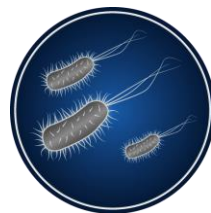


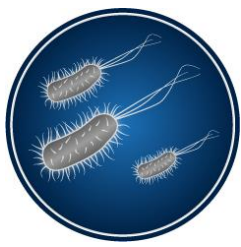
# Evaluation, Validation and Implementation of Alternative and Rapid Microbiological Methods

**A Strategy for Implementation**

Michael J. Miller, Ph.D.

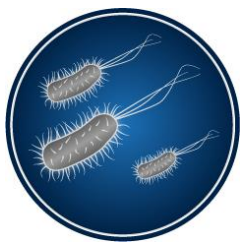


**MICROBIOLOGY**  
CONSULTANTS, LLC



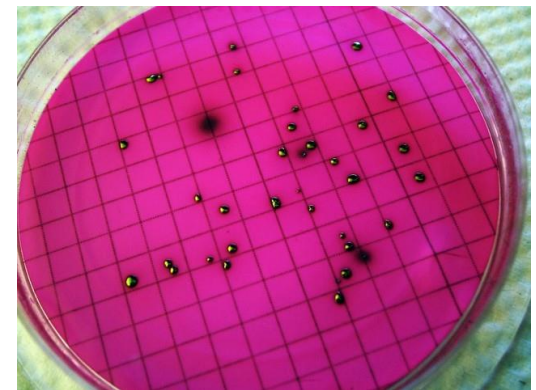
# A Strategy for Implementation

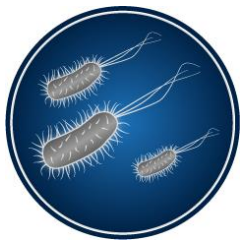
- Review current microbiological methods and identify opportunities for improvement
- Understand available and emerging RMM technologies
  - Match the technology with the intended application
- Pre-validation activities
  - Regulatory considerations
  - Business needs and return-on-investment analysis
  - Proof-of-concept or feasibility testing
  - Vendor audit or assessments
  - Risk assessment
- Develop and execute the validation plan



# Review Current Microbiological Methods

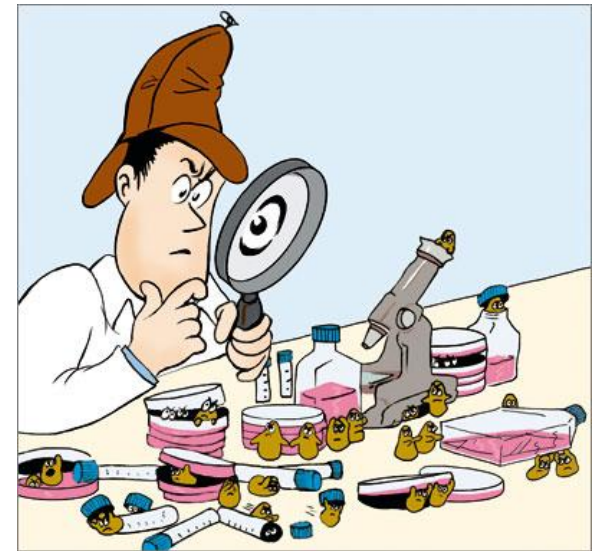
- Bioburden (raw materials, in-process), environmental monitoring, nonsterile drug testing
  - 48 to 72 hour incubation for bacterial count
  - 5 to 7 day incubation for yeast and mold count
- Purified Water Monitoring
  - 2 to 5 day incubation

