

**Microbiology Workshop
CAEAL Biennial Training – April 4-5, 2005**

CONSENSUS OR POSITION

A) Polymerase Chain Reaction (PCR) Methods – **do we have the expertise amongst us?**

- Only 5 assessors felt comfortable to assess PCR methods
- Only 4 labs indicated that they have PCR methods for microbiology
- Microbiology assessors need appropriate training
- Specific EPA checklist is available; Rhonda Schop will provide CAEAL with link to website

CONSENSUS: WE can do this with appropriate training and a specific checklist.

B) Items on the microbiology checklist that need to be clarified:

03.01 – Test Method Content

Suggestion: **add a bullet requiring use of appropriate significant figures?**

CONSENSUS: Not required. Already included in colony counting and reporting criteria

03.01 – Test Method Content

Requirement: *details on reagent preparation, storage and shelf-life*

Problem: **Some labs put “see label” in their methods; is this acceptable?**

CONSENSUS: Not acceptable. Lab must provide an SOP, reagent prep log records and objective evidence of performance acceptability

Problem: Can performance criteria be used to justify using media beyond its expiry date?

CONSENSUS: No! An expiry date is an expiry date.

03.02 – Sample History

Requirement: *QC on new, uncertified sample bottles performed, if preparing own bottles (minimum of 1 per batch for cleaned and reused bottles; minimum of 1-2% for new, non-certified bottles)*

Problem: what is acceptable QC? Is running blanks and autoclave checks enough?

CONSENSUS: No labs use uncertified or in-house prepared bottles for collecting water for microbiological analysis. In addition to manufacturers' certificate of sterility verification, at least one bottle from each lot of new, certified bottles should be verified for sterility or for influence on microbiological testing parameters. Each laboratory to determine appropriate verification procedure. Data from daily "blanks" is also useful as supplementary evidence of acceptability.

03.02 – Sample History

Requirement: *holding time of samples should not exceed time specified by appropriate method/regulation*

Problem: Most labs are referencing Standard Methods, but following regulatory holding times. Should we be asking for validation?

CONSENSUS: No! Ensure that whatever procedure the lab is following adheres to the specified holding time.

03.03 - Test Organism History

Requirement: *purity check (i.e. evidence that the culture is pure each time it is used).*

Problems:

The checklist does not specify how. A laboratory was checking for one type of colony on a selective plate; should the lab also spread *E. coli* in non-selective media and verify that there is only one type of colony?

CONSENSUS: Yes, a non-selective medium must also be used.

Should the word “culture” be replaced with “subculture”? If a lab subcultures once a week and does a purity check, but use a bit of the weekly stock to run controls daily, do they need to be doing a purification check daily?

CONSENSUS: The term subculture is more appropriate. Lab should be using weekly working subcultures, not weekly stock cultures for daily controls; but, regardless, as long as the purity of the (sub) culture has been verified each week using non-selective media, there is no need to run a daily purity check.

05.01 – Equipment

Requirement: *fridges for sample and reagent storage are maintained within the specified temperature range and temperatures monitored and recorded daily*

Question: should it be suggested to record temperatures twice daily for fridges, especially for those with samples and media?

Unfortunately, I missed this question!

Personal opinion-No. Constant or min/max temperature recording would provide better data. There is no added benefit to twice daily temperature records.

05.01 – Equipment

Requirements:

- *stereomicroscope(s), incident light and a 60 degree angle in use for counting colonies on mEndo agar*
- *Quebec colony counter(s) or stereomicroscopes in use for pour plate and spread plate method (s)*

Problem: **a large laboratory with microbiology expertise maintains that stereomicroscopes cannot be used for pour plate or spread plate methods**

CONSENSUS: *stereomicroscopes should not be used for pour plate and spread plate method (s)*

05.01 – Equipment

Requirement: *“incubators available and functioning properly; i.e., adequate humidity....”*

Problem: **Is this really only applicable for membrane filter methods. What is “adequate” humidity?**

CONSENSUS: Concept is applicable to all microbiological methods where evaporation of moisture could impact the results, e.g. MF and plating methods. Assessment of adequacy of humidity in microbiology is essentially empirical (Do plates show evidence of moisture loss during normal incubation time?)

