Community Guide to the Principles of Good Practice for the Microbiological Classification and Monitoring of Bivalve Mollusc Production and Relaying Areas with regard to Implementing Regulation 2019/627

Issue 4, September 2021

REVISION HISTORY

Issue	Date issued	Changes
1	1 June 2012	
2	13 January 2014	New Annex 2: covering requirements for areas approved for export to USA
3	16 January 2017	Glossary: inclusion of a definition of an anomalous result and definition of a holding area. Table 1: Amended to reflect Regulation (EU) 2015/2285 Sections 1, 2.1, 3.3.3, 5.3, 7 & 7.3.1: inclusion of reference to holding areas. Section 5: Changes to reflect Regulation (EU) No. 2015/2285. Section 7: Changes to reflect Regulation (EU) No. 2015/2285. Inclusion of animal waste spillages as reasons to discount anomalous results. Bibliography: removal of references not quoted in main text; inclusion of Regulation 2015/2285; removal of specific versions for standards and technical specifications.
4	September 2021	General review of the "*Community Guide to the Principles of Good Practice for the Microbiological Classification and Monitoring of Bivalve Mollusc Production and Relaying Areas with regard to Regulation 854/2004*" to conform to Implementing Regulation 2019/627

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BACKGROUND

This guide has been revised and updated in order to be adapted to the EU Implementing Regulation 2019/627. This work was carried out under the coordination of the EURLMB together with an advisory group of independent experts in microbiological control, and a working group of experts from several EU Member States with production areas of bivalve molluscs, most of whom had been involved on the elaboration of previous guides for microbiological control of bivalve molluscs production areas.

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DISCLAIMER

This guide is intended to contribute to a better understanding, and to recommend best practices for the application of EU legislation regulating the official controls in production and relaying areas for live bivalve molluscs. This is intended purely as a guidance tool, and it is necessary to take into account that only the text of the Commission Implementing Regulation (EU) 2019/627 has legal force.

GLOSSARY

Alert procedure	An official procedure demanding special control measures to assess the risk after the results of standard monitoring have been found to exceed established thresholds.[2]
Anomalous result	A result that deviates from that expected for a harvesting area for a specific and clearly identified reason that is not likely to recur.[2]
Aquaculture	The rearing or culture of aquatic organisms using techniques designed to increase the production of the organisms in question beyond the natural capacity of the environment, the organisms remaining the property of a natural or legal person throughout the rearing or culture stage, up to and including harvesting.[2]
Bivalve mollusc	Means filter-feeding lamellibranch molluscs, and by extension, echinoderms, tunicates and marine gastropods. [1,3]
Classification of bivalve mollusc harvesting areas	Assignment of harvesting areas to different classes based on an official programme to determine the extent of microbiological contamination in production and relaying areas. The requirements are given in Chapter I, of Title V to Implementing Regulation (EU) 2019/627 .[2]
Classification (established)	An official classification based on results from an extensive number of sampling occasions to ensure that potential seasonal and annual variability has been fully covered.[2]
Classification (initial)	An official classification based on results from a limited number of sampling occasions.[2]
Coliform	Gram negative, facultatively anaerobic rod-shaped bacteria which ferment lactose to produce acid and gas at 37°C. Members of this group normally inhabit the intestine of warm-blooded animals but may also be found in the environment (e.g. on plant material and soil).[2]
Combined Sewer Overflow (CSO)	A system for allowing the discharge of sewage (usually dilute crude) from a sewer system following heavy rainfall. This diverts high flows away from the sewers or treatment works further down the sewerage system and thus avoids overloading of works and flooding of properties, etc.[2]
Competent authority	Means the central authority of a Member State competent for the organisation of official controls or any other authority to which that competence has been conferred; it shall also include, where appropriate, the corresponding authority of a third country.[1]
Emergency overflow (EO)	A system for allowing the discharge of sewage (usually crude) from a sewer system or sewage treatment works in the case of equipment failure.[2]
Enteric viruses	A group of unrelated viruses that have a common characteristic of being transmitted via the faecal-oral route. The group includes norovirus and hepatitis A virus.[2]
β-glucuronidase positive *Escherichia coli*	Bacteria which, at 44 °C, form typical blue or blue green colonies on tryptone bile glucuronide medium (TBX) under the conditions specified in the reference method. [2]

Faecal coliforms	Facultative aerobic, gram-negative, non-sporeforming, cytochrome oxidase negative, rod-shaped bacteria that are able to ferment lactose with gas production in the presence of bile salts, or other surface active agents with similar growth-inhibiting properties, at 44°C ± 0.2°C within 24 hours.[2]
Flesh and intravalvular liquid (FIL)	The muscles, body and organs of a bivalve mollusc together with the liquid contained within the shells when the animal is tightly closed out of the water.[2]
Geographical information system (GIS)	A computer based system that combines mapping and data storage functions in order to store, manipulate, analyse, display and interpret spatially referenced data.[2]
Harvesting Area	The term Harvesting Area is used in this Guide to cover both Production and Relay Areas.[2]
Hepatitis A virus	This is a 27nm diameter virus that contains RNA as its nucleic acid. It is transmitted by the faecal-oral route and although most infections are asymptomatic or mild feverish episodes, it can cause inflammation of the liver resulting in jaundice.[2]
Holding Area	A part of a classified production area (i.e. sea, estuarine or lagoon area) used for the temporary storage of bivalve molluscs between harvest and processing, depuration or dispatch.[2]
Hydrodynamic models	In the context of this guide, numerical models that approximate the flow of seawater, i.e. velocities and water depths as functions of time and space. Output from these models can then be used together with a representation of diffusion processes in the water column (see Particle Transport Models below) to represent the fate and dispersion of bacteria.[2]
Investigative sample	Sample taken during an investigation period typically following a high result or pollution event. [2]
Microbiological survey	Short-term monitoring undertaken in order to help identify the position(s) for sampling point(s) for the classification monitoring programme. This will usually be undertaken at a larger number of points than will be used in the ongoing programme. [2]
Monitoring programme	Activity performed by the competent authority and based on the outcome of the sanitary survey, which involves the application of the sampling plan (number of samples, geographical distribution of sampling points and sampling frequency) to establish the classification and monitoring of a classified production area. It shall also ensure that the results obtained are representative of the area in question. [2]
Norovirus	Noroviruses are small, 27-to 32-nm, structured RNA viruses which have been implicated as the most common cause of nonbacterial gastroenteritis outbreaks.[2]
Official control	Activities performed by the competent authorities, or by the delegated bodies or the natural persons in accordance with the article 2 to Regulation (EU) 2017/625. [2]
Particle transport models	In the context of this guide, particle transport models show the diffusion (spreading) of dissolved or suspended substances in the seawater. These methods may be used to model bacterial concentrations.[2]

Production area	Any sea, estuarine or lagoon area, containing either natural beds of bivalve molluscs or sites used for the cultivation of bivalve molluscs, and from which live bivalve molluscs are taken.[1]
Relaying area	Any sea, estuarine or lagoon area with boundaries clearly marked and indicated by buoys, posts or any other fixed means, and used exclusively for the natural purification of live bivalve molluscs.[1]
Relaying	means the transfer of live bivalve molluscs to sea, lagoon or estuarine areas for the time necessary to reduce contamination to make them fit for human consumption. This does not include the specific operation of transferring bivalve molluscs to areas more suitable for further growth or fattening. [1]
Remote area	An area where no human or animal sources have been shown to impact on the fishery in the sanitary survey and where no potential changes to sources have been identified during the annual review process. The distance from the shore is not in itself a guarantee of no pollution impact. [2]
Representative sampling point	A specified geographical location from which samples are taken to represent either a single, or several, wild bivalve mollusc beds or aquaculture sites. The representative sampling point should reflect the location at highest risk of faecal pollution within the classified area.[2]
Sampler/sampling officer	In the context of this guide, a sampler is a person who takes samples of bivalve molluscs from a harvesting area for the purposes of official control testing under Implenting Regulation (EU) 2019/627. A sampling officer is a sampler directly employed by the competent authority or other control body delegated responsibility for official control sampling.[2]
Sampling plan	The activities carried out according to Article 61 to Implementing Regulation (EU) 2019/627. In practice is a formal record of the intended sampling to be undertaken in a harvesting area with respect to species(s), position of representative sampling point(s) and frequency of sampling. The components of the sampling plan are identified following the sanitary survey.[2]
Sanitary survey	The activities carried out according to Article 56 to Implementing Regulation (EU) 2019/627. In practice is an evaluation of the sources of faecal contamination and their impact in or near a harvesting area together with an assessment of the potential impact of these sources on the microbial status of the harvesting area.[2]
Sewage	A liquid that is or has been in a sewer. It consists of waterborne waste from domestic, trade and industrial sources together with rainfall from subsoil and surface water.[2]
Sewage treatment works (STW)	Facility for treating sewage from domestic and trade premises. Also known as a Wastewater Treatment Plant (WWTP).[2]
Sewer	A pipe for the transport of sewage.[2]
Sewerage	A system of connected sewers, often incorporating intermediate pumping stations.[2]
Shoreline survey	A physical survey of the shoreline and area adjacent to the harvesting area to confirm the presence of potentially contaminating sources identified through a desk-based study and to identify additional potential sources of contamination.[2]

Short-term controls	Control measures taken to reduce or negate any increased risk to public health that might arise from temporary increased contamination of harvesting areas. These controls include prohibition of harvesting, short-term reclassification and increased treatment requirement without reclassification. The control measures should address the public health risk (e.g. from sewage derived pathogens) and not simply the bacterial indicators used for monitoring purposes.[2]

[1] Definition from EU legislation.
[2] Supplementary definition
[3] The requirements of the legislation for bivalve molluscs classification of production areas, also apply to echinoderms, tunicates and marine gastropods.

GENERAL INTRODUCTION

Official controls should be on the basis of written documented procedures with appropriate documented mechanisms to verify continuously that they are effective and consistent, and take corrective action when shortcomings are identified.
The scope of this guidance is to support the Member States in their activities to perform the official controls in primary production for Live Bivalve Molluscs (LBM) and give them an harmonized approach:

1. to verify the compliance of the product according to the Regulation (EU) 2017/625 and Implementing Regulation 2019/627, and

2. for the activities to be carried out in accordance with Regulation (EU) 2017/625 and Implementing Regulation 2019/627.

Seafood can generally be considered to be a safe, healthy and nutritious food. However, consumption of raw or insufficiently cooked filter-feeding bivalve molluscs harvested from faecally contaminated waters may result in illness due to the presence of microorganisms. In the past, bivalve molluscs were associated with typhoid and paratyphoid fevers but these are now rare in developed countries. Bivalve mollusc-associated gastro-enteritis due to non-typhoid, non-paratyphoid *Salmonella* bacteria does occur from time to time but illnesses due to viruses, such as norovirus (causing gastro-enteritis) and Hepatitis A (causing infectious hepatitis) are now the most common infections associated with contaminated bivalve molluscs. Faeces from both humans and animals can be a source of pathogens that may be transmitted to man via contaminated bivalve molluscs. Although human faeces may be seen as presenting a higher risk, several pathogens that infect humans can be present in animal faeces and there is presently insufficient evidence to consider the two sources differently.

An evaluation of the sources and types of faecal contamination (human and animal) in the vicinity of harvesting areas, combined with microbiological monitoring based on the use of indicator organisms (*Escherichia coli* in the EU), provides an assessment of the risk of contamination with bacterial and viral pathogens and is the basis for public health controls.

Article 18 to Regulation (EU) 2017/625 defines specific rules on official controls and for action taken by the competent authorities in relation to the production of products of animal origin intended for human consumption and, by cross-reference, shall include the verification of compliance with the requirements with other Regulation.

In the EU, the responsibility for developing and applying official classification and monitoring programmes lies with the competent authority and the requirements are given in Implementing Regulation (EU) 2019/627 laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council and amending Commission Regulation (EC) No 2074/2005 as regards official controls.

Modernisation of European food and feed law took a further significant step forward with the replacement of Regulation (EC) No 882/2004 on official controls by Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities. The scope of EU food and feed law and its enforcement is summarised in recitals 4–15 of the new regulation which is intended to rationalise and simplify the overall legislative framework and pursue better regulation by integration of the rules applicable to official controls in specific areas. Regulation (EC) No 882/2004 and other Union acts currently governing official controls in specific areas are either repealed or amended by Regulation 2017/625 based on experience gained from their application.

