Rapid Microbiological Detection Methods in Pharmaceutical Water

Overview of Technologies and their place in Pharma / Biotech

Brian Hagopian, CPIP
Chemist and President
Clear Water Consulting, Inc.

ISPE Boston Area Chapter
February, 2017

About Clear Water Consulting, Inc.

Clear Water Consulting provides services (not equipment) to the Life Sciences Industry:

- **Optimize the operation and performance of water systems**
- **Expert, objective troubleshooting if something just isn't right**
- **Training, Education, and Certification**
  - Pharmaceutical Water
  - State Certified Industrial Wastewater Trainer
  - On-line training will be available beginning later in 2017
- **Engineering reviews**
- **Needs assessments**
- **Design and specification services**
- **Documentation to support validation, CAPA's, and OOS investigations**

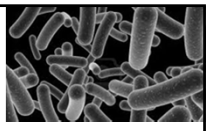
Overview of Rapid Microbiological Detection Methods in Pharmaceutical Water

What is available?

Where does it fit for me and my company?

3

Rapid Microbiological Detection Methods
Overview

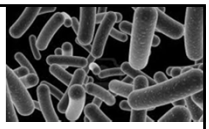


Before we get started…

Information presented today has been gathered from:

- USP General Chapter <1231> (2016)
- PDA Technical Report 33 (2013)
- EP 5.1.6
- Private communication with TC Soli, USP Expert Committee

Rapid Microbiological Detection Methods
Disclaimer

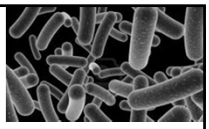


This presentation is not intended to promote, endorse, or discourage any particular technology, supplier, or vendor

This presentation is intended to provide a brief summary of the technologies available for Rapid Micro Methods without making any specific references to suppliers

Technologies are evolving so rapidly that information contained in this presentation may soon be out of date or may already be out of date

Rapid Microbiological Detection Methods
Overview



"Rapid Microbiological Detection is a potential game changer, representing the biggest breakthrough in the water purification industry in a long time" – Everyone

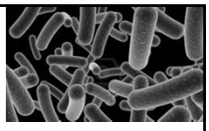
Who wouldn't want bacteria results faster???

3-5 days 2 days 1 day

16 hours 8 hours 4 hours

1 hour Instantaneous

Rapid Microbiological Detection Methods
Overview

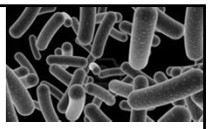


But before we get ahead of ourselves…

What are we going to use the results for ???
- Is a treatment process working properly?
- For product release purposes

Process Control vs. Quality Control

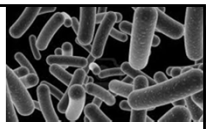
Rapid Microbiological Detection Methods
Overview



Process Control

Goal is to know the bacterial counts in the water and whether a process is working as intended
- Rinse extensively to flush and treat a sample to avoid introducing contamination
- Demonstrate that a process is “doing its job”
- Sample frequencies may change based on results
- Online analysis is suitable
- Results provide basis for process adjustments, maintenance, or sanitizations to keep the system operating in a state of control

Rapid Microbiological Detection Methods
Overview

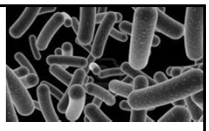


Quality Control

Goal is to know what is going into the product and for product release purposes

- Test exactly as manufacturing uses the water
- Test at point of use and not a sample valve
- Sample frequencies are established and are not usually altered
- Results provide the basis for product release
- Online analysis for bacteria does not replicate the way that manufacturing uses the water

Rapid Microbiological Detection Methods
Overview



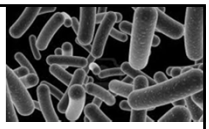
Process Control vs. Quality Control

Process Control	Quality Control
Are bacteria present	Are bacteria present
How many bacteria	How many bacteria
Don't care about species	Need to know species

