Sample collection, transport, analysis and quality of test results

Louise Stockley

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Centre for Environment Fisheries & Aquaculture Science
Food and Agriculture Organization of the United Nations
Cefas INTERNATIONAL CENTRES OF EXCELLENCE
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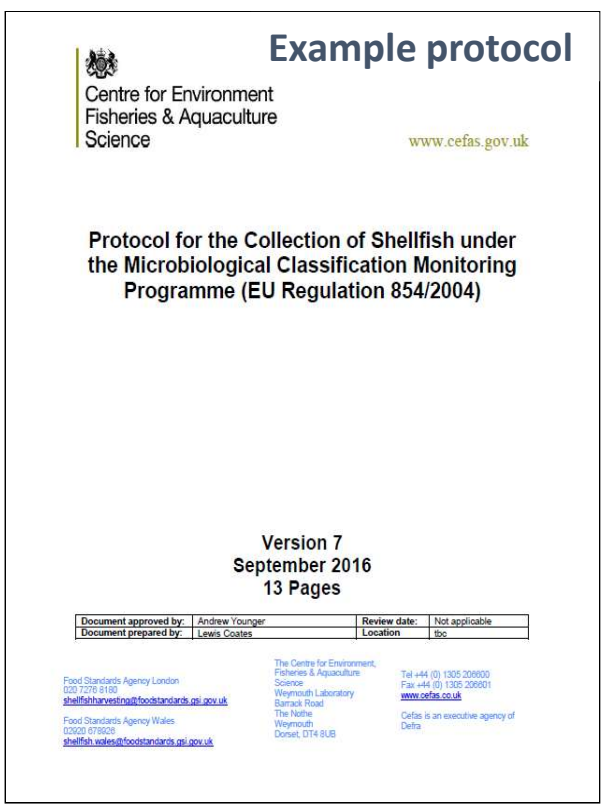
Introduction

- Harvesting area monitoring 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
- This means 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 identifier point (SIP)
- Carry out periodic audits to ensure protocols are adhered too



Example protocol

Centre for Environment
Fisheries & Aquaculture
Science

www.cefas.gov.uk

Protocol for the Collection of Shellfish under the Microbiological Classification Monitoring Programme (EU Regulation 854/2004)

Version 7
September 2016
13 Pages

Document approved by:	Andrew Younger	Review date:	Not applicable
Document prepared by:	Lewis Coates	Location	tbc

Sampling of bivalve molluscs

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

Do not immerse shellfish once collected

Wash to remove mud and debris

Place shellfish in 2 heavy duty 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

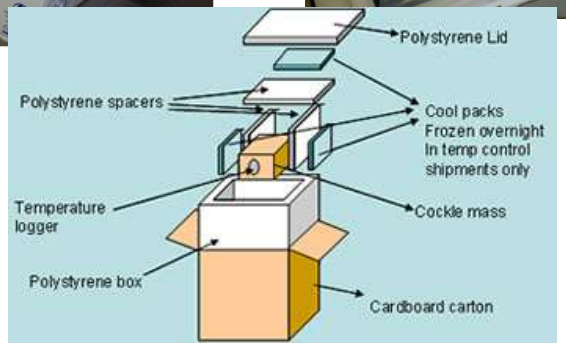
- Collect water before shellfish or sediment samples to reduce sediment disturbance
- Use a sterile glass or plastic bottle
  - Bags can be used for transporting liquid
- Take sample from middle of water column
  - Sampling pole can be used to collect sample
  - Immediately replace lid tightly to prevent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

Sample transport

- Cool packs must not be in direct contact with shellfish
- Sample transport conditions must not affect the microbiological integrity of the sample
- Inappropriate transport conditions can lead to unrepresentative results

