Rapid Microbiological Methods

Understanding the Technologies and Regulatory Expectations for Validation and Implementation

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Microbiology: Where We’ve Been

- 1683. Anton van Leeuwenhoek observes bacteria
- 1861. Pasteur disproves spontaneous generation
- 1876. Koch defines *pure culture* and *colony*
- 1881. Fanny Angelina Hesse introduces agar-agar
- 1884. Hans Christian Joachim Gram develops the Gram stain
- 1887. Julius Petri invents glass plates for bacterial growth
Microbiology: Today

- Still using 19th-Century methods...counting colonies on agar plates and Gram staining
• Classical or conventional microbiology methods are limited by slow microbial growth rates
• Variability of microorganisms in their response to culture methods
• Most microorganisms in the manufacturing environment, in-process samples and raw materials are starved, stressed or injured, and current media and incubation conditions are not optimal for the resuscitation and growth of these microorganisms
• Many times we will observe zero colony forming units (CFU) on agar plates when in fact, viable microorganisms are present
For many years, we have successfully provided pharmaceuticals to the public using batch processing with laboratory testing conducted on collected samples to ensure product quality.

Is this true?
Microbiology and Pharmaceutical Manufacturing

- Are we in control?
- Are we an industry without microbial contamination events?
- Can we respond to excursions in a timely manner?
- Do we continually improve our processes?
- Why change now?
Why Change Now?

• Our current practices are not sustainable
• We must…
  ▪ better understand our processes, and the impact on product and patient,
  ▪ reduce variability,
  ▪ reduce waste and wasteful activities, and
  ▪ increase manufacturing capacity and efficiencies.
How & What Do We Change?

- Significant opportunities exist for improving the efficiency of manufacturing and quality assurance through the application of modern process analytical tools.
- There are regulatory initiatives that are recommending changes in the way we approach microbiology testing.
- These include Rapid Microbiological Methods (RMMs).
Rapid Microbiological Methods (RMM)

- Novel technologies that provide microbial detection, quantification and identification results much faster than conventional methods
- Increased accuracy, reproducibility and sensitivity
- Automated, miniaturized and high-throughput processing
- Enhanced sampling, data handling and trend analysis
- For some technologies, results in real-time
• Many RMMs do not require microbial growth
• Enhanced detection of single cells and stressed or injured microorganisms
  ▪ Viable but non-culturable (VBNC)
• Improved microbial identification and strain differentiation
• Numerous applications, including bioburden, sterility, environmental monitoring, process water, raw materials, endotoxin, microbial identification, Mycoplasma
Contamination Control and Testing Points During Bioprocessing

Cell Line Development

Fermentation - Harvest

Purification

Formulation

Sterility
Mycoplasma

Mycobacteria
Virus (MMV and Vesivirus)
Sterility-Foreign growth
Cell mass/viability
Bioburden - Biofilm

Viral clearance/inactivation
Residual DNA Quantification
Sanitization
Bioburden

Endotoxin
Bioburden
Sterility

Environmental monitoring (air, surface, compressed gas)

Presence/absence of specific organisms or microbial identification
Contamination Control and Testing Points During Fill Finish

Components, raw material and API testing
- Sterility
- Bioburden
- Detection of indicator or objectionable organisms
- Mycoplasma
- Endotoxin

Compounding and filling, in-process testing
- Bioburden
- Personnel monitoring
- Purified water testing
- Endotoxin

Finished product release testing
- Sterility
- Microbial limits
- Bioburden
- Endotoxin

Environmental monitoring (air, surface, compressed gas)

Presence/absence of specific organisms or microbial identification
Regulatory Enablers

- International Conference on Harmonisation (ICH)
- Quality by Design (QbD)
- Process Analytical Technology (PAT)
- U.S. Food and Drug Administration (FDA)
- European Medicines Agency (EMA)
- Australian Therapeutic Goods Administration (TGA)
- Japanese Pharmaceuticals and Medical Devices Agency (PMDA)
- World Health Organisation (WHO)
ICH

• ICH Q8; Pharmaceutical Development
• ICH Q9; Quality Risk Management
• A systematic process for the assessment, control, communication and review of risk to the quality of drug product

• Implement “real time” quality control, leading to a reduction of end-product release testing
Quality by Design (QbD)

• Quality should be built-in or should be by design, and product cannot be tested into compliance
• Release testing should seldom result in an out of specification result
• If we properly design and validate our processes and include analytical testing points throughout manufacturing, release testing can be reduced or eliminated
• This is the basis for Process Analytical Technology (PAT)
FDA PAT Initiative

- A system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring predefined product quality at the end of the manufacturing process.

