Microbiological data – Importance of sample transport, sample receipting, methods and quality of test results

Louise Stockley

Introduction

- Taking regular samples from a growing area (monitoring), provides information on contamination of the area and informs controls
- Data from monitoring programmes are used to make important decisions (e.g., classification of the area and controls)
- Therefore all components of the programme should be of a high quality and traceable

Sample collection

- Important considerations
  - Protocols for sample collection and transport
  - Training for samplers taking Official Samples i.e., samples used in official monitoring programmes
  - Location of the sampling point (SP) to show traceability and consistency
  - Periodic audits by the Official Body to ensure protocols are followed

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)

Version 10
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13 Pages

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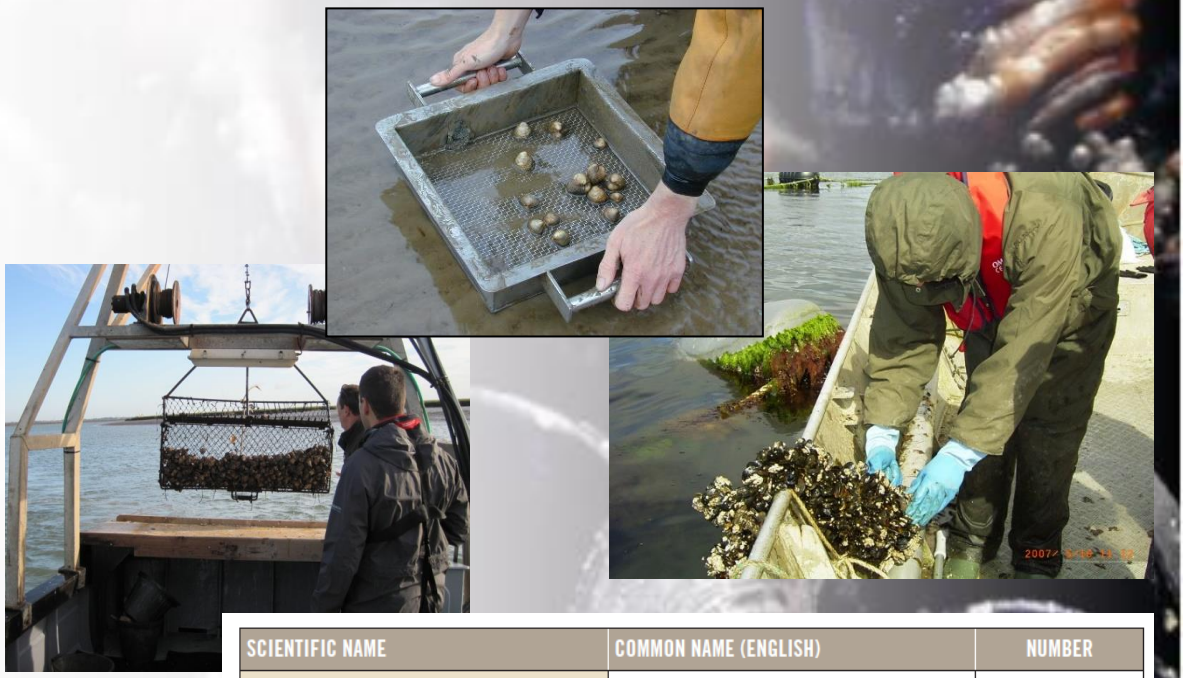
Sampling of bivalve molluscs

- Collect in same way as commercial sampling
- Ensure bivalve molluscs are alive and of a commercial size
- Select each bivalve mollusc at random to avoid bias from environmental factors
- Collect relevant environmental measurements

Do not re-immerse the sample in water once collected

Wash to remove mud and debris

Place sample in 2 strong bags



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

Sampling of water

- Collect water before bivalve molluscs or sediment samples to reduce sediment disturbance