Cool box

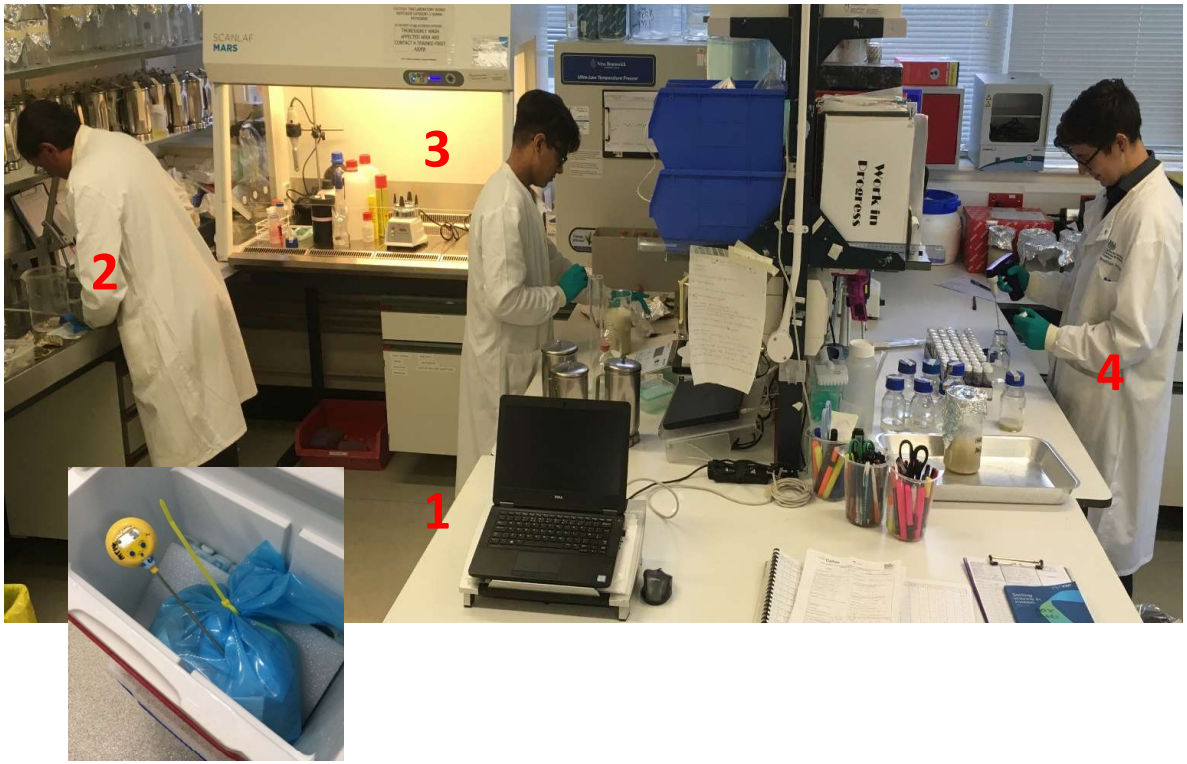
Biotherm box



Laboratory receipt and analysis

Sample Receipt

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



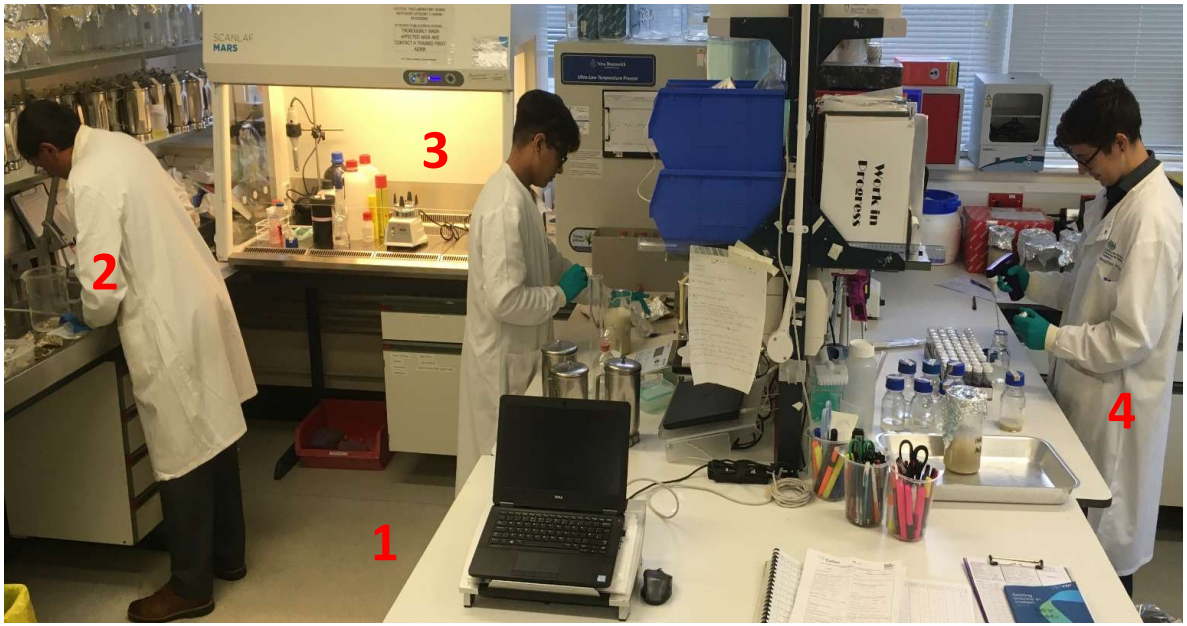
Laboratory receipt and analysis

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 2 trained staff



Laboratory receipt and analysis

Sample Receipt

- Sample submission form and temperature checked
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Sample analysis

- Shellfish shucked (2), homogenised (3) and analysed same day (4)
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Reporting of results

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

Cefas

Weymouth Laboratory,
Barrack Road,
The Nothe,
Weymouth, Dorset, DT4 8UB.
Telephone: +44 (0) 1305 206600
Direct line: +44 (0) 1305 206644

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*.

