Case study 1 on a collaboration between United Republic of Tanzania and Denmark describes training conducted as part of a broader collaboration on WGS. However, the collaboration should be established prior to finalizing the description of WGS and its incorporation into the surveillance system, to guarantee that staff will receive appropriate training as part of the implementation. Case study 2 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, what challenges they confronted during WGS implementation, and what are potential solutions.

Internships

Internships or mentoring programmes are useful for pairing students with experienced mentors from institutions that use WGS. The United Republic of Tanzania/Denmark case study (Case study 1) is an example of such internships.

Self-directed learning with 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.

ACTION

- Define the staffing requirements for the surveillance and response system.
- Determine whether new staff needs to be recruited.
- Identify training options for current laboratory staff.

Case study 1

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 had to take a lead in providing 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 2

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 disease, 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.

Measuring system performance

At the end of an outbreak, sequencing usefulness should be assessed relative to outbreak objectives and by considering the following questions.

1. What worked well?
2. What could be improved?
3. What would be the strategies to implement improvements?

After 1–2 years of implementation a formal evaluation should examine the following attributes of the system.

Usefulness
How useful was WGS for early outbreak detection 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?

Challenges
In sustaining operations, can WGS be used in future outbreaks in its current form? If not, what changes does it need?

Given that this is a new technology, the evaluation should also include qualitative data. Interviews with key individuals involved in WGS (listed below) 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 of the United States Centers of Disease Control and Prevention (US CDC) for evaluating public health surveillance systems *(10)* 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.

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 for outbreak response?**
- **Who will perform the wet lab component of WGS?**
- **How will the wet lab methods be used?**
- **Who will perform the bioinformatics analyses?**
- **What tools will be used in the bioinformatics analyses?**
- **How will WGS outputs be reported to the outbreak response system?**
- **How will epidemiologists link WGS data to epidemiological 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 resources requirements?**
- **How will existing staff be reskilled?**
- **When and how will the role of WGS in responding to outbreaks be evaluated?**

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.

Web Annex K provides a template that may be used to collate all the relevant information required to develop a business case.

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.

Local examples of WGS implementation has been used and found to be beneficial when trying to obtain a decision-makers' support. However, if WGS has not been used locally before, a previous outbreak could be retrospectively sequenced, in order to highlight the advantages WGS would have had if used at the time. Choose an outbreak where the organism and the source were known. Box 3 outlines the steps in conducting a retrospective analysis of an outbreak.

BOX 3

Steps in a retrospective analysis of an outbreak

Choose an outbreak:

- that has good coverage of epidemiological information and a representative group of samples; and
- is caused by an organism for which some historical food samples are available.

Keep a record of:

- the time it takes from deciding what outbreak to sequence to when the results combined with the epidemiological information become available; and
- the cost of sequencing and transporting of specimens.

Compare the results to the original outbreak data

Determine whether WGS has:

- linked other food items to the outbreak that were not originally detected;
- identified more or fewer cases; and
- allowed the refinement of epidemiological information, by excluding non-cases.

Outbreak studies have been conducted in which samples were retrospectively sequenced as an exercise to determine if WGS could have been useful at the time of the outbreak. Examples from Australia, the USA and Germany are provided below.

- Epidemiology and whole genome sequencing of an ongoing point-source *Salmonella* Agona outbreak associated with sushi consumption in Western Sydney, Australia 2015 *(11)*.
- Tracing origins of the *Salmonella* Bareilly strain causing a foodborne outbreak in the USA *(12)*.
- Evaluation of WGS based approaches for investigating a foodborne outbreak caused by *Salmonella* Derby in Germany *(13)*.

5.2 Estimating the cost of implementation

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

Outsourcing

When sequencing is outsourced, the costs detailed above are eliminated; however, the cost per isolate sequenced and transport costs will need to be covered.

