SciCom

SCIENTIFIC COMMITTEE

established at the

Belgian Federal Agency for the Safety of the Food Chain

OPINION 18-2021

Subject:

Whole genome sequencing for the detection of foodborne outbreaks and bacterial risk assessment

(SciCom 2020/08: self-tasking mandate)

Scientific opinion approved by the Scientific Committee on the 22th of October 2021.

Key terms:
Whole genome sequencing, foodborne pathogens, bacterial risk assessment, outbreak investigation

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Opinion 18-2021 of the Scientific Committee established at the FASFC regarding: Whole genome sequencing for the detection of foodborne outbreaks and bacterial risk assessment

Background & Terms of reference

This opinion deals with Whole Genome Sequencing (WGS), in which the DNA sequence of an organism's genome is determined starting from an isolate. "Metagenomics", in which DNA sequences are determined from a biological sample, which may contain multiple microorganisms, does not fall within the scope of this opinion. Viruses, although also important for foodborne infections, are not in the scope of this opinion. This opinion is addressing WGS of bacteria, as bacteria are also the first priority of EFSA and ECDC.

Technological development of Whole Genome Sequencing (WGS) provides new opportunities for the identification of the causes of foodborne infections and intoxications, the genotypic characterization of antimicrobial resistance, the determination of virulence, the serotyping of pathogens and the standardized exchange of data. At the same time, there are also a number of concerns about the implementation of WGS.

This opinion was prepared by the Scientific Committee using a self-tasking mandate. The following terms of reference are addressed in the opinion, with a focus on the Belgian context:

1. The benefits of using WGS for outbreak investigation
2. The benefits of using WGS for bacterial food safety risk assessment
3. Interpretation of the linking between contaminated food, human infections and the source of contamination in the food chain
4. Recommendations on the (further) implementation of WGS for managing food safety in Belgium
5. Validation of WGS methodology: importance, current status and expected evolutions
6. Requirements – technically and organizationally – to share WGS data in the context of food safety

Method

The opinion is based on information available in scientific literature and on expert opinion.

Opinion

Whole Genome Sequencing (WGS) provides new opportunities for improving bacterial food safety. At the same time, a number of concerns should be taken into account when implementing WGS in the frame of (inter)national surveillance and food safety management. The opinion focuses on different food borne pathogens including *Salmonella, Listeria monocytogenes* and Shiga Toxin-producing

Escherichia coli (STEC). These three pathogens are described in more detail, in the part on outbreak investigation in the (Annex 1), since they are the first and actual focus of the joint WGS database developed by EFSA and ECDC.

In this opinion, firstly the background of WGS is described and the technology is situated in the broader context of other bacterial and molecular methods. In the second part, the terms of reference related to the implementation of this technology are addressed with a particular focus on the Belgian context.

Conclusion

In this opinion the Scientific Committee has situated the use of WGS for the detection of foodborne outbreaks and bacterial risk assessment and reflected on the implementation of WGS in the Belgian context. In the future, WGS will become the preferred method for bacterial food safety investigation, due to its high discriminatory power and the fading out of various older typing methods at an international level. Despite that WGS methods and pipelines for data analysis are still continuously evolving and improving, WGS is ready to start being used in routine outbreak investigation and surveillance activities. The Scientific Committee formulates several recommendations regarding the implementation of WGS in a Belgian context. To facilitate the transition to WGS for the analysis of food isolates, including AMR monitoring, a transition period can be implemented. This offers the labs time to get experience and sufficient infrastructure ready. The Scientific Committee advises the FASFC to (gradually) make the transition to WGS for the analysis of food isolates.

However, despite the advantages of WGS, some limitations still need to be taken into account for routine and uniform implementation. Efforts should be made to validate the WGS methodology and to facilitate data sharing. It is recommended that WGS-based results regarding strain comparison in outbreak investigations be interpreted by a multidisciplinary team (microbiologists, molecular biologists, bioinformaticians, epidemiologists) with sufficient expertise. It is also recommended that when using WGS for subtyping strains, as part of an outbreak investigation, validated or internationally recognized WGS methods and bioinformatics tools should be used, and interpreted according to the clonality of the pathogen under consideration and taken into account the epidemiological evidence and the metadata about the strains. These metadata include features such as geolocation data, isolation source, collection date, the organization performing collection, sample and strain names). It is recommended to be vigilant of the correct interpretation and communication on the responsibilities of the different actors (competent authority, FBO, consumer) in case of an outbreak. In this, it is important to communicate that zero risk in relation to bacterial food safety does not exist.

Recommendations

The Scientific Committee makes the following recommendations:

- It is recommended to make the transition to WGS for the analysis of the food related bacterial isolates in Belgium, in the (near) future.
    a. As various older typing methods are fading out at an international level, WGS will become the method of choice for strain typing (including serotyping for *Salmonella* and Shiga toxin

producing *E. coli*) for national surveillance, source attribution and outbreak investigation. A transition period can be implemented, so that the labs can invest in appropriate infrastructure, can acquire the technical expertise needed, set-up a standardized flow for implementation of the WGS method that can be validated and built experience.

b. As WGS-AMR data can be obtained from the same WGS data used for typing, source attribution and outbreak investigation and that they can complement phenotypic data with extra information related to the genetic markers and be screened retrospectively it is recommended also for AMR monitoring to extract data from the available WGS data collected for other purposes. As it is recently been accepted as alternative method for the specific monitoring of ESBL- or AmpC- or carbapenemase-producing *E. coli* and *Salmonella* and the EURL-AR has elaborated a technical protocol to be followed, it is expected that at EU-level a further transition towards WGS-AMR can be expected.

- Sharing WGS data can be performed by centralizing the data nationally, at the Belgian level or (directly) internationally at the European level. At the Belgian level, for both the human clinical data and the agrofood data new infrastructure will need to be build. This new infrastructure is ideally concepted as a joint database or at least as two databases so that the joint data can be analyzed. By June 2022, the joint European database will be operational with communication between EFSA's WGS database (isolates from agrofood products) and ECDC's Tessy (clinical isolates from humans). The Belgian isolates from the agrofood chain and from human clinical origin will be able to be submitted to the respective databases.
- It is recommended that WGS-based results regarding the comparison of strains in outbreak investigation (e.g. performed by SNP analysis or cgMLST) are interpreted by a multi-disciplinary team (microbiologists, molecular biologists, bio-informaticians, epidemiologists) with sufficient expertise. It is not possible to define a clear threshold for the number of genetic differences between strains from a common source. The WGS-derived data should be combined with metadata informative for the epidemiological interpretation of the outbreak. Furthermore, in order to establish an effective case definition (e.g. threshold) based on WGS, WGS data should be available on the circulating strains. This will provide a population against which clusters of potential outbreak strains can be assessed. Therefore, the current use of WGS during outbreak investigation should be extended to official surveillance programs.
- In order to prevent outbreaks, especially caused by pathogens which are known to be able to persist in the processing environment (e.g. *L. monocytogenes*), it is recommended to investigate their presence in the food processing environment and to follow up the cleaning and disinfection process.
- It is recommended to follow up the process of standardisation and validation of the WGS methodology and this for both the 'wet –lab' and 'dry-lab' part. Also for this technology the performed methods need to safeguard the results conform the criteria defined in the ISO/DIS 23418 (Microbiology of the food chain — Whole genome sequencing for typing and genomic characterization of foodborne bacteria — General requirements and guidance). It is recommended to carefully document the WGS-based results with sufficient metadata, so that, also in the case databases are updated, no information will get lost and traceability to the food source or the food business operator will remain.