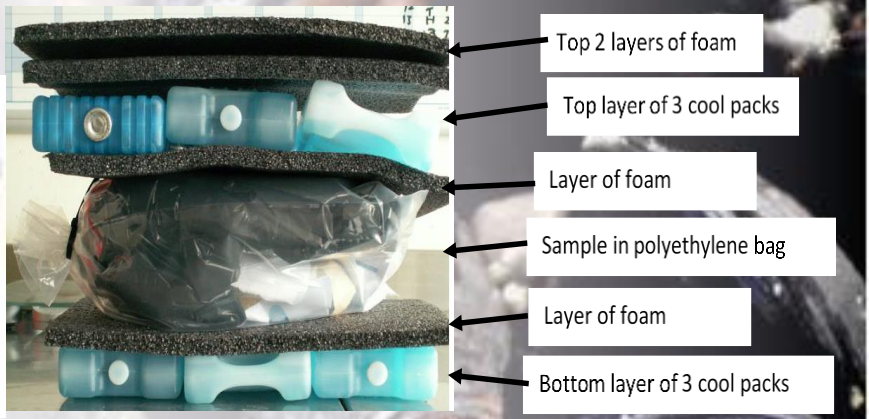
- Use a sterile glass or plastic bottle
  - Bags can be used for transporting liquid

- Take sample from middle of water column
  - Sampling pole can be used to collect sample
  - Immediately replace lid tightly to prevent leaks

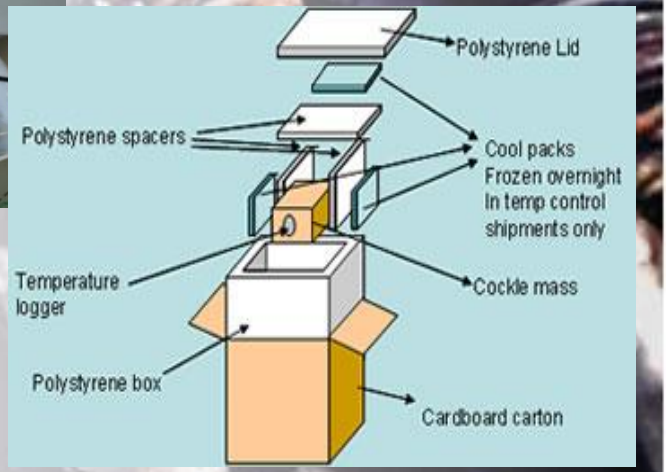
Sample transport

- Samples should be transported in a temperature controlled container
- Transport conditions must not affect the microbiological content of the samples
- Samples should be transported between 0 and 10°C. However, if the water temperature at collection is above 10°C, samples should arrive at the laboratory below the temperature at collection
- Samples should 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)**
- **Record sample information and ID number assigned**

1

Agency name:
Country region:

Growing Area: _____ Location: _____ Country municipality: _____
Date: _____ Start time: _____ End time: _____ Name of sampler: _____ Name of harvester: _____

Sample identification number	Sample point identifier	Actual sampling location	Time (24-hour format)	Type od sample				Physicochemical parameters				Type of sample		
				Seawater	Freshwater	Effluent	Bivalve molluscs (species)	Temp (°C)	pH	Oxygen	Salinity		Water/ effluent	Bivalves
												Total of Fecal coliforms		
												E. Coli		
												Salmonella spp.		
												V. parahaemolyticus		
												V. cholerae 01 or non-01		
												V. vulnificus		
												Heavy metals: Cd, Pb, Hg, As		
												Pesticides		
												Saxitoxin		