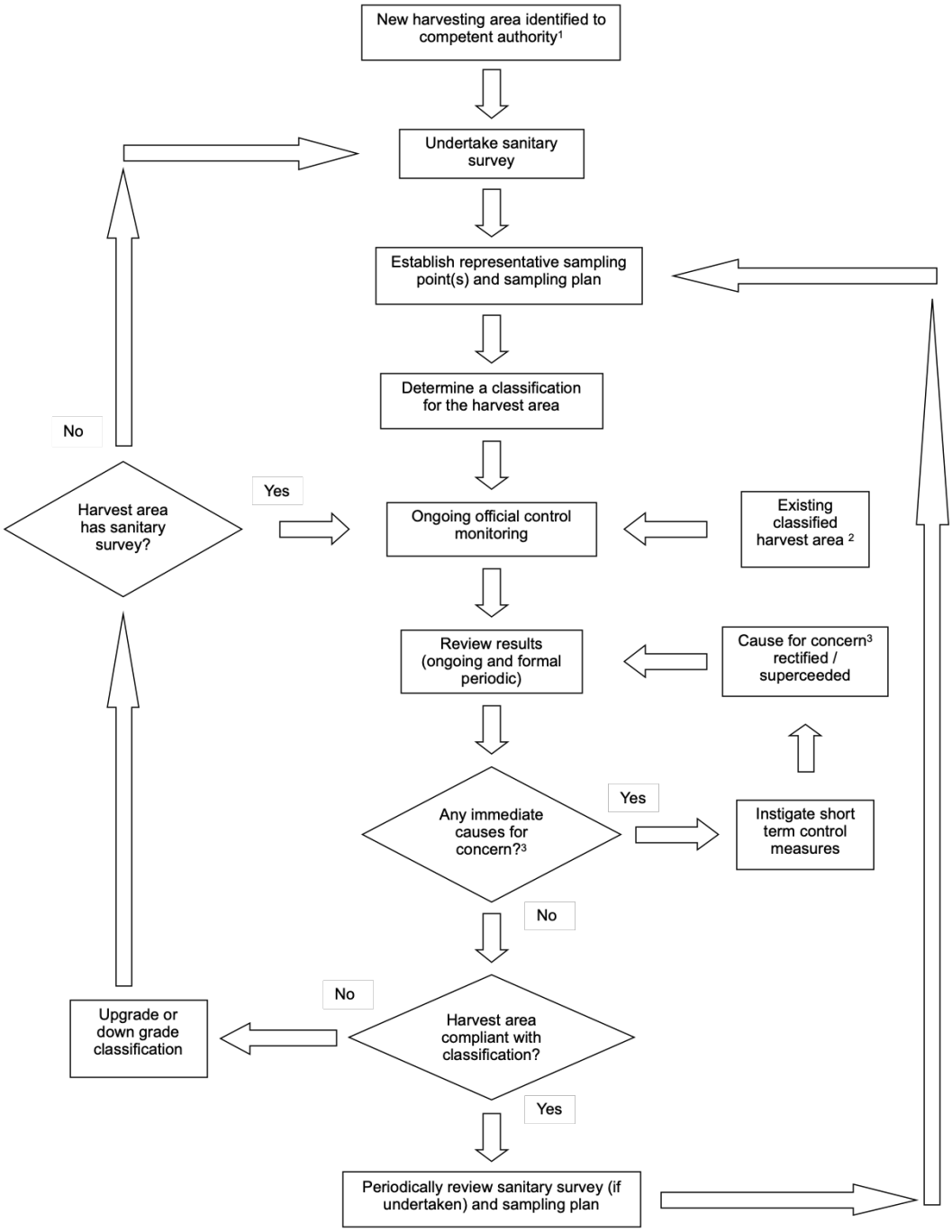
Associated requirements for the industry are given in Regulation (EC) No 853/2004 laying down specific hygiene rules for food of animal origin. The rate of uptake and removal of indicator bacteria (such as *E. coli*) by bivalve molluscs differs from that of some pathogens (particularly viral) and therefore individual *E. coli* results may not give an indication of the general risk of contamination by pathogens. Thus, in common with other food commodities, EU controls rely on preventative systems (Hazard Analysis Critical Control Points) rather than positive release of harvested batches based on microbiological testing. The testing of batches on receipt at a purification or dispatch centre provides an additional check on microbiological quality but does not replace the requirement for a properly implemented official control classification and monitoring programme.

The detailed implementation of classification and monitoring programmes following Implementing Regulation (EU) 2019/627 is the responsibility of competent authorities and may vary between Member States. The Competent authority may delegate specific tasks to a particular designated 'control body' or the natural persons subject to certain guarantees, as specified in Regulation (EU) 2017/625, and notification of the Commission. The key stages for a Competent authority in establishing and operating an Official Control programme for microbiological classification and monitoring of bivalve mollusc production, and relaying areas are given in Figure 1. These stages are further considered in the chapters of this guide. The overall objective of this Community guide is to assist competent authorities in implementing scientifically based programmes for the protection of public health and promotion of intra-community trade within the EU.

This guide is based on available scientific knowledge and experience gained from operating practical monitoring programmes in compliance with Implementing Regulation (EU) 2019/627. The guide will need to be reviewed periodically to benefit from experience with its application and to take into account any additional scientific knowledge or legislative changes. In particular, emerging knowledge on the relationship between the bacterial indicator (*E. coli*) and other relevant pathogens (e.g. norovirus, Hepatitis A virus and *Salmonella* spp) and key elements of monitoring programme design, e.g. prediction of risk, sampling practices, spatial and temporal variability, effectiveness of treatment processes, should be kept under review.

The European Union Reference laboratory for monitoring bacteriological and viral contamination of bivalve molluscs published additional technical guidance (Anon 2018) which may also assist Competent Authorities and other stakeholders (private company, association of producers, etc.).

Figure 1. Overview of key stages in official control programme for bivalve molluscs



[1] By Food Business Operator or other interested party

[2] According to point 2 of article 56 to Implementing Regulation (EU) 2019/627 The competent authorities shall carry out a sanitary survey fulfilling the requirements set out in paragraph 1 in all classified production and relaying areas, unless carried out previously

[3] Human health incidents, pollution events, anomalous results

1. SPECIFIC REQUIREMENTS FOR THE CLASSIFICATION OF PRODUCTION AND RELAYING AREAS FOR LIVE BIVALVE MOLLUSCS

1.1. Introduction

A classification is given to harvesting areas as a result of the official control assessment and this determines whether the areas can be used for harvesting and what level of post-harvesting treatment is needed to reduce the risk of microbial contamination to a level that is regarded as acceptable. Ongoing monitoring determines whether the level of risk has changed and thus whether short-term controls need to be applied or the classification status changed. Specific requirements for the classification of production and relaying areas for live bivalve molluscs are given in articles 53 (class A areas), 54 (class B areas) 55 (class C areas) of Implementing Regulation (EU) 2019/627 (see below).

1.2. Requirement

Implementing Regulation (EU) 2019/627, Title V, Chapter I, “specific requirements for the classification of production and relaying areas for live bivalve molluscs” establish that:

Article 53 Requirements for Class A areas

1. The competent authorities may classify as Class A areas those from which live bivalve molluscs may be collected for direct human consumption.
2. Live bivalve molluscs placed on the market from such areas shall meet the health standards for live bivalve molluscs set out in Chapter V of Section VII of Annex III to Regulation (EC) No 853/2004.
3. Samples of live bivalve molluscs from Class A areas shall not exceed, in 80 % of samples collected during the review period, 230 *E. coli* per 100 g of flesh and intravalvular liquid.
4. The remaining 20 % of samples shall not exceed 700 *E. coli* per 100 g of flesh and intravalvular liquid.
5. When evaluating the results for the fixed review period for maintenance of a Class A area, the competent authorities may, on the basis of a risk assessment based on an investigation, decide to disregard an anomalous result exceeding the level of 700 *E. coli* per 100 g of flesh and intravalvular liquid.

Article 54 Requirements for Class B areas

1. The competent authorities may classify as Class B areas those from which live bivalve molluscs may be collected and placed on the market for human consumption only after treatment in a purification centre or after relaying so as to meet the health standards referred to in Article 53.
2. Live bivalve molluscs from Class B areas shall not exceed, in 90 % of the samples, 4.600 *E. coli* per 100 g of flesh and intravalvular liquid.
3. The remaining 10 % of samples shall not exceed 46.000 *E. coli* per 100 g of flesh and intravalvular liquid.

Article 55 Requirements for Class C areas

1. The competent authorities may classify as Class C areas those from which live bivalve molluscs may be collected and placed on the market only after relaying over a long period so as to meet the health standards referred to in Article 53.
2. Live bivalve molluscs from Class C areas shall not exceed 46.000 *E. coli* per 100 g of flesh and intravalvular liquid.

1.3. Recommendations

In the context of microbial risks arising from human faecal contamination, a key requirement is that production areas must be classified by the competent authorities as Class A, B or C according to the level of *Escherichia coli* present in the molluscs (flesh body and intravalvular liquid) as a marker of faecal pollution. Specific recommendations for anomalous results and how to interpret them are reported in Chapter 7.

Classification is a general categorisation of the microbial contamination status of production areas using the *E. coli* results obtained from the samples of live bivalve molluscs collected over a period of time. The assessment of overall results dictates the subsequent requirements for placing molluscs on the market to manage these microbial risks. The criteria given for classification of production area are described in Implementing Regulation (EU) 2019/627 and, by cross-reference, in the Council Regulation on microbiological criteria for foodstuffs.

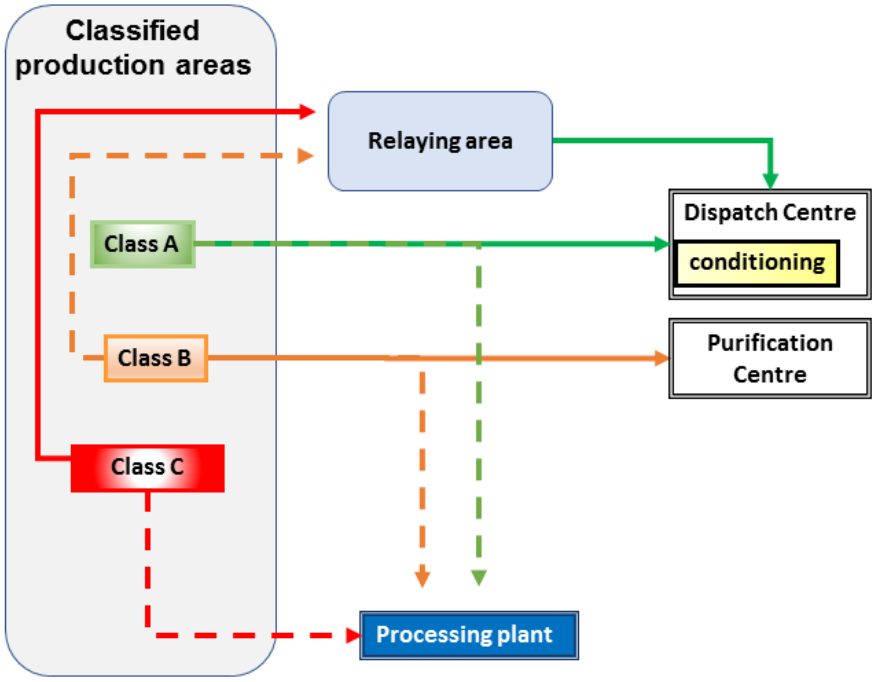
The relationship between Class, microbiological standard and post-harvest treatment required to reduce the microbiological contamination is reported in the Table 1. The figure 2 shows the schematic chain for live bivalve molluscs from primary production to the first establishment.

Table 1. Criteria for the classification of live bivalve mollusc harvesting areas

Class[1]	**Microbiological standard**[2]	**Post-harvest treatment required to reduce microbiological contamination**
A	Samples of live bivalve molluscs from these areas shall not exceed, in 80 % of samples collected during the review period, 230 *E. coli* MPN per 100 g of flesh and intravalvular liquid. The remaining 20 % of samples shall not exceed 700 *E. coli* MPN per 100 g of flesh and intravalvular liquid.[3]	None
B	Live bivalve molluscs from these areas shall not exceed, in 90 % of the samples, 4600 *E. coli* per 100 g of flesh and intravalvular liquid. The remaining 10 % of samples shall not exceed 46000 *E. coli* MPN per 100 g of flesh and intravalvular liquid.[4]	Purification, relaying or cooking by an approved method[6]
C	Live bivalve molluscs from these areas shall not exceed 46000 *E. coli* MPN per 100 g of flesh and intravalvular liquid.[5]	Relaying or cooking by an approved method[6]

[1] The competent authority has the power to prohibit any production and harvesting of bivalve molluscs in areas considered unsuitable for health reasons
[2] Annex IV to Implementing Regulation (EU) 2019/627 report the reference method for enumeration of *E. coli* in live bivalve molluscs. The method shall be the Most Probable Number (MPN) technique specified in EN/ISO 16649-3. Alternative methods may be used if they are validated against the reference method in accordance with the criteria reported in EN/ISO 16140'.
[3] Implementing Regulation (EU,) 2019/627 article 53
[4] Implementing Regulation (EU) 2019/627, article 54
[5] Implementing Regulation (EU) 2019/627, article 55.
[6] Chapter II of Section VII of Annex III to Regulation (EC) 853/2004

Figure 2. Live Bivalve Mollusc flow diagrams from classified production areas to the first establishment



Note: continous arrow: main destination from classified production areas to the first establishment approved according to Regulation 853/2004
discontinous arrow: alternative destination from classified production areas to the first establishment approved according to Regulation 853/2004

1.4. Review

Implementing Regulation 2019/627 states that:

- Article 52(3) "In order to classify production and relaying areas, the competent authorities shall fix a review period for sampling data from each production and relaying area in order to determine compliance with the standards referred to in Articles 53, 54 and 55".
- Article 59 (b) "The competent authorities shall periodically monitor production and relaying areas classified in accordance with Article 18(6) of Regulation (EU) 2017/625 in order to check: the microbiological quality of live bivalve molluscs in relation to the classified production and relaying areas".

