



Evaluation of Test Kits for Microbiological Analysis

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Microbiological Data may be Used to Assess:



- **Food Safety**
- **Verification/validation procedures in HACCP**
- **Adherence to GMP/GHP**
- **The utility (suitability) of a food or ingredient for a particular purpose**
- **The keeping quality (shelf-life) of certain perishable foods**
- **Acceptability of a food or ingredient from a source for which there is not confidence in the process**

Method Comparison / Validation Issues



- Most properties can be measured
- When 2 or more alternative methods exist for measuring the same property, how do you compare ?
- Do they really measure the same thing ?



Method Comparison / Validation Issues



- Philosophically , different methods can't measure exactly the same thing
- No measuring technique responds to only one single property
- Relationship of methods could very well depend on the material being measured

Method Validation - Terms



1. **Reproducibility** – between lab precision.
2. **Repeatability** – within-lab precision.
3. **Systematic error or bias** – deviation from the ‘true’ value.
4. **Specificity** – ability to measure what is intended to be measured.
5. **Limit of reliable measurement** – smallest increment that can be measured with confidence.
6. **Uncertainty in result**

(AOAC)

Validations Overview



- Single Lab
- Intralaboratory
- Interlaboratory



Single Lab



- One lab - one matrix – one analyte
- Matrix - analyte specific method
- Extreme validity
- Difficult reproducibility
- “In –House “ methods



Intralaboratory



- Within a lab
- Somewhat like single validation
- Typical statistical measures are used to verify performance
- Normally cover multiple matrices for one analyte
- Remember that the microbiologists performance and reliability is part of the verification process



Interlaboratory



- Throughout larger lab system
- Multiple sites
- Complex measure of ruggedness
- Both within and between lab variation measured
- Very Expensive



Validation Considerations



- **Product knowledge**
- **Previous use and standards**
 - **Hold release**
 - **Survey**
 - **Specifications**
- **Cost and Value**
 - **Balanced need**
 - **Fit for use**
- **Analysis performance**
 - **Optimized to meet certain criteria**
 - **Regulatory**
 - **Individual**

Testing



➤ Method Selection

- Analyte
- Matrix
- Time
- Money

➤ Method Execution

- Sampling
- Prep
- Test
- Result

Types of tests



→ Quantitative

- Microbiological
- Chemical

→ Qualitative

- Microbiological
- Chemical



Validation Process - Qualitative



- Determines equivalence of methods for an analyte based on a yes or no scale
- Determines proper testing conditions to achieve accurate results
 - Usually aligned by the best outcome for user
- Determines appropriate field of use



Matrix Considerations



➤ What is the matrix being tested?

- Categorize by basic characteristics
- Dry (flour , low moisture snacks , seasonings)
- Wet (liquids
- pH

➤ Are there matrix effects on the test?

- Inhibition – PCR
- Cross reactivity – immunoassay
- Background flora levels



Study Design



- Most important element of method comparison
- Qualitative or quantitative
- Choice of analyte
- Choice of samples size
- Inoculation levels
- Statistical Parameters
 - Number of samples
 - Analysis technique

Example – qualitative



- Comparison of 2 Salmonella methods
- Gold standard Cultural vs. PCR
- PCR test uses proprietary enrichment media

Question to answer “Is there a significant difference in the methods abilities to detect salmonella in X matrix ?”

What do we do?



- Determine what type of study is needed
- Determine appropriate test protocol
 - Diluents
 - Time
 - Temperature
- Determine matrices to test
 - Sample effects
- Determine inoculation levels to provide partial positive results
 - Strains and levels



Analysis



- Run each according to prescribed protocols
 - Gold standard protocol
 - PCR method protocol
- Verify quality systems around the tests
- Statistical analysis depends on initial test protocol
 - LOD
 - Method Side by Side
 - Paired or Unpaired Sample



Example - quantitative



- 2 methods to measure Aerobic plate count
- Gold standard vs. dry rehydrateable plate

- Question to answer “Is there a significant difference in the methods abilities to enumerate organisms in X matrix”

What do we do ?



➤ Determine appropriate test protocol

- Prep
- Time
- Temperature
- Enumeration procedure

➤ Determine matrices to test

- Sample effects

➤ Determine inoculation levels to give a response over a large range

- 5 to 6 log range

➤ Determine if a limit of detection study is needed



Analysis



- Run each according to desired protocols
- Verify quality systems around the tests
- Statistical analysis
 - T test to look at the mean log difference in counts over a given range
 - Samples paired

Summary



- **Methods need to be chosen that deliver the most reliable , relevant and cost effective information to a process**
- **Rigorous but relatively simple ,statistically valid protocols can be developed and run**

Summary



- Economic factors must be evaluated along with technical merit
- Validations and verifications are a key part of the testing process
- Quality systems should be in place to monitor changes in the system
- There is no perfect method !