PDA
Parenteral Drug Association

Connecting People, Science and Regulation

Rapid Microbiological Methods

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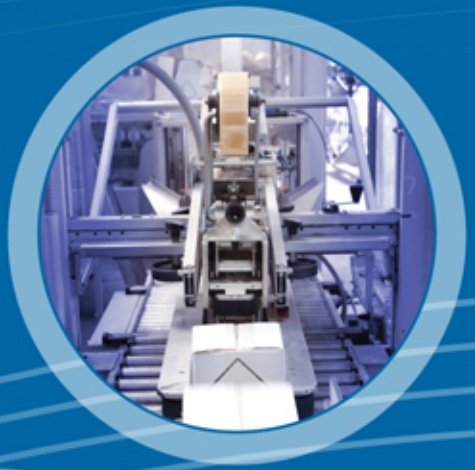


PARTICLE MEASURING SYSTEMS

ARMM Presentation Outline

- ✓ A “Rapid” History of Microbiology
- ✓ The Fundamentals of “Traditional” Microbiology
- ✓ From Cells to Molecules
- ✓ “Alternative” and “Rapid” Microbiological Methods (ARMM)
- ✓ Examples
- ✓ An “Ideal” RMM

Types of Microbiological Testing

1. Detection (Qualitative analysis of microbial contaminants [Presence / Absence])

2. Enumeration (Quantitative estimation of number of microorganisms as colony forming units [CFU's] per unit of sample)

3. Characterization / Identification of the isolated culture

Most Common Traditional Microbiological Tests

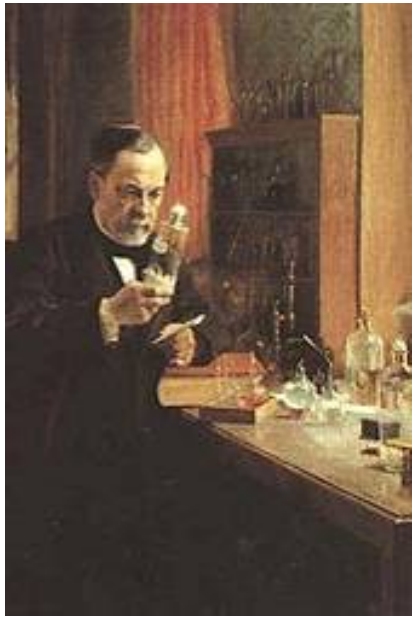
- PRODUCT TESTING
  - ✓ Microbial Limits (MLT) - USP <61>
  - ✓ Antimicrobial Effectiveness Testing USP <51> (*e.g.* preserved product)
  - ✓ Sterility - USP <71> (*e.g.,* in final product)
  - ✓ Bioburden (*e.g.*, in-process)
  - ✓ Microorganism Enumeration:
    - ✓ Direct Plate Count
    - ✓ Filtration
    - ✓ Surface inoculum
    - ✓ Most Probable Number
  - ✓ TAMC – Total Aerobic Microbial Count.
  - ✓ TYMC – Total Yeast and Mold Count
  - ✓ Absence of certain pathogens *(e.g.,* nasal, otic, vaginal, rectal, etc)

Most Common Traditional Microbiological Tests

- OTHERS MICRO TESTING
  - ✓ Container Closure Integrity: Microbial Immersion
  - ✓ Filter Integrity (*Pseudomonas diminuta*)
  - ✓ Materials bioburden
  - ✓ Microbiological Monitoring
    - ✓ Air
    - ✓ Surfaces
    - ✓ Personnel
    - ✓ Compressed gases
  - ✓ Water Activity

Traditional Microbiology

The methods used in most microbiological tests today originated in the laboratories of Koch, Lister, and Pasteur more than 100 years ago.