- The term “analytical” is viewed broadly to include chemical, physical, microbiological, mathematical, and risk analysis.
This 2004 guidance recommends the use of rapid genotypic methods for microbial identification, as these methods have been shown to be more accurate and precise than biochemical and phenotypic techniques:

- Especially valuable for investigations into failures (e.g., sterility test; media fill contamination)

Other suitable microbiological tests (e.g., rapid methods) can be considered for environmental monitoring, in-process control testing, and finished product release testing:

- Must demonstrate that these new methods are equivalent or better than conventional methods (e.g., USP)
FDA amended the sterility test requirements for biological products in their Final Rule, “Amendments to Sterility Test Requirements for Biological Products.”

Effective June 4, 2012

Many changes associated with the traditional sterility test, but also provides guidance on validating RMMs
- Specificity (panel of organisms in the test matrix)
- Ruggedness and robustness
- Limit of Detection
In August 2011, the FDA published their new strategic plan for regulatory science

“Develop sensitive, rapid, high-throughput methods to detect, identify, and enumerate microbial contaminants and validate their utility in assessing product sterility”
FDA Microbiologists Support RMMs

Alternative Microbiology Methods and Pharmaceutical Quality Control
David Hussong, Ph.D., and Robert Mello, Ph.D.
New Drug Microbiology Staff, Office of Pharmaceutical Science, Center for Drug Evaluation of Research
U.S. Food and Drug Administrations
American Pharmaceutical Review, Jan/Feb 2006

• New microbiology methods can offer advantages of speed and precision

• Quality by design principles and risk analysis methods must be extended to the development of new microbiological technologies
The use of rapid microbiology methods by the pharmaceutical industry should offer many advantages:

- Receiving microbiology test results sooner will provide for better control and understanding of the manufacturing process via faster feedback
- Industry should not feel that FDA will be a hindrance to the appropriate use of these methods

Rapid Microbiological methods in the Pharmaceutical Industry
Bryan S. Riley, Ph.D.
New Drug Microbiology Staff, Office of Pharmaceutical Science, Center for Drug Evaluation of Research
U.S. Food and Drug Administrations

American Pharmaceutical Review, Mar/Apr 2004
FDA Validation Expectations

• The FDA accepts any of the available guidance documents as a starting point for RMM validation
  - USP <1223>, *Validation of alternative microbiological methods*
  - *Ph. Eur. 5.1.6, Alternate Methods for Control of Microbiological Quality*

• You can develop your own validation strategy as long as it is scientifically sound and defendable
FDA Research Exemption

- All data generated by the RMM under investigation is for research purposes only
- All GMP decisions, including batch release, are based on the current approved validated methods
- Use an internal written document and/or comparability protocol to communicate this strategy
Can include RMMs in an NDA, ANDA, IND, BLA

Existing products may require a post-approval change or prior-approval supplement in the relevant CMC sections

Formal changes can include the use of a comparability protocol and approval through the FDA PAT Team

Use of a Special Report (usually no data, but a summary may be required for certain products such as biologics)

Reduced reporting category; CBE-0 or CBE-30
FDA Submissions and Approvals

- For in-process tests and methods that are not in a regulatory dossier, a formal submission may not be necessary
  - Environmental monitoring, purified water testing, in-process or pre-filtration bioburden testing
- Review current submissions to determine if there will be method and/or specification changes associated with the RMM being implemented
• RMMs clearly have the potential to be used to support QbD (Riccardo Luigetti, 2009 PDA RMM Conference)

• The introduction of such methods are in general supported by the regulatory competent authorities in the European Union

• The implementation of the revision of the variations regulations simplifies the introduction of changes to Marketing Authorisations, including the introduction of RMMs
EMA Submissions and Approvals

• EU approvals have been considered (by many companies) to be more burdensome than in the U.S.