This ensures that the basis for determining the class of each classified production area is clear and is based on the results obtained over time.

Recommendations on the steps necessary to meet these requirements are on chapters 2 and 3 of this guide. The frequency of the review, and the period of data used for the review should be explicitly recorded so that the Competent Authority can demonstrate compliance with the requirement.

Recommended sampling frequency for the classification and the alternative methods for analysis of *Escherichia coli* are reported in the annex 1

1.5. Outcome

This chapter explains the requirements that the competent authority shall consider to assign a class to any production area in accordance with articles 52, 53 and 54 to Implementing Regulation (EU) 2019/627. The class assignment is based on the evaluation of the results obtained during the sanitary survey (see Chapter 2), the monitoring of production and relaying areas, and the additional sampling for classification, in accordance with the recommended frequencies and periods reported in Annex 1.

2. SANITARY SURVEYS

2.1. Introduction

Sanitary surveys involve the identification of potential sources of faecal contamination of bivalve mollusc harvesting areas and an assessment of the likely impact of the sources on the microbiological quality of the fisheries. A sanitary survey is the first step in establishing a microbiological monitoring programme for a bivalve mollusc production or relaying area providing an overview of pollution influences and thus a scientific basis for subsequent establishment of representative sampling points and a sampling plan. Faecal contamination may arise from a variety of sources including sewage discharges (continuous or discontinuous), farm animals, wildlife and shipping. The impact will be affected by the amount of dilution of the source in the receiving water and the way that currents take the contamination towards, or away from, the bivalve mollusc fishery(ies).

Faeces from both humans and animals can be a source of pathogens that may be transmitted to man via contaminated bivalve molluscs. Although human faeces may be seen as presenting a higher risk, several pathogens that infect humans can be present in animal faeces and there is presently insufficient evidence to consider the two sources differently.

As much information as possible should be obtained from existing data sets and other government bodies in order to minimize the resources needed. Shoreline surveys should be undertaken in order to determine whether all significant sources of contamination have been revealed by these existing data sets and whether previously identified sources are still present.

The depth of water and currents in an area will affect the extent of dilution of contaminants and also the way that these contaminants will impact on nearby bivalve molluscan shellfisheries. This will markedly influence the level of microbiological contamination of the bivalves and, with regard to currents, how this varies with time (due to tidal and wind effects, etc.). Knowledge of these effects is therefore important in interpreting the information on sources of pollutants obtained for the sanitary survey.

Qualitative or quantitative assessment of the effects of contaminating sources is complicated due to the large number of factors that may modify the impact. Even after undertaking a sanitary survey, it may not be clear where representative sampling points should be located. A time-limited microbiological survey at several potential points may provide such information. Samples need to be taken on a number of different occasions to reflect differing environmental conditions (e.g. spring/neap tidal cycles, periods of wet/dry weather, etc.).

The sources of contamination in an area may change with time, e.g. due to the implementation of sewage improvement schemes, or changes in farming practices, and therefore the information, and consequent recommendations, including the sampling plans, should be subject to periodic review.

2.2. Requirement

Implementing Regulation (EU) 2019/627, Title V, Chapter I, article 56 “Sanitary survey requirement” establish that:

> 1. Before classifying a production or relaying area, the competent authorities shall carry out a sanitary survey that includes:
>
> (a) an inventory of the sources of pollution of human or animal origin likely to be a source of contamination for the production area;
>
> (b) an examination of the quantities of organic pollutants released during the different periods of the year, according to the seasonal variations of human and animal populations in the catchment area, rainfall readings, wastewater treatment, etc.;
>
> (c) determination of the characteristics of the circulation of pollutants by virtue of current patterns, bathymetry and the tidal cycle in the production area.

2. the competent authorities shall carry out a sanitary survey fulfilling the requirements set out in paragraph 1 in all classified production and relaying areas, unless carried out previously.

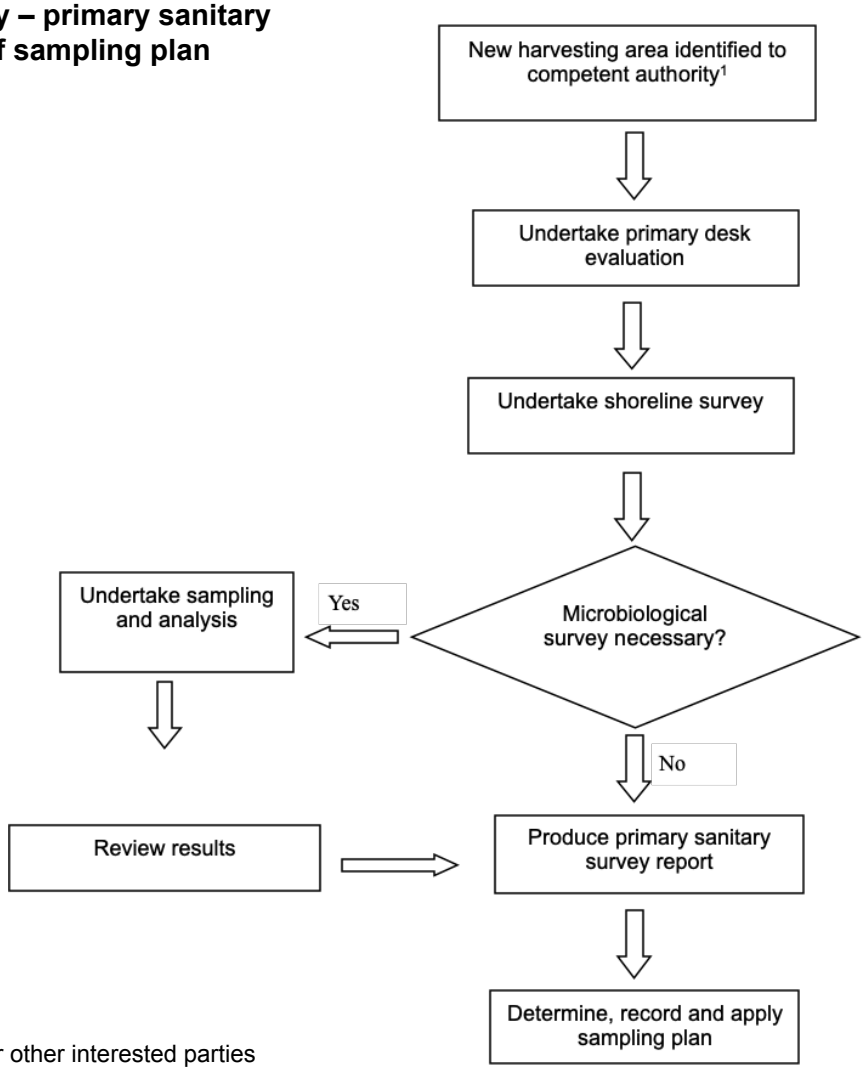
2.3. Recommendations

The requirements in Article 56 to Implementing Regulation (EU) 2019/627 shall apply in all classified production and relaying areas. Producers, and producer affiliated organisations, may assist the competent authority to undertake sanitary surveys.

Figure 1 of Introduction represents the key stages for a Competent authority in establishing and operating an Official Control programme for microbiological classification and monitoring of bivalve molluscs production and relaying areas. Figure 3 represents the key stages for a Competent authority to undertake sanitary survey with as a final result the determination, the recording and the application of a sampling plan.

In the case of an extension of a production area and/or incorporation of new species to the shellfishery, a new sanitary survey is required, or a review/update of an existing survey is required (Fig 4).

Figure 3. Sanitary survey – primary sanitary survey and production of sampling plan



[1] By Food Business Operators or other interested parties

2.3.1. Content of the sanitary survey

It is recommended that the survey comprises the following elements:

- Desk study
- Shoreline Survey
- Bathymetry/Hydrodynamics
- Microbiological Survey

NOTE: Several of these elements may be progressed in parallel which will shorten the time necessary for completion of sanitary surveys

2.3.2. Desk Study

This should address the following topics:

- **Characterisation of shellfishery(ies)**
  - Location and extent
  - Bivalve species for human consumption
  - Aquaculture or wild stocks
  - Growing method (e.g. bottom, trestle, rope, bouchot)
  - Capacity of area
  - Area Destination: production area or relaying area
  - Seasonality of harvest
  - Harvesting techniques
  - Any conservation controls
- **Information on potential sources of pollution**
  - Continuous sewage discharges (Including trade-related discharges with a significant sewage content)
  - Wastewater treatment (eg. primary, secondary, tertiary treatments) discharges
  - Rainfall-dependent sewage discharges (combined sewer overflows or storm tank overflow) and other rainfall-dependent discharges (stormwater discharges)
  - Emergency discharges (e.g. for pump failure at sewage works)
  - Land use
  - Farm animals
  - Wildlife
  - Ships and Boats
  - Any seasonal variations in above factors (e.g. application of manure to land, tourism, farm animals, etc)
- **Information on potential environmental pressure of pollution**
  - Meteorological condition (rainfall readings, thermal amplitude, wind pattern, etc)
  - Hydrography: hydrographic charts, currents and tides (not to be considered if it is justified that the tides do not have a significant impact)
  - Salinity readings (if available)
  - Any other factors pressuring the area

2.3.3. Shoreline Survey

This should aim to:

- Confirm the information obtained on the location and extent of the shellfisheries
- Confirm the information obtained on the location and nature of potentially polluting sources
- Identify additional potential sources of pollution
  - Where possible, samples for *E. coli* analysis should be taken from:
    - ➢ any previously unidentified sewage or surface water discharges
    - ➢ any watercourses discharging near harvesting areas
    - ➢ bivalve molluscs from near the potential impacting sources

Note: not all potential contaminating sources will necessarily be identified during a single survey due to:

- seasonal effects
- rainfall-dependent discharges

NOTE: methods of analysis of freshwater, seawater and sediment are not covered by EU Food legislation.

2.3.4. Hydrography

The level of complexity necessary for this component will depend on local circumstances, including the presence of potentially significant sources of faecal contamination, the proximity of these to the fishery(ies), and the hydrodynamic characteristics of the area.
This component may consist of one or more of the following:

- Reference to hydrographic and bathymetrics charts
- Reference to tidal charts/tidal stream software
- Hydrodynamic modeling
- Alternative or complementary approaches such as
  - dilution estimation (which may include output from dye dosing or salinity studies)
  - tracer studies

NOTE: Further information is available on EU inspire theme register (https://inspire.ec.europa.eu/theme)

2.3.5. Analysis of historical microbiological data

This should be undertaken:

- Where historical microbiological data are available for the immediate vicinity or nearby area. The analysis may inform the overall assessment and recommendations of the sanitary survey but not override other elements of the sanitary survey.
- For this:
  - Geographical and temporal (including seasonal) variation should be considered
  - If sufficient data are available, statistical analyses to consider the effect of environmental factors may be possible.
  - Care should be taken to ensure that analyses inform the outputs of the sanitary survey.

2.3.6. Microbiological survey

This should be undertaken:

- If the best location for one or more representative sampling points for an area is not clear after doing the desk-based study, shoreline survey and historical microbiological data analysis.
  For this:
  - Several potential points should be identified from the results of the desk-based study, shoreline survey and historical microbiological data analysis
  - At least 3 samples are taken from each site at intervals not closer together than fortnightly and tested for *E. coli*.
  - If differences in the concentration of *E. coli* exist in a sampling point along the depths where molluscs are being harvested (e.g. on ropes or bouchots), the depth that generally yields the highest *E. coli* results should be selected.
  - Taking seawater and/or surface sediment samples as well as bivalve mollusc samples may provide additional information.
  - Target sampling towards conditions that are considered to increase the risk of contamination of bivalve molluscs in the specific area (e.g. rainfall, specific tidal conditions).
  - Calculate the geometric mean and ranges of results and record along with the raw data.
  - The sampling point or points showing the highest peak *E. coli* concentrations should be selected for the monitoring programme.
  - Where the peak concentrations are similar, the point or points showing the highest geometric mean *E. coli* concentration should be selected.