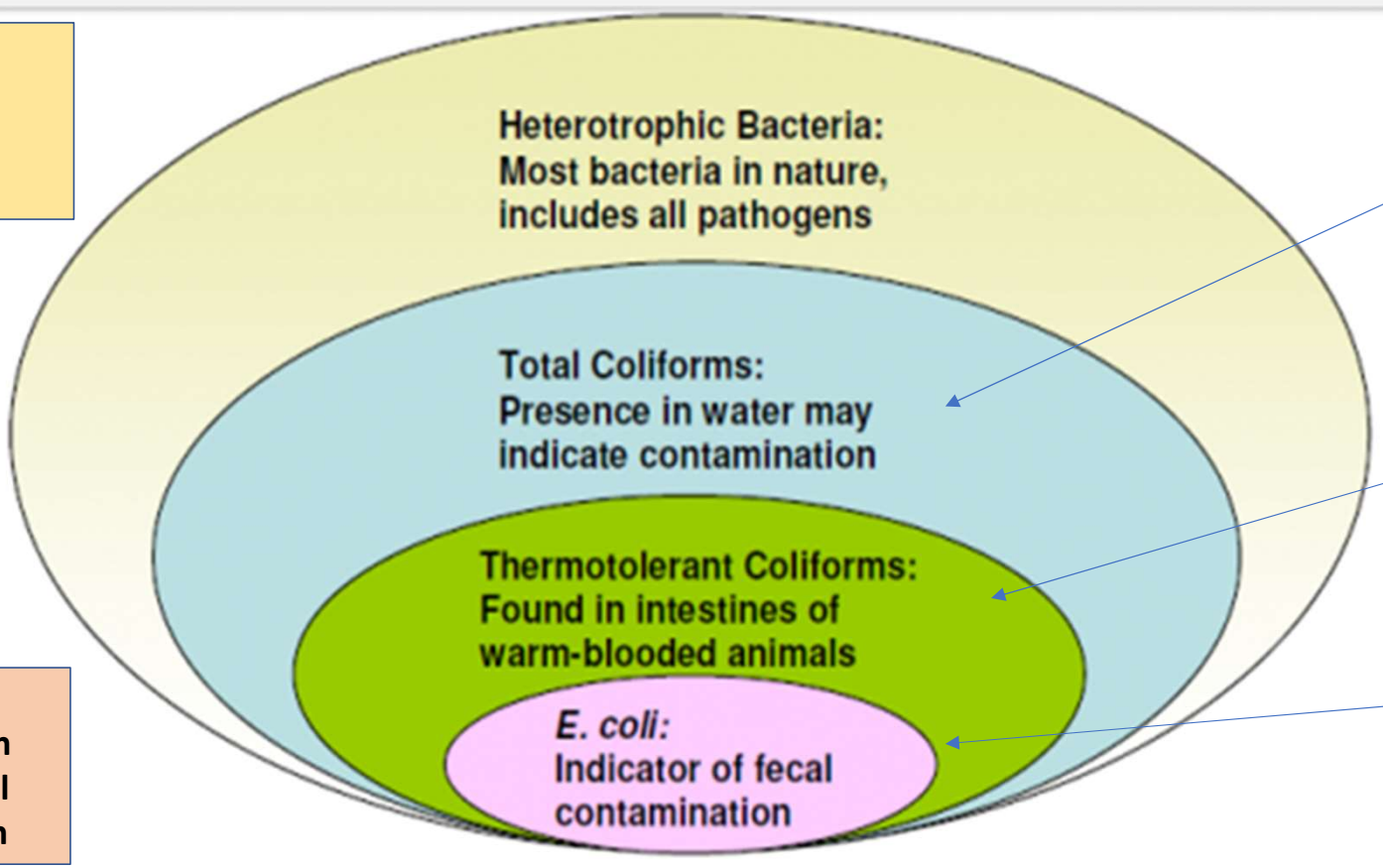
Comments

Recognised microbiological methods used in bivalve mollusc sanitation programmes around the world

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

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

Choice of indicators



Very broad taxonomic group, found in soil, animal/human guts

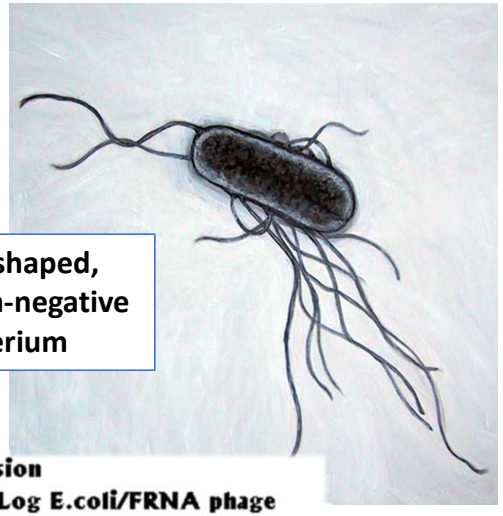
Smaller taxonomic group, generally but not all FCs derived from warm blooded animals

Presence of *E. coli* in foodstuffs is always indicative of contamination with faecal pollution

