Food and Agriculture Organization of the United Nations

Cefas

VIRTUAL REGIONAL WORKSHOP ON BIVALVE MOLLUSCS SANITATION

9, 10, 11 December 2020

Laboratories - Sample collection, transport, analysis and quality of test results

Louise Stockley

Introduction

- Monitoring a harvesting area provides evidence for the presence of, and concentration of faecal indicators and/or specific hazards in the growing area
- Monitoring data used to inform classification, should be of the highest quality
- Controlling the collection, transport and analysis of a sample is essential, as well as being able to demonstrate the quality of the test results

Sample collection – Local authorities responsibility

- Provide protocols for sample collection and transport requirements
- Provide training to Sampling Officer in the relevant sampling techniques
- Specify the location of the sampling point (SP)
- Carry out periodic audits to ensure protocols are adhered too

Example protocol

Centre for Environment Fisheries & Aquaculture Science

Food Standards Agency food.gov.uk

Protocol for the Collection of Shellfish under the Microbiological Classification Monitoring Programme (EU Regulation 627/2019)

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13 Pages

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Sampling of bivalve molluscs

- Collect in same way as commercial sampling
- Ensure shellfish are alive, healthy and of a commercial size
- Select shellfish at random to avoid bias from environmental factors

SCIENTIFIC NAME	COMMON NAME (ENGLISH)	NUMBER
Pecten maximus	Atlantic great scallop King scallop	12 – 18
Aequipecten opercularis *Chlamys (Aequipecten) opercularis (Linnaeus)*	Queen scallop	18 – 35
Crassostrea gigas	Pacific oyster	12 – 18
Ostrea edulis	European flat oyster Flat oyster	12 – 18
Mercenaria mercenaria	northern quahog = Hard clams	12 – 18
Tapes philippinarum	Manila clam	18 – 35
Ruditapes decussatus	Grooved carpet shells	18 – 35
Spisula solida	Thick trough shells	35 – 55
Mya arenaria	Sand gapers	12 – 18
Ensis spp.	Razor clams	12 – 18
Mytilus spp.	Mussels	18 – 35
Cerastoderma edule	Cockles	35 – 55
Donax spp.	Bean clams	40 – 70

Do not immerse shellfish once collected

Wash to remove mud and debris

Place shellfish in 2 heavy duty bags

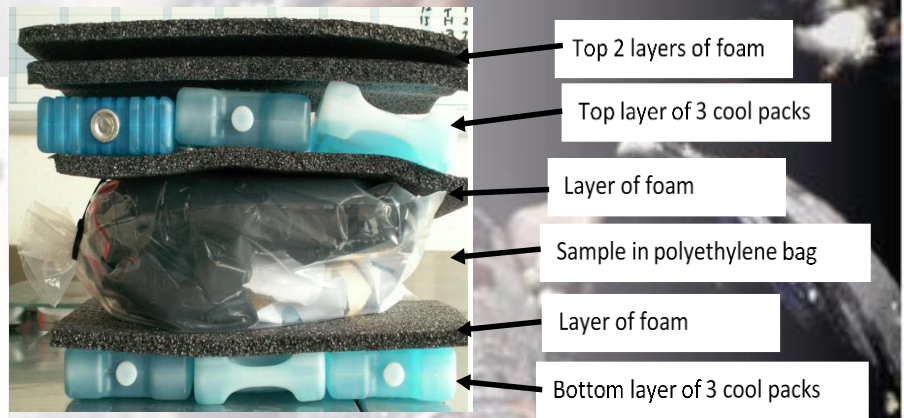
Sampling of water

- Collect water before shellfish or sediment samples to reduce sediment disturbance
- Use a sterile glass or plastic bottle
  - Bags can be used for transporting liquid
- Take sample from middle of water column
  - Sampling pole can be used to collect sample
  - Immediately replace lid tightly to prevent leaks