2.3.7. Assessment of sanitary survey data

This may be at one of three levels, as appropriate to the data and the local situation:

- *Qualitative assessment*
  - For each potential source, an assessment should be made as to whether it will contribute to the microbial load at the bivalve mollusc fishery
- *Semiquantitative assessment*
  - For each potential source, a relative assessment is made of the contribution it will make to the microbial load at the bivalve mollusc fishery
- *Quantitative assessment*
  - Where the contribution from a source cannot be discounted on the basis of a qualitative assessment
  - Will normally require the use of quantitative modelling

2.3.8. Contents of the Sanitary Survey Report

The report should contain at least the following:

- Characterisation of shellfishery(ies)
  - Location and extent
  - Bivalve species for human consumption
  - Aquaculture or wild stocks
  - Growing method (e.g. bottom, trestle, rope, bouchot) Capacity of area
  - Bathymetry
  - Area Destination: production area or relaying area
  - Seasonality of harvest
  - Harvesting techniques
  - Any conservation controls
- Location, size and treatment level of human sources of contamination
- Location and estimated volume/load of agricultural sources of contamination
- Significant wild animal/bird populations
- Hydrography
- Maps, seasonality effects, for these factors
- Records of shoreline surveys
- Records of microbiological survey results
- Assessment of effect on contamination of shellfish
- The recommended sampling plan (see Chapter 3)
- A recommendation on the extent of the production area (geographic delineation)
- Including any specific considerations relating to impacting sources
- Recommended classification (if sufficient data available – see 7.3.1)

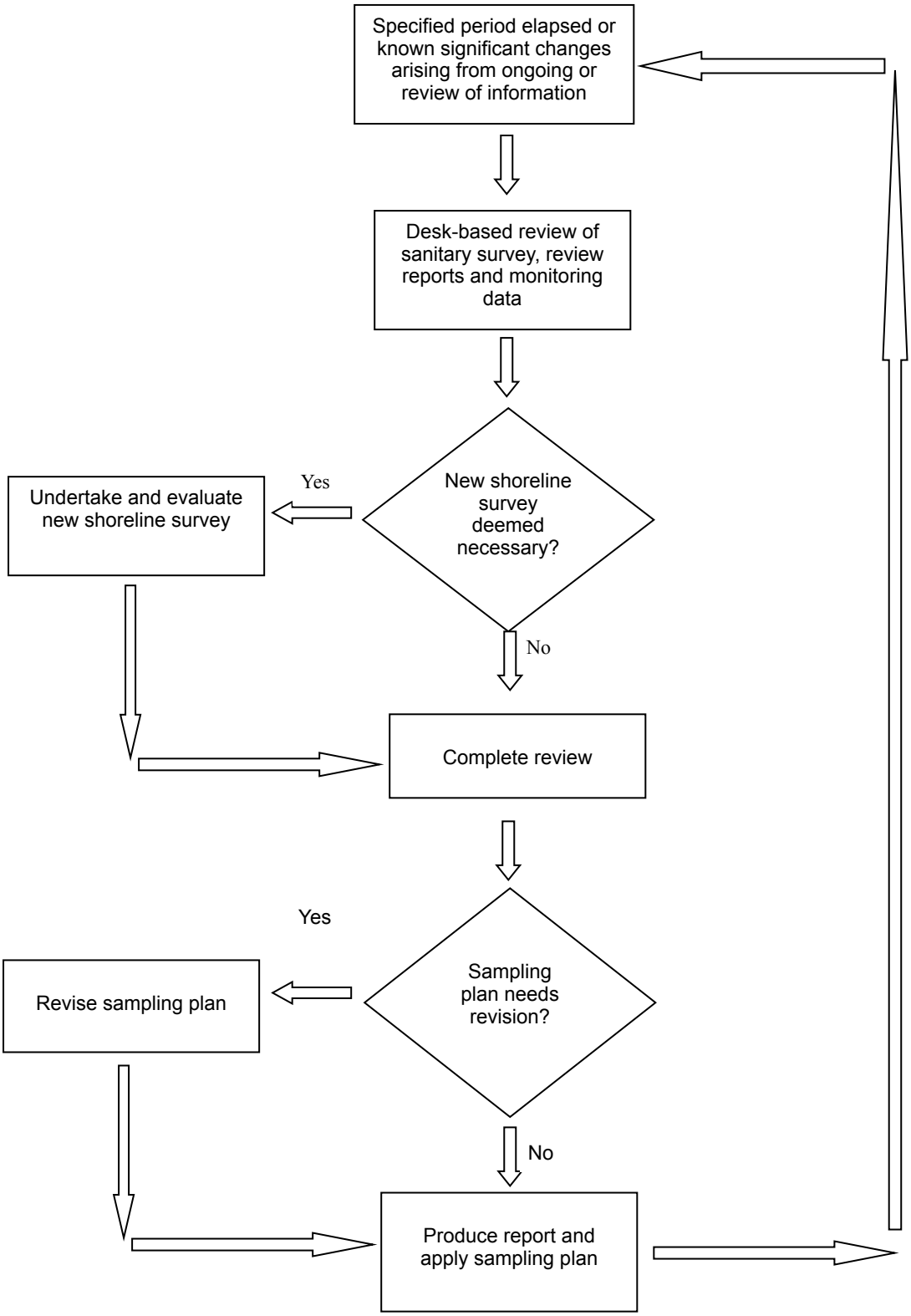
2.4. Review

The content and conclusions of the sanitary survey should be reviewed on a periodic basis (the suggested frequencies are given in Annex 1).
The stages in the review of a sanitary survey are shown in Figure 4.

2.5. Outcome

At the conclusion of this stage the competent authority should have a comprehensive understanding of the proposed harvest area, and the faecal contamination sources impacting the area, and therefore to establish a sampling plan.

Figure 4. Review of sanitary survey and sampling plan



3. ESTABLISHMENT AND RECORDING OF SAMPLING PLANS

3.1. Introduction

Effective public health protection relies on representative results being obtained from microbiological monitoring programmes. Key factors in the design and implementation of an effective programme are the species sampled, the location of representative sampling points (primarily in relation to sources of contamination), the frequency of sampling, timing of sampling (largely in relation to environmental variables) and the way that the data is assessed (period of time, tolerance allowed). Sub-optimal approaches to these variables can lead to unrepresentative datasets and thus inappropriate classification decisions.

The sampling plan constitutes a formal record of the intended sampling to be undertaken in a harvesting area with respect to species(s), position of representative sampling point(s) and frequency of sampling. The components of the sampling plan are identified following the sanitary survey. A number of other items of information, e.g. the responsible authority and the designated sampler(s) also need to be recorded in order to ensure that the sampling plan is put into effect.

Sampling plans are necessarily a balance between the scientific assessment of the requirements necessary to properly reflect the level of microbiological contamination in a harvesting area (with a view to protecting public health) and the practicalities of obtaining, transporting and analysing the samples. This balance has to be taken into account when interpreting the resulting data (see section 7).

All those involved in the microbiological monitoring programme need to be aware of the sampling plans for the part(s) of the programme in which they are involved in order that the work can be carried out properly. This can only be achieved if the plans are formally recorded and made available to those concerned. It also provides the means by which the monitoring actually undertaken can be audited against that which was expected.

3.2. Requirements:

Implementing Regulation (EU) 2019/627, Title V, Chapter I, establishes that:

Article 57. Monitoring programme

The competent authorities shall establish a monitoring programme for live bivalve mollusc production areas that is based on an examination of the sanitary survey referred to in Article 56. The number of samples, geographical distribution of sampling points and sampling frequency for the programme shall ensure that the results of the analysis are representative of the area in question.

Article 58

The competent authorities shall establish a procedure to ensure that the sanitary survey referred to in Article 56 and the monitoring programme referred to in Article 57 are representative of the area considered.

Implementing Regulation (EU) 2019/627, Title V, Chapter II establishes that:

Article 59. Monitoring of classified production and relaying areas

The competent authorities shall periodically monitor production and relaying areas classified in accordance with Article 18(6) of Regulation (EU) 2017/625 in order to check:

(a) that there is no malpractice with regard to the origin, provenance and destination of live bivalve molluscs;
(b) the microbiological quality of live bivalve molluscs in relation to the classified production and relaying areas;

Article 61. Sampling plans

1. For the purposes of the checks provided for in points (b), (c) and (d) of Article 59, the competent authorities shall draw up sampling plans providing for such checks to take place at regular intervals, or on a case-by-case basis if harvesting periods are irregular. The geographical distribution of the sampling points and the sampling frequency shall ensure that the results of the analysis are representative of the classified production or monitoring area concerned.

2. Sampling plans to check the microbiological quality of live bivalve molluscs shall take particular account of:
(a) the likely variation in faecal contamination;
(b) the parameters referred to in Article 56(1).

3.3. Recommendations

The intent of the legislation is to ensure that sampling plans, and thus the resulting microbiological data, are as representative of the area being monitored as possible. The recommendations given below assist competent authorities to meet these requirements through a systematic scientifically based approach.

A sampling plan should consist of the following elements:

3.3.1. Sampling Plan Considerations

The following items need to be addressed within a sampling plan:

- Bivalve species to be sampled
- Selection of location and number of representative sampling points
  - Should be based on the outcome of the sanitary survey
  - For off-shore areas (>5 km from shore) not impacted by point discharges (according to the sanitary survey) random sampling points within the classified area, centroid point or other specified criteria may be used
- Geographical identification of representative sampling points
  - Identify to sufficient accuracy
- Depth of sampling
  - Where relevant (e.g. for bivalves grown on ropes or bouchots)
  - Sample at the depth that yields the highest *E. coli* results
- Sampling frequency
  - Classification (initial)
    - ➢ areas should be monitored at a high frequency[a] that will enable a relatively rapid assessment of classification status
    - ➢ samples should not be taken so close together in time as to produce a correlation between results
    - ➢ sampling should be undertaken over sufficient part of a year to reflect variability associated with short-term and seasonal effects
  - Classification (established)
    - ➢ monitoring should be continued at a high frequency[b] until sufficient data are established on the effects of seasonal variation
    - ➢ ongoing monitoring should be sufficient[c] to detect fluctuating levels of *E. coli*
  - Stable areas
    - ➢ areas defined as stable[d] with regard to their *E. coli* results may be monitored at a reduced frequency
- Seasonality of sampling - where there are clear seasonal patterns to commercial activity
  - Monitoring may be considered for a reduced period of the year
  - Monitoring should start prior to the harvesting season in order to confirm the microbiological status of the area before harvesting commences.
- Time of sampling:
  - With respect to the factors that may affect the microbiological quality of the sample

3.3.2. Timing of sampling of relay areas

- A sufficient period of time should elapse between the depositing of the bivalve molluscs in the relay area and any sampling to allow the animals to take on the microbiological quality of the area.

3.3.3. Recording of sampling plans

Plans should be explicitly recorded and should include the following:

- Production or relay area
- Site Name
- Site Identifier
- Bivalve species
- Geographical location (grid reference and/or latitude/longitude)
- Allowed maximum distance from identified sampling point
- Depth of sampling (if relevant)
- Frequency of sampling
- Responsible authority
- Authorised sampler(s): name(s) and reference number(s)

Other relevant information

3.4. Outcome

At the conclusion of this stage the competent authority should have considered the various practical factors effecting the establishment of a scientifically based sampling plan and should have consolidated these into a formal sampling plan record.

4. SAMPLING AND SAMPLE TRANSPORT

4.1. Introduction

Bivalve molluscs for the official control microbiological monitoring of harvesting areas need to be taken from the designated representative sampling point (as dictated by the sampling plan) and under the appropriate controlled conditions in order to ensure that the results are representative. Depending on the type of bivalve mollusc fishery, sampling may necessitate the use of a boat. Packaging, temperature control during transport, and time between sampling and testing are also important factors. Both sampling and sample transport need to be carefully planned and sufficient resources made available to ensure that the data obtained from the sampling programme is in accordance with the sampling plan.

The recommendations given in this section are intended to ensure that the results obtained from samples are as representative as possible. Sampling and sample transport protocols are an important basis for ensuring the standardisation of these procedures and therefore that the results obtained from the samples are representative of the bivalve molluscs in the harvesting area. In order to ensure that the protocols are applied, they should be available to all involved in the management of the classification and monitoring programme and the taking and transport of samples.

4.2. Requirements

Regulation (EU) 2017/625 states that:

(27) For the performance of official controls aimed at verifying the correct application of Union agri- food chain legislation, and of the other official activities entrusted to Member State authorities by Union agri-food chain legislation, Member States should designate competent authorities which act in the public interest, are appropriately resourced and equipped, and offer guarantees of impartiality and professionalism. Competent authorities should ensure the quality, consistency and effectiveness of official controls.

(28) The correct application and enforcement of the rules falling within the scope of this Regulation require appropriate knowledge of both those rules and the rules of this Regulation. It is therefore important that the staff performing official controls and other official activities are regularly trained on the applicable legislation, in accordance with their area of competence, as well as on the obligations resulting from this Regulation.