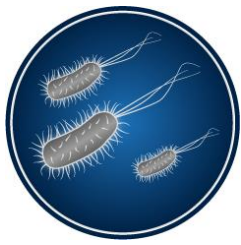




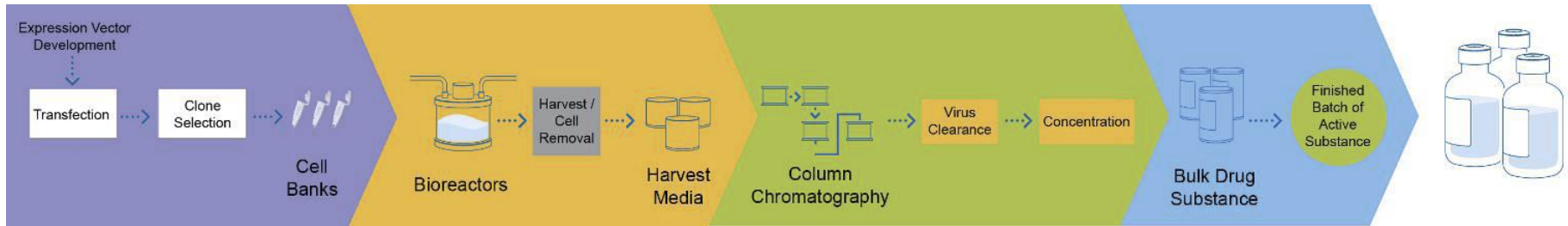
# Review Current Microbiological Methods

- Sterility Testing
  - 14 day incubation
  - Additional incubation (4 days) when sub-culturing if the product being evaluated renders the media turbid
- Microbial Identification
  - Average 3 to 14 days
- Bacterial Endotoxin Testing
  - 90 minutes
- Mycoplasma testing
  - 28 days using conventional methods





# Contamination Control and Testing Points During Bioprocessing

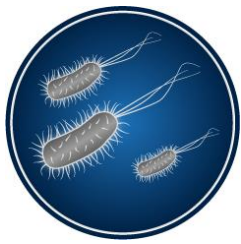


Cell Line Development	Fermentation - Harvest	Purification	Formulation
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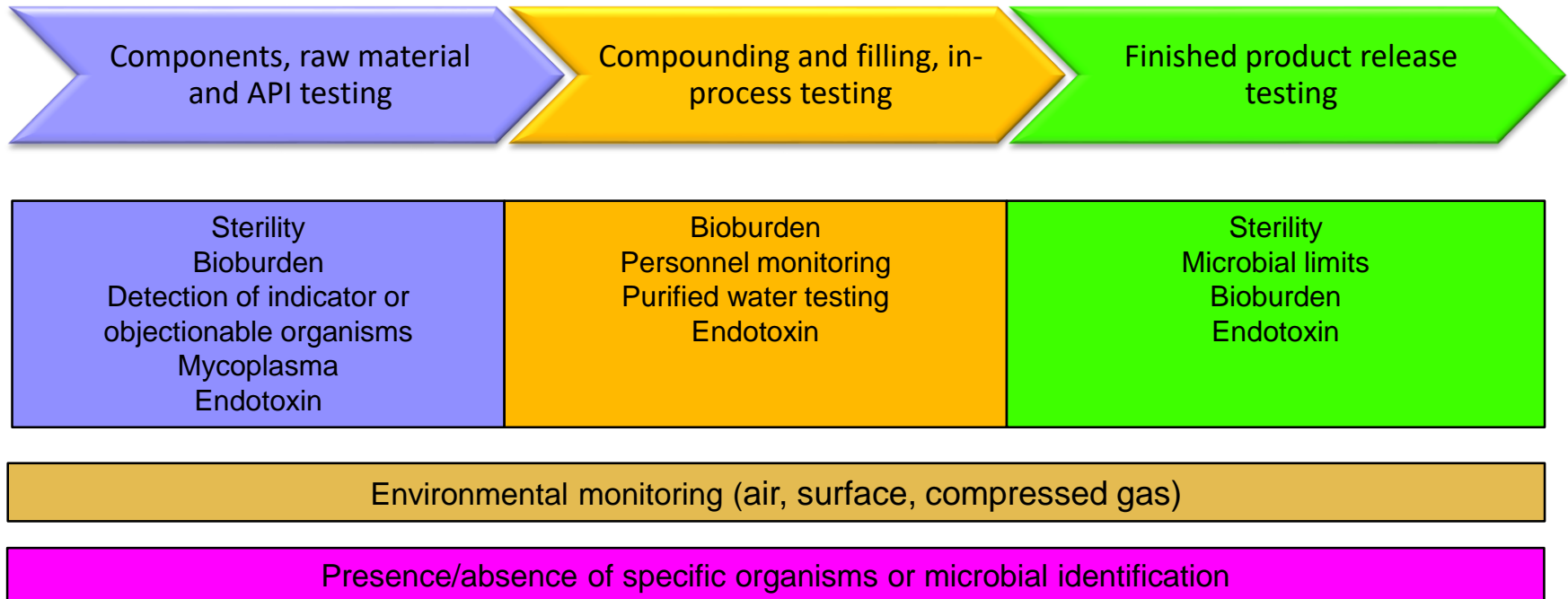
Sterility Mycoplasma	Mycoplasma Virus (MMV and Vesivirus) Sterility-Foreign growth Cell mass/viability Bioburden - Biofilm	Viral clearance/inactivation Residual DNA Quantification Sanitization Bioburden	Endotoxin Bioburden Sterility
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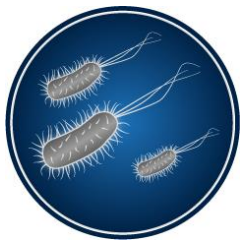
Environmental monitoring (air, surface, compressed gas)