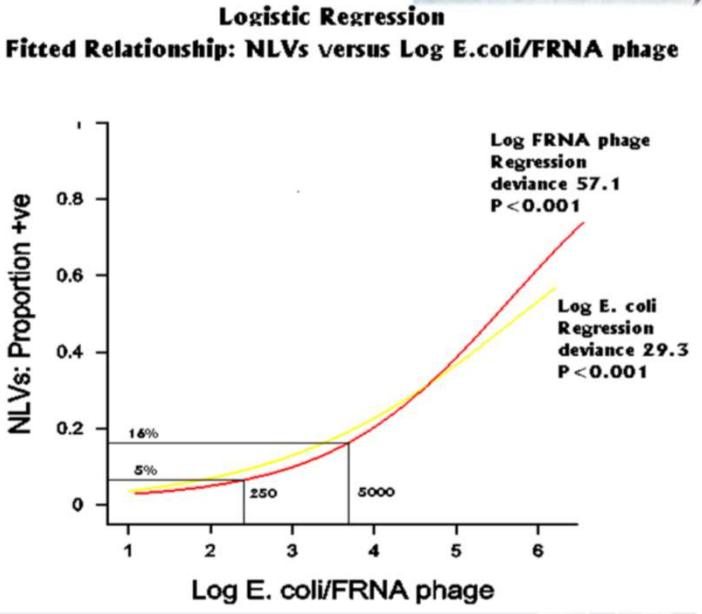
Best quality information on human/animal contamination

Choice of indicators - *E. coli*

- *E. coli* was first used as an indicator of faecal water pollution to predict the risk from *S*. Typhi
- Presence of *E. coli* in foodstuffs is evidence of contamination with faecal pollution
- Strong association between *E. coli* levels in a harvesting areas, pollution and the risk of norovirus presence
- Low *E. coli* results does not guarantee the absence virus in shellfish