- It is recommended to be vigilant of the correct interpretation and communication on the responsibilities of the different actors (competent authority, FBO, consumer) in case of an outbreak. In this, it is important to communicate that zero risk in relation to bacterial food safety does not exist.

1. Terms of reference

1.1. Question

Technological development of Whole Genome Sequencing (WGS) provides new opportunities for the detection of the causes of foodborne infections and intoxications, the prevention of foodborne outbreaks, the genotypic characterization of antimicrobial resistance, the determination of virulence, the serotyping of pathogens, the comparison and standardized exchange of sequence data and risk assessment. At the same time, a number of concerns should be taken into account when implementing WGS in the frame of (inter)national surveillance and food safety management. The opinion focuses on different pathogens including *Salmonella, L. monocytogenes* and human pathogenic Shiga toxin producing *E. coli*. These three pathogens are the first and actual focus of the joint WGS database developed by EFSA and ECDC.

This opinion was prepared by the Scientific Committee using a self-tasking mandate. The following points will be addressed in the opinion:

1. The benefits of using WGS for outbreak investigation.
2. The benefits of using WGS for bacterial food safety risk assessment.
3. Interpretation of the linking between contaminated food, human infections and the source of contamination in the food chain
4. Recommendations on the (further) implementation of WGS for managing food safety in Belgium.
5. Validation of WGS methodology: importance, current status and expected evolutions.
6. Requirements – technically and organizationally – to share WGS data in the context of food safety

1.2. Method

The opinion is based on information available from scientific literature and on expert opinion. The advances of the use of WGS in the context of outbreak investigation is summarized taking into consideration the established ECDC expert opinion paper of 2016, the ECDC corporate document for public health microbiology strategy 2018-2022 and the Scientific opinion of the EFSA Panel on Biological Hazards (EFSA BIOHAZ panel) from 2019. An in-depth compilation of literature on outbreak investigations was based on a search in PubMed for original articles published in English between 2017 and 2020, using the key words “whole genome sequencing” AND “outbreak” AND [“*Listeria*” OR “*Salmonella enterica*” OR “STEC”]. The records were finely tuned to consider solely outbreaks reports. Traditional phylogeny of *L. monocytogenes* was based upon Orsi *et al*., 2011.

2. Abbreviations and Acronyms

Following abbreviations are used (definitions are given in **Appendix 1**):

AMR	Antimicrobial resistance
ANSES	Agence Nationale Sécurité Sanitaire Alimentaire Nationale (France)

CC	Clonal complex(es)
CDC	Centers for Disease Control and Prevention
CFU	Colony forming unit
cgMLST	Core genome MLST
DDBJ	DNA Data Bank of Japan
ddNTPs	Dideoxy-nucleotides
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EFSA BIOHAZ panel	EFSA Panel on Biological Hazards
ENA	European Nucleotide Archive
EURL	European Union Reference Laboratory
FASFC	Belgian Federal Agency for the Safety of the Food Chain
FBO	Food Business Operator
FDA	Food and drug administration
FWD	Food- and waterborne disease
GWAS	*Genome Wide Association Study*
ISO	International Organization for Standardization
kb	Thousand base pairs
LAMP	Loop-mediated isothermal amplification
LPS	Lipopolysaccharide
Mb	Million base pairs
MOST	Metric Oriented Sequence Typer
MLST	Multilocus sequence typing
MLVA	Multilocus variable-number tandem repeat analysis
NCBI	National Center for Biotechnology
NGS	Next generation sequencing
NRC	National Reference Center
NRL	National reference laboratory(ies)
PCR	Polymerase Chain Reaction
PFGE	Pulse-Field Gel Electrophoresis
PHE	Public Health England
rMLST	Ribosomal MLST
ROA	Rapid Outbreak Assessment
SciCom	Scientific Committee established at the FASFC
SRA	Sequence Read Archive
STEC	Shiga toxin-producing *Escherichia coli*
SNP	Single-nucleotide polymorphism
SNV	Single-nucleotide variant
ST	Sequence type
ToR	Terms of Reference
WGA	Whole Genome Amplification
wgMLST	Whole genome MLST
WGS	Whole genome sequencing

Considering the discussions during the working group meeting on 26 May, 26 August, 9 October and 4 December 2020, 18 January, 12 February, 5 March, 16 April and 4 October 2021, and during the plenary sessions of the Scientific Committee on 29 May 2020, 26 March, 23 April, 25 June and 22 October 2021,

the Scientific Committee gives the following scientific opinion:

3. Context

This opinion deals with Whole Genome Sequencing (WGS), in which the DNA sequence of an organism's genome is determined starting from an isolate. "Metagenomics", in which DNA sequences are determined from a biological sample, which may contain multiple microorganisms, does not fall within the scope of this opinion. Viruses, although also important for foodborne infections, are not within the scope of this opinion. This opinion is addressing WGS of bacteria, as bacteria are the first priority of EFSA and ECDC.

Technological development of Whole Genome Sequencing (WGS) provides new opportunities for the detection of the source of foodborne infections and intoxications, the prevention of foodborne outbreaks, the genotypic characterization of antimicrobial resistance, the determination of virulence, the serotyping of pathogens, the comparison and standardized exchange of sequence data and risk assessment. Besides the advantages, some limitations and challenges still need to be taken into account before routine and uniform implementation.

Due to the offered benefits, there is no doubt that WGS will become the preferred method for bacterial food safety investigations by food safety authorities, the scientific community and food business operators.

In this opinion, the background of the WGS is described and situated in the broader context of other bacterial and molecular related methods, including phylogeny which is important in foodborne outbreak investigation. In a second part, the terms of reference related to the implementation of this technology are addressed with a particular focus on the Belgian context. The opinion focuses on different pathogens including *Salmonella, L. monocytogenes* and human pathogenic Shiga toxin producing *E. coli* (STEC). These three pathogens are included because they are the first and actual focus of the joint WGS database developed by EFSA and ECDC.