Regulatory Guidance

- USP <1223> Validation of Alternative Microbiological Methods
- EP 5.1.6 Alternative Methods for Control of Microbiological Quality
- PDA TR No. 33 The Evaluation, Validation and Implementation of New Microbiological Testing Methods (update in progress)

Regulatory Strategy – Performance Step

Validation Parameters by type of Microbial Test

– Quantitative Tests

VALIDATION PARAMETERS FOR QUANTITATIVE TESTS			
REFERENCE Parameters	Pharm. Eur. 5.1.6	USP <1223>	PDA TR N°33
Accuracy	**Yes**	**Yes**	**Yes**
Precision	**Yes**	**Yes**	**Yes**
Repeatability	**Included in precision study**	**Yes**	**Yes**
Specificity	**Yes**	**Yes**	**Yes**
Detection Limit	**No**	**Yes**	**Yes**
Quantification Limit	**Yes**	**Yes**	**Yes**
Linearity	**Yes**	**Yes**	**Yes**
Operational range	**Yes**	**Yes**	**Yes**
Robustness	**Yes**	**Yes**	**Manufacturer**
Ruggedness	**No**	**Yes**	**Yes**
Equivalence	**No**	**No**	**Yes**

Regulatory Strategy – Performance Step

Validation Parameters by type of Microbial Test

– Qualitative Tests

VALIDATION PARAMETERS FOR QUALITATIVE TESTS			
REFERENCE Parameters	Pharm. Eur. 5.1.6	USP <1223>	PDA TR N°33
Accuracy	**Yes**	**No**	**No**
Precision	**Yes**	**No**	**No**
Repeatability	**Included in precision study**	**Yes**	**Yes**
Specificity	**Yes**	**Yes**	**Yes**
Detection Limit	**Yes**	**Yes**	**Yes**
Quantification Limit	**No**	**No**	**No**
Linearity	**No**	**No**	**No**
Operational range	**No**	**No**	**No**
Robustness	**Yes**	**Yes**	**Manufacturer**
Ruggedness	**No**	**Yes**	**Yes**
Equivalence	**No**	**No**	**Yes**

Regulatory Strategy – Performance Step

Validation Parameters by type of Microbial Test

– Identification Tests

VALIDATION PARAMETERS FOR IDENTIFICATION TESTS			
REFERENCE Parameters	Pharm. Eur. 5.1.6	USP <1223>	PDA TR N°33
Accuracy	**Yes**	**NOT IN THE SCOPE**	**NO DETAILS**
Precision	**Yes**		
Repeatability	**Included in precision study**		
Specificity	**No**		
Detection Limit	**No**		
Quantification Limit	**No**		
Linearity	**No**		
Operational range	**No**		
Robustness	**Yes**		
Ruggedness	**No**		

Traditional Microbiology

Are the traditional methods aligned with the requirements for the “Alternative methods” listed in the Pharmacopoeias?

Traditional Microbiological Methods

The time required to obtain results from classical compendial methods varies according to the nature of the test / sample, but is generally very long relative to the speed of today processes.

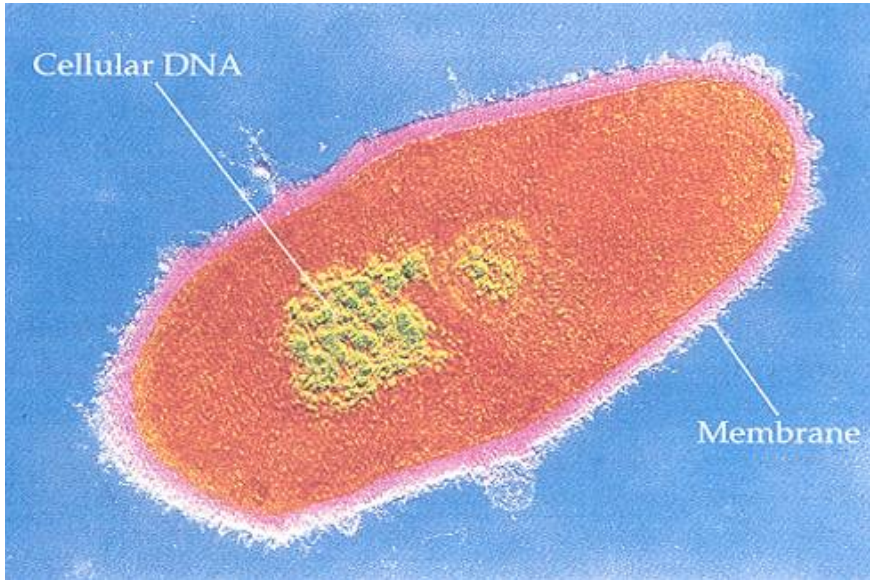
Why is this?