Rod-shaped, Gram-negative bacterium



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

1. Preparation of shellfish

- Dilute shellfish 1:3 with 0.1% P

2. Recovery step – MMGB

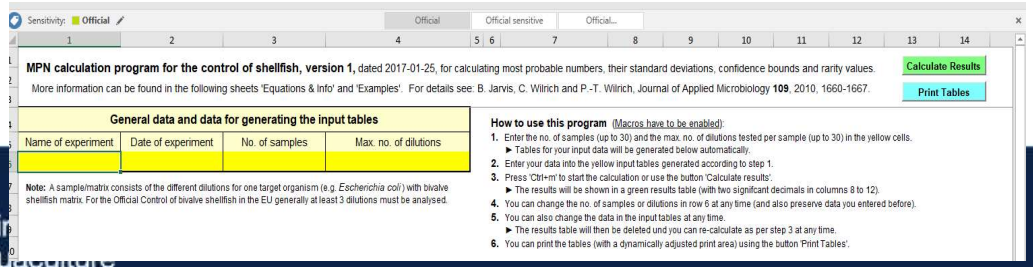
- Inoculate 5 tube x 3 format, incubate 37±1°C for 24±2h

3. Plating confirmation – Chromogenic medium

- Inoculate TBX plates with acid producing tubes - detects presence of β-glucoronidase enzyme (not pathogenic serotypes), incubate 37±1°C for 21±3h

4. Interpretation of MPN/ 100g shellfish flesh

- Confirmation of *E. coli* and generation of MPN tube combination e.g. 2, 0, 0



Appendix 1:

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

MPN of organisms: table for multiple tube methods using 5 × 1g, 5 × 0.1g, 5 × 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

Choice of indicators – Male specific coliphage

- FRNA bacteriophage are found abundantly in shellfish waters impacted by sewage effluent and agricultural waste
- Group of single-stranded RNA viruses that infect bacteria
- Have similar physical and genomic properties to human enteric viruses, making it a good alternative indicator to *E. coli*

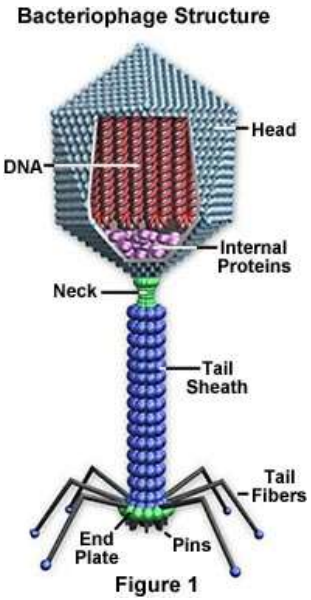
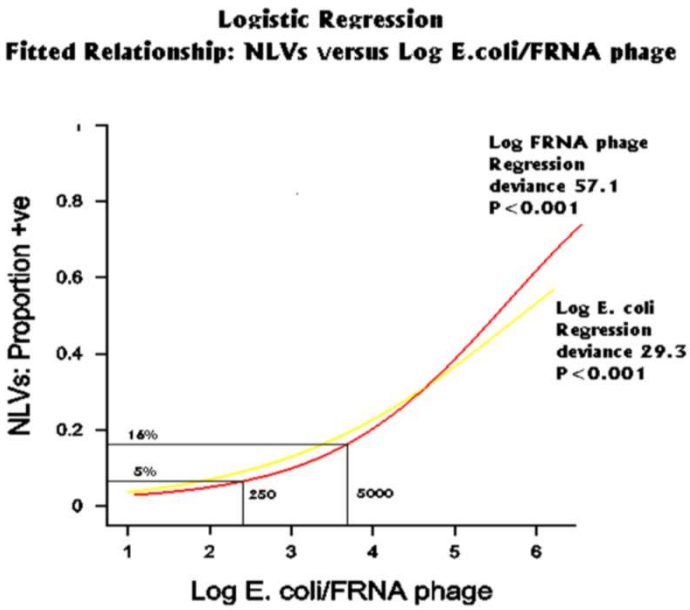


Figure 1



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

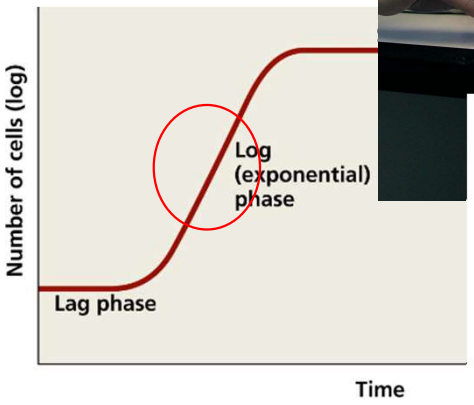
- *S. typhimrium* (WG49) - genetically modified with *E. coli* sex pili
- Grow host in TYGB to obtain 7 – 40 x 10^7 cfu/ml