Current state of WGS implementation and data exchange in Belgium

The implementation of WGS in routine surveillance and monitoring applications has not occurred in Belgium in a harmonized manner. Only certain laboratories have introduced the technique.

At present the Belgian WGS data for clinical human isolates are locally stored in laboratory databases and shared on a voluntary basis in public databases such as Enterobase, NCBI and ENA. The WGS data available for the agrofood area are limited and stored locally by the labs performing the analyses. Since no centralized WGS database for agrofood isolates exists in Belgium, WGS data are only used for specific data exchange between laboratories in the frame of specific investigations.

Due to the very selective and targeted approach in the use of WGS for food isolates in Belgium, the technique is at the moment mostly used as a high resolution method to confirm an outbreak and only in specific cases to proactively detect a potential future one.

4. Brief background of WGS in the context of bacterial food safety

A detailed background of WGS in the context of bacterial food safety is available in Appendix 1 (in English).

DNA sequencing is the process by which the sequence of nucleotides, the building blocks of DNA, in the genome is determined. WGS determines the whole genomic sequence of a bacterial isolate and is increasingly replacing traditional bacterial typing and characterization techniques, providing faster and more accurate responses.

WGS is performed in two processes: 1) a first "wet-lab" part dedicated to the selection of isolates, DNA extraction, library preparation, and sequencing that will generate raw sequenced data ; 2) a second "dry-lab" part where different bioinformatics tools are used for quality control, for the assembly of the genome and for further analysis (e.g. to demonstrate the relatedness of strains for outbreak investigation, screening for the presence of antimicrobial resistance (AMR) genes, genes associated with virulence and pathogenicity, determination of serotype). WGS also creates the possibility to store the data over time according to the FAIR-principles (Findable, Accessible, Interoperable and Reusable; https://www.nature.com/articles/ng.3910).

WGS can be used to:

A) predict serotype and antimicrobial resistance.

Serotyping is most often an early step in a typing scheme and has been of importance during investigations and surveillance of pathogens worldwide. Furthermore, it has been incorporated in EU regulations. Several WGS-based solutions have been described to replace conventional serotyping.

- For *Salmonella* the web-based *Salmonella* Typing Resource (SISTR) database and SalmonellaTypeFinder are freely available with a high degree of accuracy compared to the conventional serotyping (> 90% for > 85-90 % of the serotypes).
- WGS-based serotyping of *E. coli* is enabled by the web based SeroTypeFinder tool and database which are freely available and are able to confirm conventional serotyping results and clarify some doubtful results obtained with conventional serotyping.
- Serogrouping of *L. monocytogenes* can be performed based on WGS data using the same genes as those used by the developed conventional multiplex PCR method.

In most cases WGS has been shown to accurately predict phenotypic AMR properties allowing to rapidly screen isolated strains for a multitude of known genes involved in resistance. Another advantage of using WGS for AMR typing is the fact that also retrospective analyses on stored WGS data remain possible. This allows the analysis for the presence of resistance genes or mechanisms based on newly discovered AMR resistance mechanisms.

B) refine risk assessments.

WGS is also offering opportunities to refine the actual risk assessment for important foodborne bacterial pathogens. Bacterial risk assessment – in a context of food safety – is a structured science-based process to determine the health risk associated with a specific bacterial hazard in food. Bacterial risk assessment consists of four steps: hazard identification, hazard characterization, exposure assessment and risk characterization, and as part of the risk analysis, it has the overall objective to minimise foodborne risks to the consumers (FAO/WHO, 2007, EFSA[1]). Thanks to its high resolution, WGS can be used to describe, through a Genome Wide Association Study (GWAS), markers/indicators predicting phenotypes associated with pathogenicity, growth and survival characteristics of bacterial foodborne pathogens (EFSA, 2019). With this, WGS has the potential to impact the hazard identification and narrow down the risk assessment process and regulatory consequences to certain strains belonging to the same species. WGS can also impact the exposure assessment.

The opportunities of WGS for future risk assessment is illustrated by two examples (for more info, see appendix 1 part 1.5):

- For the "*Bacillus cereus* group" (this group contains several *Bacillus* species including *B. cereus, B. anthracis* and *B. thuringiensis*), WGS, using genetic markers, would have the capacity to discriminate potential pathogenic strains (those involved in diarrhoeal and/or emetic syndrome) from non-pathogenic ones.
- For *L. monocytogenes* WGS would have the potential to link certain strains containing certain genetic markers to properties as pathogenicity, virulence, stress resistance and to predict how virulence can be impacted for a subset of strains by physicochemical parameters such as temperature, pH, osmotic stress or fat content.

Besides the advantages, some limitations still need to be taken into account for routine and uniform implementation. There is the current lack of consensus for the optimal methodology to analyze WGS data and the applied methods. Also only 'known' resistance determinants can be searched for as unknown or novel resistance genes or mutations are not present in the database. Therefore it is possible that some strains have been classified as susceptible to certain antibiotics while they are actually resistant. This limitation will be progressively resolved thanks to continuous sequencing efforts and updating databases with genes or mutation related to phenotypic data.

C) detect foodborne outbreaks.

Clusters of human cases could trigger an outbreak investigation leading to a targeted sampling of the potential food source based on interviews looking for common food consumption. Conventional typing methods or WGS are used to link the food or environmental isolates to the human cluster. This model is fully based on epidemiological data. With the introduction of much higher resolution WGS, the detection of a cluster including both human isolates and food or environmental isolates could trigger an outbreak investigation and thereby steering the epidemiological investigation, complementing and reversing the former model.

[1] https://www.efsa.europa.eu/en/interactive-pages/riskassessment/RiskAssessment, accessed Jan. 12, 2021

D) define outbreak clusters.

In outbreak investigation it is important to find the origin of the infection, identify the involved cases and determine the transmission routes. Therefore the first step of most foodborne outbreak investigations is the isolation and identification of the pathogen from a suspected food source, the environment, as well as the patients. Typing methods should differentiate the strains well below species or subspecies level to verify whether the strains are identical or not.

