# Adapting to Annex 1: Quality Risk Management for Sterile Products

**Alan Hoffmeister**

Senior Global Scientific Portfolio Specialist

Charles River Laboratories – Microbial Solutions

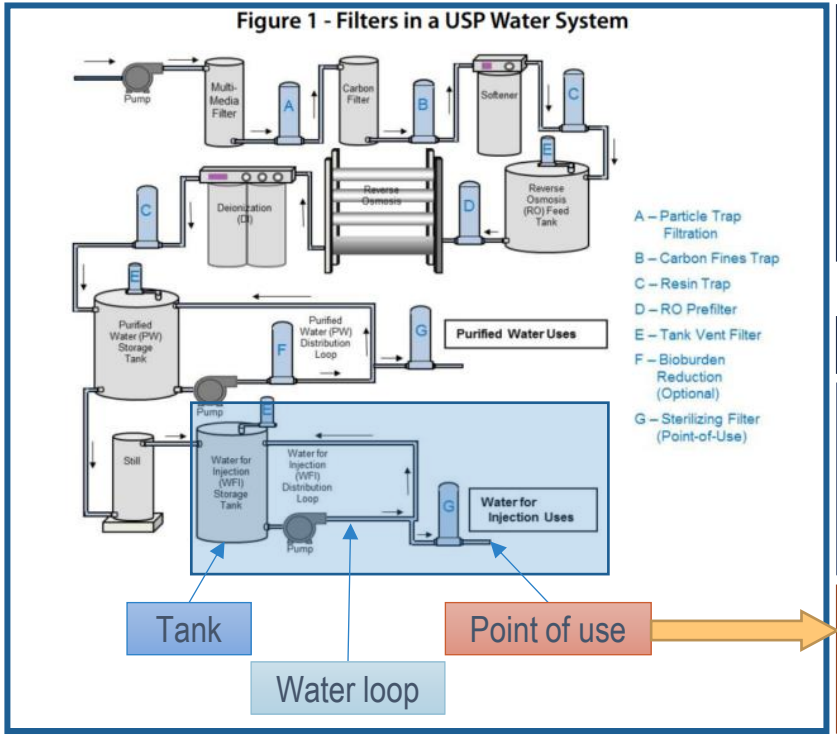


**PDA Aseptic Processing of Biopharmaceuticals Conference 2024**

# Contents

# Annex 1 - Focus on Endotoxin

# ANNEX 1 – Critical Utilities




ANNEX 1

MANUFACTURE OF STERILE PRODUCTS

WFI (water for injection) systems are critical utilities for manufacturing pharmaceutical sterile products.

CHAPTER 6 - UTILITIES

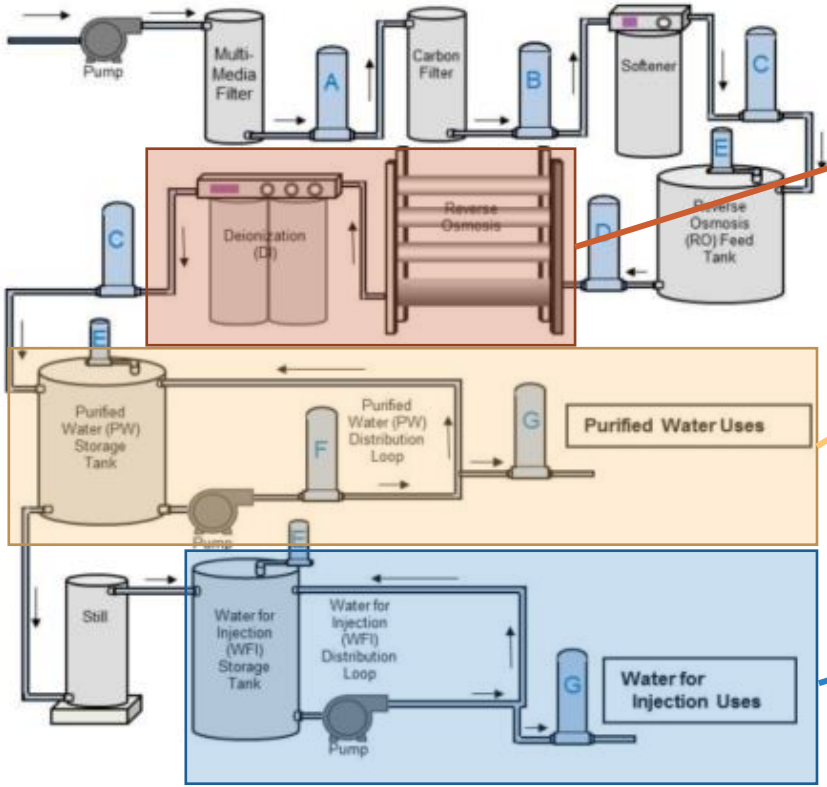
Critical utilities are described in point 6.2.:

- Directly contact product.
- Contact materials that will ultimately become part of the product.
- Contact surfaces that come into contact with the product.

RAW MATERIAL

RINSE SOLUTION

# WFI as a Critical Utility



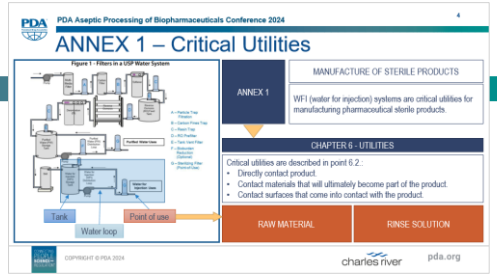

## Microbiological requirements

| Deionized water | |
|---|---|
| Bioburden limit | NA |
| Endotoxin limit | NA |

| Purified water (EP GM0008 / USP 1231) | |
|---|---|
| Bioburden limit | **<100cfu/mL (10000cfu/100ml)** |
| Endotoxin limit | NA |

| Water for injections (EP GM0169 / USP 1231) | |
|---|---|
| Bioburden limit | **<10cfu/100mL (not sterile)** |
| Endotoxin limit | <0.25EU/mL |

**Water Quality increase cascade**




# WFI Microbiological Characteristics

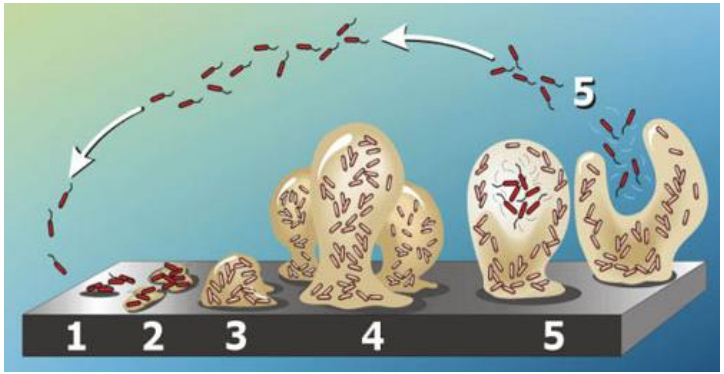
1. Due to lack of nutrients, continuous flow and high temperature (>70°C) in WFI systems not all microorganisms are able to grow in this environment
2. Gram negative rods are more adapted to these environments than other bacteria because they have resistance forms, called BIOFILM
3. As a resistance form, a BIOFILM, has the task to ensure colony survival, is extremely difficult to eliminate and this is why a highly sensitive method for early biofilm detection is critical
4. WFI systems have a bioburden acceptance criteria (less than 10cfu/100ml) this means that these systems are NOT STERILE a certain amount of bioburden is allowed, so BIOFILM formation is a potential (rare, but not impossible) event that has a huge impact to business continuity

# Annex 1 – Chapter 6 Utilities – Water systems 6.7.

Due to WFI systems criticality and that WFI requirements accept a certain amount of bioburden coming from the systems that supply water to them, these WFI systems must be:

QUALIFIED

MONITORED




**MINIMIZE RISK** microbial contamination/proliferation and pyrogens

+

**PREVENT** formation of BIOFILMS

**ENSURE** A reliable source of water of an appropriate quality

# Microbial Monitoring of CRITICAL UTILITIES - Water Systems

## WFI Contamination Control Strategy (CCS) - SAMPLING PLAN

### QUALIFICATION

#### INITIAL

#### PERIODIC

6.8. Water systems should be qualified to maintain the appropriate levels microbial control, taking seasonal variation into account.