- ✓ Microbial tests rely on the growth of organisms in culture media, a process that can take several days to visualize
- ✓ Variability of microorganisms in their response to culture methods.
- ✓ Many microbes do not grow or grow slower in TSA or SDA (slow cultures and VBNC)!
- ✓ Many microorganisms in pharmaceutical materials are stressed or dormant and require extra incubation phase to allow recovery.
- ✓ Many species present lag phases longer than others in which cells activate their metabolism for growth in the new environment provided for testing.

Traditional Microbiology

- Growth-based
  - in liquid or on agar with defined media under controlled conditions
  - Slow!
- Simple
  - - colonies or turbidity visible to the unaided eye
- Inexpensive
- Sensitive (upon time of incubation)
  - - LOD 1 CFU
- Accepted

From Traditional to Rapid Microbiology

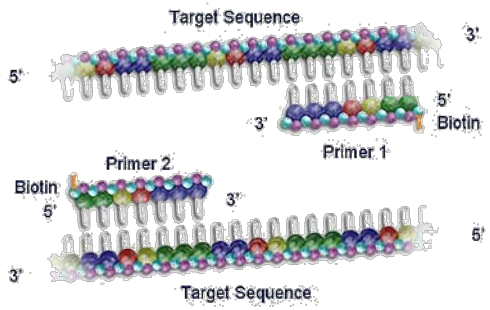


From Cells to Molecules

Rapid micro instrumentation is based on the application of molecular microbiology concepts developed over several decades of spectacular discoveries at both the research and the technology levels

The Birth of ARMMs
1990's

A myriad of new microbiological methods emerged through the application of technological advances in molecular biology, chemistry and biochemistry, immunology and immunochemistry, nanotechnology, physics, electronics and computer-aided imaging.



What are ARMMs?

The new methods are collectively known as Alternative or Rapid Microbiological Methods (ARMM).

- Molecular-based assays combined with new detection technologies
- More sensitive, detection requiring fewer microbial cells in relatively shorter time
- Results are, in most cases, faster, more accurate, and informative than those obtained through the use of traditional methods.
- Some of the instruments are also semi-automated, offering improved throughput and precision.

What are ARMMs?

These methods may be applied to detecting, enumerating and identifying microbial contaminants present in all sort of pharmaceutical samples and environments:

- Active ingredients, Intermediate and final Products, Excipients and other Raw Materials
- Equipment, Personnel, and Manufacturing environments (Air, Surfaces)

Types of ARMMs based on Detection Technology

Metabolic- or Growth Based Technologies

- They measure the growth and viability of microorganisms directly or through biochemical, physiological ,or physical parameters that indicate metabolism.
- They require proliferating microorganisms, either on a solid or liquid medium, in order to be detected and/or quantified
- The time required in the enrichment phase is often significantly shorter that the time required to visualize growth in the traditional method

Types of ARMMs based on Detection Technology

Metabolic- or Growth Based Technologies

- Electrochemical detection by changes in impedance (conductivity) due to the production of charged metabolites
- Colorimetric reactions with metabolic by-products
- Microcalorimetry (heat generated by metabolic activity)
- Monitoring of microbial growth by enhanced turbidimetry and microplate-based Laser Nephelometry
- Phage plaque formation and subsequent detection of specific intracellular components released from lysed cells. (Applicable to Presence / Absence of specified microbes)

Types of ARMMs based on Detection Technology

Metabolic- or Growth-Based Technologies (continues)

- Growth-based: Early detection of micro-colonies growing on solid phase by using microscopy & digital imaging
- Gas production (CO_2), consumption (O_2)
- Detection of metabolites natural fluorescence (NADPH, riboflavin) by laser excitation
- Detection of ATP, a molecule universally associated with living cells, by bioluminescence.
- Automated biochemical assays using advanced colorimetry in a miniaturized, disposable test card format
- Metabolic fingerprint based on Carbon utilization

Types of ARMMs based on Detection Technology

Viability-based Technologies (aka Direct Measurement Technologies)

- Do not require growth for detection, but can count and differentiate living from dead cells and particulates.
- May utilize specific stains and imaging technology.
- These technologies are based on fluorescent cell labeling and laser scanning, combined in either:
  - Solid Phase Cytometry and Microscopy
  - Flow Cytometry