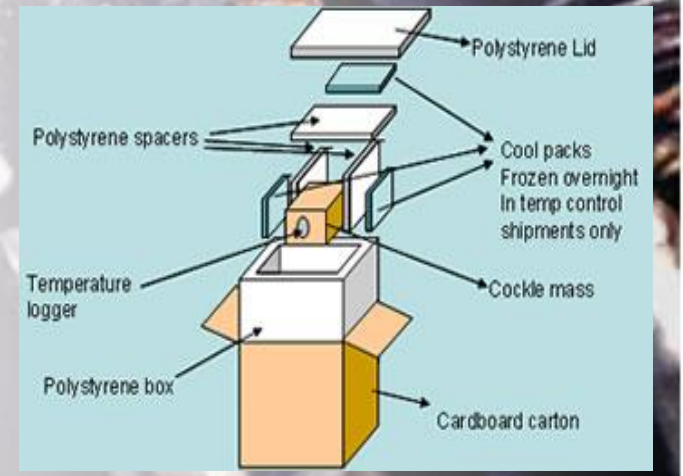
Sample transport

- Transport conditions must not affect the microbiological integrity of the samples
- Cool packs must not be in direct contact with the packed samples
- Samples must be stored below 10 °C if transport is over 4 hrs from sample collection
  - If samples arrive within 4 hrs from collection, arrival temp. must be below the sample collection temp.
- Samples must be analysed within 24 hrs of being collected
  - This can be extended if studies have shown samples can be left for longer

Cool box



Biotherm box

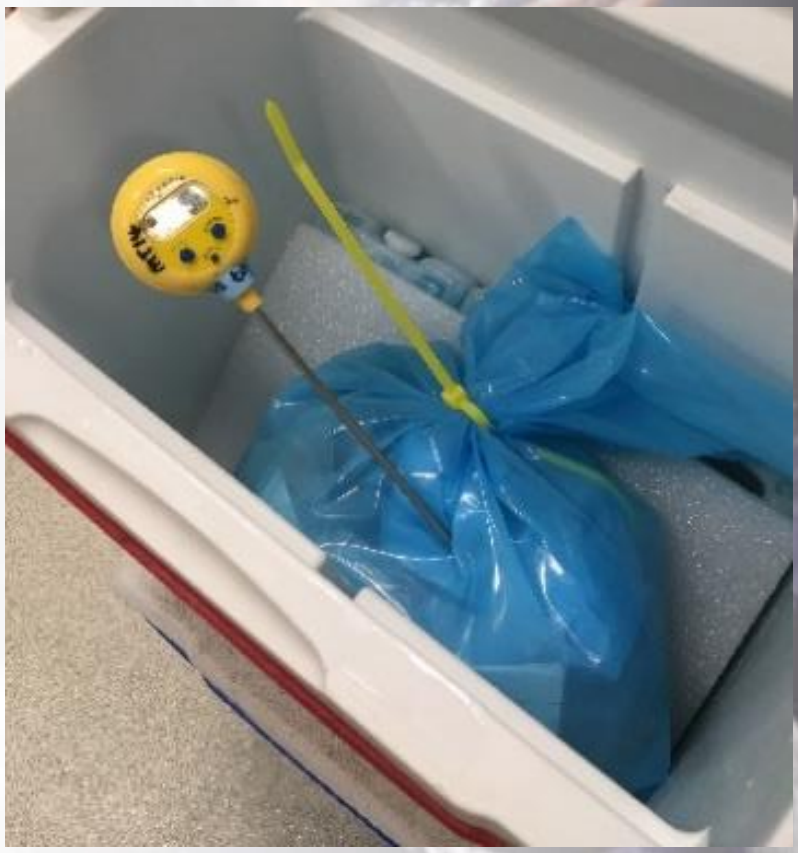


Laboratory receipt and analysis

Sample Receipt

- Sample submission form and temperature checked (1)
- Sample information recorded and ID number assigned