### DAILY

6.13. Regular ongoing microbial monitoring of water systems should be performed.

i. All points of use, at a specified interval (QRM).

ii. A sample from the point at the end of the distribution loop each day that the water is used.

# Biofilms in guidelines – EMA Q&A

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

1 August 2017
EMA/INS/GMP/443117/2017
GMP/GDP Inspectors Working Group

Questions and answers on production of water for injections by non-distillation methods – reverse osmosis and biofilms and control strategies
Final

***PART II - BIOFILMS AND CONTROL STRATEGIES***

**2. What approach should be taken to maintain control over systems which can be affected by biofilms?**

A control strategy should be developed to assess the risks associated with the current manufacturing processes and to determine acceptability of existing control measures. The effectiveness of the sampling and testing regimes employed at the site should also be critically assessed in conjunction with the development of a control strategy.

https://www.ema.europa.eu/en/documents/other/questions-answers-production-water-injections-non-distillation-methods-reverse-osmosis-biofilms_en.pdf

# Biofilms in guidelines – EMA Q&A

## *PART II* - BIOFILMS AND CONTROL STRATEGIES

**3. What is a control strategy in the context of biofilm and contamination control?**

A control strategy should take account of the design of the process, the mechanisms required to be put in place to control and ultimately prevent or minimise the risk of contamination.

Such a strategy requires the following thorough process knowledge and understanding taking account of all aspects of contamination control and prevention, including:

- Design
- Water system qualification
- Personnel qualification/training
- Raw Materials, e.g.
- Control strategy including in-process controls applied to

  - Raw Materials - Feed water system - Treatment system
- Monitoring systems (qualification/calibration) used in the control strategy
- Preventative maintenance to a standard that will not add significant risk from a contamination view-point
- Robust QMS

  - Deviation handling - Root cause analysis (investigations) – CAPA

Contamination control and steps taken to minimise the risk of contamination are a series of successive linked events/measures. Quality Risk Management tools along with scientific judgement can be applied in determining critical control points.

A contamination control strategy would integrate all of these measures to ensure a more comprehensive approach is taken with respect to prevention and control of microbiological contamination.

Such a strategy should lead to the introduction of a control programme which is an iterative process taking into account all information throughout the lifecycle of the products and processes.

# Biofilms in guidelines – EMA Q&A

## *PART II* - BIOFILMS AND CONTROL STRATEGIES

**6. Are there any additional measures which should be considered in order to increase the probability of detecting the presence of biofilms?**

A robust sampling plan is a requirement. Such a sampling plan forms part of the assessment of the effectiveness of the control strategy employed to minimise such risks of biofilm and general contamination issues. Each potential source of contamination should be incorporated into such a sampling regime. Ongoing evaluation to determine the appearance of an adverse trend should be performed, however, the seasonal variation that occurs can only be determined during the annual trend assessment. The effectiveness of an environmental monitoring programme should be formally assessed at minimum on an annual basis.

Sampling programmes for water systems should take account of the quality of the water supply to the system as well as assessing points throughout water generation. Water quality is best assessed through a pre-determined, systematic approach. The loop return should be sampled each day of use of the system in order to provide additional assurance of the quality of water utilised in the manufacturing processes. All points should be sampled on a rotational basis to ensure that the entire system user points are sampled at least once per week.

Routine identification of contaminants isolated during monitoring activities is critical in order to ascertain if there is any shift or change in the flora present within a facility or if certain specific species become more prevalent.

Use of more sensitive endotoxin detection methods should also be taken into account. Alert levels should be set based on the capability of the system and any change or adverse trend should be appropriately investigated.

The frequency of trend analysis and use of trend data is critical. The use of rapid microbiological test methods and systems should be considered in order to improve or increase the probability of early detection and allow timely action to be taken.