Types of ARMMs based on Detection Technology

Technologies Based on Cell Component Analysis

- Detection of specific cellular components

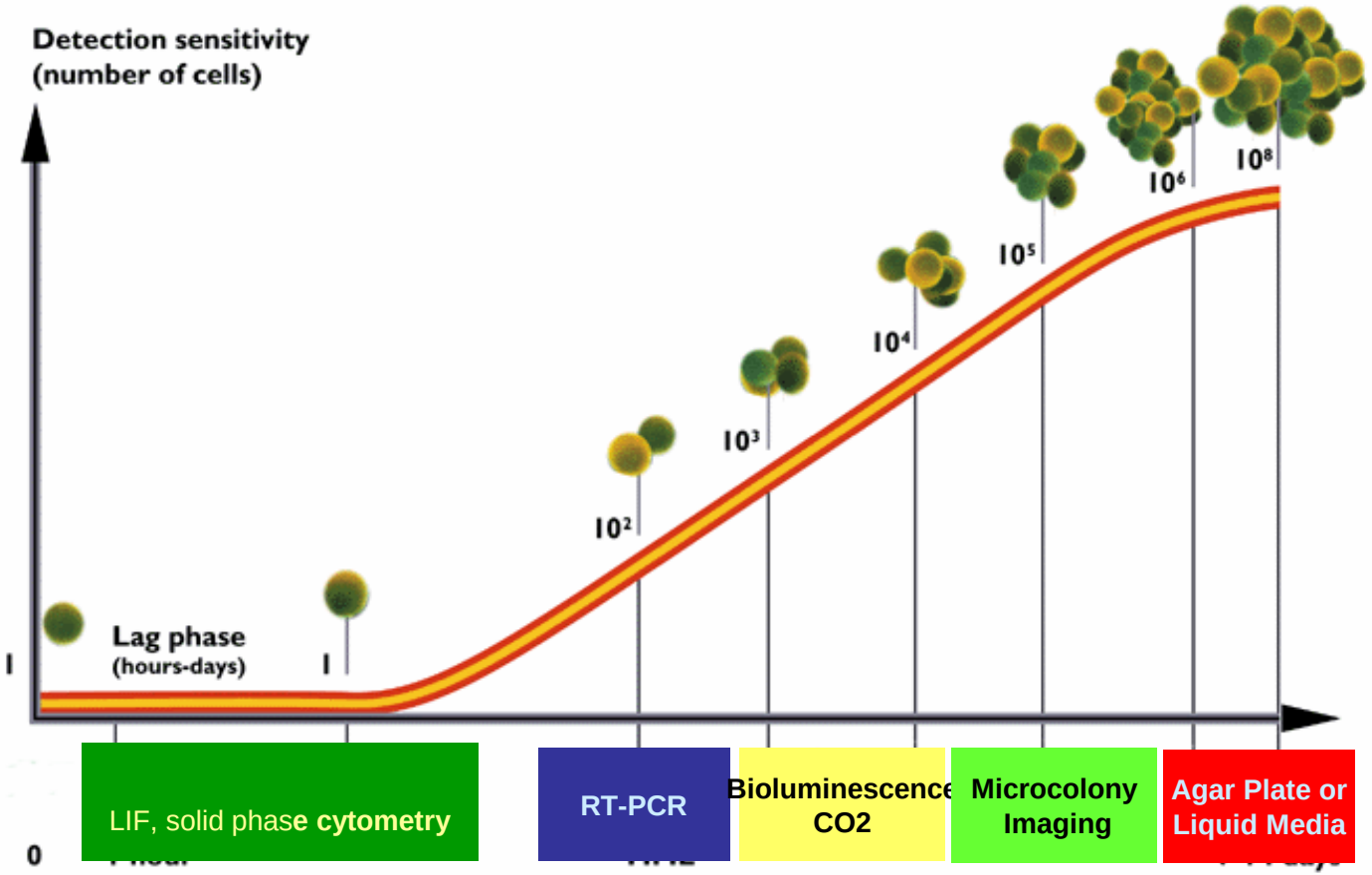
- Phenotypic
  - ✓ Enzyme-Linked Immunosorbent Assays (ELISA)
  - ✓ Fatty Acid Methyl Esters (FAME) profile by gas chromatography
  - ✓ MALDI-TOF-MS (Mass Spectrometry, whole cell analysis)
  - ✓ Fourier Transformation of Infrared Spectroscopy (FTIR) pattern analysis
  - ✓ Detection of endotoxin by using disposable multi-cartridge in conjuction with a handheld spectrophotometer