(29) To ensure the reliability and consistency of official controls and other official activities across the Union, the methods used for sampling and for laboratory analyses, tests and diagnoses should meet scientific standards, satisfy the specific analytical, testing and diagnostic need of the laboratory concerned, and offer sound and reliable analytical, test and diagnostic results. Clear rules should be established for the choice of the method to be used where more than one is available from different sources, such as ISO, the European and Mediterranean Plant Protection Organization (EPPO), the International Plant Protection Convention (IPPC), the World Organization for Animal Health (OIE), European Union and national reference laboratories, or national law.

And (Title II; Chapter I):

Article 5. General obligations concerning the competent authorities and the organic control authorities

1. The competent authorities and the organic control authorities shall:

(a) have procedures and/or arrangements in place to ensure the effectiveness and appropriateness of official controls and other official activities;

(b) have procedures and/or arrangements in place to ensure the impartiality, quality and consistency of official controls and other official activities at all levels;

(c) have procedures and/or arrangements in place to ensure that staff performing official controls and other official activities are free from any conflict of interest;

(d) have, or have access to, an adequate laboratory capacity for analysis, testing and diagnosis;

(e) have, or have access to, a sufficient number of suitably qualified and experienced staff so that official controls and other official activities can be performed efficiently and effectively;

(f) have appropriate and properly maintained facilities and equipment to ensure that staff can perform official controls and other official activities efficiently and effectively;

4. Staff performing official controls and other official activities shall:

(a) receive, for their area of competence, appropriate training enabling them to undertake their duties competently and to perform official controls and other official activities in a consistent manner;

(b) keep up-to-date in their area of competence and receive regular additional training as necessary; and

(c) receive training in the subject matters set out in Chapter I of Annex II and on the obligations of the competent authorities resulting from this Regulation, as appropriate.

And (Title II; Chapter IV):

Article 34. Methods used for sampling, analyses, tests and diagnoses

1. Methods used for sampling and for laboratory analyses, tests and diagnoses during official controls and other official activities shall comply with Union rules establishing those methods or the performance criteria for those methods.

2. In the absence of the Union rules as referred to in paragraph 1, and in the context of official controls and other official activities, official laboratories shall use one of the following methods according to the suitability for their specific analytical, testing and diagnostic needs:

(a) available methods complying with relevant internationally recognised rules or protocols including those that the European Committee for Standardisation (CEN) has accepted; or

relevant methods developed or recommended by the European Union reference laboratories and validated in accordance with internationally accepted scientific protocols;

(b) in the absence of the suitable rules or protocols, as referred to in point (a), methods which comply with relevant rules established at national level, or, if no such rules exist, relevant methods developed or recommended by national reference laboratories and validated in accordance with internationally accepted scientific protocols; or

relevant methods developed and validated with inter or intra-laboratory methods validation studies in accordance with internationally accepted scientific protocols.

5. Samples shall be taken, handled and labelled in such a way as to ensure their legal, scientific and technical validity.

Implementing Regulation (EU) 2019/627, Chapter III, Article 65 states that:

2. When deciding on the classification, reclassification, opening or closure of production areas in accordance with Articles 52, 62 and 63, competent authorities may take into account the results of checks carried out by food business operators or organisations representing food business operators, only if the laboratory carrying out the analysis is designated by the competent authorities, and the sampling and analysis are performed in accordance with a protocol agreed upon jointly by the competent authorities and food business operators or organisation concerned

4.3. Recommendations

4.3.1. Sampling and sample transport protocols

Sampling officers should be provided with instructions containing the following details:

- The location to be sampled
- The species to be sampled
- The means of sampling
  - Including the avoidance of contamination over that which might be caused by normal commercial practices
- Number and minimum weight of individual animals forming the sample (by species)
- Cleansing of the exterior shells of samples
- Sampling record
  - Including use of sample submission form
- Sample containers and outer packaging to be used
- Recording of air or seawater temperature at the sampling site
- Stipulated method for the use of coolpacks or other means of temperature control
  - To maintain the temperature within the range specified by EN ISO 6887-3 to ensure stability of *E. coli* during transport.
  - The maximum time between sampling and commencement of the laboratory analysis specified by EN ISO 6887-3 to ensure stability of *E. coli*.

4.3.2. Sample submission form

It is important to use appropriate sample submission forms in order to prevent loss of data, and to ensure traceability.

The following should be recorded on the sample submission form:

- Sampling point identification
- Map co-ordinates (grid reference and/or latitude/longitude) of actual sampling location
- Time and date of collection
- Species sampled
- Method of collection (hand-picked, dredged, etc)
- Seawater temperature (or air temperature for intertidal species exposed at time of sampling).
- Any other information deemed relevant (e.g. unusual events, adverse weather conditions etc) should also be recorded
- Wind direction and speed, tide, current direction (if relevant)

An example form is shown in Table 2.

Table 2. Example of a sample submission form

Programme code/description	
Sampler's reference number	
Sampler's name	
Sample reference number	
Date	
Time	
Actual sampling point number	
Actual sampling point name	
Representative sampling point location (grid ref or lat/ long)	
Bivalve species	
Collection method (please circle)	Dredged Hand-picked Hand-raked Diver-gathered Other (please specify)
Tidal Phase (please circle)	Spring Neap
	High Ebb Low Flood
Water temperature (if shellfish covered)	
Air temperature (if shellfish exposed)	
Wind (direction and speed)	
Rainfall in last 48 hours	Yes / No
Observations[1]	
Lab arrival date	
Lab arrival time	
Accepted by lab (if No, please given reason)	Yes / No

[1] e.g. Animals/birds/overflows operating/vessels in area/tourists/etc.

4.3.3. Control of the sampling programme

- All samplers should receive formal training
- Samplers should be provided with relevant sampling and safety equipment.
- Sampling should be audited by means of:
  - An ongoing review of sampling records and
  - A periodic physical audit of sampling and sample transport procedures
  - With the frequency determined on the basis of a risk assessment
- Identified deviations in sampling procedures should be rectified
  - Including retraining of samplers, where necessary

4.3.4. Provision of samples or sample results by industry where authorised by the Competent Authority

- Where officers of the competent authority, or other authorized official bodies, cannot obtain samples
  - Members of the industry may provide them
    - ➢ if all of the requirements for submission by official samplers are met
    - ➢ wherever possible, such sampling is under the supervision of an authorised officer
      - otherwise occasional samples are taken by, or under the supervision of, an authorised officer
    - ➢ if sampling is conducted in accordance with a protocol that has been agreed between the competent authority and the industry
  - Procedures should be instituted to ensure that any possible deviations from protocols are identified at the time of sample submission and not after the laboratory result is known.
- Provision of sample results by the industry.
  - The location(s) and timing of samples should be such as to adequately represent the level of contamination in the area.
    - ➢ assessed with respect to the outcome of the sanitary survey.
  - Sampling and sample transport procedures should conform to protocols issued by the competent authority or Control Body managing the monitoring programme.
  - Sampling and sampling transport procedures should also conform to the guidance given above in Sections 4.3.1 - 4.3.3.
  - Laboratories should be designated by the competent authority.
  - Laboratory analyses should conform to the recommendations given in Section 5 of this guide.
  - A formal procedure should be in place to ensure that all results of samples taken for this purpose are available to the competent authority.

4.3.5. Receipt at the laboratory:

Samples should only be tested if:

- They are transported in accordance with 4.3.1
- The minimum number and weight of flesh of live animals meet the stipulations of the competent authority and the absolute minimum of 10 animals required by Regulation (EU) 2015/2285 and as specified in EN ISO6887-3.
- Samples are received in the specified containers and bags.
- The sample container/bag is adequately labelled.
- The sample is received in a satisfactory condition.
- The sample is accompanied by a completed sample submission form (see 4.3.2).
- The temperature and elapsed time meets the limits specified by the competent authority.

4.4. Outcome

At the conclusion of this stage the competent authority should have put in place robust formally recorded arrangements for the taking of official control samples and for the transporting of these samples to the testing laboratory.

5. MICROBIOLOGICAL TESTING

5.1. Introduction

The quality of analytical results is a critical consideration for official control monitoring programmes and it is necessary to pay particular attention to this aspect to avoid introduction of test bias. EU regulations contain a number of important stipulations concerning the quality framework for official control testing including requirements on methods used, laboratory accreditation, proficiency testing, and appropriate supervision by a reference laboratory. Some of the different methods available for the enumeration of *E. coli* in foodstuffs have been shown to give markedly disparate results when applied to bivalve molluscs. In particular, it is necessary to use a method that gives adequate recovery of marine-stressed bacteria. The use of an inappropriate method may yield inaccurate low results that will lead to a classification that is not sufficiently protective of public health. EN/ISO 16649-3 is the stipulated reference method given in EU legislation for the enumeration of *E. coli* in bivalve molluscs (see below). It is a two-stage, five tube by three dilution MPN method. The first stage of the method is a resuscitation requiring inoculation of minerals modified glutamate broth (MMGB) with a series of diluted bivalve mollusc homogenates and incubation at 37±1°C for 24±2 hours. *E. coli* is subsequently confirmed by subculturing tubes showing acid production onto tryptone bile glucuronide agar (TBGA) and detecting β-glucuronidase activity by the presence of blue or blue-green colonies. EN/ISO 16649-3 cross-refers to EN/ISO 7218 for determination of the most probable number from the combination of positive and negative tubes.

Details of the laboratory method are given in EN/ISO 16649-3. Methods for the preparation of samples can be found in EN ISO 6887-3.

Regulation (EU) 2017/625 identifies that “The designation of Community and national reference laboratories should contribute to a high quality and uniformity of analytical results. This objective can be achieved by activities such as the application of validated analytical methods, ensuring that reference materials are available, the organisation of comparative testing and the training of staff from laboratories.”

5.2. Requirements

5.2.1. Specified method for *E. coli*

Annex IV to Implementing Regulation 2019/627 specifies the reference method for analysis of *E. coli* as “the detection and Most Probable Number (MPN) technique specified in EN/ISO 16649-3” Alternative methods may be used if they are validated against this reference method in accordance with the criteria in EN/ISO 16140. Alternative *E. coli* methods for which the validation has been accepted as satisfactory by the EURL are specified [k,l] in Annex 1 of this guide.

5.2.2. Designation and accreditation of laboratories

Regulation (EU) 2017 /625 states that:

(50) Laboratories designated by the competent authorities to carry out analyses, tests and diagnoses on samples taken in the context of official controls and other official activities should possess the expertise, equipment, infrastructure and staff to carry out such tasks to the highest standards. To ensure sound and reliable results, those laboratories should be accredited for the use of these methods according to standard EN ISO/IEC 17025 on ‘General requirements for the competence of testing and calibration laboratories’. The accreditation should be delivered by a national accreditation body operating in accordance with Regulation (EC) No 765/2008 of the European Parliament and of the Council

And (Chapter IV):

Article 37. Designation of official laboratories

1. The competent authorities shall designate official laboratories to carry out the laboratory analyses, tests and diagnoses on samples taken during official controls and other official activities, in the Member State in whose territory those competent authorities operate or in another Member State or a third country that is a Contracting Party to the Agreement on the European Economic Area.

4. The competent authorities may only designate as an official laboratory a laboratory which:
(a) has the expertise, equipment and infrastructure required to carry out analyses or tests or diagnoses on samples;
(b) has a sufficient number of suitably qualified, trained and experienced staff;
(c) ensures that the tasks conferred upon it as set out in paragraph 1 are performed impartially and which is free from any conflict of interest as regards the exercise of its tasks as an official laboratory; (d) can deliver in a timely manner the results of the analysis, test or diagnosis carried out on the samples taken during official controls and other official activities; and
(e) operates in accordance with the standard EN ISO/IEC 17025 and is accredited in accordance with that standard by a national accreditation body operating in accordance with Regulation (EC) No 765/2008.

The competent authority may delegate specific tasks to a particular designated ‘control body’ subject to certain guarantees in accordance with the Article 28 “Delegation by the competent authorities of certain official control tasks” to Regulation (EU) 2017/625.

5.2.3. Comparative testing and supervision by the national reference laboratory

Laboratories designated for the official control shall take part in inter-laboratory comparative tests or proficiency tests that are organised by the European Union reference laboratory or national reference laboratory according to the Regulation 2017/625.

Regulation (EU) 2017/625 (Title III) states that:

Article 101. Responsibilities and tasks of national reference laboratories

1. National reference laboratories shall, in their area of competence:

(a) collaborate with the European Union reference laboratories, and participate in training courses and in inter-laboratory comparative tests organised by these laboratories;

(b) coordinate the activities of official laboratories designated in accordance with Article 37(1) with a view of harmonising and improving the methods of laboratory analysis, test or diagnosis and their use;

(c) where appropriate, organise inter-laboratory comparative testing or proficiency tests between official laboratories, ensure an appropriate follow-up of such tests and inform the competent authorities of the results of such tests and follow-up;

5.3. Recommendations

In establishing and conducting monitoring programmes for *E. coli* in bivalve mollusc production or relaying (including holding) areas, competent authorities should ensure that the above legislative requirements on laboratories are complied with.