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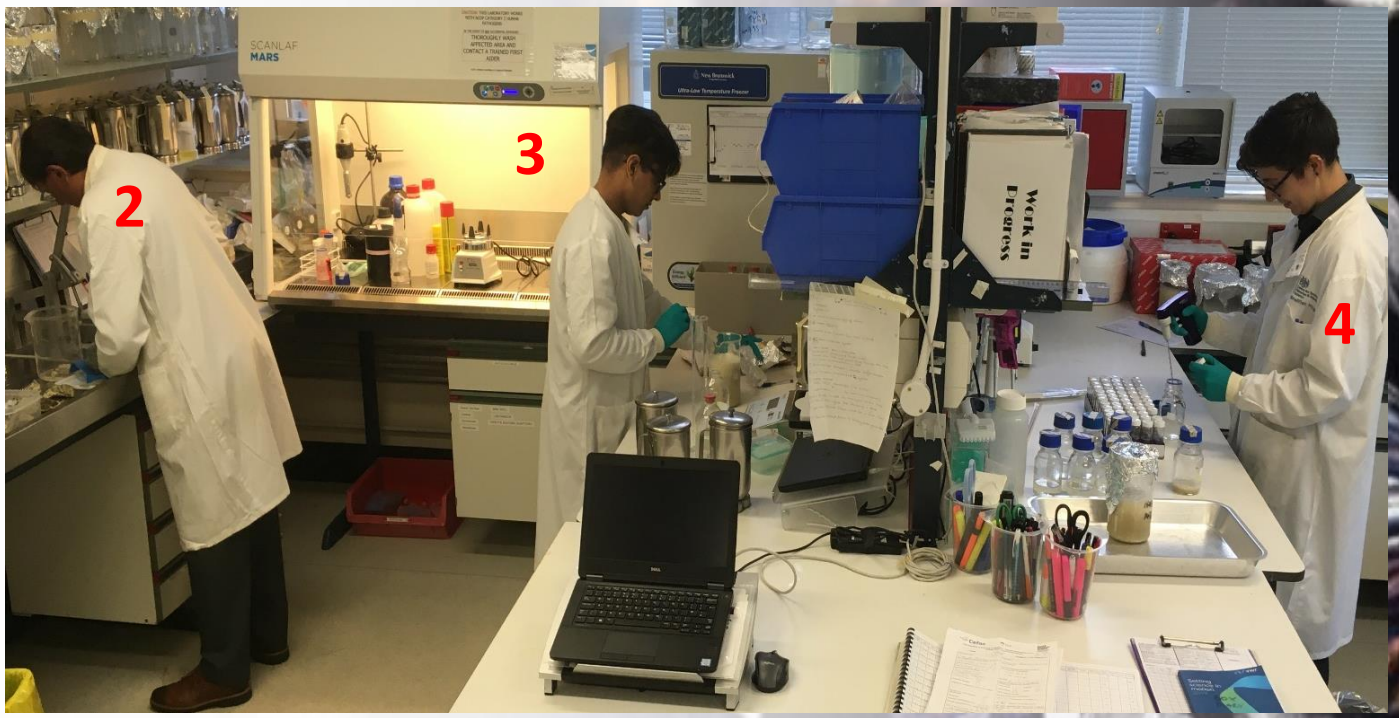
Sample analysis

- Bivalve molluscs are shucked (opened) (2), homogenised (3) and analysed same day (4)
- Results checked by 2 trained staff



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Reporting of results

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

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UKAS TESTING
2293

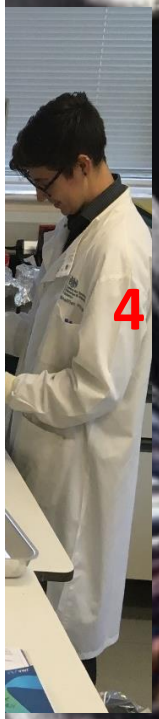
RESULTS OF MICROBIOLOGICAL EXAMINATIONS OF SHELLFISH HYGIENE SAMPLES

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

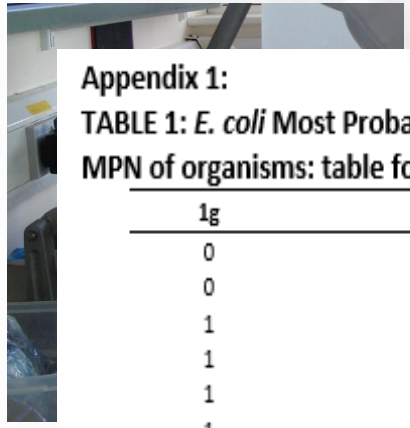
- Methods listed can be used in the monitoring programme – method will require verification in the laboratory before use
- Alternative methods can be used but should be validated against a method listed

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 bivalve molluscs**
   - Dilute bivalve molluscs 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 bivalve mollusc 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 bivalve molluscs

- Dilute shellfish 1:3 with 0.1% P

2. Preparation of bacterial host

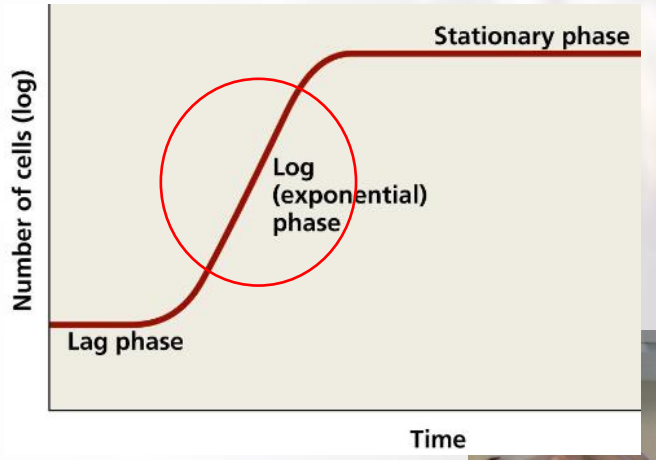
- *S. typhimrium* (WG49) - genetically modified with *E. coli* sex pili
- Grow host in TYGB to obtain 7 – 40 x 10^7 cfu/ml