05.01 – Equipment

Requirement: *is there a regularly scheduled service/maintenance program for each piece of equipment?*

Problem: **Must this be “regularly scheduled”?**

CONSENSUS: Service scheduled as required. Maintenance must be scheduled where appropriate

05.04 – Equipment Requiring Calibration

Requirement: ensure all equipment requiring calibration is labeled to indicate calibration status and date of calibration verification.

Problem: **What equipment in microbiology is applicable to this requirement?**

CONSENSUS: Balances; semi-automated pipettes; thermometers; biological safety cabinets

06.01 – Supplies

Requirement: *Records of reference standard/material certificates*

Problem: **Is the documentation that comes with Cultiloops and Bacterol Plus Tubes adequate? These do not come with a certificate of ATCC purity.**

CONSENSUS: No! Still need to check ID and purity of culture

06.01 - Supplies

Reagent water for use in microbiology tests continues to be a controversial item. Please see excerpt from comments from an assessor:

The major issues revolve around hospital labs that purchase water, small labs that purchase distilled water, conductivity testing, and chlorine testing.

Task: come up with clear requirements that meet intent of Standard Methods.

CONSENSUS: Modify checklist (***do NOT have to check conductivity if lab is distilling water in-house or purchasing distilled water***) "and receives a certificate of analysis from the supplier for parameters as per table 9020ii in Standard Methods". Distilled water prepared in-house must conform to the same requirements as reagent water prepared in-house.

06.01 – Supplies –Availability

Requirement: *quality control of filters done (e.g., inhibitory effects, hydrophobicity)*

1. Hydrophobicity – does a statement on water flow rate on a certificate meet this requirement, or does the laboratory need to further testing? If so, what is acceptable?

CONSENSUS: Statement on flow rate is inadequate. Must conduct “charcoal” test or confluent culture.

2. Inhibitory effects – does a statement of recovery on a certificate meet this requirement, or does the laboratory need to further testing? (Note: by comparing positive cultures on selective and non-selective media, isn't the laboratory is, in effect, testing inhibitory effects, and just needs to track the lot numbers of media?).

CONSENSUS: No! See specific information in training binder.

3. If we're accepting certificates for sample bottles, why are we not accepting certificates filters?

CONSENSUS: Bottles must be verified, so must MF's

06.05 – Supplies – Labware

Requirement: *Procedures in place to monitor air contamination; e.g., exposure of plate count agar plates (minimum monthly).*

Problem: heard opinion more than once that this isn't scientifically valid

CONSENSUS: Should be discontinued as a requirement. Weekly environmental swabbing of working surfaces for contamination of “target organisms” is recommended.

07.01 – Record Keeping

Requirement: *Records of Volumetric Traceability*

Problem:

1. For volumes of 100 mL, are records of volumetric traceability required? If so, frequency? (e.g., on delivery? Annually?)

CONSENSUS: No! Small variations in 100 mL volumes of water contributes minimally to uncertainty of measurement in microbiology.

2. Table: Should the temperature requirements for total coliforms be changed to 36 +/- 1 degree, or would the change be media-dependent?

CONSENSUS: This item was discussed and recommended for change 2 year's ago. Unless required by regulatory authorities, incubation temperature of 36 +/- 1 C is acceptable. Table on page 9, appended to CAEAL Microbiology Checklist should be changed Regulatory authorities that specify 35 +/- 0.5 C should be requested to reconsider this prescriptive requirement. ISO and HC accept 36 +/- 1 C!

Other Issues:

Definition of and analytical methods for Biosolids (Dewatered Sludge)

CONSENSUS: Dewatered sludge should be regarded as wastewater. For accreditation purposes, labs should be allowed to use the existing methodologies that apply to wastewater on dewatered sludges.