What it means for Rapid Microbiological Detection Methods

Can destroy the bacteria	Need to culture bacteria*

Rapid Microbiological Detection Methods
Overview



An Important Consideration

Bacteria are not uniformly distributed in a water system

Bacterial results from one location are not representative of results in all other locations

Therefore, online rapid microbiological analysis data collected from one location is not suitable for Quality Control and Product Release at other locations

Rapid Microbiological Detection Methods
Current Methods for Microbiological Detection

Limitations of current microbiological assay techniques

- It takes days to get results
  - **Then it takes days to identify**
- Some bacteria are alive but don't grow in the test media – Viable But Not Countable (VBNC)
- Errors range from 18-100% vs. actual (USP<1227>)
- But, we are familiar with the technique

Rapid Microbiological Detection Methods

Brief History on Rapid Microbiological Detection Methods

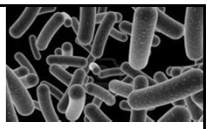
- Some techniques have existed for 30-40 years
- Development has not been driven by Pharma / Biotech, but by
- Food and Beverage – food safety
- Microelectronics – product yields
- Homeland Security – pathogenic organisms, bioterrorism
- Adoption by Pharma / Biotech is lagging behind other industries, but there are reasons for this

Rapid Microbiological Detection Methods

Technical Considerations on various Rapid Microbiological Detection Methods

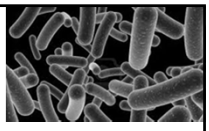
- Some techniques are more ACCURATE
- Some techniques are more PRECISE
- Some techniques are more SENSITIVE
- Some techniques are more REPRODUCEABLE
- All techniques are FASTER

Rapid Microbiological Detection Methods
Summary of Results Obtained



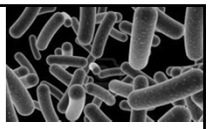
QUALITATIVE	Are bacteria present
QUANTITATIVE	How many bacteria
IDENTIFICATION	Need to know species

Rapid Microbiological Detection Methods
Classification of Destructiveness of Techniques



DESTRUCTIVE	May or may not quantify Cannot identify Usually the fastest
NON-DESTRUCTIVE	May or may not quantify Can identify
HYBRID	May destroy May quantify May allow for identification

Rapid Microbiological Detection Methods
Classify by Technologies Utilized



GROWTH BASED Metabolism generates “stuff” that can be detected

VIABILITY BASED Stain and laser excitation

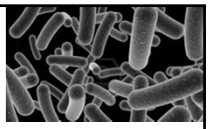
CELL MARKER BASED Most are destructive

OPTICAL SPECTROSCOPY Some are destructive

NUCLEIC ACID AMPLIFICATION Usually destructive

MICRO-ELECTRO-MECHANICAL SYSTEMS (MEMS)

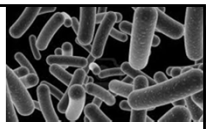
Rapid Microbiological Detection Methods
Specific Technologies for RMM



GROWTH BASED TECHNOLOGIES

- Electrochemical- Bacterial metabolism creates more ions, which are detected
- Carbon Dioxide- Bacterial metabolism creates CO_2, which is detected
- Biochemical and Substrate Marking
- Digital Imaging- Blue light to detect colonies before we can see them
- Fluorescence Staining
- Gas Pressure- Monitors gas evolution
- Heat Production- Monitors temperature changes
- Selective Media- Developed to detect a specific Genus/Species/Pathogen

Rapid Microbiological Detection Methods
Specific Technologies for RMM



VIABILITY BASED TECHNOLOGIES

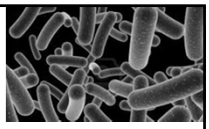
Detects living and Viable But Not Culturable (VBNC) cells