1



Laboratory receipt and analysis

Sample Receipt

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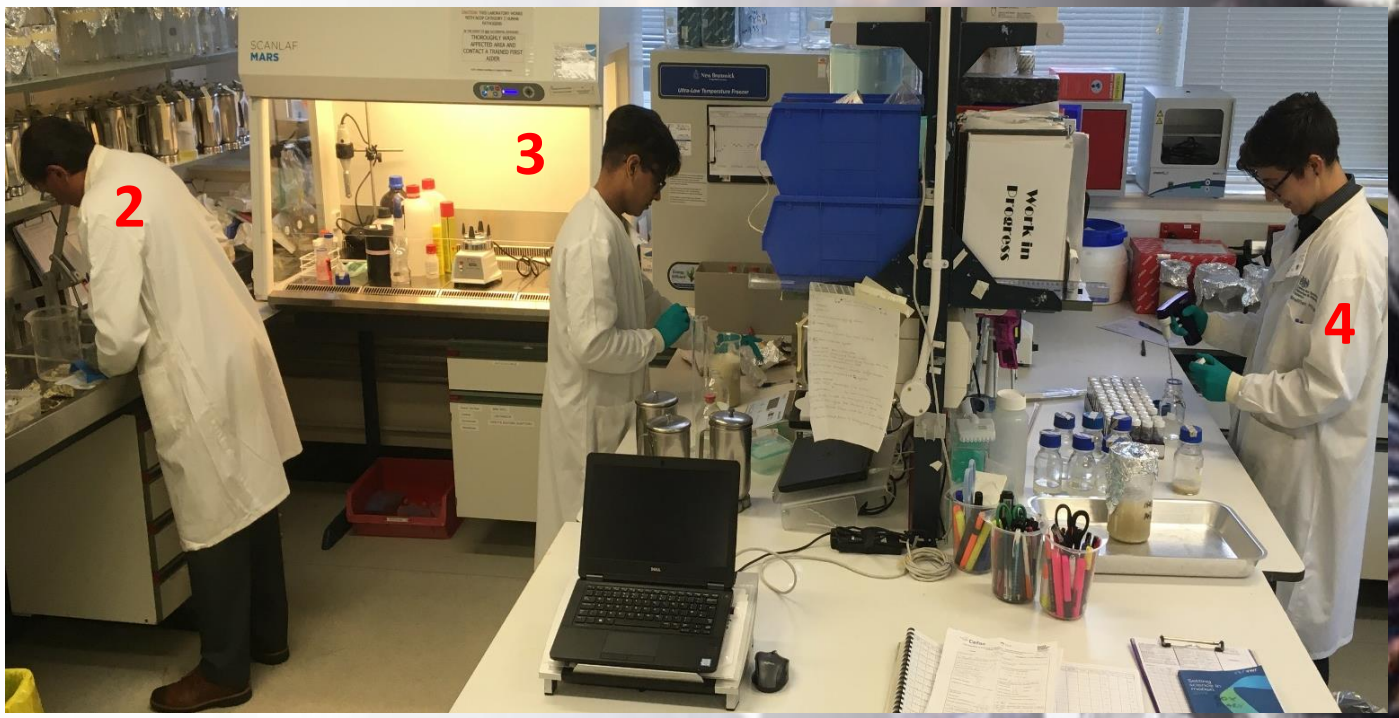
Sample analysis

- Shellfish shucked (2), homogenised (3) and analysed same day (4)
- Results checked by 2 trained staff



Laboratory receipt and analysis

Sample Receipt

- Sample submission form and temperature checked
- Sample information recorded and ID number assigned (1)

Sample analysis

- Shellfish shucked (2), homogenised (3) and analysed same day (4)
- Results checked by trained staff

Reporting of results

- Results recorded on computer (5)
- Results reported to customer

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UKAS TESTING
2293

RESULTS OF MICROBIOLOGICAL EXAMINATIONS OF SHELLFISH HYGIENE SAMPLES

Name of client:	
Address of client:	
Cefas sample number:	
Your reference:	**Species:**
Date received:	**Date of analysis:**

E. coli MPN/100g	*Salmonella* spp. in 25 g	*Vibrio parahaemolyticus* in 25 g

SOP 1172 - 'General procedure for receipt, opening and homogenisation of shellfish'
SOP 1175 - 'Enumeration of *Escherichia coli* in bivalve molluscan shellfish using the Most Probable Number technique' was used for the analysis of *E. coli.*
SOP 1176 - 'Detection of *Salmonella* spp. in bivalve molluscan shellfish' was used for the analysis of *Salmonella* spp.. This excludes *Salmonella* Typhi.
SOP 1333 - 'Detection of *Vibrio parahaemolyticus* in bivalve molluscan shellfish' was used for the analysis of *V. parahaemolyticus*.

Comments

Recognised microbiological methods for indicators and pathogens

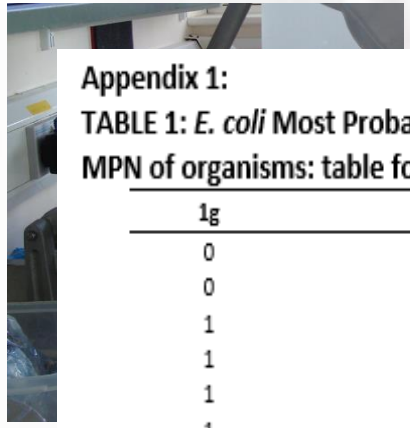
- Methods listed can be used in the sanitation programme – method will require verification in the laboratory before use
- Alternative methods can be used but should be validated against a methods listed