Presence/absence of specific organisms or microbial identification



# Contamination Control and Testing Points During Fill Finish



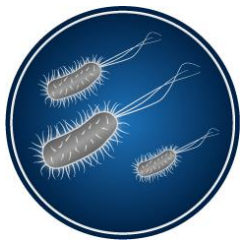


# Understand Available RMM Technologies

- It is important to understand what technology platforms are available, in order to appropriately match the RMM with its intended application
- Consider the technical or method requirements
  - Do you need to detect, enumerate and/or identify microorganisms?
  - Is the RMM compatible with your samples or product?
  - Do you need to detect different type of microorganisms?
  - What is the required level of sensitivity or limit of detection/quantification?
  - What sample sizes are required?
  - Data management requirements?
  - Operator qualification requirements?



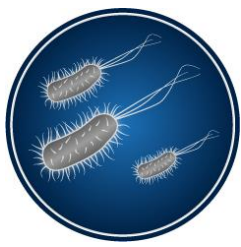




# Understand Available RMM Technologies

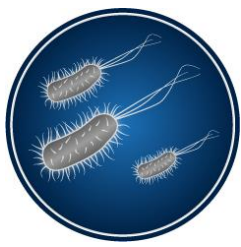
- RMMs can provide qualitative, quantitative and/or microbial identification data
- Qualitative
  - Information on the presence or absence of all microorganisms or the presence of specific microbial species
- Quantification
  - The number of microorganisms present in a sample
- Microbial identification
  - The identity of microorganisms, either at the Genus, species or strain level





# Understand Available RMM Technologies

- RMMs may be based on a wide variety of detection principles
  - Fluorescence techniques to rapidly detect growing microorganisms on conventional media
  - The use of viability stains and laser excitation for the detection and enumeration of microorganisms without requiring cell growth
  - The detection of cellular components or markers (e.g., ATP)
  - Optical spectroscopy, intrinsic fluorescence and Raman
  - Amplification of nucleic acids (e.g., PCR)
  - Microarrays, biosensors, Lab-On-A-Chip and nanotechnology



# Disclaimer

- The examples provided in this course are not meant to endorse any specific technology
- More than 60 different RMMs have been implemented or reviewed by various industry sectors; we will discuss some of them
- For an in-depth review of RMM technologies, workflow, and other relevant information, please visit the RMM Product Matrix at [rapidmicromethods.com](http://rapidmicromethods.com)