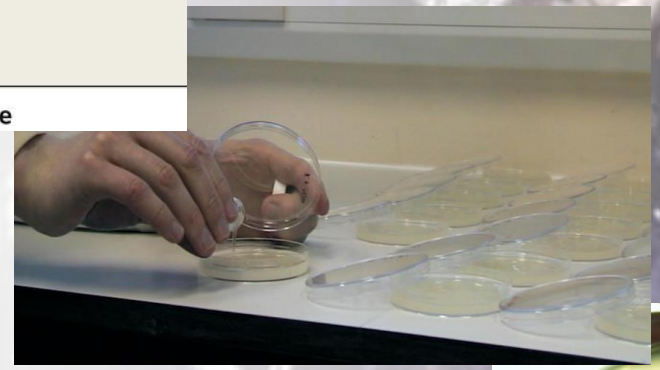
3. Agar overlay

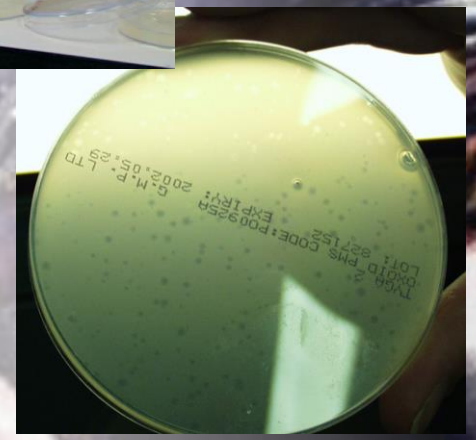
- Mix bacterial host, molten agar (TYGA) and sample, form an overlay, incubate 37±1°C for 18±4h

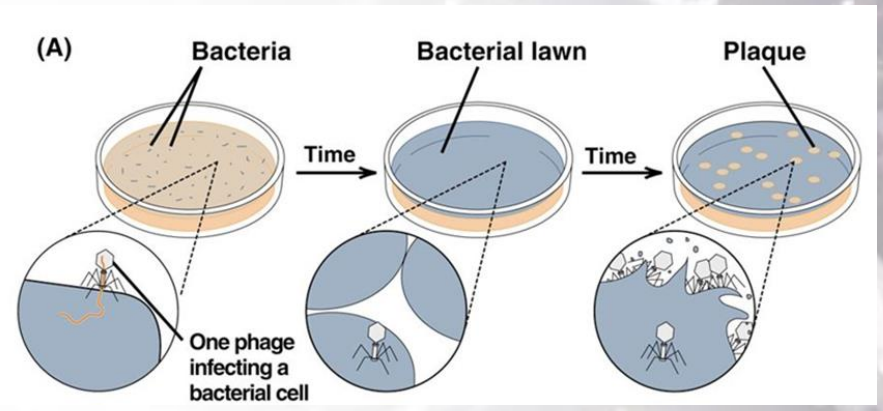
4. Interpretation of cfu / 100g bivalve mollusc flesh