MATRIX	TARGET ORGANISM	METHOD
Bivalve molluscs	Sample preparation for all bacteriological methods	ISO 6887-3
	Preparation of dilutions of homogenized samples for all bacteriological methods	ISO 6887-1
	E. coli	ISO 16649-3 (5 tube format)
	MSC	EURL generic protocol (EURL 2007) FDA MSC Method
	Salmonella spp. (detection)	ISO 6579-1
	Salmonella spp. (quantification)	ISO 6579-3
	Pathogenic vibrios	See FAO/WHO (2016)
	Hepatitis A virus and norovirus (quantification)	ISO/TS 15216-1
	Hepatitis A virus and norovirus (qualitative detection)	ISO/TS 15216-2
Water	Faecal coliforms and presumptive *E. coli* by membrane filtration	ISO 9308-1
	Faecal coliforms and presumptive *E. coli* by Most Probable Number (MPN)	ISO 9308-2
	MSC	ISO 10705-1
	Standard Methods for the Examination of Water and Wastewater (APHA, 1985)	APHA

E. coli detection method – ISO 16649-3:2015

1. **Preparation of shellfish**
   - Dilute shellfish 1:3 with 0.1% P
2. **Recovery step – MMGB**
   - Inoculate 5 tube x 3 format, incubate 37±1°C for 24±2h
3. **Plating confirmation – Chromogenic medium**
   - Inoculate TBX plates with acid producing tubes - detects β-glucoronidase enzyme presence, incubate 44±1°C for 21±3h
4. **Interpretation of MPN/ 100g shellfish flesh**
   - Confirmation of *E. coli* - β-glucoronidase +ve (blue-green colonies)
   - MPN generated from tube combination e.g. 2, 0, 0

ISO 16649-3 is the EU reference method. This is the method expected to be used for exporting to Europe

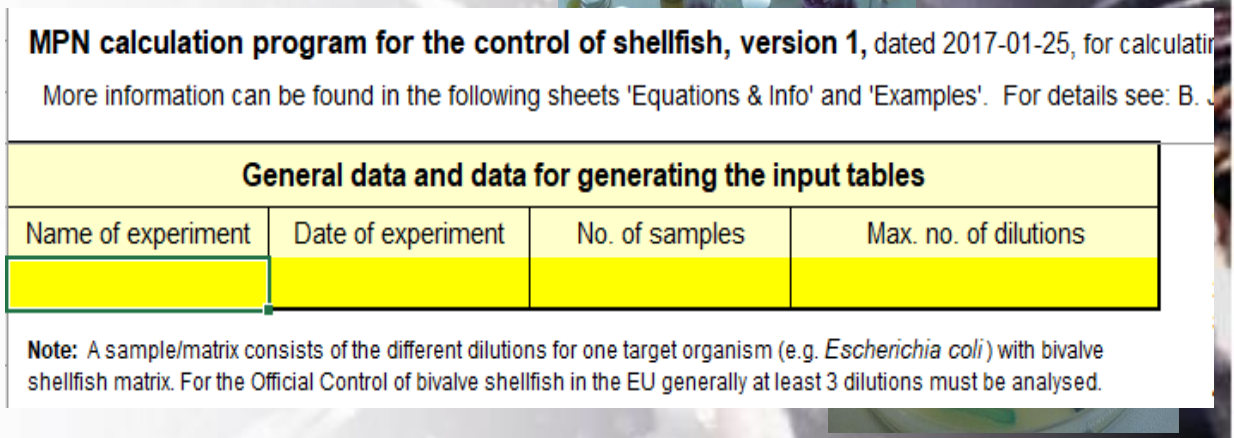


Appendix 1:

TABLE 1: *E. coli* Most Probable Number (MPN)

MPN of organisms: table for multiple tube methods using 5 x 1g, 5 x 0.1g, 5 x 0.01g.

1g	0.1g	0.01g	MPN/100g	Category
0	0	0	<18 [1]	1
0	1	0	18	1
1	0	0	20	1
1	0	1	40	2
1	1	0	40	1
1	2	0	61	2
2	0	0	45	1
2	0	1	68	2
2	1	0	68	1
2	1	1	92	2
2	2	0	93	1
3	0	0	78	1



MPN calculation program for the control of shellfish, version 1, dated 2017-01-25, for calculati

More information can be found in the following sheets 'Equations & Info' and 'Examples'. For details see: B.

General data and data for generating the input tables			
Name of experiment	Date of experiment	No. of samples	Max. no. of dilutions

Note: A sample/matrix consists of the different dilutions for one target organism (e.g. *Escherichia coli*) with bivalve shellfish matrix. For the Official Control of bivalve shellfish in the EU generally at least 3 dilutions must be analysed.

