Transformation of Microbiological Methods

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General Dynamics Information Technology

In the Beginning…….

- Media prepared from scratch
- Laboratory supplies were reusable
- Pipets were washed and plugged
- Glassware sterilized by boiling or pressure cooker
- Limited safety precautions in place
- Exposure to hazardous chemicals common
- Mouth pipetting was acceptable

In the Beginning……. (cont.)

- Most environmental methods were not formally validated
- Methods could be vague
- Quality control (QC) criteria were not available to assess analyst proficiency
- Analytical QC checks consisted of positive and negative controls and sterility checks
- Lack of standards or precise QC controls

As Time Progressed Things Got Better

- Commercially prepared media
- Disposable laboratory supplies
- Plastic labware; no longer reliant on glass
- Better equipment
- Additional safety precautions implemented
- Material Safety Data Sheets (MSDS)
- Mouth pipetting is no longer acceptable

Early Environmental Monitoring Methods

- Generally culture-based
- Required expertise
- Labor intensive
- Resource intensive (e.g., media, glassware)
- Days to results
- Detect and enumerate indicator organisms

Most Probable Number (MPN) Methods

- Multiple media and transfer steps depending on target
- Multiple configurations
  - 5 tubes
  - 10 tubes
  - 15 tubes - 5 tubes per volume/dilution
- Applicable for liquid, semisolid and solid samples
- Wide analytical range
- Bacterial density is determined using MPN tables or calculators
- Not very precise
- 72 – 96 hours to results
- Required analytical expertise

Membrane Filtration (MF) Methods

- Multiple media and transfer steps depending on method
- Direct Counts
- Narrow analytical range
- Sample volume based on estimated bacterial densi
- Ideal plate counts range between 20-80 or 20-60
- High turbidity may clog filters
- Considered more precise than MPN methods
- 24 – 48 hours to results
- Required analytical expertise

Improvements to Environmental Methods

- Ease of Use
- Rapidity
- Formal validation of methods
- Inclusion of QC criteria to demonstrate proficiency and assess matrix issues
- Availability of standardized QC materials
- Development of hybrid methods (e.g., RV-PCR)

Enzyme Substrate Methods

- Use chromogenic and fluorogenic substrates
- Multiple configurations
  - 5 or 10 tubes
  - 15 tubes - 5 tubes per volume/dilution
  - Multiple Well (51 and 97 wells)
- Wide analytical range
- Bacterial density is determined using MPN tables
- 18 – 24 hours to results
- Vendor provided comparators
- UV light used to determine fluorescence

Traditional Polymerase Chain Reaction (PCR)

- Rapid
- Fairly low cost
- Analytical expertise required
- Requires a post-PCR step for detection or visualization of the DNA
- PCR reaction is confirmed by size as compared to a DNA ladder
- Limitations include low sensitivity and non-quantitative results

Real Time PCR or qPCR

- Rapid same day results
- Quantitative
- PCR product can be detected in real time
- Analytical expertise required
- Quantitation is based on DNA standards or calibrators of known concentration
- Limitations: increased reagent costs and specialized thermocyclers

Droplet Digital PCR (ddPCR)

- High precision and sensitivity
- High throughput
- Quantitative
- Sample is partitioned into 20,000 nanoliter-sized droplets
- Amplification is carried out within each droplet
- Poisson statistics are then applied to the fraction of “positive” droplets to calculate the absolute concentration of the template DNA
- Analytical expertise required

Method Validation

- EPA1600-series Microbiological Methods have all been validated
  - Minimum of 10 laboratories per study and matrix of interest
  - Reference matrix and matrices of interest
  - Assessed method performance and developed QC criteria
- Methods 1103.1 and 1106.1 have also been multi-laboratory validated
- Validated methods are posted on the CWA webpage

Method Validation (cont.)

- QC Criteria
  - Results from the analysis of spiked reference matrix samples were used to develop initial precision and recovery (IPR) and ongoing precision and recovery (OPR) criteria
  - Results from the analysis of spiked matrix of interest samples were used to develop matrix spike/matrix spike duplicate (MS/MSD) criteria
  - For qualitative methods results from spiked reference matrix samples were used to develop initial demonstration of capability (IDC) and ongoing demonstration of capability (ODC) criteria
- In addition, to QC criteria the methods also include detailed method-specific QC checks and verification procedures

Standardized QC Materials

- Flow-sorted Spikes
  - Developed for *Cryptosporidium* and *Giardia* analysis
  - Used the technology to develop bacterial spikes
  - EPA evaluated the bacterial prototype BioBall® for *Salmonella* during the MLV study of Method 1682
  - Subsequent EPA validation studies have included both laboratory-prepared and BioBall® spikes
- The use of standardized QC materials are now an integral part of many laboratories Quality Assurance Programs

Rapid Viability-PCR (RV-PCR)

- Qualitative
- Viability determination
- Enrichment media is added to the samples
- Prior to incubation the T_0 aliquot is removed; after incubation the T_{24} is removed
- T_0 and T_{24} aliquots are run on the same PCR plate
- Lower cycle threshold for the T_{24} aliquot indicates the presence of viable organisms
- Analytical expertise required

Some Things to Think About

- On the surface all of these changes have been positive but there have been trade-offs
- For example, the availability of disposable supplies

Disposable Labware and Supplies	
Advantages	**Disadvantages**
Reduced burden on laboratory staff	The amount of laboratory generated waste has increased
Products are sterile and ready for use	Potential staff reduction
Reduced safety concerns by switching from glass to plastic	
Reduced costs	

Some Things to Think About

Availability of real-time PCR methods for water quality monitoring

Real-Time PCR Methods	
Benefits	**Limitations**
Same day results	PCR-inhibitory substances
Better sensitivity than culture	Not able to differentiate between viable and non-viable organisms
Detect bacteria and viruses that are non-culturable	Small sample volumes
Reduced risk	Sample preparation procedures can be labor intensive and time consuming
Automated, results instrument derived	Instrumentation can go down

What's Trending?

- Monitoring for pathogens, not just indicators, in the environment to track disease prevalence
- Microbial source tracking methods
- Automated microbial detection systems controlled by smartphone apps
- Continuous microbial monitoring systems