In summary, laboratories must:

- Be designated by the competent authority
- Use the correct method for *E. coli* analysis
- Be accredited for that method
- Participate in proficiency testing for *E. coli* in bivalve molluscs
- Be supervised by the National Reference laboratory

Particular additional specific points to address include:

- The dilution ranges to be used for the MPN test
  - To yield a value rather than a greater than (>) result
- Validation of alternative methods to EN ISO 16140
  - If the reference method, EN ISO 16649-3, is not to be used
- Ongoing checking of accreditation status of laboratories
- Internal quality control procedures
- Determination of measurement uncertainty
- Means of comparative testing
  - Participation in appropriate EQA schemes and NRL ring trials
  - Remedial measures if results are outside target values
- The mechanism for supervision of laboratories by the NRL

5.4. Outcome

By following this guidance the competent authority can be assured that Offical Control samples are tested in accordance with the legislative requirement and thus produce scientifically meaningful data on which to base risk management decisions.

6. DATA HANDLING AND STORAGE

6.1. Introduction

Proper management of the microbiological monitoring programme, and subsequent analysis of the data, requires that the relevant information and results are stored in a secure, well-organised and easily accessible form. In general, the most effective and versatile way to achieve this is in the form of a database. Since much of the information from the programme will have a geographical element programme management can also be assisted through the use of a geographical information system (GIS) preferably linked to the database.

The microbiological monitoring programmes for Member States or Regions with more than a few fisheries will rapidly accumulate large amounts of data. It is important that these data are properly validated and readily accessible to allow assessment and analysis as necessary. The use of a dedicated database, preferably linked to a Geographic Information System to enable proper display of geographical data, will enable these requirements to be more easily achieved.

6.2. Requirements

Storage of laboratory data may be covered by the accreditation body. However, there are no legislative requirements in the EU in relation to the storage of the monitoring programme data itself.

6.3. Recommendations

- Data from the monitoring programme should be stored in a secure database which has tables containing the following:
  - Information on the sampling plans (see Section 3.3.1)
  - Information relating to the samples
  - Results of the testing of samples
- The following may also be considered for inclusion in the database:
  - Results of the sanitary survey
  - Information on pollution events
  - Results of investigations into pollution events and anomalous *E. coli* results
- Access should be password protected and users are individually assigned read only or write permissions according to organisational need.
- Data should be subject to appropriate verification procedures,
- Retrieval of data
  - Sampling plans should be accessible by both harvesting area and sampling point. *E. coli* results should be at least retrievable by sampling point and date range.
- Data audit
  - A traceability system should be introduced so that any changes to data are recorded together with an identifier of the person making the change and the reason therefore.
- Integration with the mapping functions
  - Where a GIS is used instead of hard copy maps, the general content of sampling plans should be available via the mapping functionality
- Web-based data publication
  - May be considered by the competent authority as an effective means of dissemination of relevant information.
- Electronic systems (databases) should incorporate appropriate quality assurance routines to ensure data is verified.

6.4. Outcome

This section assists the competent authority to ensure that data associated with the offical control programme is held in a secure, well-organised and easily accessible form. This greatly assists ongoing risk management activities as well as supporting audit and review processes.

7. INTERPRETATION OF MONITORING PROGRAMME DATA

7.1. Introduction

The microbiological monitoring data generated by the programme (as described in previous sections) is utilised to establish and maintain a classification for the production or relay area. The classification yields an assessment of risk of contamination based on the presence of faecal indicator bacteria and determines the subsequent treatment to which harvested bivalve molluscs must be subjected prior to placing on the market. Classification assessment is based on historical time series data and provides a prediction of that risk of contamination for a period into the future. In this sense, there is no special interest in historical compliance in itself, only its use in predicting the potential future risk. In practice, faecal pollution is likely to vary markedly in the environment and thus between sampling occasions – this variation can occur over a period of hours in areas with fluctuating concentrations of *E. coli* in the contaminating sources, or in areas subject to strong currents or marked rainfall influences. Pathogen occurrence will also vary according to other factors such as relative environmental persistence, occurrence in the community, etc. Consequently, various studies have noted the lack of correlation between individual sample *E. coli* results and pathogen occurrence. Classification should therefore be based on a sufficient number of results obtained over time and a range of environmental conditions to establish a representative classification which can reasonably predict the pollution status of, and thus risk from, future harvested products. For these reasons it is not appropriate to classify areas on a sample-by-sample basis – samples containing low *E. coli* counts from areas previously more polluted cannot be assumed to have a comparable low risk of pathogen occurrence. Conversely, unexpectedly high results may indicate a specific faecal contamination event, and thus elevated risk, and should be proactively investigated and control actions taken if appropriate.

The interpretation of the data needs to take into account characteristics of the area (such as those demonstrated in the sanitary survey) and the influence of environmental conditions such as season and rainfall. Environmental factors tend to increase the variability of the monitoring data. Variability in classification status due to these external factors can be reduced using data sets containing large numbers of results obtained over a longer period of time. Conversely, the use of small data sets, or short periods of monitoring, will tend to increase the variability of classifications based on them.

An outline of the data interpretation process is shown in Figure 5.

7.2. Requirements

Regulation (EU) 2019/627 (Title V) states that:

Article 52. Classification of production and relaying areas for live bivalve molluscs

1. The competent authorities shall fix the location and boundaries of the production and relaying areas that they classify in accordance with Article 18(6) of Regulation (EU) 2017/625. They may, where appropriate, do so in cooperation with the food business operator.

2. The competent authorities shall classify production and relaying areas from which they authorise the harvesting of live bivalve molluscs as Class A, Class B and Class C areas according to the level of faecal contamination. They may, where appropriate, do so in cooperation with the food business operator.

3. In order to classify production and relaying areas, the competent authorities shall fix a review period for sampling data from each production and relaying area in order to determine compliance with the standards referred to in Articles 53, 54 and 55.

And (Chapter I)

Article 53. Requirements for Class A areas

1. The competent authorities may classify as Class A areas those from which live bivalve molluscs may be collected for direct human consumption.

2. Live bivalve molluscs placed on the market from such areas shall meet the health standards for live bivalve molluscs set out in Chapter V of Section VII of Annex III to Regulation (EC) No 853/2004.

3. Samples of live bivalve molluscs from Class A areas shall not exceed, in 80 % of samples collected during the review period, 230 *E. coli* per 100 g of flesh and intravalvular liquid.

4. The remaining 20 % of samples shall not exceed 700 *E. coli* per 100 g of flesh and intravalvular liquid.

5. When evaluating the results for the fixed review period for maintenance of a Class A area, the competent authorities may, on the basis of a risk assessment based on an investigation, decide to disregard an anomalous result exceeding the level of 700 *E. coli* per 100 g of flesh and intravalvular liquid.

Article 54. Requirements for Class B areas

1. The competent authorities may classify as Class B areas those from which live bivalve molluscs may be collected and placed on the market for human consumption only after treatment in a purification centre or after relaying so as to meet the health standards referred to in Article 53.

2. Live bivalve molluscs from Class B areas shall not exceed, in 90 % of the samples, 4 600 *E. coli* per 100 g of flesh and intravalvular liquid.

3. The remaining 10 % of samples shall not exceed 46 000 *E. coli* per 100 g of flesh and intravalvular liquid.

Article 55. Requirements for Class C areas

1. The competent authorities may classify as Class C areas those from which live bivalve molluscs may be collected

2. Live bivalve molluscs from Class C areas shall not exceed 46 000 *E. coli* per 100 g of flesh and intravalvular liquid.

And (Chapter III)

Article 62. Decisions following monitoring

1. Where the results of the monitoring provided for in Article 59 indicate that the health standards for live bivalve molluscs are not met or that there may otherwise be a risk to human health, the competent authorities shall close the classified production or relaying area concerned, preventing the harvesting of live bivalve molluscs. However, they may reclassify a production or relaying area as being of Class B or C if it meets the relevant criteria set out in Articles 54 and 55 and presents no other risk to human health.

2. Where the results of microbiological monitoring show that the health standards for live bivalve molluscs referred to in Article 53 not met, competent authorities may, on the basis of a risk assessment, and only on a temporary and non-recurring basis, permit

continued harvesting without closure or reclassification subject to the following conditions:

(a) the classified production area concerned and all approved establishments receiving live bivalve molluscs from it are under the official control of the same competent authorities;

(b) the live bivalve molluscs concerned are subjected to appropriate restrictive measures such as purification, relaying or processing.

3. The accompanying registration document, as referred to in Chapter I of Section VII of Annex III to Regulation (EC) No 853/2004, shall include all the information concerning the application of paragraph 2.

4. The competent authorities shall establish the conditions under which paragraph 2 can be availed of in order to ensure, for the production area concerned, maintenance of the compliance with the criteria established in Article 53.

Article 63. Re-opening of production areas

1. The competent authorities may re-open a closed production or relaying area only if the health standards for live bivalve molluscs comply once again with the relevant requirements of Chapter V of Section VII of Annex III to Regulation (EC) No 853/2004 and present no other risk to human health.

2. Where the competent authorities have closed a production or relaying area because of the presence of plankton or levels of toxins in live bivalve molluscs that exceed the regulatory limit for marine biotoxins laid down in point 2 of Chapter V of Section VII of Annex III to Regulation (EC) No 853/2004, they may re-open it only if at least two consecutive analytical results separated by at least 48 hours are below the regulatory limit.

3. When deciding whether to re-open a production or relaying area, the competent authorities may take account of information on phytoplankton trends.

4. Where there are robust data on the dynamic of the toxicity for a given area, and provided that recent data on decreasing trends of toxicity are available, the competent authorities may decide to re-open an area with results below the regulatory limit in point 2 of Chapter V of Section VII of Annex III to Regulation (EC) No 853/2004 obtained from a single sampling.

Article 64. Control system

1. The competent authorities shall set up a control system to ensure that products of animal origin harmful to human health are not placed on the market. The control system shall comprise laboratory tests to verify food business operators' compliance with the requirements for the end product, including live bivalve molluscs and any products derived from them, at all stages of production, processing and distribution.

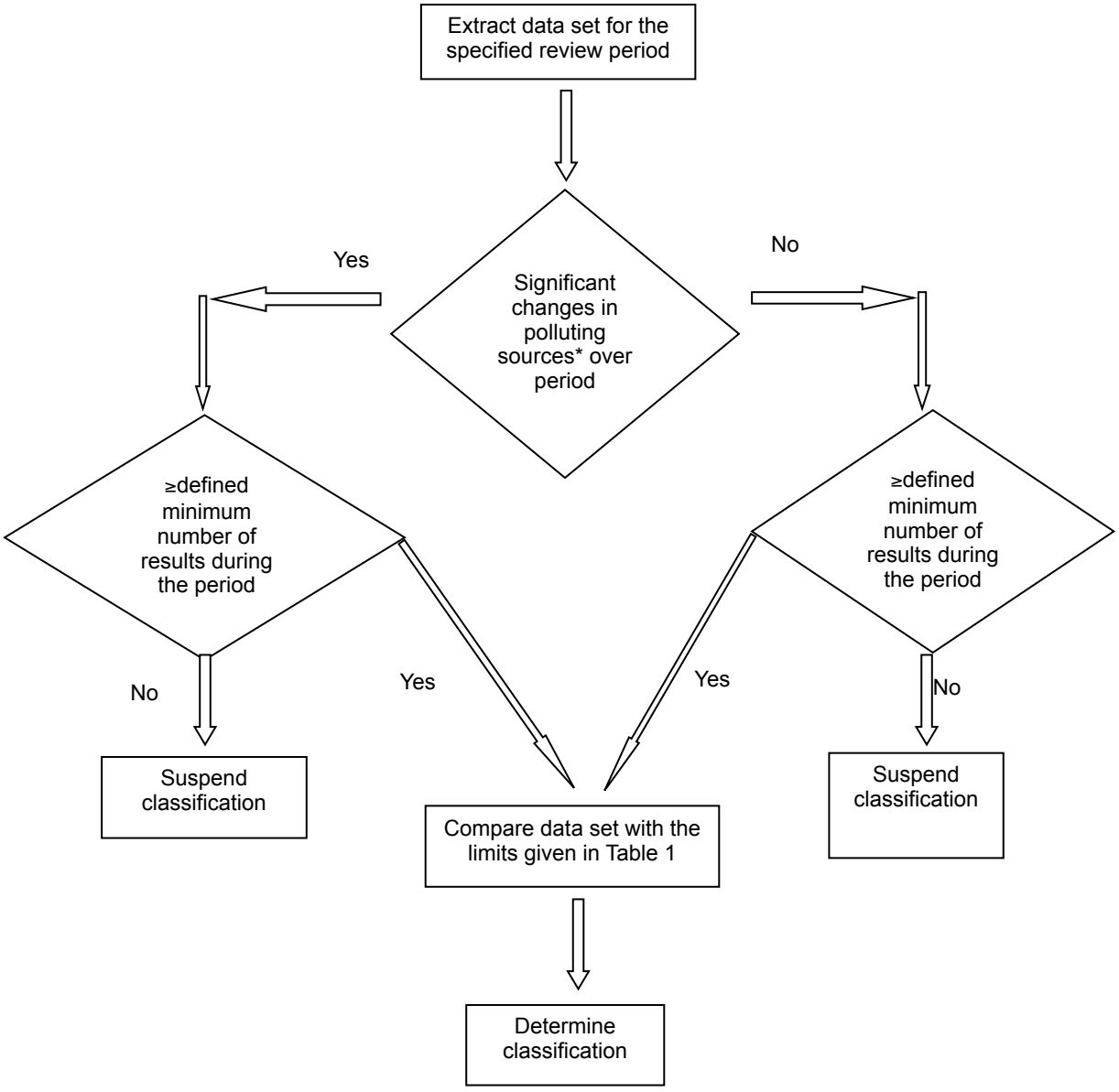
2. This control system shall verify, where applicable, that the levels of marine biotoxins and contaminants do not exceed safety limits and that the microbiological quality of the molluscs does not constitute a hazard to human health.