FRNA bacteriophage detection method – ISO 10705-1:1995

1. Preparation of shellfish

- Dilute shellfish 1:3 with 0.1% P

2. Preparation of bacterial host

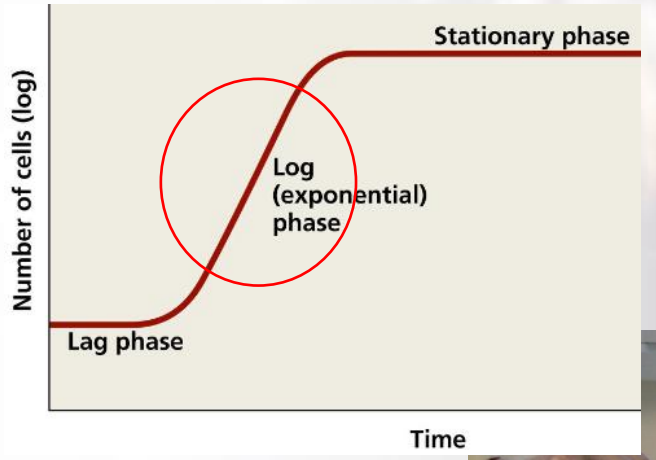
- *S. typhimrium* (WG49) - genetically modified with *E. coli* sex pili
- Grow host in TYGB to obtain 7 – 40 x 10^7 cfu/ml

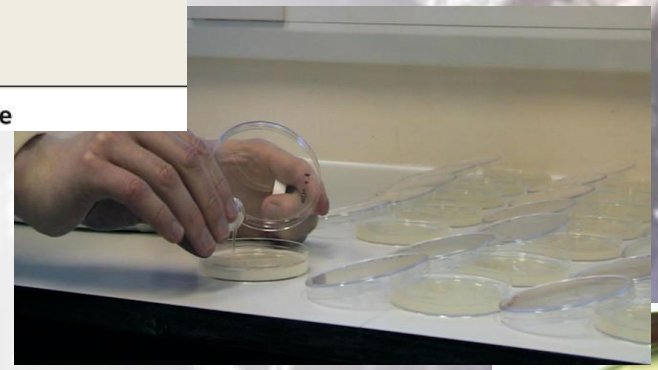
3. Agar overlay

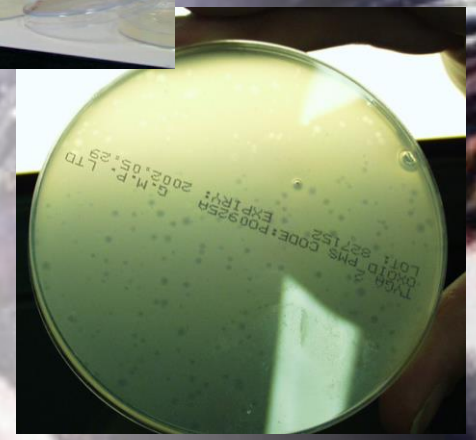
- Mix bacterial host, molten agar (TYGA) and sample, form an overlay, incubate 37±1°C for 18±4h

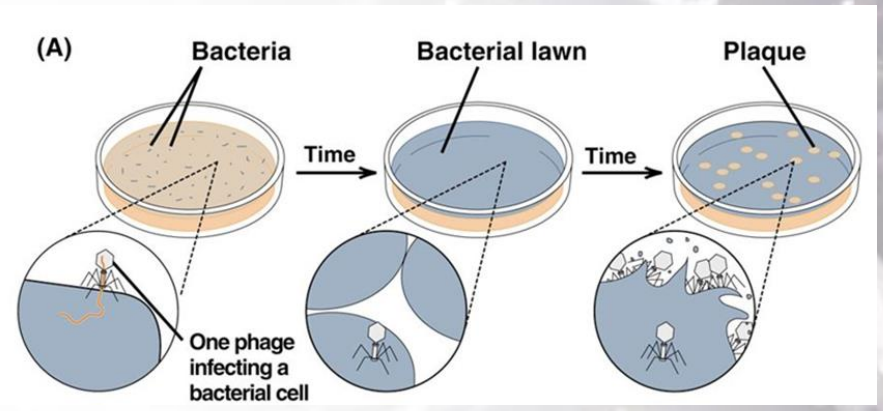
4. Interpretation of cfu / 100g shellfish flesh

- Count plaques – Bacteriophage attach to sex pili of *E. coli,* cells lyse causing visible holes in bacterial lawn









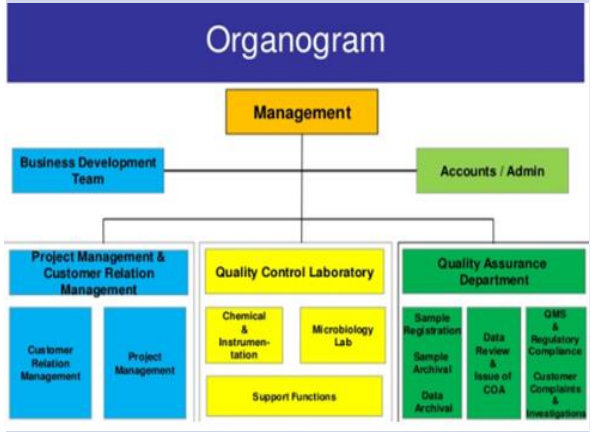
‘Rapid’ methods for *E. coli* enumeration in shellfish