When outsourcing WGS, there is no need to have IT equipment, nor storage for sequencing data and subsequent analyses. Nonetheless, a platform is required to combine epidemiological information with genomic outputs. For small outbreaks, this might be done with standard software such as Microsoft Excel. However, if the number of cases in an outbreak is high and the expectation is to sequence as many isolates as possible, automated processes will be required.

Costs

Once the system description is final, it will be important to estimate the associated costs of sequencing across the outbreak response system, including the cost of each component of WGS implementation that must be acquired. Obtain quotes from local, regional or international suppliers, use estimates in the landscape paper *(4)*, as well as knowledge from any existing operational and laboratory costs. See a template for estimating the costs in Web Annex L.

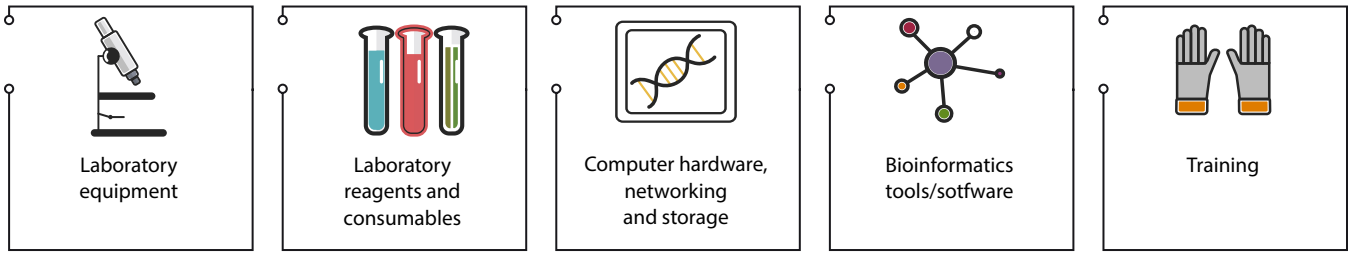
When the sequencing and bioinformatics components are outsourced, a cost-savings section should be added to emphasize the costs not incurred in purchases, maintenance of laboratory equipment and other inputs. Costs that may still need to be considered, depending on the outsourcing arrangements, are shown 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/sotfware	Training
Sequencing platform equipment Ancillary equipment Personnel	Propietary reagent procurement or generic reagent procurement production Consumables	Desktop hardware High performance computing/cloud access Data storage facilityand/or cloud access Internet connections, speed and bandwith Personnel	License fees (commercial) Personnel	Training for microbioogists, epidemiologists, managers, policy-makers, strakeholders: courses, workshops, visiting sccientists, online training

Ongoing costs

Laboratory equipment	Laboratory reagents and consumables	Computer hardware, networking and storage	Bioinformatics tools/sotfware	Training
Service contracts Maintenance & repair Expected lifespan before replacement Personnel	Need to know: Volume of isolates expected to be sequenced Proprietary reagent procurement Generic reagent procurement or production Consumables	Expected lifespan before replacement High performance computing and storage and/or cloud access Internet connections, speed and bandwidth Personnel	License fees (commercial) Open source: maintenance and upgrades, troubleshooting Personnel	Staff turnover Expansion of WGS utilization requires more personnel with suitable knowledge in al related sectors

ACTION

- Estimate the costs of WGS based on the system's description.

5.3 Cost–benefit analysis

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

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.

Case study 3 is an example of a cost–benefit analysis. A bibliography of pertinent publications is provided in this module.

Case study 3

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) *(14)*. 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* and *Salmonella* (depending on volume/throughput). That 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.

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 programme. 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 and/or jurisdictions that are most similar to yours. Conduct your own analysis using cost and benefit elements that are specific to your programme. It is critical to be able to demonstrate that your programme 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
- useful for solving other problems as well (e.g. obtain support from policy-makers).

Wherever possible, express benefits and costs in units that are most relevant to established local priorities (Table 4).

Table 4

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.

5.4 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. In addition, depending on the type of outbreak, WGS, as well as the number of samples to be sequenced may be limited.

