

# New Standards in Microbiological Methods

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# Sub title

- How legislation can make life difficult for the analyst

# Why do Microbiological Tests ?

- Raw material quality
  - Quality/Safety
- Production hygiene
  - Hygiene of equipment etc
- Final product quality
  - Quality
  - Safety

# Microbiology of Finished Product

- Is microbiological quality assured by testing ?
- HACCP
- So why test ?
- Verification

# Food Microbiology Testing and Legislation

- In the past very little legislation in the UK has covered microbiological testing of food products
- Food Safety Act
  - Safe to eat– microbiologically there is no definition of safe
  - Tests to be done are not defined
- A few EU Vertical Directives

# EU Criteria

- Commission Regulation 2073/2005
- On Microbiological Criteria for Foodstuffs
- Gives a background and criteria for testing foods for microbial contaminants
- Safety is ensured by HACCP
- Criteria are used in validation and verification of HACCP

# Regulation 2073

- Consists of a number of descriptive articles
- And 2 Chapters of Criteria
  - Chapter 1 -Food Safety Criteria
    - Acceptability of a product placed on the market
  - Chapter 2 –Process Hygiene
    - Acceptable functioning of the production process

So what's this got to do with  
test methods?

# Methods in EU Regulation-An Example. Annex 1

Category	Micro-organism	n	c	Limits	Method	Where Criterion Applies
1.2. Ready-to-eat foods able to support the growth of L. monocytogenes, other than those intended for infants and for special medical purposes	Listeria monocytogenes	5	0	100 cfu/g	<b>EN/ISO 11290-2</b>	Products placed on the market during their shelf Life
		5	0	Abs in 25g	<b>EN/ISO 11290-1</b>	Before the food has left the immediate control of the food business operator, who has produced it

# Other Methods In Chapter 1 of the Regulation

- Salmonella EN/ISO 6579
- Staph toxin European screening method  
CRL Milk (commercial ELISA)
- E.sakazakii ISO/DTS 22964
- E.coli(count shellfish) ISO TS 16649-3
- APC ISO 4833
- Enterobacteriaceae ISO 21528-2
- E,coli count (meat) ISO 16649-1 or 2
- Coagulase + Staphylococci EN/ISO 6888-1 or 2

# Methods are Specified

- The Microbiological methods within Regulation 2073 are EN/ISO
- They are standard microbiological tests
- They take a long time to give a result

# An Example- Salmonella

25g food + Broth	18h
Subculture into 2 <sup>nd</sup> Broth	24h
Put onto Agar plate	24h
	<b>3 days</b>

Negative or Presumptive



Isolate	24h
Biochemical	24h
	<b>2 days</b>

# But we do have some Rapid methods !

- Immunoassays – 50h
- PCR - 30h
- Commercial kits
- Proprietary methods
- How can we use these if testing within the Regulation ?

# Article 5

- Rules for sampling and testing
- FBOs may use other sampling & testing procedures- if they can demonstrate to the satisfaction of the Competent Authority that they give at least equivalent guarantees
- The use of alternative methods is acceptable – if----

# Article 5

- The methods are validated against the reference method and if a proprietary method, certified by a 3<sup>rd</sup> party according to ISO Standard 16140 --- or other internationally accepted similar protocols is used
- If not- methods shall be validated according to internationally accepted protocols & their use authorised by the competent authority

# So: for Proprietary Methods

- If the method is to be used to test under the regulation, it must be:
  - certified by a third party in accordance with the protocol set out in EN/ISO standard 16140 or other internationally accepted similar protocols, is used.
- EN/ISO 16140: The Microbiology of Food & Animal Feedingstuffs-Protocol for the Validation of Alternative Methods

# So Who is the Third Party ?

- A validating body
  - AFNOR
  - MicroVal
  - NordVal
- All 3 use ISO 16140 as the basis of validation
- Interpretations are different between different validating organisations
- So are they all equally acceptable ?

# Some issues to remember

- ISO 16140 has a scope
- Methods can be validated against different criteria- read the small print
- E.g. from AFNOR- one method for *Salmonella*
- AFNOR Certificate 10/4-05/04 valid until 7/5/12
- Certificate on AFNOR website in French
- Meat, egg, milk, seafood, animal food, environmental sample
- Detects motile salmonella not non-motile salmonella
- How can this be equivalent to the ISO reference method that detects motile salmonella as well?

# The value of ISO 16140 Validation

- It is an International Standard thus has International Standing
- A method validated & certified to ISO 16140 can be used for testing under EU Micro Criteria Regulation
- It is interpreted differently by different certification bodies-This could be a problem
- As yet, no challenge to any certified method – but it could happen.
- The scope of the validation is important and needs to be checked

# What about Other Validations

- E.G. AOAC etc
- ‘if a proprietary method, certified by a third party in accordance with the protocol set out in EN/ISO standard 16140 or other internationally accepted similar protocols’
- What are similar protocols ? – no definition thus open to interpretation

# Non-officially validated Methods

- If not- methods shall be validated according to internationally accepted protocols & their use authorised by the competent authority
- We have no direction on authorisation by the Competent Authority

# Conclusions

- When introducing criteria its essential to have :
  - The limits
  - Where those limits apply
  - What they apply to
  - An analytical method (s)
  - Actions to be taken if outside of the limits
- In the EU Microbiological Criteria Regulations we have all of these

# Conclusions

- But
- If we wish to move away from the specified method we have some problems
- Interpretation of what the Regulation says
- Interpretation of the validation standard
- Understanding of what methods are valid to be used to test under the regulation.