Method name	Pros	Cons	Comments
TBGA-MPN (EU reference)	• “Gold-standard” • Established, well- characterised	• ~2 days for results	Reference method in European legislation
Impedance	• Validated • Rapid (24 hours)	• Expensive • Uses proprietary consumables	Mostly used in France
Pour-plate	• Validated • Rapid (24 hours) • Cheap (ish)	• High detection limit (200 CFU/100 g) • Availability of media?	Mostly used in Netherlands
PCR-MPN	• Rapid (30 hours) • Sensitive • Equipment and consumables commonly available	• Not validated • Needs more work • May be expensive	Not recommended for use yet

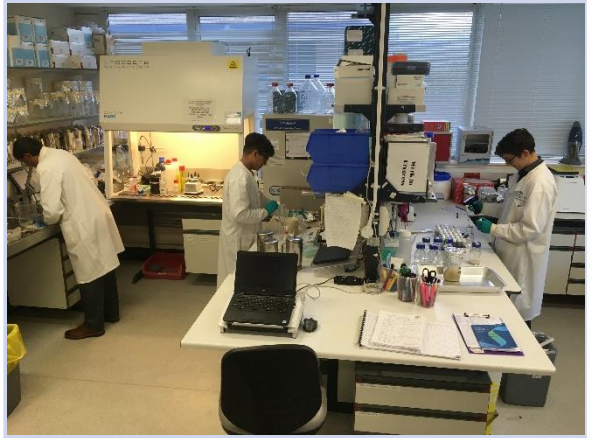
Accreditation to ISO 17025:2017

- Holding accreditation demonstrates a laboratory can operate competently and generate valid results, thereby promoting confidence in their work
- **ISO/IEC 17025 - General requirements for the competence of testing and calibration laboratories**

