“Uncertainty in Micro Lab"

Uncertainty Formula $(u) = \sqrt{\frac{\Sigma(X_i - \mu)^2}{n * (n-1)}}$

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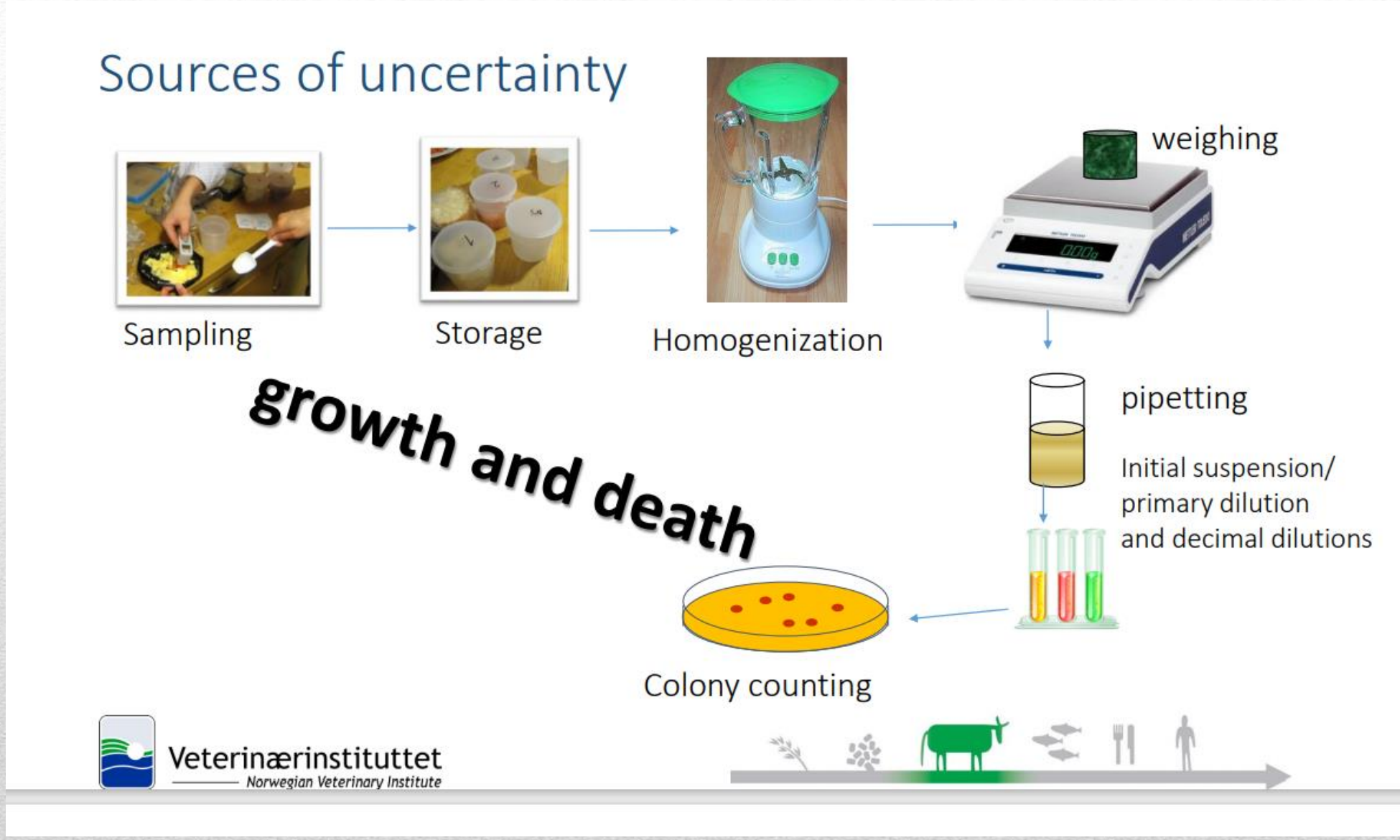
Sources of Uncertainty

“level of uncertainty” is “level of confidence”



Why are pathogens hard to detect:

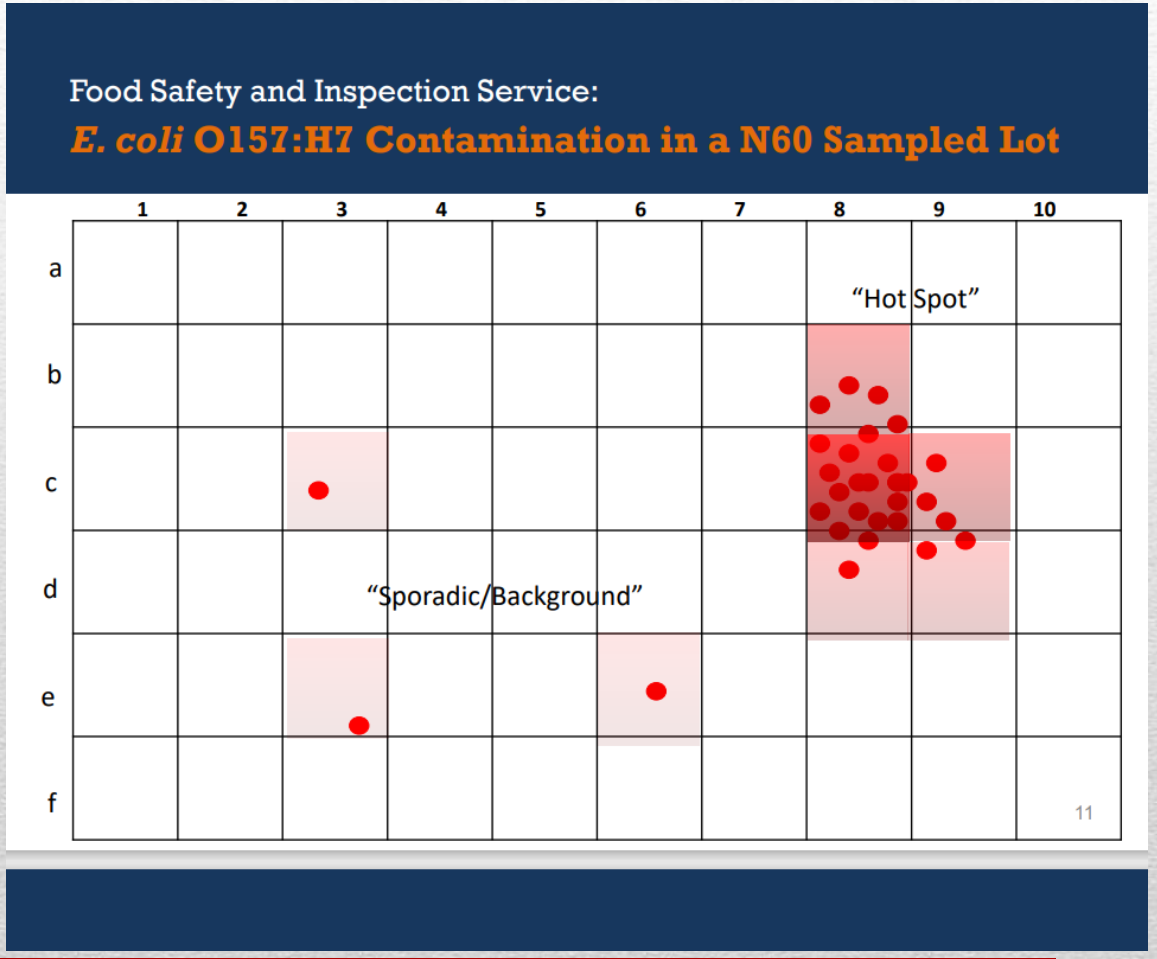
- Typically not evenly distributed
- Low levels
- Often injured when found in the product
- May be inhibited by food matrix

– Example: high amounts of fat may inhibit PCR assays;