• Historically, changes to a method in a Marketing Authorisation required a separate Type Variation (for each product/process) to be submitted, which could be very costly and time consuming

• Now, companies can group variations under the same or different Marketing Authorisations such that they can all be assessed at the same time
• 2011: Post Approval Change Management Protocol (PACMP)
• Similar to FDA’s comparability protocol (CP)
• Formal review and approval prior to the start of testing
• But, the data must be submitted
• Reduced reporting structure, similar to FDA’s CBE-0 or CBE-30
EMA Validation Expectations

- Use Ph. Eur. chapter 5.1.6 as a starting point for RMM validation
- The guidance document does not need to be followed exactly, yet all deviations from the guidelines should be clearly stated and reasons provided
- The use of environmental isolates and stressed organisms is strongly recommended
World Health Organisation (WHO)

- GMPs for Sterile Pharmaceutical Products (Annex 6; 2011)
- The use of rapid microbiological methods to replace the traditional microbiological methods, and to obtain earlier results on the microbiological quality of, for example, water, the environment or bioburden, could be considered if appropriately validated and if a comparative assessment of the proposed rapid method is performed against the pharmacopoeial method.
RMM Technology Examples

- The use of viability stains and laser excitation for the detection and enumeration of microorganisms without requiring cell growth
- Optical spectroscopy, such as light scattering
- Detection of cellular components (e.g., ATP and endotoxin)
- The amplification of nucleic acids and detection of specific genetic sequences
- The use of fluorescence techniques to rapidly detect the growth of microorganisms on conventional media
- Micro-Electro-Mechanical Systems (MEMS), such as microarrays, biosensors, Lab-On-A-Chip and nanotechnology
Disclaimer

- Although I consult with many RMM suppliers, the examples provided in the following slides are not endorsements of the technologies disclosed.
- There are more than 60 different RMMs that have been implemented or reviewed by various industry sectors.
- We will review a very small number of these.
- For an in-depth review of RMM technologies, workflow, and other relevant information, please visit the RMM Product Matrix at http://rapidmicromethods.com.
• Automated enumeration of bacteria, yeast, mycoplasma and spores (bacterial and mold) as early as 4 minutes using flow cytometry
• Accurate counting of 10 – 10^6 organisms per mL
• Fully automated, robotic arm processes samples
• Microorganisms are captured on a membrane, which are stained with a non-fluorescent substrate
• The substrate is enzymatically cleaved to release a fluorochrome
• A laser scans the membrane
• A viable count is displayed
• Bacteria, yeast, mold and spore count within 90 minutes
Rap.ID Particle Systems

• Viability staining and Raman spectroscopy
• Particles are collected on metal foil and enumeration in 4 minutes.
• Viable cells are then subjected to Raman. The spectral signature is compared with a database and an ID provided within 5 seconds. 150 bacteria and spores
• 300-600 ID’s per hour; non-destructive
Millipore Milliflex Rapid Microbiology Detection System

- Utilizes a filter membrane to capture individual cells, allow them to grow into micro-colonies
- Add luciferin and luciferase; ATP bioluminescence is detected and micro-colonies are enumerated
Bruker microflex and MALDI BioTyper Software

- Intact cells from a pure culture are added to a stainless steel target plate and UV-absorbing matrix is added
- The plate is placed into the mass spectrometer and exposed to a laser
- Ionized proteins and peptides are arranged in a spectrum with increasing mass
- Within seconds, mass spectra are compared with an internal database
• qPCR to detect multiple organisms
• After an enrichment period (e.g., 16 hrs), cells are lysed and DNA is purified
• DNA, polymerase and deoxynucleotides are added to the GeneDisc plate
• Primers will amplify DNA sequences, if present, and fluorescent signals are monitored
greiner bio-one CytoCheck

- Detection and identification of 40 Mycoplasma species
- DNA is extracted and PCR performed; the DNA fragments are hybridized to a microarray chip, which contains probes for both species-specific targets and a universal probe for all Mycoplasma
Summary

- The implementation of RMMs represents significant progress toward the acceptance of microbiological PAT and QbD solutions for the industry.
- Regulatory agencies understand and encourage RMMs.
- There are many guidance documents and regulatory policies that enable RMM validation and implementation.
- Many technologies and applications.
- Marked increase in RMM implementation over the past few years.
For More Information

Encyclopedia of Rapid Microbiological Methods
• Guidance on validation, regulatory expectations, technologies and ROI
• Extensive reference list
• Product Matrix Chart
• The RMM Blog and News Page
• Calendar of events
• Newsletter
• LinkedIn Discussion Forum
Thank You!

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