Rapid Microbial Methods (RMM)

Christopher A Bravery
cbravery@advbiols.com

Pharmacopoeial Methods

- 14d (full results)
- Validated (subject to matrix/interferance)
- USP 71; EP 2.6.1 etc.

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EP 2.6.27 Microbial control of cellular products

2.6.27. MICROBIOLOGICAL CONTROL OF CELLULAR PRODUCTS

This test has been shown to be preferable to the test for sterility (*2.6.1*) for certain cellular products, since it has better sensitivity, has a broader range, and is more rapid. It is applied instead of the test for sterility (*2.6.1*) where prescribed in a monograph. It may be carried out manually or using an automated system.

- Not less than 7 days. (7d automated, 14d visual)
- Requires some validation (LoD/matrix)

Potential Benefits of RMM

Dependent on actual method; will need to be evaluated

- In-process
  - Result before next step
  - Smaller sample
- Autologous (same may be useful for allogeneic)
  - Result before shipping/before administration
  - In-process results available at release
  - Smaller sample
- Stability testing
  - Smaller sample

Possible Methods (1/4)

- Growth-based
  - Electrochemical Measurement
  - Detection of Carbon Dioxide (C02)
  - Utilization of Biochemical and Carbohydrate Substrates
  - Digital Imaging and Auto-fluorescence of Micro-Colonies
  - Fluorescent Staining and Laser Excitation of Micro-Colonies
  - Use of Selective Media for the Detection of Specific Microorganisms
  - Measurement of Change in Head Space Pressure
  - Microcalorimetry

Possible Methods (2/4)

- Viability-based
  - Flow Cytometry
  - Laser Scanning Solid Phase Cytometry
  - Direct Epifluorescence Filter Microscopy
- Optical Spectroscopy
  - Light Scattering/Intrinsic Fluorescence
  - Raman Spectroscopy

Possible Methods (3/4)

- Cellular Component-based
  - ATP Bioluminescence
  - Fatty Acid Profiling
  - Matrix Assisted Laser Desorption Ionization Time of Flight
  - (MALDI-TOF) Mass Spectrometry
  - Surface Enhanced Laser Desorption Ionization Time of Flight
  - (SELDI-TOF) Ma.ss Spectrometry
  - Fourier Transform-Infrared (FT-IR) Spectrometry
  - Endotoxin Detection

Possible Methods (4/4)

- Optical Spectroscopy
  - Light Scattering/Intrinsic Fluorescence
  - Raman Spectroscopy
- Nucleic Acid Amplification
  - Polymerase Chain Reaction (PCR)
  - Reverse Transcriptase (RT) PCR
  - Ribotyping
  - Gene Sequencing
  - PCR and MALDI-TOF Mass Spectrometry

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Potential advantages of RMM -1

- For cell products released fresh (or that need to be released immediately), the possibility to release with final product with;
  - Interim or full sterility results
  - Full in-process test results
- Potential for reduced in-process microbiology testing and finished product release cycle times
- Reduction in risks associated with forward processing (e.g., bacterial contamination of mammalian cell cultures)
- Increases in laboratory automation and reductions in manual testing, sample handling and/ or data management
- Reduced overhead and/ or headcount for sampling and/ or testing

Potential advantages of RMM -2

- Ability to make immediate microbiology decisions on the state of microbial control
- Faster response to contamination events or microbial data deviations, and the initiation of investigations
- Reduced repeat testing, lot rejection, reprocessing and rework
- Reduction in plant downtime and investigations
- Reduced raw material, in-process and finished goods inventory holdings
- Reduced warehousing space/ cost and work-in-process (WIP).

Should I consider a RMM?

The operating costs associated with the existing method and the alternative or RMM can be identified, as well as the potential cost savings/cost avoidances and the investment costs for validation and implementation. These values can then be used in for financial cost modeling to calculate either a potential financial benefit or loss.

Examples of operating costs

- Cost per test (e.g., consumables)
- Labour time and labour costs
- Equipment depreciation, calibration, qualification and maintenance
- Laboratory overhead
- Data management and storage
- Additional testing (e.g., if the RMM is not approved in all countries).

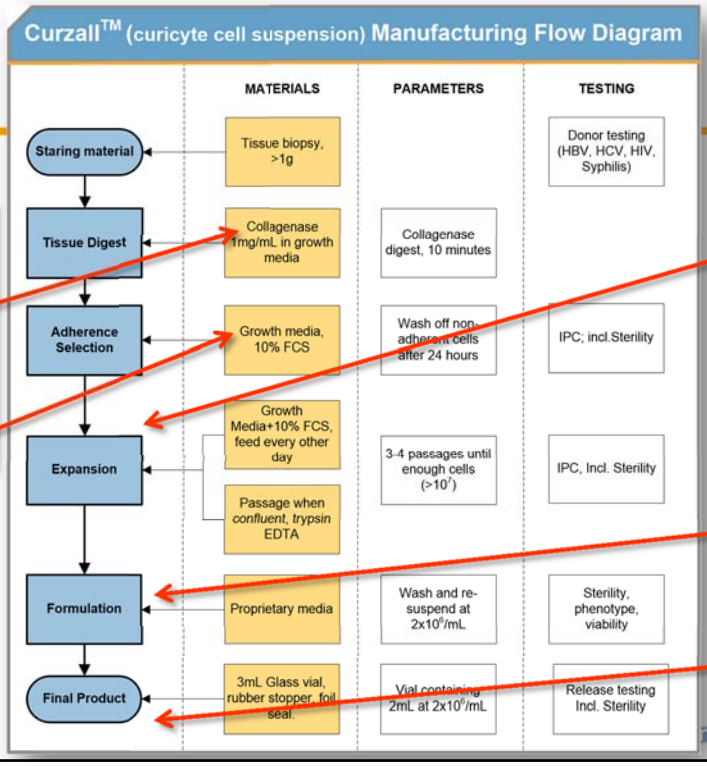
Examples of investment costs

- The capital costs for the new technology
- Software updates
- Training
- Validation
- Regulatory filings and associated costs, when applicable.

Examples of possible uses

Raw materials testing;

- Incoming materials
- Prepared buffers, media etc.



In process tests and controls;

- Could allow result before next process step

Release testing;

- Result from bulk DP before release
- Result from DP before administration

Useful Literature

- PDA Technical Report No. 33 (Revised 2013) Evaluation, Validation and Implementation of Alternative and Rapid Microbiological Methods
  - Excellent review of methods and practical and commercial considerations.
- Mastronardi et al; Evaluation of the BacT/ALERT 3D system for the implementation of in-house quality control sterility testing at Canadian Blood Services. Clin Chem Lab Med 2010;48(8):1179–1187
  - Example of the work involved
- FDA: Guidance for Industry Validation of Growth-Based Rapid Microbiological Methods for Sterility Testing of Cellular and Gene Therapy Products (2008) – *withdrawn in 2012*