- spices, salt, acidulants can affect isolation and detection

Positive samples out of sampled lot

- Zero tolerance vs rate of contamination in sampled lot



Probability of occurence

- Distribution of *E. coli* contamination in ground beef

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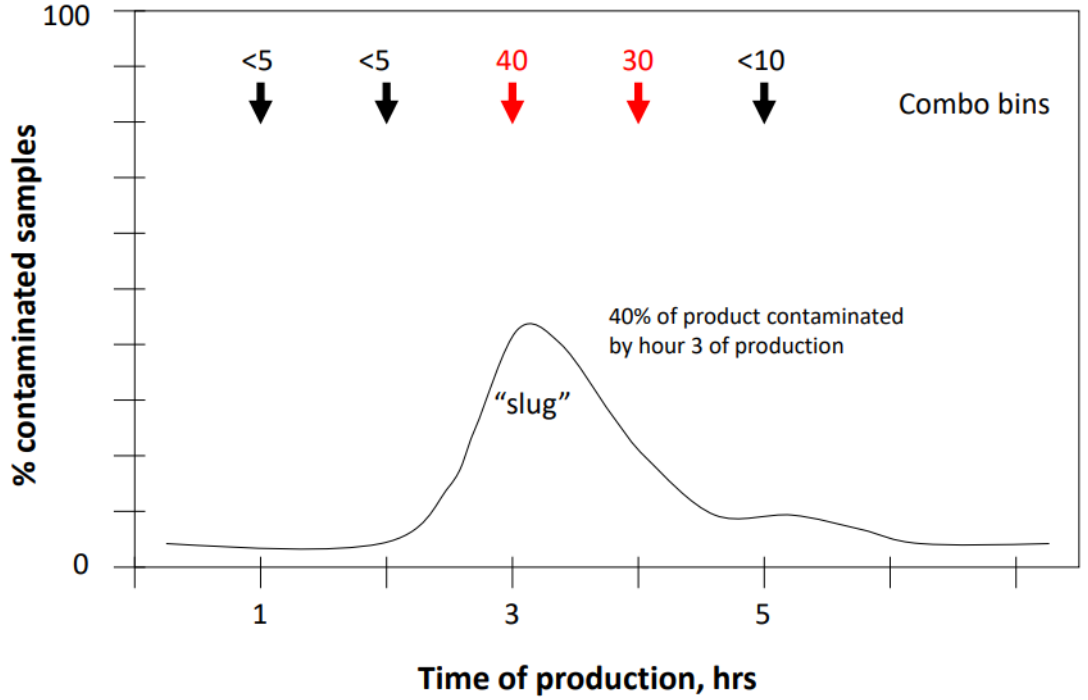
E. coli O157:H7 Contamination in Ground Beef



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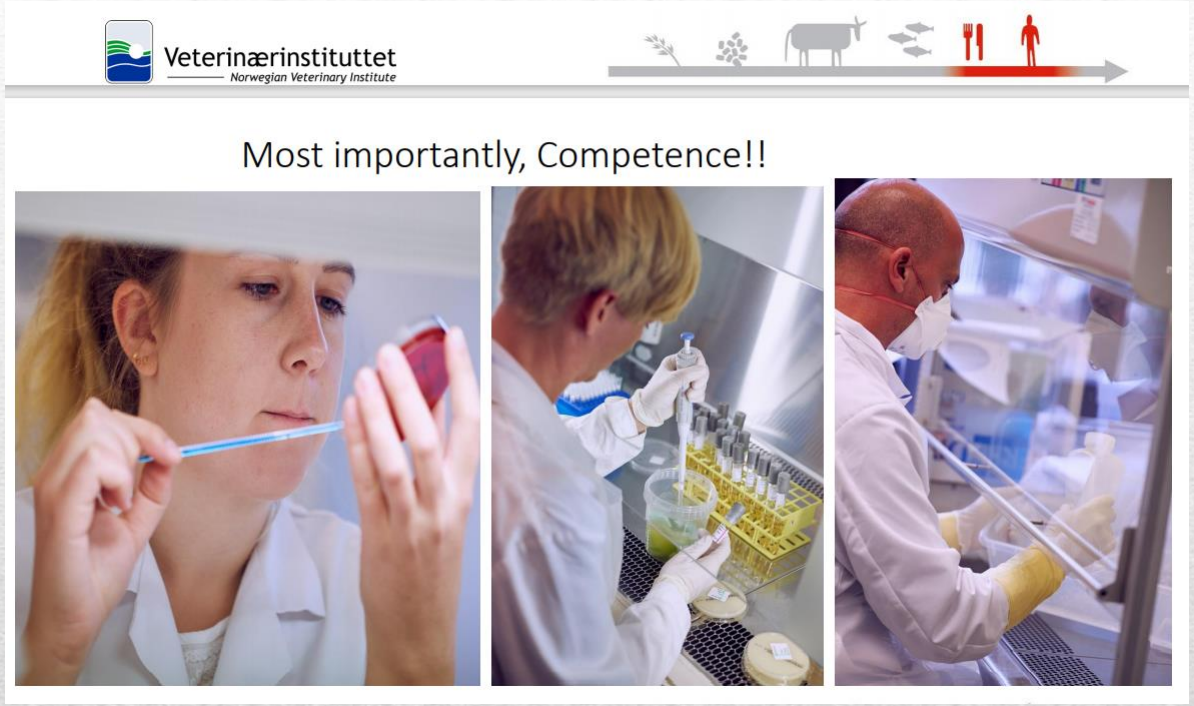
Steps in detection methods

