Validating new microbiological methods: How equivalent is equivalent?

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Validation of new coliform/ *E. coli* methods

- Compare with LTB
- Use disinfected samples
- Confirm with BGLB and ECMUG
- Coliforms must produce gas
- *E. coli* must produce gas and cleave MUG

The changing face of definitions

- Coliforms originally defined as lactose fermenters that grew in the presence of bile salts and produced gas
- 100 years later virtually no regulators (or microbiologists) use that definition
- Currently ISO defines coliforms as members of the *Enterobacteriaceae* that produce the enzyme β-D-galactosidase

So why are definitions important?

- In sewage-polluted water about 25-30% more organisms meet the ISO definition
- This means methods based upon galactosidase detect 25-30% more target organisms
- OR
- Methods based on galactosidase give 25-30% FALSE POSITIVES

So how do we compare methods?

- The choice of definition is critical
- The choice of confirmation procedures is paramount
- How can an *E. coli* that does not produce gas be of less hygienic significance than one that does?
- How can a non lactose-fermenting *Klebsiella* be of less significance than one that does?

New methods moving forward

- Mostly based on galactosidase and glucuronidase
- Ignore the ability to produce gas
- Ignore the presence of lactose permease
- Aim to produce faster results
- Aim to be more accurate

Why the fuss about definitions?

- Would a method that missed *Salmonella* in 25-30% of food samples be accepted?
- Do we care if *Campylobacter* occurs in ready to eat foods 30% more often than reported?
- IS it OK to learn that water was contaminated over 100 hours after it was sampled?
- We need to use pragmatic and accurate definitions to facilitate develop of more useful methods!!

So what is a Reference Procedure?

- A reference procedure is one which other methods are compared against
- It should be accurate, meaning it is both sensitive and specific
- Time to result or “user friendliness” are unimportant
- Reference procedures should be constantly reviewed to ensure they represent the most accurate procedure available!!

Some practical field data

- The EPA reference procedure was compared to another method in an attempt to determine its performance
- Both specificity and sensitivity were compared
- The range of organisms recovered was studied
- Previous “equivalence” data were examined

Bravo for the reference procedure!

	18 hours	24 hours	48 hours	Confirmed
New Method Total coliforms	20 (21)	22 (23)	-	27
Reference Method Total coliforms	-	-	21 (25)	25
New Method *E. coli*	11	13	-	13
Reference Method *E. coli*	-	-	9	9

How did the “new method” perform?

- No difference from reference procedure for total coliforms
- Detected >40% more *E. coli*
- Small numbers of samples
- All samples NON DISINFECTED
- According to EPA procedure the new method had a false positive rate of >30% for *E. coli* although all isolates were confirmed!!

Maybe not so good!!

	18 hours	24 hours	48 hours	Confirmed
New Method Total coliforms	276	293	-	301
Reference Method Total coliforms	-	-	212	226
New Method *E. coli*	198	209	-	211
Reference Method *E. coli*	-	-	167	172

New method looks bad!!

- Performance is dependent on method of analysis of the results!!
- Either the new method detected 30% more coliforms
- OR
- New method had 30% false positive coliforms

How about *E. coli*?

- WHOOPEE!!
- We found 18% more *E. coli* with the new method
- OR
- POOH
- The new method is bad, it gives too many false positive *E. coli*

And here is the extreme!! 4 log reduction chlorination

	18 hours	24 hours	48 hours	Confirmed
New Method Total coliforms	11/60	32/60	-	45/60
Reference Method Total coliforms	-	-	4/60	5/60
New Method *E. coli*	14/60	22/60	-	29/60
Reference Method *E. coli*	-	-	6/60	8/60

Injured bugs don’t do well in the reference method

- The more severe the chlorination, the more damaged the remaining organisms are
- Organisms do not recover well in LTB
- It is extremely easy for relatively poor methods to become approved

Another new method!!

	16 hours	18 hours	24 hours	48 hours
New method Total coliforms	101	115	126	137
Reference Total coliforms	-	-	-	118
New method *E. coli*	101	114	121	133
Reference *E. coli*	-	-	-	112

You'd never approve this method at 16 hours, right??

- Method approved for 16 hours incubation
- Detects >30% more positives at 48 hours than at 16 hours
- Detects 10% less *E. coli* and nearly 20% less total coliforms at 16 hours than the reference method

Where are we at with things?

- The current Alternate Test Procedure needs updating
- It uses a lab procedure that is out of date
- There is no realistic procedure for demonstrating that a new method is more sensitive
- Definitions need revision in line with scientific best practise
- We need a standardized procedure that states what levels of sensitivity and specificity are required!