Phenotyping techniques detect features expressed by the microorganism (examples are serotyping, AMR profiling and toxin detection), while molecular typing techniques are based on the analysis of the genome of the organism. They can be non-sequence based, e.g. Restriction Fragment Length Polymorphism (RFLP), Pulsed Field Gel Electrophoresis (PFGE), repetitive-sequence-based (rep)-PCR, and Random Amplified Polymorphic DNA (RAPD). Sequence based techniques are comparing the whole genome for Single-Nucleotide Polymorphisms (SNPs) or compare differences between genes for a given subset of genes (MLTS or Multi-Locus Sequence Typing). This can be genes belonging to the core genome (cg) (cgMLST) that is shared by all analyzed bacteria of the same species (usually > 1000 genes), or belonging to the whole genome (wg) (wgMLST) (usually > 3000 genes). For comparison, conventional MLST compared the sequence information of seven genes. However, no 100 % correlation could be found between some conventional methods, such as PFGE, and WGS-based typing results. In the transition period where conventional and WGS based methods are both applied, the discrepancy between the different methods could hamper the outcome of strain comparison made with different methodologies. It is foreseen that most conventional typing methods will be replaced by WGS based methods.

WGS for source tracing and outbreak investigation relies on the comparison of WGS of clinical and agrofood-related strains. The use of either SNP-based or MLST-based analyses has shown high concordance when assessing the number of clusters identified, even though SNP-based analyses showed a higher resolution as it takes into account every nucleotide polymorphism in the analysis. It uses no typing scheme but depends on a well-chosen optimal reference genome. Important is the search for a threshold which defines how many nucleotide or allelic differences are allowed between isolates to be still considered as belonging to a single cluster. However, this threshold should not be absolute, but remains adjustable based on re-evaluation of all WGS data and the linkage between WGS data and epidemiological information. These metadata include information about where and when a sample was collected, the type of environment it came from and the sample's properties. These types of features include, but are not limited to, geolocation data, isolation source, collection date, the organization performing collection and sample and strain names (Chang *et al.,* 2016). Furthermore, in order to establish an effective case definition (e.g. threshold) based on WGS, WGS data should be available on the circulating strains. This will provide a population against which clusters of potential outbreak strains can be assessed.

Considered per pathogen, the following attention points are raised in the context of source tracing and outbreak investigation based on a literature review:

- WGS for *L. monocytogenes* is particularly useful for source tracing.
- The number of studies using WGS for STEC analysis are yet limited to provide a strong comparison with traditional methods for subtyping.
- WGS of *Salmonella* for outbreak investigation is gradually replacing traditional methods for subtyping as WGS provides higher resolution, which is needed because e.g. some serovars are highly clonal. However, due to the high number of serovars present within the *S. enterica* species, establishment of a consensus pipeline is challenging.
- Human pathogenic *Y. enterocolitica* biotype strains are rather clonal, making differentiation of strains difficult using traditional genetic typing methods. WGS could therefore be useful to detect a greater variation between the strains. Currently, only a selection of human *Yersinia* strains are being sequenced in Belgium.

E) share data at a national and international level.

Collaborating networks to exchange conventional typing data between primary, food and public health sectors have been disrupted with the introduction of WGS since specific laboratories have introduced the technique, while others were unable to do so. More and more attention is given to restore this data exchange, focusing on exchange of WGS data, by giving sufficient attention to the inter-operationality of the various data systems and this at the national, EU and global level.

Due to the very selective and targeted approach in the use of WGS for food isolates in Belgium, the technique is at the moment mostly used as a high resolution method for outbreak confirmation, rather than outbreak detection. Since no centralized WGS database for agrofood isolates exists in Belgium, WGS data are stored in local databases, only used for specific data exchange between laboratories in the frame of specific investigations.

Currently, in the frame of outbreak investigation, European WGS data are communicated via the European Reference Laboratory (EURL) networks or via ECDC's Epidemic Intelligence Information System on Food- and Waterborne Diseases and Zoonoses (EPIS-FWD) network. In 2021, a new jointed ECDC–EFSA database for typing data, focusing on WGS, should be operational, allowing the member states to submit data to either EFSA's WGS database (for agrofood related strains) or ECDC's Tessy (for clinical human strains).

Globally, several public databases are available for nucleotide sequences, such as the National Center for Biotechnology Information (NCBI) GenBank, the European Nucleotide Archive (ENA) and the DNA Data Bank of Japan (DDBJ). Other public (e.g. Enterobase) and commercial (Ridom SeqSphere+, BioNumerics) databases are available to facilitate the end user in the introduction and use of WGS analyses. In the United States, both public health (CDC's PulseNet) and primary and food (FDA's GenomeTrakr) sectors have already made the transition to WGS surveillance, setting up inter-operable systems between agencies, with publicly accessible databases.

F) identify 'persistent strains' along the food chain.

Food products can be contaminated by pathogenic bacteria via employees, contaminated food ingredients or due to contamination from the food producing environment. In many cases the contamination of food products from the food producing environment can be the origin of a recurrent contamination, often at an irregular frequency. This recurrent contamination may occur if the pathogen is persistent in the food production environment. The strains responsible for these recurrent contaminations are called "persistent strains[2]" and the process is called "persistence". It are especially these persistent strains, occasionally causing human disease, which could be discovered by the implementation of the WGS methodology because WGS is especially competent to link strains over time and over large geographical regions. Eliminating persistent strains is challenging for food business operators. It includes extensive environmental testing to detect and identify the persistent strains and to find the environmental source combined with eradication and mitigation actions such as intensified cleaning and disinfection protocols. For the identification of persistent strains the food business operator can apply different typing methods and would not necessarily rely on WGS.

5. Response to the terms of reference

Reference term 1: Benefits of using WGS for outbreak investigation

Currently in Belgium an outbreak investigation can be triggered by the isolation of clustered human clinical isolates leading to a possible food source based on epidemiological information. If a food isolate can be obtained, molecular typing permits the identification of the link between the human and the food isolate. At this time, WGS is used to confirm an outbreak and only in specific cases to proactively detect a potential future one.

WGS has clear advantages for improved outbreak investigation. For this it is required that WGS of food isolates is performed on a routine basis like it is now already done for human clinical strains. This WGS efforts, independent from a human clinical outbreak, has the potential to proactively detect the outbreak without the need for clustering clinical cases first, e.g. based on the identity of the WGS of a food isolate with the WGS of a clinical human isolate present in the database. As such previously undiscovered outbreaks can be detected and human and economic harm can be prevented. This is due to the superior potential of WGS to cluster human and food isolates.

WGS has the power to link isolates over a **longer period of time** (also retrospective) and over a much **larger geographical region** (EFSA BIOHAZ panel, 2019). This is due to the ability to store the WGS data in databases, data that can be easily shared and analyzed by bioinformatic tools for matches between isolates (human, food, animal and/or environment). When **new clinical cases** are identified, it is then possible to re-analyze **historical data**. This can be important e.g. to discover outbreaks spanning a long time period. Due to the use of WGS, sporadic cases can be linked over a large geographical region and over a longer time to the same food products and are, as of recently, considered as outbreaks. These findings can steer the epidemiological investigation rather than the other way round.