- Sample collection
- Sample preparation
- Enrichment for the pathogen
- Screening of the Pathogen
- Confirmation of the Pathogen

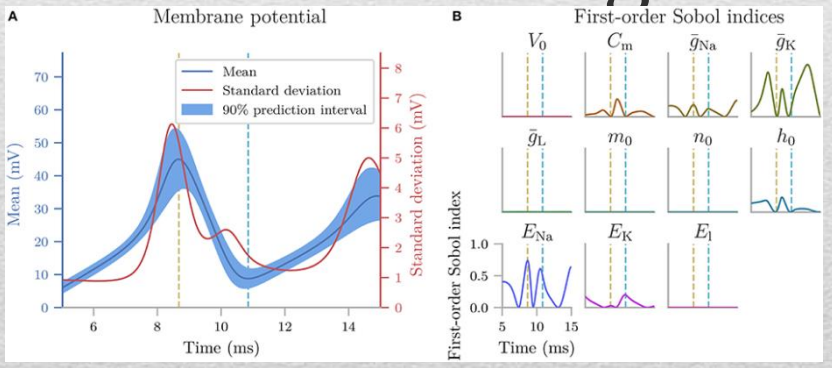


Consideration for testing methods

- Fit for the intended purpose of the analysis?
- Optimized and experimentally validated for sensitive detection of pathogens?
- Laboratory complying to the validated method protocol?

Assessing fitness for purpose

- Test portion appropriate to meet the need?
- Enrichment-based with the intent to detect the lowest possible numbers of stressed pathogen cells?
- Food matrix been validated for the method used?
- Confirmation procedures appropriate for determining true negative samples?



Test portion

- Laboratory sample preparation => “test portion” – “analytical unit” or “analytical portion”
- – Definition: the part of the “sample” that is actually tested by the laboratory
- The test portion determines the theoretical (i.e., best possible) sensitivity of the test
- – e.g., 1 cell/test portion
- – 25-gram test portion: detecting 0.04 cells/gram is possible
- – 325-gram test portion: detecting 0.003 cells/gram is possible

Enrichment

- Test portion is incubated 8-48 hours in a culture broth – Why?
- Contamination levels are too low for detection without enrichment
- Must grow to high levels so very small volumes have enough pathogen present for later detection steps
- Different pathogens require different enrichment media (broth)
- – One vs. two-stage enrichment
- Primary enrichment vs. secondary enrichment
- – Resuscitation vs. selective growth

Proper enrichment ??

