Single laboratory verification of new microbiological methods

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ISO 13843

- Currently under review
- Covers initial demonstration of performance characteristics and single lab validation
- Initial demonstration of performance characteristics are now essential for new ISO standards
- Single laboratory validation offers guidance on how laboratories should demonstrate their ability to perform a method

Single laboratory validation

- Sensitivity
- Specificity
- Efficiency
- Selectivity
- False positive rate
- False negative rate
- Repeatability
- Uncertainty of counting

Single lab validation

		Presumptive		
		+	-	
Confirmed count	+	a	b	a+b
	-	c	d	c+d
		a+c	b+d	n

Determining values

- Sensitivity = a / (a+b)
- Specificity = d / (c+d)
- False positive rate = c / (a+c)
- False negative rate = b / (b+d)
- Selectivity = a/n
- Efficiency = (a + d)/n

Repeatability

- The design for determining the repeatability performance of a method consists of 10 replicates of the same sample which are analysed in repeatability conditions, i.e. by the same technician on the same day, at the same approximate time and all samples incubated in the same incubator.
- A minimum of three sets of repeatability data should be prepared using different sources of target organisms. Naturally contaminated samples are preferable. The three sets of data are then collected and examined using the following procedure.

Uncertainty of counting

The reliability of the counts is determined by repeated counting of the colonies of the same plates within a short time. The observations on counting uncertainty will give an indication of potential problems with use of the method. Uncertainty of counting can be determined with single or multiple analysts. If multiple analysts routinely perform the test then uncertainty of counting should be determined with multiple analysts.

Upper limit for uncertainty should normally be less than 0.1.

The issue of false positive rate

- False positive rates are determined by confirmation of presumptive colonies
- Therefore the false positive rate is dependent both on the primary culture AND the confirmation procedure.
- The confirmation procedure is critical to the false positive rate value
- Ideally confirmation should be performed using definitive microbial identification (?16S)

The value of false positive rate

- The false positive rate should never be viewed in isolation
- Data must be evaluated while considering the effectiveness of the confirmation procedure
- Comparing the FPR from two methods (in isolation) does not always yield useful information

The value of false negative rate

- Again FNR is dependent upon the effectiveness of the primary culture method AND the confirmation procedure
- It is less dependent upon the confirmation procedure than the FPR
- Comparing the overall recovery of two methods helps to put FNR into perspective

Elevated false negative rates

- The FNR is determined by calculating the proportion of colonies/samples that do not appear typical on the primary isolation but that are found to contain the target organism
- Ideally samples that contain the target organism should yield a positive appearance in the primary test
- However, in some circumstances methods that yield elevated false negative rates may be superior to methods that have low false negative rates
- The important factor is the number of samples found to be positive

Elevated false positive rates

- No-one wants to see high false positive rates!!
- High false positives lead to a lack of confidence in the method
- The FPR is influenced by the primary isolation medium, the confirmation procedure and the definition of the target organism

Coliform data

- Reference procedure assumed to be 100% sensitive and 100% specific!!
- Calculated false positive rate for test procedure 8.3%
- Calculated false negative rate 10.2%
- Total positives by test procedure 152% of those obtained by reference procedure!
- True false positive rate (using bacterial identification) 0.2%

E. coli data

- Calculated false positive rate for test procedure 25%
- Calculated false negative rate 9.3%
- Total positives by test procedure 175% of those obtained by reference procedure!
- True false positive rate (using bacterial identification) 0%

Some “false positive” scenarios

- A strain of *E. coli* that does not grow at 44 C but the confirmation procedure utilizes this temperature
- A strain of *E. coli* that does not produce gas but the definition includes gas production
- A coliform strain that does not produce gas but the definition includes gas production
- A coliform strain that does not ferment lactose within 48 hours but the definition is based upon lactose fermentation

Things to consider!

- A reference document is being produced that describes single laboratory validation
- It is not and will not be accepted by all regulatory authorities
- Having a poor performance with one particular parameter does not make a method a bad method
- Be cautious with false positive and false negative values. They can be extremely misleading!!
- The true value of a test can only be determined when the purpose of the test is considered.