This is particularly important for *L. monocytogenes,* previously known as causing mainly sporadic infections and less outbreaks. The reason behind is that human listeria infections are for a great part

[2] Strains responsible for recurrent contaminations observed along the food chain.

related to the immune status of the consumer (EFSA BIOHAZ panel, 2018). So, a contaminated food product will only infect a very limited number of susceptible consumers in a certain time span and geographical region. In addition, the incubation period after ingestion of the contaminated food can be days or even weeks, making it challenging to link human cases to a specific source. Epidemiological data as a starting point for outbreak investigations are, and were in most cases, not able to link these sporadic cases based on the consumption of a common food product. For this reason single listeriose cases were removed from the list of notifiable infections in Flanders, while the investigation is still attempted in the regions of Brussels and Wallonia. Nowadays rare infections can by the use of WGS data be clustered over time and over an extended geographical region. Important is also that *L. monocytogenes* has the potential to persist in the environment of the food processing plant for years and that from this environment it can contaminate the food product with the same strain for a long period of time, even years. By WGS it becomes possible to link human cases caused by these persistent *L. monocytogenes* strains to the contaminated food products.

Reference term 2: Benefits of using WGS for bacterial food safety risk assessment

- One single WGS-workflow has the potential to be used for outbreak investigation, **serotyping and AMR analysis** and does not require substantial extra resources. It is probable that there will be a shift to WGS for serotyping and determining AMR (antimicrobial resistance) at the international level. WGS has been shown to rapidly screen isolated strains for a multitude of known genes involved in resistance allowing to accurately predict phenotypic AMR properties. There is a potential risk that in some cases isolates are identified as susceptible to antibiotics based on the sequence data because lack of functional expression of the genes while they are actually resistant. This limitation will be progressively resolved thanks to continuous sequencing efforts associated with phenotypic observation and evolutions in data analytics.
- It will be possible to perform **retrospective queries** to screen for the presence of resistance genes or mechanisms based on newly discovered AMR resistance mechanisms. This enables the investigation of the presence of newly discovered antimicrobial resistance genes, in our region spanning the time period of the historical data. So, investment of performing WGS is also worthwhile in the light of interesting future developments.
- Another opportunity is that, based on WGS data via a Genome Wide Association Study (GWAS), information can be collected on genetic markers/indicators for **predicting phenotypes** related to the pathogenicity of particular strains belonging to a certain species (EFSA, 2019). This is thought to have potential in the case of the fine-tuning of the risk assessment narrowed down to strains with the highest pathogenic potential. For the future, this could lead to a full, or partial, replacement of animal toxicity/pathogenicity tests by WGS data. It should be emphasized though that the mere presence of genes, involved in virulence or toxin production, does not mean these genes will be expressed. Determining the probability of gene expression relevant to pathogenicity based on only DNA sequencing data is complicated. WGS is not a suitable tool for detecting expression levels; other omics technologies (e.g. transcriptomics or proteomics) are better suited for this.
- Although, at the moment the evolution is yet difficult to predict, WGS could help to better **discriminate between non-pathogenic/useful and dangerous bacteria** in groups of closely related

species. This could be especially interesting for pathogens as *L. monocytogenes,* members of the *B. cereus* group and *Yersinia* spp.

- For example, for *L. monocytogenes*, data are collected linking phenotypical data on virulence and growth potential at low temperature to genetic markers (Fritsch *et al.,* 2019). The genetic markers associated to at least some of these important phenotypic features have already been clarified.
- Another example are members of the *Bacillus cereus* groups that contain the valuable strains of *Bacillus thuringiensis*, used as biopesticides for more than 60 years, and the pathogen *Bacillus anthracis* or the emetic and diarrheic strains of *B. cereus* responsible for foodborne illnesses. Similarly, the question of what makes a bacterium pathogenic for humans and which genetic markers are involved could be further investigated using WGS combined with other omics techniques, cell culture assays, simulations in gastro-intestinal gut models or epidemiological data.

  For the *Yersinia* spp. strains there is a long list of possible virulence genes available, but no (good) correlation with their potential pathogenicity and, as such, the meaning for human health is often unsure. It is likely that various virulence factors still go undetected at present. Comparison of WGS data of clinical and agrofood isolates could help for clarifying the genetic markers of pathogenicity for *Yersinia* spp. but will need a large data set of strains with appropriate metadata and context available.

- WGS could also play an important role in **pathogenicity assessment** of certain pathogens, such as STEC. An EFSA opinion has clearly stated in 2019 that all STEC strains are potentially pathogenic in humans and capable of causing (severe) illness. While serotyping has been for many years the main instrument to assess pathogenicity of these bacteria, the subtyping of the Shiga toxin encoding genes and accessory virulence genes has proven more reliable. WGS is the ideal technique to perform these genomic characterization in one single workflow.

Reference term 3: Interpretation of the linking between contaminated food, human infections and the source of contamination in the food

The EFSA opinion stated that the link made between contaminated food and human infections should always be made by a **combination of WGS data and epidemiological data** (EFSA BIOHAZ panel, 2019). WGS analysis alone would not be sufficiently conclusive to undoubtfully link two strains only on the basis of a well-defined threshold for the extent of genetic differences (EFSA BIOHAZ panel, 2019). In addition, defining this threshold is dependent upon a variety of factors such as the species or subspecies, isolation date and evolutionary rate. Since these factors may be unknown, setting a threshold remains challenging. To start, a sufficiently large number of a given species or subspecies needs to be sequenced to create a collection of strains, which circulate in the relevant ecosystems. Once this population has been established, this can support to define threshold values of genetic differences for inclusion or exclusion of isolates within an outbreak.

It is possible that an individual consumer becomes infected by consuming a food product contaminated with a number of pathogenic bacteria below the legal microbiological criterium (e.g. 100 CFU/ml or g for *L. monocytogenes*) at the moment of consumption (EFSA BIOHAZ panel, 2020) and at the same

time other (healthy) consumers are exposed to higher numbers and do not develop disease or severe symptoms. The risk to get infected by a foodborne pathogen is not directly linked to a well-established dose-response relationship between the level of contamination in the food products and human illness.

- **The susceptibility of the host and thus host-related factors** are extremely relevant since certain people (YOPI – young, old, pregnant, immunosuppressed) are more susceptible to infection and illness as result of the consumption of the contaminated food product. It should be noted that the immune capacity and/or the medication taken by the consumer (e.g. the intake of proton pump inhibitors) are at least as important as the level of contamination of the pathogen in the food product to be infected (HGR/SciCom, 2016, Kvistholm Jensen *et al.,* 2017).
- The **survival/growth of the pathogen** can be influenced by multiple factors and can also be due to misconduct by the consumer, outside the responsibility of the food producer. The treatment of the food product by the consumer (e.g. well heated for non-ready-to-eat food products compared to moderately warmed; consumption of frozen vegetables intended to be further cooked as raw products) will also make a difference. Also the type of food has an impact. An example are the low moisture fatty foods as chocolate, peanut butter promoting gastrointestinal survival of low levels of *Salmonella* and therefore provoking infection (Finn *et al.,* 2013). Also, the time of ingestion can make a difference, as when you start eating the first things pass fast through the stomach (acid environment) and are more likely to survive (Lehmacher *et al.,* 1995, Tompkins *et al.,* 2011).