Article 65. Decision by the competent authorities

1. The competent authorities shall act promptly where a production area must be closed or reclassified, or may be re-opened, or where live bivalve molluscs are subject to the application of measures as referred to in Article 62(2).

2. When deciding on the classification, reclassification, opening or closure of production areas in accordance with Articles 52, 62 and 63, competent authorities may take into account the results of checks carried out by food business operators or organisations representing food business operators, only if the laboratory carrying out the analysis is designated by the competent authorities, and the sampling and analysis are performed in accordance with a protocol agreed upon jointly by the competent authorities and food business operators or organisation concerned.

Figure 5. Data interpretation for classification of harvesting areas



*See Anon 2018 section 7.3.5 for further guidance

7.3. Recommendations:

7.3.1. Interpretation of monitoring programme data

With the aim of establishing and/or maintaining the classification of a production or relay area

- All results should be assessed to determine compliance with the criteria given in Table 1.
- Each separately classified harvesting area should:
  - be defined by specific geographical limits
  - constitute a homogenous area with respect to the fishery and microbiological quality
  - constitute a separately enforceable area
- Review periods should be explicitly defined and recorded:
  - for initial and established classifications
  - specifying the frequency with which data will be reviewed
  - and the period of data to be reviewed.
- Criteria for making an initial classification of a new harvesting area:
  - The competent authority should identify a minimum number of results, and a minimum period of time over which these should be taken, to ensure that an initial classification adequately reflects the microbiological quality of the bivalve mollusc fishery(ies)
  - Bivalve mollusc test results obtained from relevant representative monitoring points during a sanitary survey may contribute towards this data set
- Monitoring following initial classification
  - Data obtained from the sampling should be reviewed on an ongoing basis in order to determine whether the preliminary classification should continue to apply.
  - The competent authority should identify a minimum number of results, and a minimum period of time over which these should be taken, to ensure that an established classification adequately reflects seasonal and environmental effects
- Established classifications:
  - Results from each sampling point should be reviewed on a periodic basis
  - The competent authority should identify a minimum number of results necessary for review and maintenance of the classification
  - The competent authority may identify a lower minimum number of results necessary for continuation of the classification of a harvesting area designated as stable.
  - Where no results are available for sampling occasions identified within the sampling plan, the reasons for the absence of results should be explicitly documented.
  - If significant changes in contaminating sources (e.g. significant known changes in sewage discharge arrangements) have occurred, then only the data obtained since the change(s) should be included in the review.
- Classifications reflecting consistent seasonal variations (‘seasonal’) should, if used:
  - be based on an extended data set of at least 2 years showing clear and consistent differences in the extent of contamination between different periods of the year
  - incorporate an in-built equilibration period, hereafter refered to as ‘buffer period’, prior to the period classified as the least contaminated in order to allow for the natural depuration of pathogens to reflect the new classification. Such buffer period should be of at least two months after a class C period or one month after a class B period. Similarly, a buffer period at the end of the season should be incorporated (i.e. effectively making the harvestable season shorter) to allow for natural temporal shift in the season and variation due to the timing of sampling in the month (i.e. sampling can take place anywhere from the beginning to the end of the month)
- Data assessment
  - For initial, established and seasonal classifications, monitoring data should be assessed against, and be compliant with, the criteria given in the Title V of Regulation (EU) 2019/627.

7.3.2. Single versus multiple representative sampling points in a classified harvesting area

- Single sampling point
  - The classification of the area should be determined on the results from the single point as described above
- Multiple sampling points
  - The results from each point should be assessed as described in 7.3.1
  - If a difference is seen between the points, the classification for a species in an area should be based on the worst classification obtained from all of the sampling points (i.e. the most contaminated) for that species or the indicator species by which it is represented

7.3.3. Anomalous results

Results may be identified as anomalous and excluded from the dataset used for determining classification status if the result is affected by any of the following:

- Failure to comply with the sampling protocols (e.g. temperature or time requirements not complied with) and where the authority responsible for the monitoring programme deems that this may have significantly affected the microbiological result;
  - an additional sample should be included in the sampling plan for the year on a random basis.
  - for this criterion, all results (low as well as high) for samples that failed to meet the requirements of the protocol should be excluded from the dataset.

- Failure of the sewerage or sewage treatment systems that have been rectified and where the authority responsible for controlling pollution identifies that such a failure is not expected to recur.

- Failure of an animal slurry storage facility or other animal waste disposal practices that has been rectified and where the authority responsible for controlling pollution identifies that such a failure is not expected to recur.

Or

- A rainfall event with a return period of 5 years or greater (approximately equal to an event greater than the 99.9%ile value of a long-term daily rainfall data set) where the authority responsible for the monitoring programme deems that this has, or may have, significantly impacted on the microbiological status of the harvesting area.
  - in this case consideration should be given to the taking of further investigative samples and to the imposition of short-term control measures on the harvesting area.

The competent authority should fully document the outcome of investigations and of the risk assessment. Where it is decided that an anomalous result should be disregarded from the classification process the reason for this decision should be clearly documented.

7.3.4. Alert monitoring procedures

An alert procedure should be initiated:

- If the following values are exceeded at a sampling point:
  - Class A: 230 *E. coli*/100 g of F.I.L.
  - Class B: 4600 *E. coli*/100 g of F.I.L.
  - Class C: 46000 *E. coli*/100 g of F.I.L.

This should include the results of own-checks monitoring by the industry at dispatch or purification centres or the results of audit samples taken by the competent authority.

The investigative actions will depend on the magnitude of the result and on the classification status of the area.

- ➢ Results within the compliance tolerance of the classified area (for Class A results of >230 - ≤ 700 and for Class B >4600 – ≤ 46,000 *E. coli*/100 g of F.I.L.).
  - ❖ Compliance with the assigned classification should be checked by review of the results dataset against the defined review period for the area. If the assessment indicates potential or actual non-compliance the Competent Authority should either reclassify the area or instigate an investigation to determine whether the classification is still appropriate.

- ➢ Results exceeding the compliance threshold for the area (for Class A >700, for Class B > 46,000, for Class C >46,000 *E. coli*/100 g of F.I.L.).
  - ❖ The Competent Authority should instigate an alert procedure as soon as the result is known.

- If a pollution event or extreme adverse weather conditions have occurred in an area
- If information is received regarding the association, or possible association, of the harvesting area with an outbreak of illness
- If end-product failures suggest gross contamination of a harvest area

or:

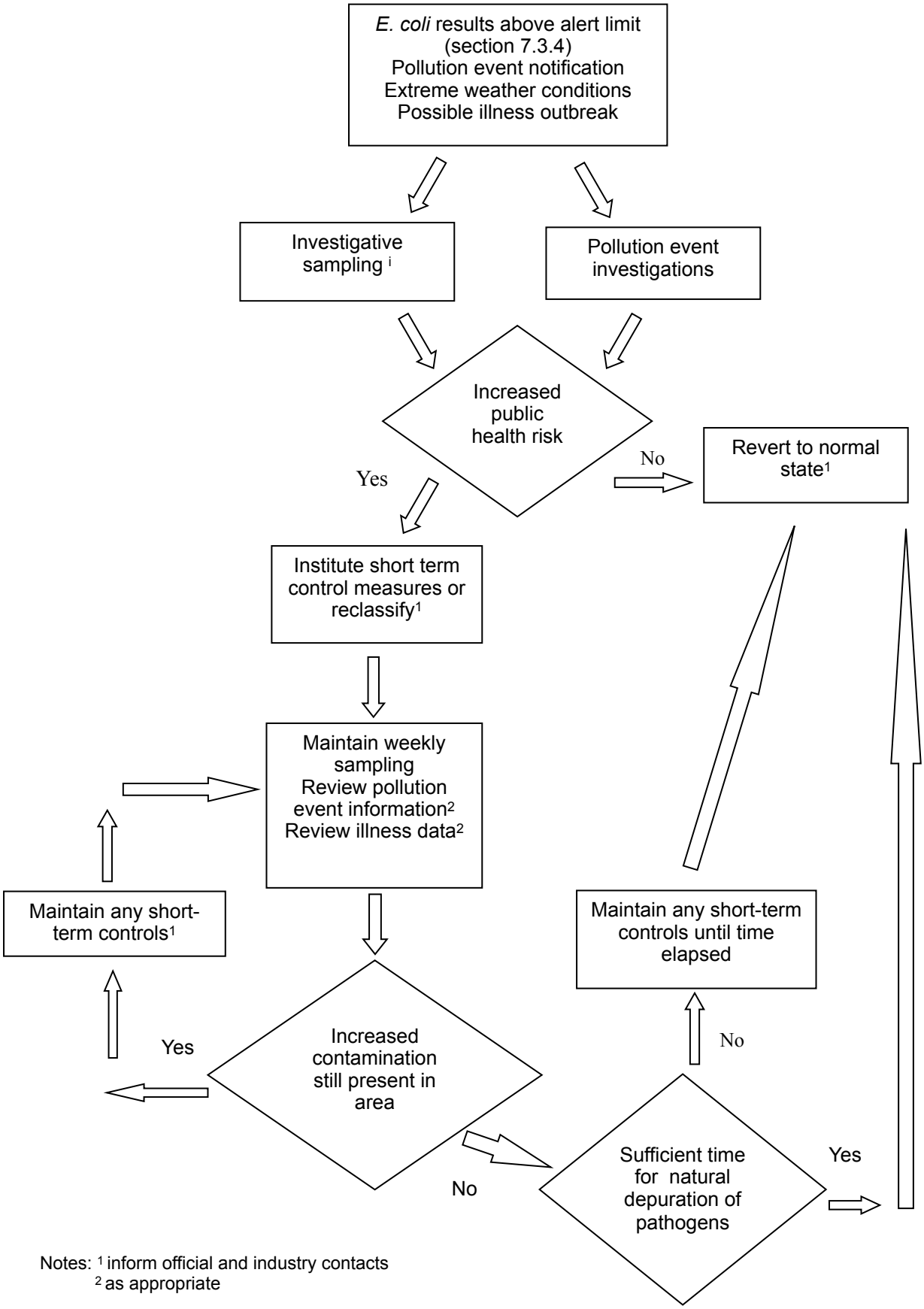
- If a pollution event or extreme adverse weather conditions have occurred in an area
- If information is received regarding the association, or possible association, of the harvesting area with an outbreak of illness
- If end-product failures suggest gross contamination of a harvest area

The alert procedure should involve:

- Conduct a risk assessment to determine the need for short-term controls (e.g. harvest area closure) to protect public health
- Instigate pollution event investigations
- Immediate follow up investigative sampling and, depending on the results, further sampling at a minimum of weekly frequency to determine whether a contamination event persists
- An investigation to determine if the sample result may be anomalous
- A review of the classification status of the area informed by the above investigations
- Consideration of short-term controls to protect public health
- Notification of relevant official and industry bodies at the national, regional and local level.

Figure 6 shows an example flow diagram of an alert procedure.

Figure 6. Alert monitoring procedures - example flow diagram



Notes: [1] inform official and industry contacts
[2] as appropriate

7.4. Outcome

This section assists the Competent authority in reviewing Offical Control monitoring data and in determining a scientifically robust classification or necessary change of classification. It also covers implementation of additional short term controls in the event of unusual high results, pollution events or human health incidents.