Types of ARMMs based on Detection Technology

Technologies Based on Cell Component Analysis

- Detection of specific cellular components
- Genotypic (Detection, Enumeration and ID)
  - ✓ Automated Ribotyping (genetic fingerprinting)
  - ✓ Nucleic Acid Amplification–based Technologies (NAAT)
    - o DNA or cDNA amplification
      - End point PCR, fragment analysis
      - Real time PCR, Reverse Transcription-PCR, Quantitative PCR
      - Rep PCR (genetic fingerprinting)
    - o RNA amplification (Transcription Mediated Amplification [TMA])
    - o 16S rRNA Genes Sequencing

Limit of Detection

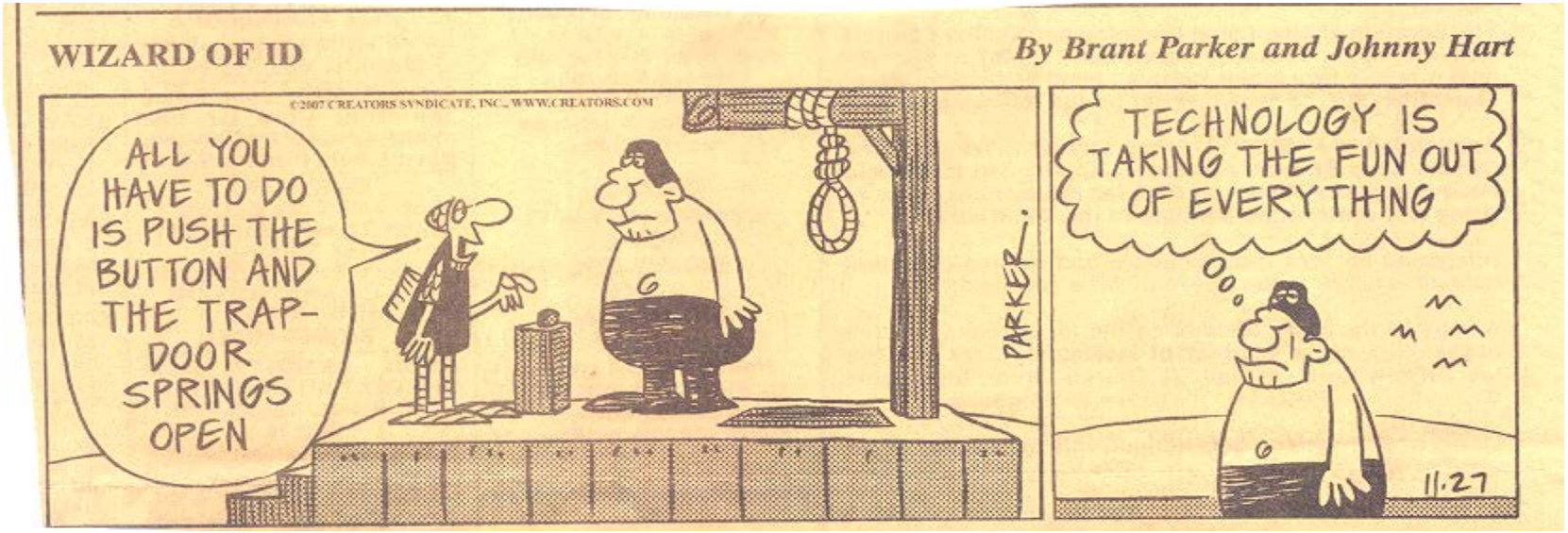


An Ideal RMM Platform

Attribute	Comments	Check (√)
Appealing design and good technology supporting it		
Enrichment no needed or short		
Sample Prep with few steps		
Hands-on time minimal		
Semi/Fully automated or operationally independent		
Disposables and Reagents low cost or justified		
Sensitive equal or close to 1 CFU equivalent		
Quantitative		
LOQ equal or close to 1		
Complies with all required validation parameters		
Complies with all regulatory requirements (including 21 CFR part 11)		
Easy to train personnel		
Simple to Operate		
Calibration available (Standards)		
New units correlate with CFU or add more valuable information		
Unambiguous interpretation of results		
Differentiates live vs. dead cells		
Results in Real Time or in very short time		
Vendor support (troubleshooting, equipment qualifications, service, warranty)		
Reasonable priced according to technology innovation		

Denoya, 2014, Jan-Feb, American Pharmaeutical Review

New Technologies



Questions?

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