It is not easy to collect the data cited above and this can make it unclear how far the food producer is to be hold responsible for such foodborne infections. There are limitations to the 'reasonably foreseen abuse' principle for a food business operator. Food safety is a shared responsibility throughout the food chain, from farm to fork.

Reference term 4: Recommendations on the (further) implementation of WGS for managing food safety in Belgium

A broad implementation of WGS is feasible and will become inevitable in an international context. It should be noted that the previous internationally recognized PFGE technique as typing method for several bacterial pathogens is no longer supported. In addition, various older typing methods are fading out at an international level. So, it is important to make the transition to WGS of the food related isolates.

Epidemiological information could trigger a decision to perform WGS on a selections of strains in the frame of national surveillance programs and this can be pathogen dependent and dependent on the purpose of the monitoring/surveillance. For *L. monocytogenes* the number of clinical isolates and food isolates obtained from the FASFC control program is limited (on annual basis ca. 70 human clinical isolates and ca. 150 food isolates). The situation of pathogenic *E. coli strains* is comparable (on annual basis ca. 100 human clinical isolates and ca. 100 food isolates). For *Salmonella* there are a lot more isolates (on annual basis ca. 3000 human clinical isolates, ca. 350 food isolates and ca. 850 food producing animal isolates).

There are two possibilities to achieve the transition from the traditional typing methods towards WGS:

- Combination of WGS and conventional typing methods, possibly used in a transition period. Sequencing can be performed on a selection of agrofood isolates and this selection can possibly be enlarged over time. This selection can be based on the results obtained by conventional typing methods or based on epidemiological data. The actual practice for the typing of human strains can be used as guidance. For strains from pathogens linked to high numbers of human clinical cases a selection of strains for WGS is actually made based on the use of older typing techniques. This is for example the case for the typing of *Salmonella* Enteritidis and Typhimurium strains which is performed by MLVA. The same practice could be proposed for agrofood strains. Therefore, the older techniques will still remain in use for some time. A transition phase has the advantage for the labs to get experience and sufficient infrastructure ready. It is also an advantage for food business operators to get appropriately informed and prepared. The disadvantage is that the combined use of different techniques during a transition phase will complicate cluster analysis between human and agrofood strains and even between agrofood strains themselves.
- A direct switch to the use of WGS as the only pathogen typing technique. This would require that sufficient infrastructure and competence (wet and dry lab) would be made available at once. This requires a substantial initial investment. A switch for serotyping would also need to be validated and to be accepted based on the EU legislation.

For both scenarios, the need for big data storage cannot be overlooked, since this will require a further investment, either on a local or centralized level. To be able to use historical data in WGS databases, it is important to consider comparability of old data with more recent ones when technological improvements in the WGS technology are implemented.

It is recommended to harmonize the analyses and databases for strains from agrofood and human origin, so that comparison of the data from both areas can be performed. The database would need to be able to interact with the database containing the WGS data from clinical human isolates to be useful for confirmation of foodborne infections and for outbreak investigation. Epidemiological data are important and should be used as metadata together with the WGS data.

For the food business operators, other simpler and cheaper typing methods are still valuable for identifying strains that persist in their processing environment. They can be supplemented with selective WGS analysis where desired and useful. The isolates from the production environment can be typed, helping to pinpoint the control points and in response improving the HACCP procedures. To get a clear picture of what is happening in the company an appropriate processing environment monitoring plan should be put in place and a sufficient and consistent number of environmental samples in a defined time period will be needed.

Transparency, moral responsibility and juridical liability are important aspects that need to be reflected upon when further implementing WGS for managing food safety. Back tracing using WGS, combined with epidemiological data and information on the consumption of specific foods, will uncover links between products/companies and human cases previously unknown. It will undoubtedly become less likely for companies to never get associated with human cases. It should be noted that even in a clean production environment, the (sporadic) presence of a pathogen cannot be fully excluded. It is also possible that conform products are responsible for a human infection e.g. due to misconduct by the

consumer including not adhering to instructions for storage and use indicated on the package and/or his weakened immune status. As such, information on good practices to handle and store food safely are a prerequisite for both public health authorities, food companies and other organization of health care practitioners in contact with the general public or defined susceptible target groups is needed. In addition, in case of a recall or outbreak investigation a good (crisis) communication and swift response to correct the problem will be essential.

Training and education are also an important aspect that needs to be addressed to ensure that the food business operators are sufficiently aware of the possibilities and consequences of the implementation of WGS.

A good communication towards the general public is also essential. Zero risk does not exist in relation to bacterial safety of food products. It is needed to explain that not all factors contributing to human foodborne illness are the exclusive responsibility of the food producers but also that occasional (low) presence of pathogens in the agrofood supply chain cannot be avoided in particular for raw or minimal processed food stuffs. The element of the immune capacity and/or medication of the consumer, the moment (and manner) of consumption, and the possibility of misconduct by the consumer need to be envisaged in the assessment when food becomes unsafe for consumption.

Reference term 5: Validation of WGS methodology: importance, current status and expected evolutions

At the international level guidance for ensuring the inter-operationally of data and databases are increasingly agreed on with the ISO standard (ISO/DIS 23418: Microbiology of the food chain – Whole genome sequencing for typing and genomic characterization of foodborne bacteria – General requirements and guidance) as a general standard for the quality of WGS. There are also increasing efforts at the European EURL level for elaborating the validation system for the WGS methodology. Following information obtained from the European Reference Laboratory for *E. coli* (Stefano Morabito ECDC National Focal Point Italy, personal communication, February 2021) accreditation of the WGS workflow is, at this time, not yet accomplished. The ISO 23418 standard can be a guidance for the validation of the wet part of the workflow, by treating the sequencing quality parameters and their influences on the downstream applications. For the dry part, specific guidance for standardizing the process is not be expected soon and validation of this process will rely on the classical standards of the ISO 16140 series. To validate the bioinformatic workflow, there are documents, which are being produced by the inter EURL Working group in WGS, established by the European Commission. These documents aim at being a guidance in the different segments of the WGS workflow and there are some under preparation meant to guide the benchmarking of the software, for instance. These documents will be published soon on the EURLs websites and can be found on https://www.iss.it/e.-coli-genomics/-/asset_publisher/. Also research groups are actively developing validation strategies of WGS workflows. Validation of the bioinformatics workflow for the characterization of Shiga toxin-producing *E. coli* isolates has been reported with the aim of routine usage from laboratories operating under a quality system (Bogaerts *et al.,* 2021).