- Resuscitation (lag phase) can require 2-3 hours before log-phase growth begins
  - Some samples support slower growth

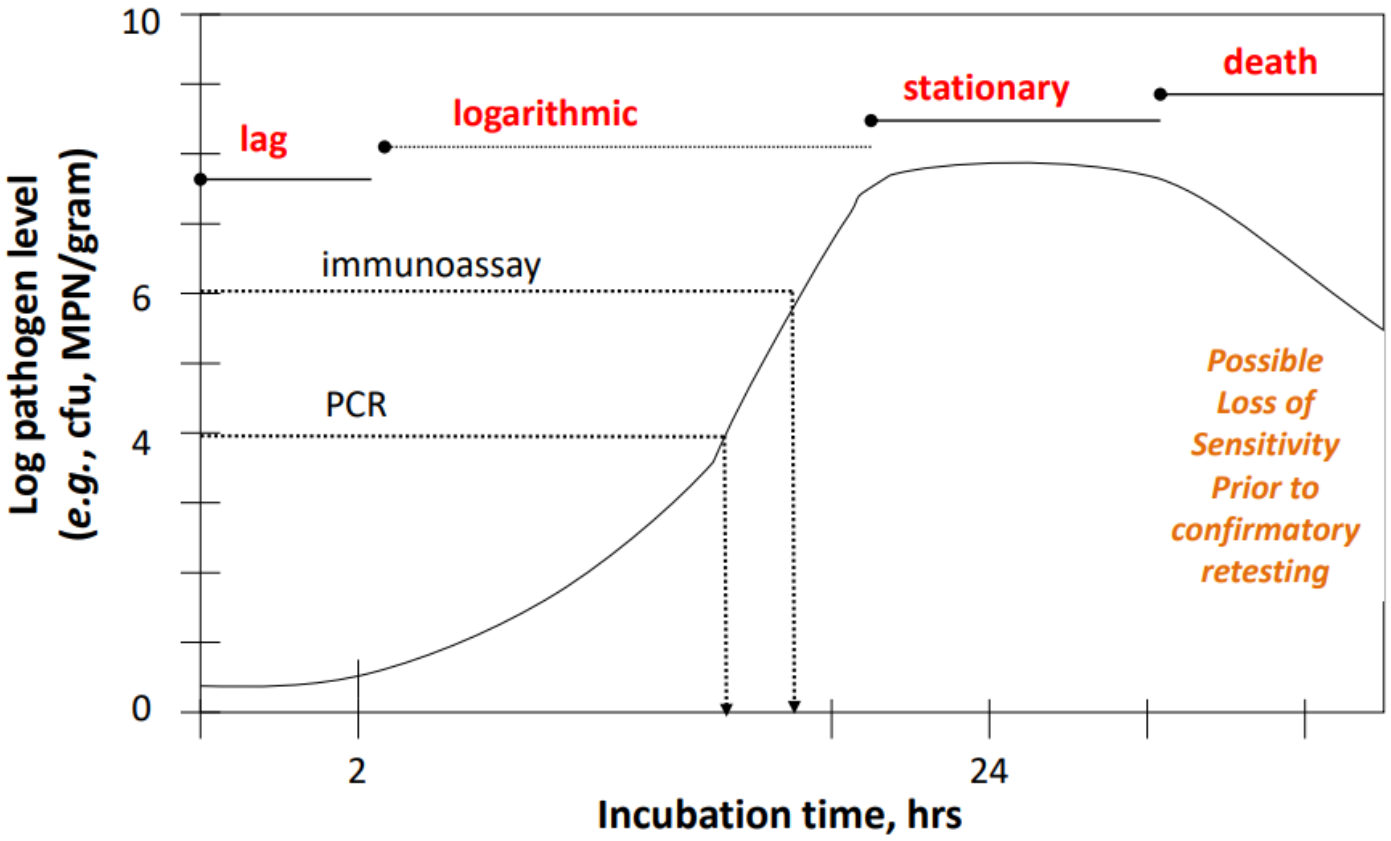
- Enrichment broth tempered to warm temperature prior to incubation?
  - Particularly critical for large test portions or shorter incubation periods

Pathogen growth during enrichment

Different screening tests require different levels of enriched pathogen

- Shorter incubation periods (<15 hours) may warrant additional scrutiny of laboratory compliance to the validated protocol
- Has enrichment/screening combination been validated for a larger test portion?
  - Particular concern for large test portions incubated for shorter periods
  - e.g., 375-gram test portion incubated for 8 hours
- Proposed incubations <8 hours may warrant OPHS review