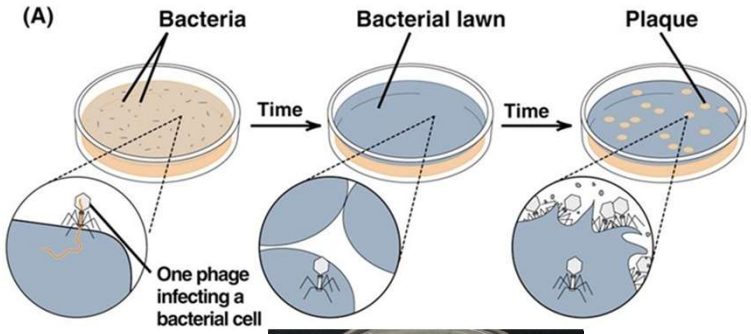
3. Agar overlay

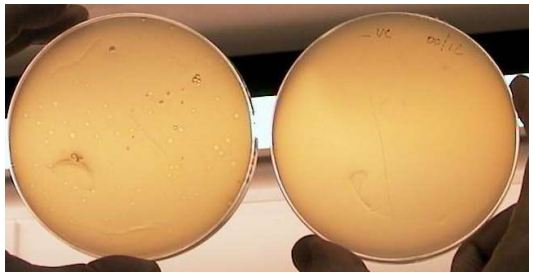
- Mix bacterial host, molten agar (TYGA) and sample, form an overlay, incubate 37±1°C for 18±4h

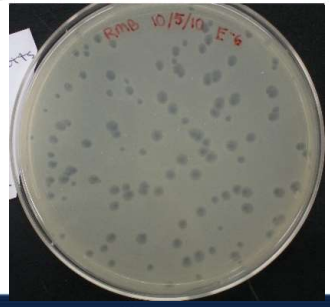
4. Interpretation of cfu / 100g shellfish flesh

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









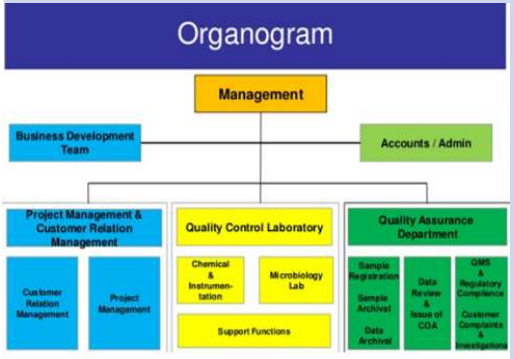
‘Rapid’ methods for *E. coli* enumeration in shellfish