An inquiry of some NRLs of our neighboring countries at the start of 2021 has shown that many NRLs perform WGS analyses for foodborne outbreak investigation. While they participate in working groups and in international proficiency tests, the WGS analyses are seldom accredited. Some NRLs have

indicated that validation studies based on ISO 16140 are planned for specific WGS analyses, such as *Salmonella* serotyping. The Austrian NRL for STEC and *Listeria* has a fully-validated WGS method for the characterization of all STEC and *Listeria monocytogenes* strains isolated during official control programs. In first line agrofood laboratories the interest to introduce this new technology is great, but accreditation is not being pursued at present and this is also not asked in specific national legislations. Nevertheless, the methods are validated internally.

Reference term 6: Requirements – technically and organizationally – to share WGS data in the context of food safety

The "One Health" approach should be pursued. For this purpose, WGS data for different types of isolates (human, food, animal and environment) should be shared. Sharing is possible at the national, European and international level. Ideally data from different sources could be centrally collected. Data can come from government agencies (official controls ,..), clinical reference laboratories and private actors in the agrofood chain.

The establishment of a central database or another system for data exchange (interaction between different databases) is important for outbreak investigation and for improving microbiological food safety governance. Agreements should be made on what (part of the) data will be shared and how this can be used. The question of data ownership should also be addressed. An agreement should be made on the minimum information of metadata to be included in the database. The accompanying metadata should be made as uniform as possible. It should be noted that the methods and pipelines for WGS data analysis are still continuously evolving and improving. As such it is not possible to identify one 'optimal' method and pipeline to be used for WGS.

Sharing WGS data can be performed by centralizing the data nationally, at the Belgian level or directly internationally at the European level.

- Currently, at the national level in Belgium, there is no regular data exchange between the database containing the human isolates and the one containing the agrofood isolates (which contains actually only a limited number of isolates). At present the Belgian WGS data for clinical human isolates are locally stored in laboratory databases and shared on a voluntary basis in public databases such as Enterobase, NCBI and ENA. The WGS data available for the agrofood area are actually in Belgium limited and stored locally by the labs performing the analyses. For both the human clinical data and the agrofood data new infrastructure at the Belgian level will need to be built. This new infrastructure is ideally concepted as a joint database (human and agrofood) or at least as two databases which can communicate and able to query the common data. To realize this approach one organization or a consortium of organizations will have to be established at a national level tasked with the development and maintenance of this novel infrastructure. Clear rules on data ownership will have to be agreed upon to ensure a nationwide participation.
- In case a data sharing system on the national level cannot be developed, the exchange can be exclusively done at the European level. The main drawback of this approach will probably be the limited number of recognized users (NRLs and NRCs) able to access these databases. When there is a Belgium infrastructure set up, exchange of NRL data (official control data) at the European level will be complementary. At the European level EFSA and ECDC received a joint mandate from the

European commission to develop a joint WGS database. ECDC already developed the "Tessy" database for zoonotic isolates in which the National reference centers (NRCs) can already share their WGS data of clinical human isolates. By June 2022, the joint database will be operational, allowing the member states to submit data to either EFSA's WGS database or ECDC's Tessy. Both databases will be able to communicate and available to query the data.

6. Uncertainties

Related to this opinion the most relevant uncertainties are the following:

1. The technological evolution related to the determination of the WGS of bacterial isolates which could create new opportunities for data acquisition and data analysis but also new challenges for data comparison, interoperability and validation of WGS results.
2. Uncertainty remains how the sharing of the databases will evolve and this on the national, European and global level.
3. At this time, it is difficult to estimate when and how far the food business operators will shift to WGS in their self-checking system and how they will be willing to share data, with or without anonymizing information and this in relation to transparency along the agrofood chain.
4. WGS is only collecting information on the DNA sequence, which is, as such, not informative on the phenotypic expression of the genetic information. Interpretation of this information e.g. for serotype prediction, virulence, toxin expression or AMR depend on the information available in the databases. Even when a match is found with genes in the databases, uncertainty remains on the degree of expression and on the biological relevance.
5. Interpretation of the relatedness of strains in outbreak investigation has to be performed with great care because there is a substantial uncertainty on how much genetic differences is needed to considered two strains as different. It is not possible to define a clear threshold for the amount of genetic differences (e.g. SNPs) between strains from a common source. As such WGS-derived data should always be combined with metadata.

7. Conclusion

In this opinion the Scientific Committee has situated the use of WGS for the detection of foodborne outbreaks and bacterial risk assessment and reflected on the implementation of WGS in the Belgian context. In the future WGS will become the preferred method for bacterial food safety investigation, due to its high discriminatory power and the fading out of various older typing methods at an international level. Despite that WGS methods and pipelines for data analysis are still continuously evolving and improving, WGS is ready to start being used in routine outbreak investigation and surveillance activities. The Scientific Committee formulates several recommendations regarding the implementation of WGS in a Belgian context. To facilitate the transition to WGS for the analysis of food isolates, including AMR monitoring, a transition period can be implemented. This offers the labs time to invest in infrastructure, acquire technical expertise and built experience with the WGS methodology and its interpretation. The Scientific Committee advises the FASFC to (gradually) make the transition to WGS for the analysis of food isolates.

However, despite the advantages of WGS, some limitations still need to be taken into account for routine and uniform implementation. Efforts should be made to validate the WGS methodology and to facilitate data sharing. It is recommended that WGS-based results regarding strain comparison in outbreak investigations be interpreted by a multidisciplinary team (microbiologists, molecular biologists, bioinformaticians, epidemiologists) with sufficient expertise. It is also recommended that when using WGS for subtyping strains, as part of an outbreak investigation, validated or internationally recognized WGS methods and bioinformatics tools should be used, and interpreted according to the clonality of the pathogen under consideration and taken into account the epidemiological evidence and the metadata about the strains. These metadata include features such as geolocation data, isolation source, collection date, the organization performing collection, sample and strain names). It is recommended to be vigilant of the correct interpretation and communication on the responsibilities of the different actors (competent authority, FBO, consumer) in case of an outbreak. In this, it is important to communicate that zero risk in relation to bacterial food safety does not exist.