A country may implement WGS to support foodborne disease outbreak investigation, therefore, the benefits and drawbacks of using WGS in outbreaks should be recorded. This information can be used to communicate the benefits of WGS to decision-makers and other stakeholders. Communication can prepare presentations or reports that may be published as journal articles. In addition, the information will be useful if the country has a long term goal of transitioning to WGS for foodborne disease surveillance.

5.5 Risks

It is important to state the risks associated with WGS implementation, and those of not establishing WGS in support of foodborne disease outbreak investigations, so that 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.

Risks of not implementing

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

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

A clear demonstration of the specific local benefits of WGS for foodborne disease outbreak investigations is critical for:

- building buy-in from management and stakeholders;
- increasing the chances of securing new funding; and
- convincing decision-makers to re-allocate existing funds from other priorities to WGS (Case study 4).

When communicating with decision-makers, 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.

Web Annex M contains some general talking points for communicating key aspects of WGS to decision-makers.Developing a business case ensures that you have a clear and concise description of your intent from the early stages. This will be useful when briefing senior officials, building stakeholder buy-in and applying for funding. In addition, regular information should be provided on the progress of WGS implementation to decision-makers, through memos and other means.

Case study 4

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 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 *(15)*. Decision-makers at the US FDA requested additional evidence regarding the degree of variation, and a second study was conducted to evaluate variability *(16)*. This was achieved by determining the level of genetic diversity when different isolates were sequenced, the changes related to thaw and refreezing, and the 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 *(17)*, 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 World Health Organization (WHO)'s reports *(18)* 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 5). Data from the whole food chain will provide a better understanding of foodborne disease epidemiology and 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 5

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 *(19)*. 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 WGS inclusion in the outbreak response system (Fig. 4). Box 4 highlights some key differences between the description of the outbreak response 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

What is the difference between a pilot study plan and the description of the outbreak response system?

The pilot study plan:

- should use elements from the description of the outbreak response 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 outbreak response system:

- defines how the entire system will function, from specimen collection to public health action;
- has objectives are about the functioning of the entire surveillance and response system;
- is intended to reflect practice into the future with no time limits; 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 the outbreak response system should be utilized during the pilot. The team should meet regularly in the early stages to ensure that 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 for outbreak response. 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 (e.g. 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. Is the discriminating power of WGS higher than that of other typing methods?
5. What will be the impact on investigation resources?
6. Does WGS produce data timely enough to inform a public health response in outbreak management?

Suitability and acceptability

7. How fast are WGS results available in comparison to current typing methods?
8. Are results compatible with current public health databases?
9. Can the WGS process accommodate changing demands?
10. Are public health staff able to use WGS to guide action?
11. Does 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 its use for outbreak investigations on a smaller scale, and over a defined time period. For example, if implementing WGS throughout the country, the pilot study 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 a single 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 5 lists the advantages and disadvantages of prospective and retrospective study designs.

Table 5

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 • 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 6 outlines examples of elements of a communication plan.

Table 6

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 outbreak, this may just occur at the mid-point of the pilot
Report and/or manuscript	All partners and stakeholders	

7.6 Step 6. Develop evaluation criteria

Evaluation criteria should be developed prior to starting the pilot, as there are data to be recorded from the beginning of the project. Criteria should be consistent with the pilot project's planned objectives.

As the pilot study advances, identified barriers or constraints will need to be recorded. Some barriers may be easily overcome, but others may be difficult to be removed and may even change the potential description of the outbreak response 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
Record 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.

Case specificity
Were any cases included or excluded based on sequencing results? Did this help refine epidemiological data?

Usability of WGS output
Could the output be integrated into the outbreak response?

Flexibility
Could the WGS process meet the demands during an outbreak?

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:

- the method providing the greatest discrimination for detecting similarities between isolates
- the timelier method
- 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 O. Case study 6 is an example of approaches to pilot studies.

Case study 6

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 *(20)*.

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 monitor and see through each step in the implementation. The working group can then follow the guidance document and plan accordingly.

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9789240021242