Pathogen Growth During Enrichment



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Enrichment Period

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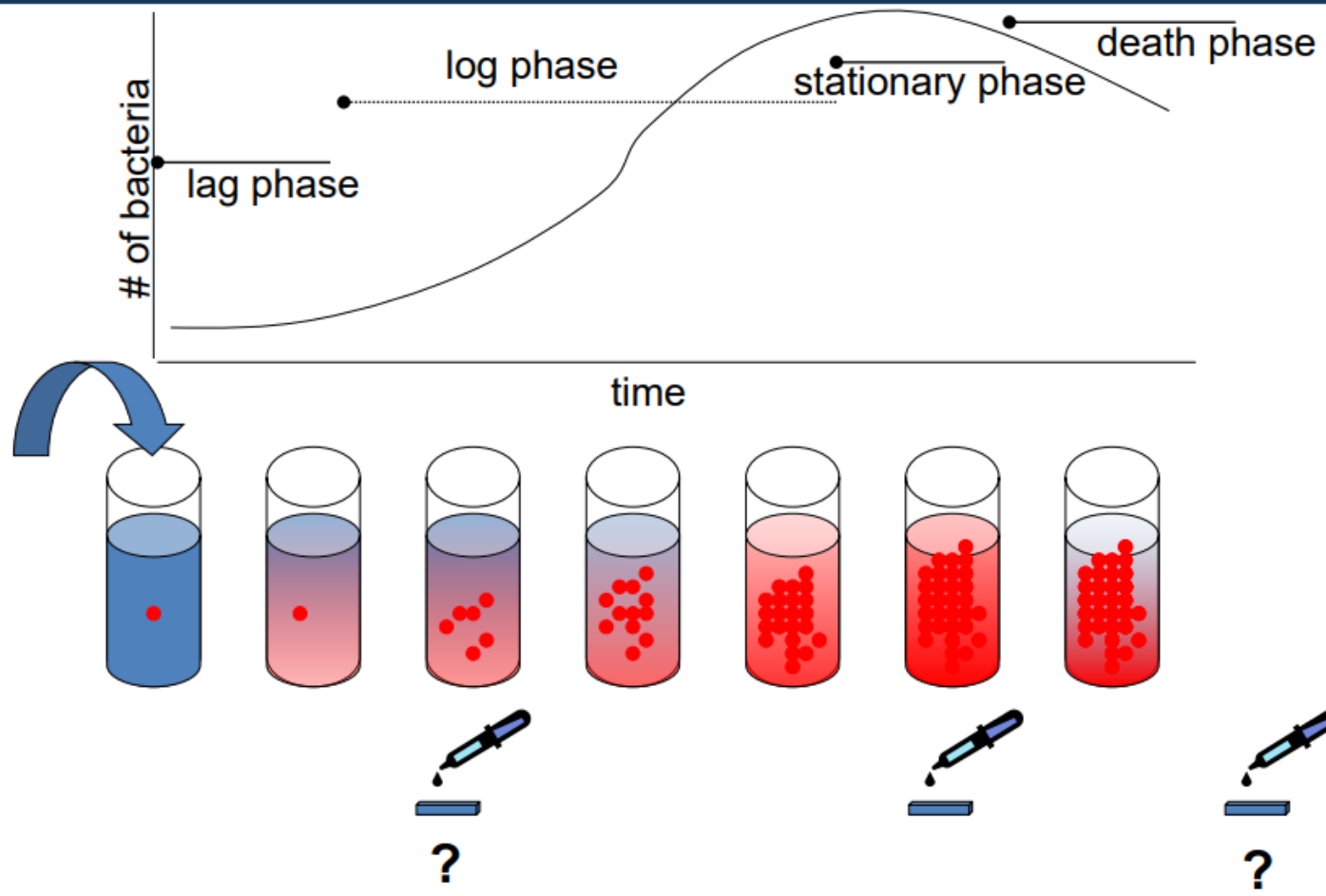
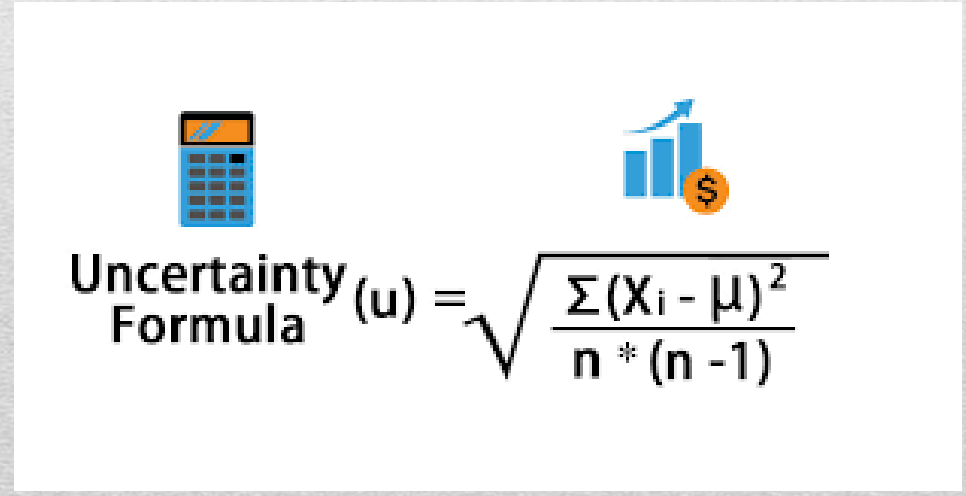
Role of Enrichment