8. SUMMARY

The recommendations given in this Guide form the framework for a systematic scientifically based official control microbiological monitoring programme for bivalve mollusc harvesting areas in accordance with Regulation (EU) 2019/627. This Community level guidance for competent authorities is intended to help ensure that Member State programmes provide equivalent levels of public health protection and facilitate free trade within the EU. The former European Reference Laboratory for monitoring bacteriological and viral contamination of bivalve molluscs published additional technical guidance (Anon 2018; downloadable from https://www.cefas.co.uk/media/jyzhl1si/good-practice-guide-issue-7.pdf) which provides further practical assistance on meeting the principles established in this guide. Competent authorities and other organisations involved in the application of microbiological monitoring programmes for bivalve molluscs can seek additional advice from the respective National Reference Laboratory (see https://www.aesan.gob.es/en/CRLMB/web/home.html for NRL contact details).

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ANNEX 1. RECOMMENDED FREQUENCIES, PERIODS AND ALTERNATIVE *E. COLI* METHODS

Text note	Recommended frequency
a	For initial classification of an area – at least 12 samples taken from each sampling point over at least a 6month period with the interval between two sampling occasions being not less than 2 weeks. If the sanitary survey determines no significant sources of pollution (remote area) – at least 6 samples taken over at least a 3 month period with the interval between two sampling occasions being not less than 1 week. If harvesting occurs only during a restricted and enforceable period then the above sampling can be confined to this period immediately before harvesting (2 months prior for class C, 1 month prior for class A and B)
b	Following initial classification – areas should be monitored at least fortnightly until a full year data is available (including data used for establishment of the initial classification). Alternatively, monthly monitoring should be supplemented with additional sampling targeted at worse case events (e.g. high rainfall, storm events, high river flows). For harvesting occurring only during restricted and enforceable period see also note a (above).
c	Ongoing monitoring – at least monthly for sites with <3 years data. For harvesting occurring only during a restricted and enforceable period see note a (above).
d	Stable areas – for sites that have >3 years data, and are considered stable (see Anon 2018), frequency may be reduced to each 2 months. For harvesting occurring only during a restricted and enforceable period see note a (above).
e	Initiation of sampling in relay areas – not before 2 weeks following deposition of animals.
f	Minimum review dataset for maintenance of classification – at least 24 results for a 3 year period (or pro rata for shorter periods).
g	Minimum review dataset for maintenance of classification for areas designed as stable – at least 12 results over a 3 year period (or pro rata for shorter periods).
h	In built equilibration (relay) period for seasonal classifications – 2 months for class C moving to class B, 1 month for class B moving to class A.
i	Investigative monitoring – at least weekly monitoring is recommended.
k	Impedance method: EURL generic protocol - Enumeration of *Escherichia coli* in live bivalve molluscan shellfish by the direct impedance technique using Bactrac 4300 series analyser. Current issue. https://www.iss.it/documents/5430402/0/E+coli_enumeration-BacTrac_impedance_+technique_v1.pdf/8d4555fe-c8ba-8c4a-5b7a-86cfe134c986?t=1623939678855
l	Colony count method: EURL generic protocol - Enumeration of *Escherichia coli i*n bivalve molluscan shellfish by the colony count technique (based on ISO 16649-2). Current issue. https://www.iss.it/documents/5430402/0/E+coli_TBX_16649-2.doc/08fc3edc-6e4c-6654-ff14-aa4e796f8fb0?t=1623940298343

ANNEX 2. ADDITIONAL REQUIREMENTS FOR PRODUCTION AREAS FROM WHICH LBMS ARE HARVESTED FOR EXPORT TO THE USA

A2.1 Introduction

The EU and the USA have agreed official terms for reciprocal trade of live bivalve molluscs under the respective legal framework of the European Regulations (as specified throughout this Guidance) and the USA National Shellfish Sanitation Programme (FDA, 2013). The trade agreement relates to specific EU and US production areas designated, listed, and agreed between, the authorities of DG Sante of the EU Commission for EU areas, and the US Food and Drug Administration (US FDA), for US areas. In agreeing this trade both EU and US authorities have required some additional guarantees to ensure compliance with various aspects of the receiving blocks legislation. This annex sets out the additional requirements for bivalve molluscs produced in the EU and exported to the USA under this trade agreement. The additional requirements for US products exported to the EU are set out elsewhere. In relation to protection against faecal pollution the US FDA has required additional guarantees which have been agreed by both the EU Commission and Member States wishing to export. The additional guarantees are that all live bivalve molluscs exported to the USA from the EU will have:

1. Originated from a specifically listed and agreed production area;
2. The listed production area of established year-round Class A status with a minimum data set of 24 samples to establish and maintain the classification;
3. All aspects of the guidance set out in both this Community Guidance and the Guide to Good Practice: Technical Application (Anon 2018), including a full sanitary survey, will have been implemented for the listed production areas;
4. The additional requirements listed in this annex regarding buffer zones will have been implemented prior to any exports from listed areas.

It should be noted that the US FDA have indicated that they would intend to perform an on-the-spot audit of listed production areas, to check compliance with the above requirements, prior to accepting exports.

This annex sets out the additional requirements regarding item 4: buffer zones. In keeping with the general principles adopted in community guidance this Annex outlines the requirements while detailed technical recommendations as to how to comply with those requirements are given in annex 5 of the Guide to Good Practice: Technical Application (Anon 2018). The US FDA has agreed the text of both this annex and annex 5 of the Technical Guide.

Buffer zones around point source inputs of human wastewater (such as sewer pipes or marinas), where harvesting is not permitted, are an explicit requirement of the US National Shellfish Sanitation Programme Manual of Operations (NSSP MO) (FDA, 2013). Their designation is a preventative public health measure principally aimed at protection against contamination of molluscs with human enteric viruses such as norovirus and hepatitis A virus. Their designation reflects the fact that routine faecal indicator monitoring cannot necessarily be relied upon to indicate the public health risk in such circumstances - particularly where the discharge is of treated effluent. It is well established that faecal indicator bacteria have different survival characteristics to enteric viruses both during sewage treatment processes and in the marine environment. Such buffer zones are not currently an explicit requirement of EU legislation but may be considered to be covered by the general provision in Reg. (EU) 2019/627 (Title V, chapter III, Article 62: C.1) that ‘where the results of sampling show that the health standards for molluscs are exceeded, or that there may be otherwise a risk to human health, the competent authority must close the production area concerned, preventing the harvesting of live bivalve molluscs’.

A2.2 Requirement for buffer zones around wastewater discharges

The US legal requirement for buffer zones around wastewater discharges that the US FDA will audit against is set out in the NSSP MO (FDA, 2013) Section II, Chapter IV .03E(5) as follows:

(5) Wastewater Discharges.

(a) An area classified as prohibited shall be established adjacent to each sewage treatment plant outfall or any other point source outfall of public health significance.

(b) The determination of the size of the area to be classified as prohibited adjacent to each outfall shall include the following minimum criteria:
(i) The volume flow rate, location of discharge, performance of the wastewater treatment plant and the bacteriological or viral quality of the effluent;
(ii) The decay rate of the contaminants of public health significance in the wastewater discharged;
(iii) The wastewater's dispersion and dilution, and the time of waste transport to the area where shellstock may be harvested; and
(iv) The location of the shellfish resources, classification of adjacent waters and identifiable landmarks or boundaries.

By default, the buffer zone calculations are based on an assumption of failure conditions of the discharge (e.g. failure of treatment at a sewage treatment plant). If the buffer zone is sized according to the protection afforded by treated effluent (e.g. from a sewage treatment plant) then there must also be a formal written 'management plan'. The legal requirement is set out in the NSSP MO (FDA, 2013) Section II, Chapter IV @.03 C(2)(a) as follows:

(2) Management Plan Required. For each growing area, a written management plan shall be developed and shall include:

(a) For management plans based on wastewater treatment plant function, performance standards that include:
(i) Peak effluent flow, average flow, and infiltration flow;
(ii) Bacteriological or viral quality of the effluent;
(iii) Physical and chemical quality of the effluent;
(iv) Conditions which cause plant failure;
(v) Plant or collection system bypasses;
(vi) Design, construction, and maintenance to minimize mechanical failure, or overloading;
(vii) Provisions for monitoring and inspecting the waste water treatment plant; and
(viii) Establishment of an area in the prohibited classification adjacent to a wastewater treatment plant outfall in accordance with §E. Prohibited Classification;

(b) For management plans based on pollution sources other than waste water treatment plants:
(i) Performance standards that reliably predict when criteria for conditional classification are met; and
(ii) Discussion and data supporting the performance standards.

A2.3 Requirement for buffer zones around marinas

The US legal requirement for buffer zones around marinas that are adjacent to shellfish growing areas is set out in the NSSP MO (FDA, 2013) Section II, Chapter IV @.05 Marinas as follows:

@.05 Marinas.

A. Marina Proper. The area within any marina which is in or adjacent to a shellstock growing area shall be classified as:

(1) Conditionally approved;
(2) Conditionally restricted; or
(3) Prohibited.

B. Adjacent Waters. Waters adjacent to marina waters classified under §A. may be impacted by pollution associated with the marina.

(1) A dilution analysis shall be used to determine if there is any impact to adjacent waters.
(2) The dilution analysis shall be based on the volume of water in the vicinity of the marina.
(3) The dilution analysis shall incorporate the following:
(a) A slip occupancy rate for the marina;
(b) An actual or assumed rate of boats which will discharge untreated waste;
(c) An occupancy per boat rate (i.e., number of persons per boat);
(d) A fecal coliform discharge rate of 2 x 10^9 fecal coliform per ninth power per day; and
(e) The assumption that the wastes are completely mixed in the volume of water in and around the marina.
(4) If the dilution analysis predicts a theoretical fecal coliform loading greater than 14 fecal coliform MPN per 100 ml, the waters adjacent to the marina shall be classified as:
(a) Conditionally approved;
(b) Restricted;
(c) Conditionally restricted; or
(d) Prohibited.
(5) If the dilution analyses predicts a theoretical fecal coliform loading less than or equal to 14 fecal coliform MPN per 100 ml, the waters adjacent to the marina may be classified as:
(a) Approved; or
(b) Conditionally approved.
(6) If the Authority chooses not to determine a specific occupancy per boat rate by investigation in specific areas or sites, the Authority shall assume a minimum occupancy rate of two persons per boat.

A2.4 Recommendation

According to the agreements concluded between DG Sante of the EU Commission and the US FDA during trade negotiations Competent Authorities of EU Member States exporting bivalve molluscs to the USA are required to:

- Designate and delineate the area intended for export
- Conduct a sanitary survey of the designated area according to the guidance contained both here (chapter 2) and in the Guide to Good Practice: Technical Application (Anon 2018)
- Operate sampling, testing and classification procedures in accordance with this guide and also with the Guide to Good Practice: Technical Application (Anon 2018)
- Establish buffer zones around point sources of waste water discharges impacting the designated area as identified in the sanitary survey.
- Buffer zones should be sized according to the dilution required to meet a bacteriological standard of 14 faecal coliforms or *E. coli* per 100ml of water, according to a theoretical calculation, at the nearest point of the designated zone
  - as a default calculations should be based on the worst case loadings i.e. for untreated effluents in the case of sewage treatment plant discharges
  - if it is instead desirable to perform dilution calculation loadings for treated effluents then:
    - ➢ a management plan must be established detailing how risk management actions will prevent product being exported in the event of treatment failure

  - ➢ the buffer zone must incorporate either a minimum effluent dilution of 1:1000 in all cases, or;
  - ➢ utilise other documented measures that provide equivalent levels of protection against enteric viruses (for example direct viral assessment)
- Exclude marinas from any area designated for export
- Establish buffer zones in waters adjacent to marinas according to the same principles as used for waste water discharges (theoretical calculation to meet a 14 faecal coliforms/*E. coli* per 100ml of water standard)
- Demonstrate that the designated area meets the criteria for permanent class A classification in bivalve mollusc flesh using a minimum data set of 24 samples to establish and maintain the classification

Further technical details on establishing buffer zones according to the requirements of USA legislation (including permitted calculation assumptions) are set out in annex 5 of the Guide to Good Practice: Technical Application (Anon 2018).

A2.5 Outcome

At the conclusion of this stage the competent authority should have designated and delineated areas compliant with trade agreements for export to the USA. These will have been subject to a sanitary survey and sampled and classified in accordance with Community guidance. In addition buffer zones will have been established around point source human wastewater inputs, and around marinas, such that all areas of the harvest area designated for export to the USA can be demonstrated to be compliant with the US water standard for approved areas. If necessary this will include the establishment of management plans setting out the procedures for control of pollution where buffer zones have been established conditional on such controls.

A2.6 Reference

Anon 2018. Microbiological Monitoring of Bivalve Mollusc Harvesting Areas - Guide to Good Practice: Technical Application. Issue 6. Available at https://www.cefas.co.uk/media/jyzhl1si/good-practice-guide-issue-6.pdf

FDA, 2013. National Shellfish Sanitation Program (NSSP): Guide for the Control of Molluscan Shellfish. 2011 Revision. U. S. Department of Health and Human Services, Public Health Service, Food and Drug Administration.