8. Recommendations

The Scientific Committee makes the following recommendations:

- It is recommended to make the transition to WGS for the analysis of the food related bacterial isolates in Belgium, in the (near) future.
    a. As various older typing methods are fading out at an international level, WGS will become the method of choice for strain typing (including *Listeria monocytogenes*, *Salmonella* and Shiga toxin producing *E. coli*) for national surveillance, source attribution and outbreak investigation. For other foodborne pathogens WGS data are also of interest. A transition period can be implemented, so that the labs can invest in appropriate infrastructure, can acquire the necessary technical expertise, set-up a standardized flow for implementation of the WGS method that can be validated and built experience.

    b. As WGS-AMR data can be obtained from the same WGS data used for typing, source attribution and outbreak investigation and that they can complement phenotypic data with extra information related to the genetic markers and be screened retrospectively it is recommended also for AMR monitoring to extract data from the available WGS data collected for other purposes. As it is recently been accepted as alternative method for the specific monitoring of ESBL- or AmpC- or carbapenemase-producing *E. coli* and *Salmonella* and the EURL-AR has elaborated a technical protocol to be followed, it is expected that at EU-level a further transition towards WGS-AMR can be expected.
- Sharing WGS data can be performed by centralizing the data nationally, at the Belgian level or (directly) internationally at the European level. At the Belgian level, for both the human clinical data and the agrofood data new infrastructure will need to be build. This new infrastructure is ideally concepted as a joint database or at least as two databases so that the joint data can be analyzed. By June 2022, the joint European database will be operational with communication between EFSA's WGS database (isolates from agrofood products) and ECDC's Tessy (clinical isolates from humans). The Belgian isolates from the agrofood chain and from human clinical origin will be able to be submitted to the respective databases.

- It is recommended that WGS-based results regarding the comparison of strains in outbreak investigation (e.g. performed by SNP analysis or cgMLST) are interpreted by a multi-disciplinary team (microbiologists, molecular biologists, bio-informaticians, epidemiologists) with sufficient expertise. It is not possible to define a clear threshold for the number of genetic differences between strains from a common source. The WGS-derived data should be combined with metadata informative for the epidemiological interpretation of the outbreak. Furthermore, in order to establish an effective case definition (e.g. threshold) based on WGS, WGS data should be available on the circulating strains. This will provide a population against which clusters of potential outbreak strains can be assessed. Therefore, the current use of WGS during outbreak investigation should be extended to official surveillance programs.
- In order to prevent outbreaks, especially caused by pathogens which are known to be able to persist in the processing environment (e.g. *L. monocytogenes*), it is recommended to investigate their presence in the food processing environment and to follow up the cleaning and disinfection process.
- It is recommended to follow up the process of standardisation and validation of the WGS methodology and this for both the 'wet –lab' and 'dry-lab' part. Also for this technology the performed methods need to safeguard the results conform the criteria defined in the ISO/DIS 23418 (Microbiology of the food chain — Whole genome sequencing for typing and genomic characterization of foodborne bacteria — General requirements and guidance). It is recommended to carefully document the WGS-based results with sufficient metadata, so that, also in the case databases are updated, no information will get lost and traceability to the food source or the food business operator will remain.
- It is recommended to be vigilant of the correct interpretation and communication on the responsibilities of the different actors (competent authority, FBO, consumer) in case of an outbreak. In this, it is important to communicate that zero risk in relation to bacterial food safety does not exist.

For the Scientific Committee,

Dr. L. Herman
Chairwomen
The 22/10/2021

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Presentation of the Scientific Committee established at the FASFC

The Scientific Committee is an advisory body established at the Belgian Federal Agency for the Safety of the Food Chain (FASFC) that provides **independent scientific opinions** on risk assessment and risk management in the food chain, and this at the request of the Chief Executive Officer of the FASFC, the Minister competent for food safety or at its own initiative. The Scientific Committee is administratively and scientifically supported by the Staff direction for Risk Assessment of the Agency.

The Scientific Committee consists of 22 members who are appointed by royal decree on the basis of their scientific expertise in areas related to the safety of the food chain. When preparing an opinion, the Scientific Committee can call on external experts who are not a member of the Scientific Committee. Similar to the members of the Scientific Committee, they must be able to work independently and impartially. To ensure the independence of the opinions, potential conflicts of interest are managed transparently.

The opinions are based on a scientific assessment of the question. They express the view of the Scientific Committee which is taken in consensus on the basis of a risk assessment and the existing knowledge on the subject.

The opinions of the Scientific Committee may contain **recommendations** for food chain control policy or for the stakeholders. The follow-up of these recommendations for control policy is the responsibility of the risk managers.

Questions on an opinion can be directed to the secretariat of the Scientific Committee: Secretariat.SciCom@afsca.be.

Members of the Scientific Committee

The Scientific Committee is composed of the following members:

A. Clinquart, P. Delahaut, B. De Meulenaer, N. De Regge, J. Dewulf, L. De Zutter, A. Geeraerd, N. Gillard, L. Herman, K. Houf, N. Korsak Koulagenko, L. Maes, M. Mori, A. Rajkovic, N. Roosens, C. Saegerman, M.-L. Scippo, P. Spanoghe, K. Van Hoorde, Y. Vandenplas, F. Verheggen, S. Vlaeminck

Conflict of interest

No conflicts of interest were identified.

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Composition of the workgroup

The workgroup was composed of:

Members of the Scientific Committee:	L. Herman (reporter), L. De Zutter, A. Geeraerd, M. Mori, N. Roosens[3], K. Van Hoorde
External experts:	N. Botteldoorn (DGZ), V. Delcenserie (ULiège), J. Mahillon (UCLouvain)[4], B. Verhaegen (Sciensano), M. Uyttendaele (Ugent)[5]
Dossier manager:	K. Feys

The activities of the workgroup were attended by the following members of the administration (as observers): V. Cantaert (FASFC) and B. Pochet (FASFC)

Open consultation

In order to increase the transparency, but without diminishing the independent position of the Scientific Committee, the members of the Advisory Committee were invited to provide their comments on the opinion. The comments received as well as the answer to these comments are included in the Annex to the opinion (Annex 3).

Legal framework

Law of 4 February 2000 on the creation of the Federal Agency for the Safety of the Food Chain, in particular article 8;
The Royal Decree of 19 May 2000, on the composition and operating procedures of the Scientific Committee, as established at the Federal Agency for the Safety of the Food Chain;
The Internal Rules as mentioned in Article 3 of the Royal Decree of 19 May 2000, on the composition and operating procedures of the Scientific Committee, as established at the Federal Agency for the Safety of the Food Chain, approved by the Minister on 8 June 2017.

Disclaimer

The Scientific Committee at all times reserves the right to modify the opinion by mutual consent, should new information and data become available after the publication of this version.

[3] Member of the working group starting from January 2021
[4] Member of the Scientific Committee until January 2021
[5] Member of the working group until October 2020