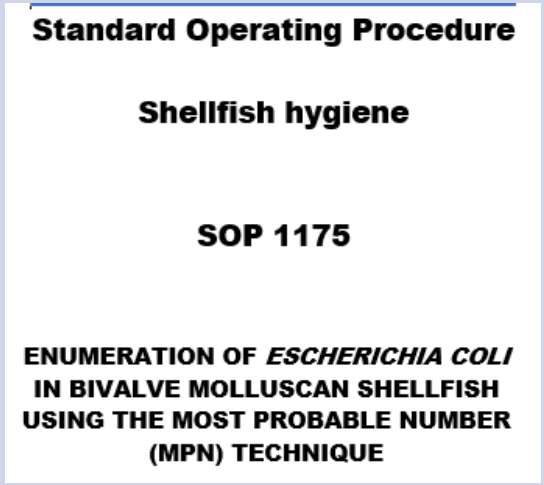
General structure



Resource



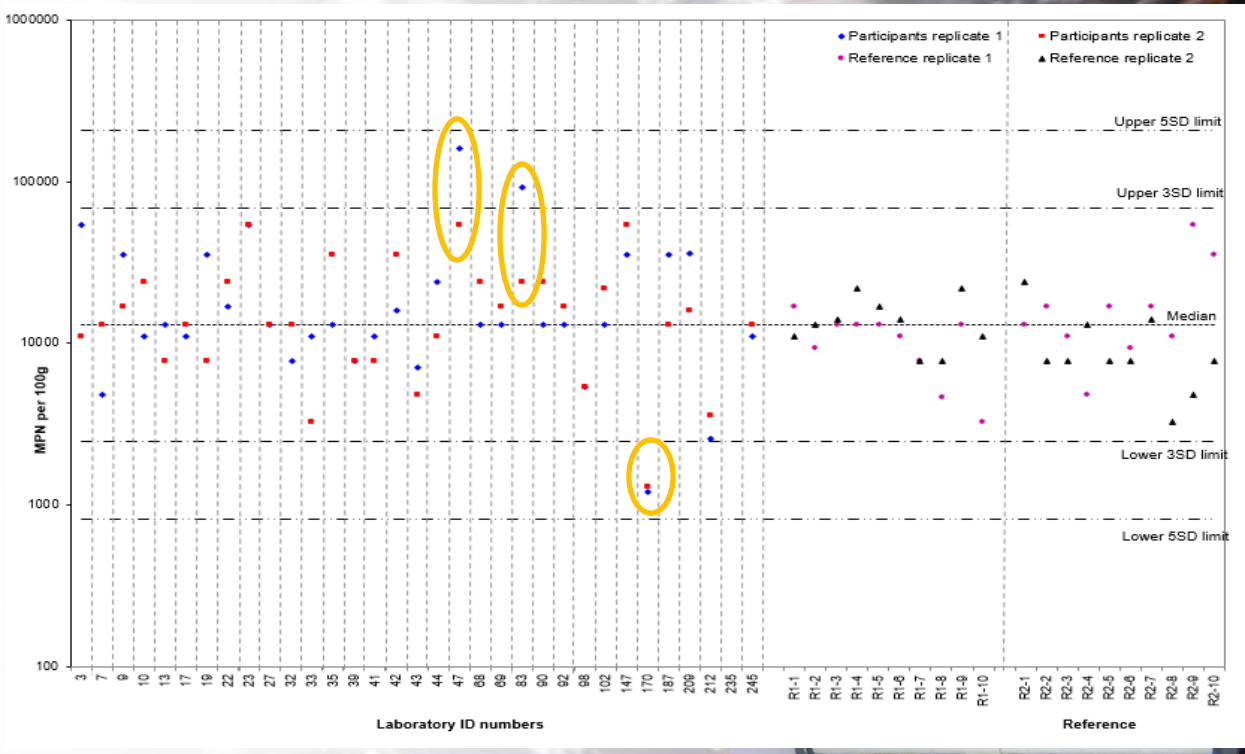
Process



Management system

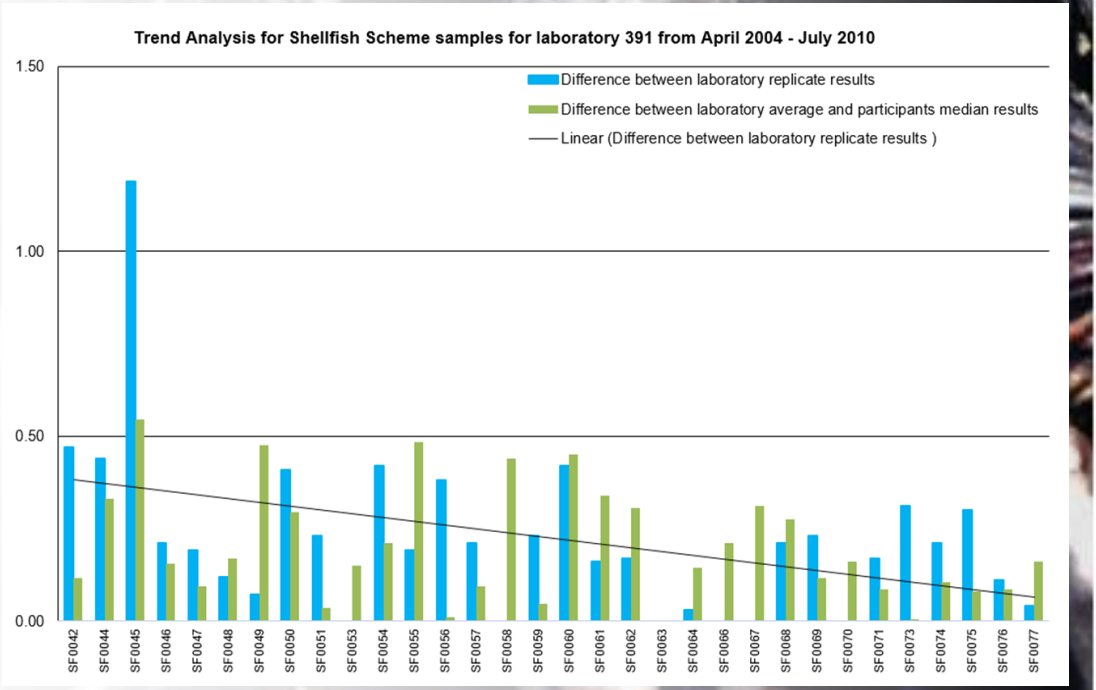
Proficiency testing (PT)

- PT or external quality assessment (EQA) is a valuable tool to assess the performance and verifies the accuracy and reliability of a lab's test result
- Regular participation:
  - Demonstrates a lab's commitment to maintaining and improving performance
  - Provides proof of competence to the customers
- PT samples should be analysed in same way as routine samples
- Allocated scores helps to identify a problem from a single distribution and over time (rolling)