Confirmatory testing

- **Non-culture confirmation (e.g., PCR)**
- **Culture confirmation** (e.g., FSIS confirmation)
- – Plating the enrichment on selective and differential agar media
- – Immunomagnetic separation (IMS) necessary prior to plating for E. coli O157:H7 and non-O157 STECs
- Suspect colonies = “presumptive positive”
- – Purification and confirmatory identification tests including:
- Biochemical (e.g., identifies “E. coli”)
- Serological (e.g., identifies “O157” and “H7”)
- Genetic (e.g., identifies “stx” = Shiga toxin genes)



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Value of Validation

- Determines performance characteristics of the method in comparison to a gold standard (reference) method (*e.g.*, usually FSIS or FDA method)
- Independent evaluation provides credibility
- Rigor varies (multilab vs. single lab, # tests, etc)
- Still must consider fitness for purpose and how the method is applied
  - *e.g.*, some AOAC-validated methods are not consistent with FSIS goals or Compliance Guidelines

Testing Method Validation

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Testing Method Specifications

- Sensitivity: probability that truly positive samples are detected as positive by analytical test
  - 100 – false negative rate
- Specificity: probability that truly negative samples detected as negative by analytical test
  - 100 – false positive rate
- Level of detection (LOD): lowest level of contamination reliably detected by analytical test
  - LOD expressed as ratio of organisms to quantity tested material (*e.g.*, CFU per gram, MPN per mL, CFU per square-ft) but definitions vary (e.g., LOD95, POD)

Recommendations to min uncertainty

- 1. personnel
- 2. equipment
- 3. Diluents and media
- 4. Incubation
- 5. Primary sampling
- 6. Analytical or test sample
- 7. Examining culture and recording data
- 8. Quality monitoring

British Standards Institute,
the International Standards Organisation (ISO),
Codex Alimentarius,
the International Dairy Federation (IDF),
the Nordic Committee for Microbiological Standardisation (NMKL)
and
AOAC International

seeking to define and to provide measurements of uncertainty associated with methods used for the examination of foods for pathogenic and other micro-organisms.

References

- NMKL Procedure No. 8, 4th Ed., 2004: Measurement of uncertainty in quantitative microbiological examination of foods
- NMKL Procedure No. 32, 2017 Verification of microbiological methods
- ISO/NP 19036:2016. Microbiology of the food chain – Guidelines for the estimations of measurement uncertainty for quantitative determinations
- Eurachem – AOAC Europe Workshop 29 May 2017