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

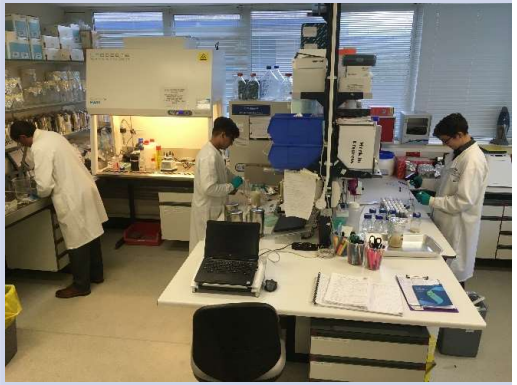
Accreditation to ISO 17025:2017

- 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


INTERNATIONAL STANDARD

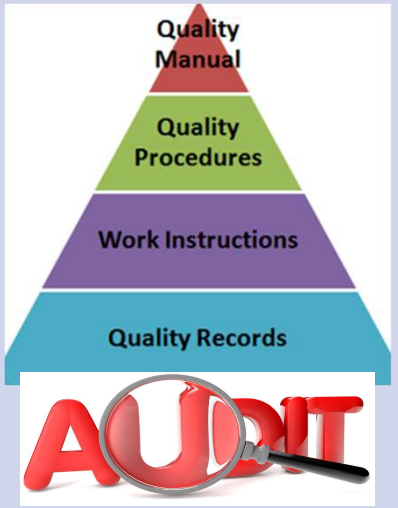
ISO/IEC 17025

Third edition 2017-11

General requirements for the competence of testing and calibration laboratories

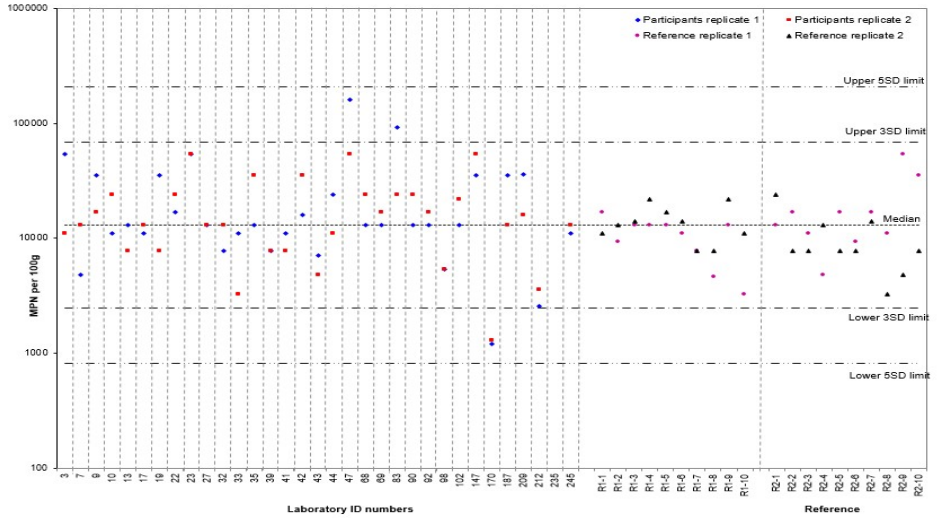
Exigences générales concernant la compétence des laboratoires d'étalonnages et d'essais

Management system



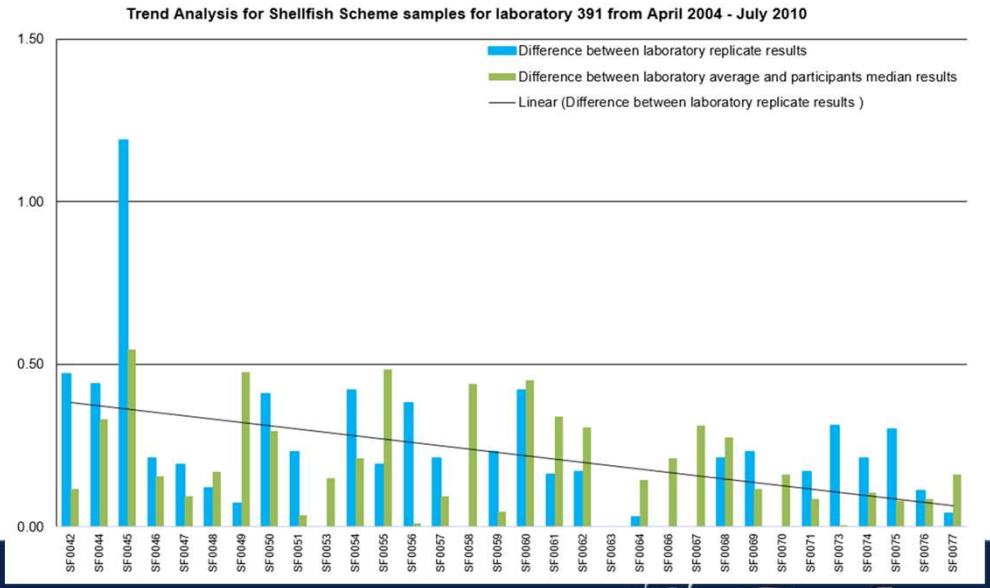
Proficiency testing

- Proficiency testing (PT), or external quality assessment (EQA) is a valuable tool for assessing laboratory performance and verifying the accuracy and reliability of test results
- Regular participation demonstrates a commitment to the maintenance and improvement of a laboratories performance and provides valuable proof to customers of their competence
- PT samples should be analysed in same way as routine samples
- Reported results are scored to help in identifying problems and allow individual distributions and over time (rolling)



PT benefits

- Provides an independent assessment of a laboratory 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
- Help generate data to support method development and validation
- Periodically testing of matrix samples is important to test aspects of methods not challenged by Lenticule™ samples



Summary

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

Thank you for listening

Any questions?