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





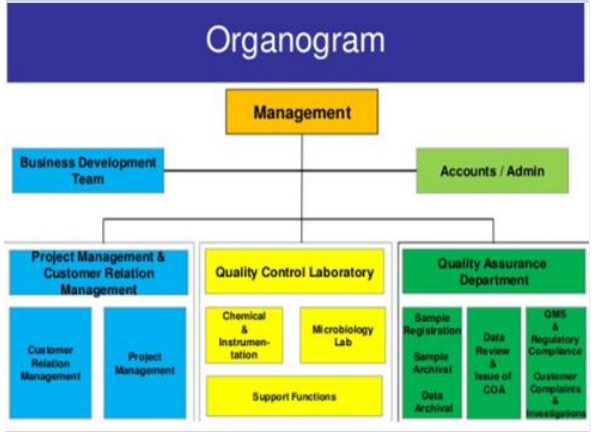




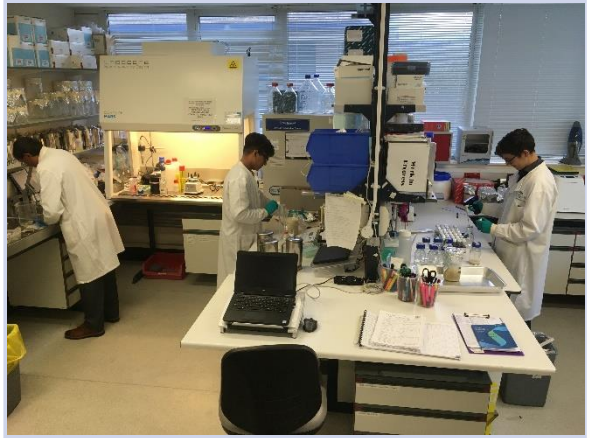
Accreditation to ISO 17025:2017

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

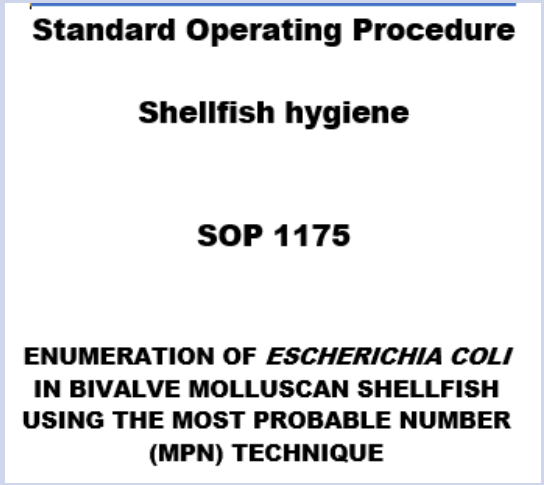
General structure



Resource



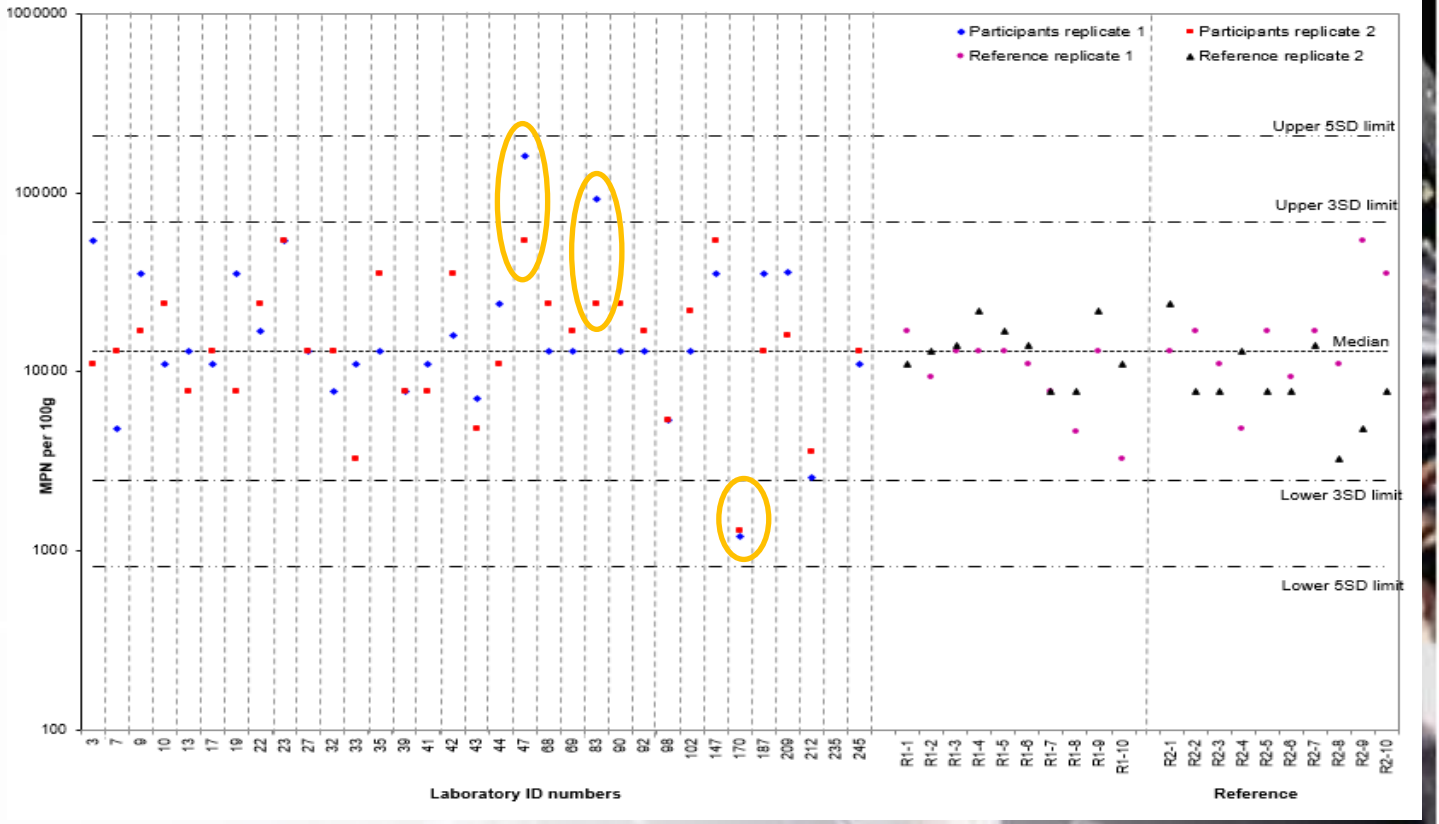
Process



Management system

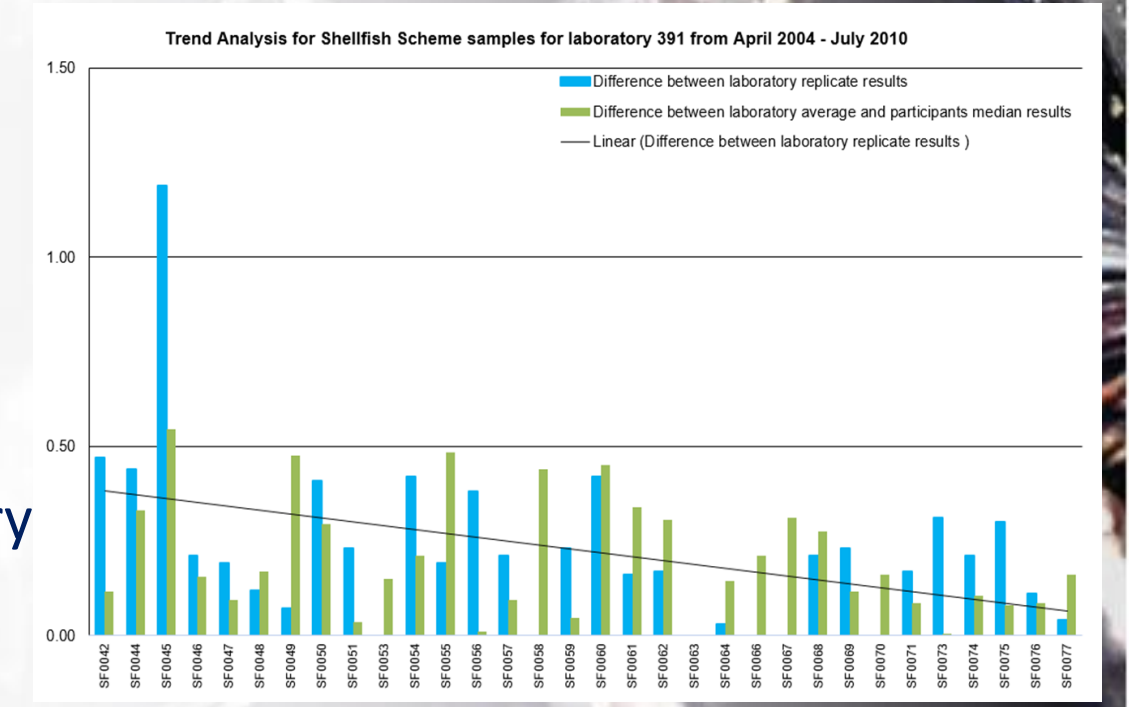
Proficiency testing (PT)

- PT or external quality assessment (EQA) is a valuable tool to assess the performance, accuracy and reliability of a laboratory's test result
- Samples should be analysed in the same way as routine samples
- Laboratory's results are compared with other participating laboratories
- Allocated scores can be used to assess performance from a single distribution and over time (rolling)