Flow Cytometry- Add marker then pass through a laser

Solid Phase Cytometry- Done on a filter media

Direct Epifluorescence- Living and dead emit different colors

Rapid Microbiological Detection Methods
Specific Technologies for RMM



CELL MARKER BASED TECHNOLOGIES

Some techniques destroy the bacteria to detect their presence

ATP Bioluminescence- ATP plus enzyme = photon of light, which is detected

Fatty Acid Profiling- Extract then analyze by GC, compare to a library to ID

MALDI-TOF* Mass Spectrometry- Laser + Mass Spec, compare to library to ID

SELDI-TOF* Mass Spec- Bind specific proteins/molecules, then MALDI-TOF to ID

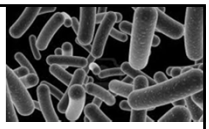
FT-IR- Uses IR to generate spectra to ID

Endotoxin- LAL (15-20 minutes)

MALDI-TOF = Matrix Assisted Laser Desorption Ionization – Time of Flight

SELDI-TOF = Surface Enhanced Laser Desorption Ionization – Time of Flight

Rapid Microbiological Detection Methods
Specific Technologies for RMM



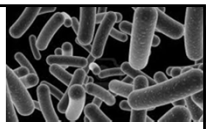
OPTICAL SPECTROSCOPY BASED TECHNOLOGIES

Use light scattering/optics to detect without the need for growth

Light Scattering/Fluorescence- Detects particles and Viable bacteria

Raman Spectroscopy- Bacteria plus ID against a known library

Rapid Microbiological Detection Methods
Specific Technologies for RMM



NUCLEIC ACID AND GENE AMPLIFICATION BASED TECHNOLOGIES

Can be used to ID or find specific organisms – Some methods can quantify counts

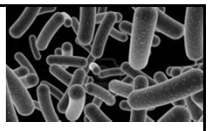
PCR using DNA- DNA amplification, can ID a specific organism

Reverse Transcriptase PCR- Uses RNA to make DNA

Ribotyping- Uses unique rRNA to ID based on an existing library

PCR and MALDI-TOF Mass Spec- ID after Gene amplification

Rapid Microbiological Detection Methods
Specific Technologies for RMM



MICRO-ELECTRO-MECHANICAL SYSTEMS (MEMS) BASED TECHNOLOGIES

Lab on a Chip / Microfluidic- Sample Prep, fluid handling, analysis, detection using nano or pico liter volumes, may ID

Microarrays- Specific arrangement of proteins, RNA, DNA, or other biological materials on a substrate to rapidly detect specific organisms (flu, disease, etc.)

Practical Considerations before Implementation

Of Rapid Microbiological Detection Methods (RMM)

Rapid Microbiological Detection Methods
Practical Considerations before Implementation

Regulatory agencies are acknowledging the value of RMM

- No agencies have prohibited the use of RMM
- Many agencies are establishing guidelines for their use

Know and understand the sensitivity or limit of detection of a technology (i.e. 2 cfu/ml or about 20 cfu/ml) before moving forward

RMM for Process Control is easier to implement

RMM for Quality Control requires a greater effort to implement

Rapid Microbiological Detection Methods
Practical Considerations before Implementation

Do we need to validate a Rapid Micro Method (RMM)?

- Do not expect results to correlate exactly – they won't
- They should show the same trends

What if RMM shows more bacteria than traditional methods?

- System is less "under control" than originally thought
- Why would I change ?

Rapid Microbiological Detection Methods
Practical Considerations before Implementation

Why wouldn't we want to get a more accurate count?
Why wouldn't we want to get a more representative count?
Why wouldn't we want to get readings faster?

Early adopters and the bleeding edge
- May prompt initial use for process control
- Until QA/QC "gets used to" the approach

Rapid Microbiological Detection Methods
Summary and Path Forward

RMM is coming, whether we like it or not
Regulatory authorities acknowledge the value of RMM
Many different techniques are available, evolution is rapid
Do your homework on RMM technologies
Determine if you are comfortable with a technology
Then figure out where/how you want to start
Different organizations will have different criteria to determine when to take the plunge

Questions?

Please use the microphone indicated so our recording includes audio of your question

29

For further information, please contact
Brian Hagopian, CPIP
at brian@clear-water-consulting.com

Brian Hagopian, CPIP
Chemist and President
Clear Water Consulting, Inc.
(978) 888-3082
brian@clear-water-consulting.com