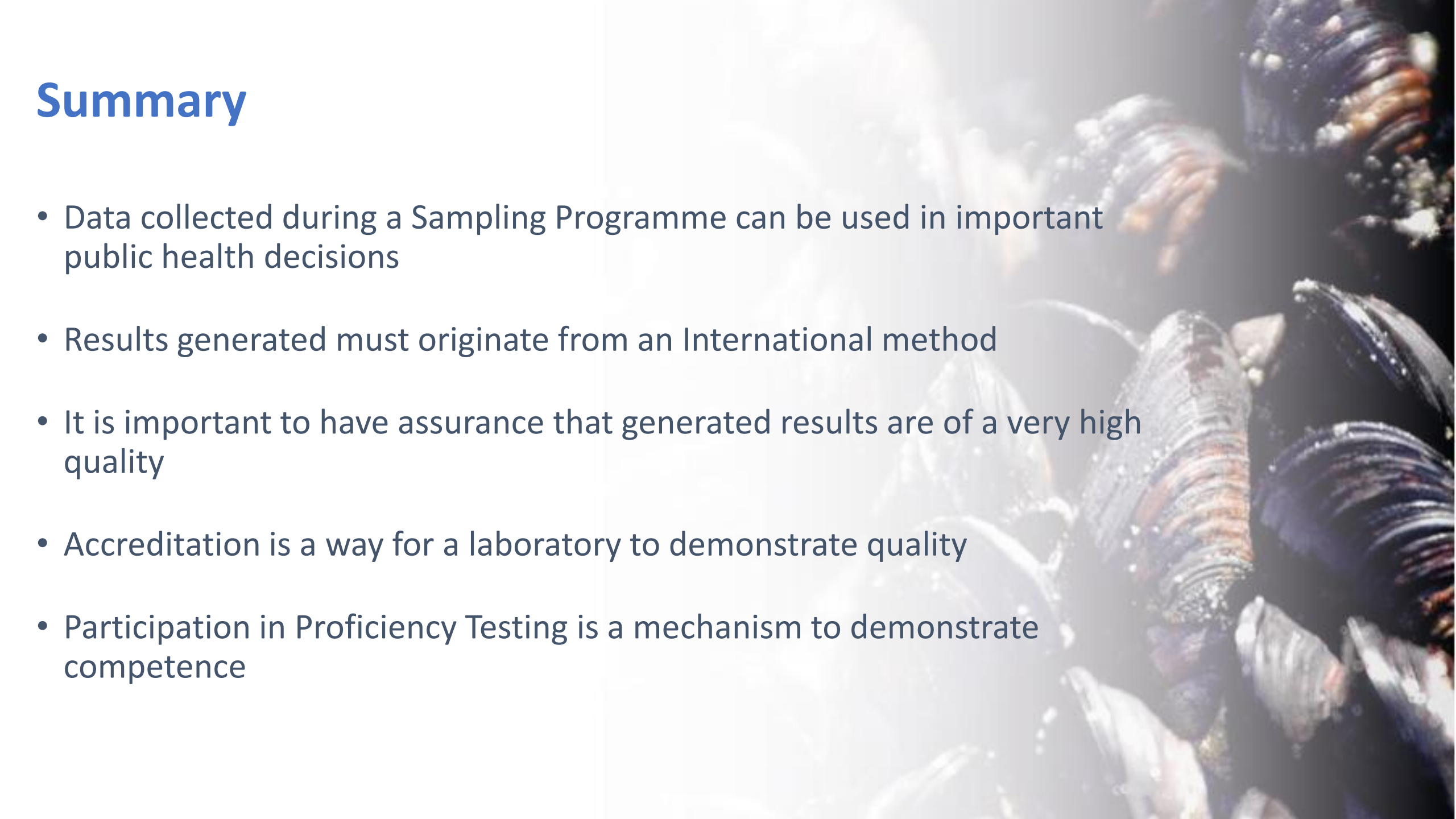
PT benefits

- Provides an independent assessment of a laboratory's performance
- Provides a performance comparison with other participant laboratories
- Helps to identify areas where there may be problems
- A requirement for auditing bodies (for quality and trade)
- Used to train staff and assess ongoing competency
- Can be used to generate data to support method development and validation
- Periodic testing of matrix samples is important to test aspects of the method not challenged by laboratory constructed material (e.g. Lenticule™)



Summary

- Data collected during a Sampling Programme can be used in important public health decisions
- Results generated must originate from an International method
- It is important to have assurance that generated results are of a very high quality
- Accreditation is a way for a laboratory to demonstrate quality
- Participation in Proficiency Testing is a mechanism to demonstrate competence



Thank you