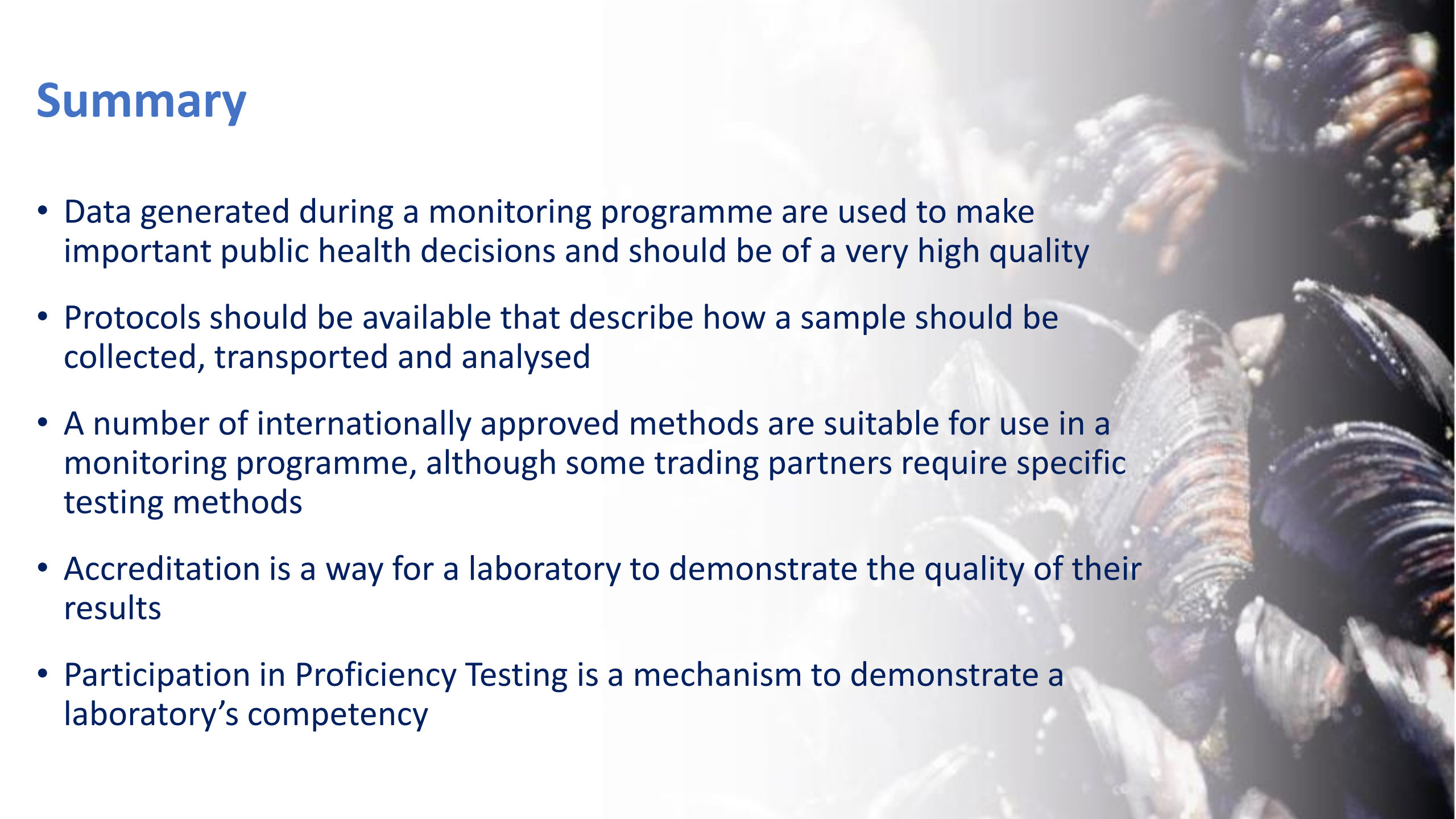
PT benefits

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



Summary

- Data generated during a monitoring programme are used to make important public health decisions and should be of a very high quality
- Protocols should be available that describe how a sample should be collected, transported and analysed
- A number of internationally approved methods are suitable for use in a monitoring programme, although some trading partners require specific testing methods
- Accreditation is a way for a laboratory to demonstrate the quality of their results
- Participation in Proficiency Testing is a mechanism to demonstrate a laboratory's competency



Thank you