# Endotoxin testing as a detection method for bacterial biofilms

- American Pharmaceutical Review Article | Dr Tim Sandle

*Article Summary: Endotoxin testing can play a role in the earlier detection of biofilms than is possible using conventional bioburden tests. This is on the assumption, albeit one supported by most literature, that much of the bacterial contamination of water systems, and to an extent medical implants, is by Gram-negative bacteria, which on lysis would release endotoxin.*

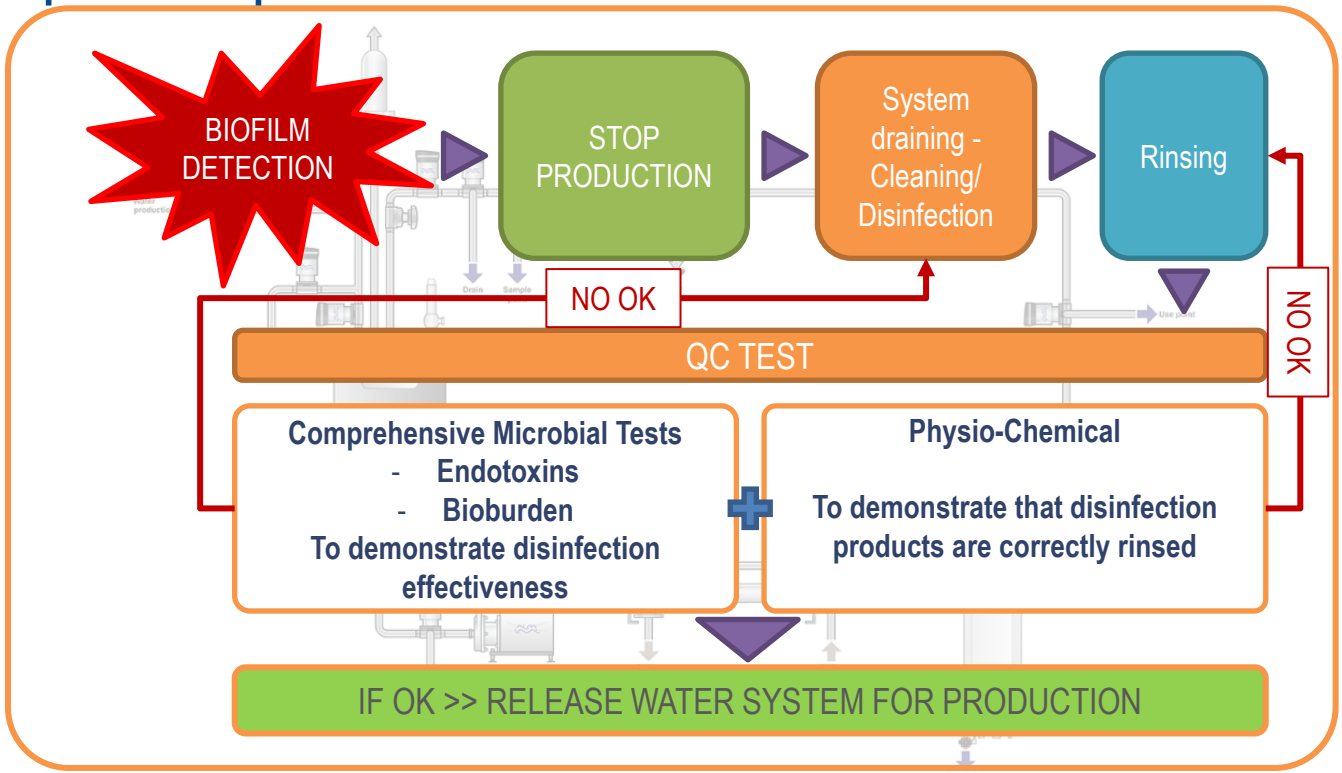
Endotoxin Testing as a Detection Method for Bacterial Biofilms | American Pharmaceutical Review - The Review of American Pharmaceutical Business & Technology

# Improve Biofilm Detection to Minimize Impact

# Biofilm impact to production

15 days
to
1 month

COST



# GOAL: EARLY BIOFILM DETECTION

To minimize production impact, EARLY BIOFILM DETECTION whilst complying with Annex 1 requirements is needed:

- LESS spread => LESS BIOFILM focus => easier disinfection
- LESS batches impacted

To keep critical utilities under the required quality, CCS requires:

- an effective sampling plan
- and a test method that is specific and sensitive enough to optimize contaminant detection

Methods to assess microbiological quality for WFI systems:

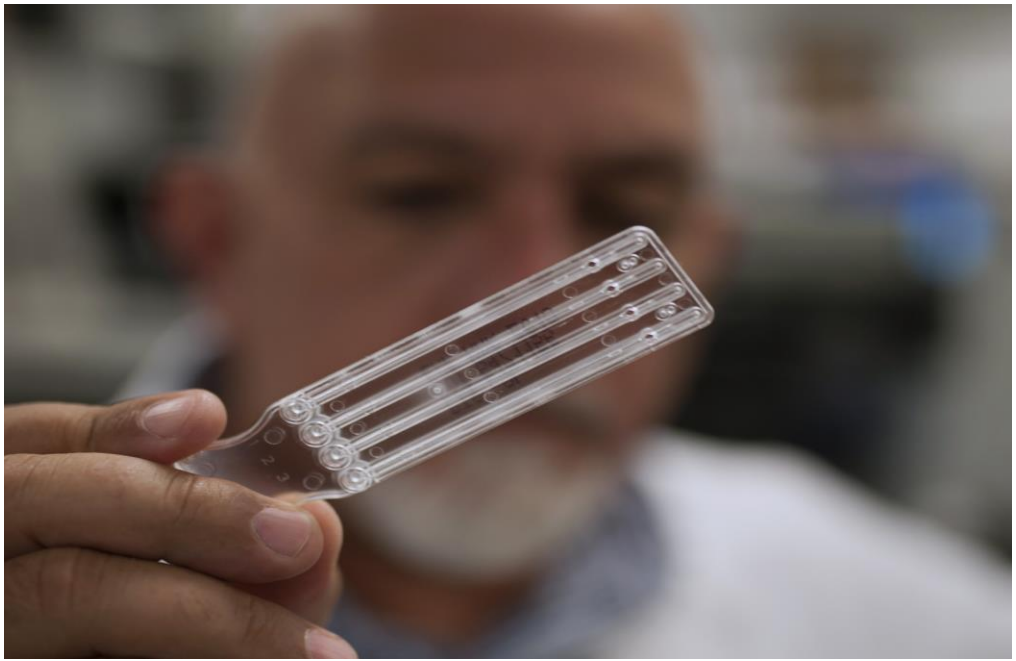
CLASICAL BIOBURDEN METHOD

ENDOTOXIN TESTING AS AN EARLY DETECTION METHOD FOR BACTERIAL BIOFILMS

# Methods to assess microbiological quality for WFI systems

| | Bioburden | Endotoxin testing |
|---|---|---|
| Analyte | Viable microorganisms | Non-viable, highly resistant and stable microorganism particles |
| Limit of detection | 1CFU/200mL | Sensitivity up to 0.005EU/mL<br>parts per trillion (ppt; $10^{-12}$) |
| Specificity | All microorganisms that can grow in the media used (R2A?) | High specificity to gram-negative bacteria |
| Time to result | ▪ minimum 5 days of incubation<br>▪ ~1-day MANUAL data analysis and report | ▪ 15 min to result (CARTRIDGE TECH)<br>▪ MCS (20 samples/hour)<br>▪ Cortex data analysis software |

# BET Cartridge Technology Improving Biofilm Detection



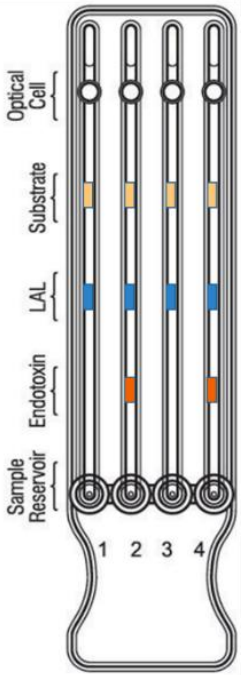
# Improving Biofilm Detection in WFI Systems Using Endosafe® Cartridge Technology

## Flexibility

- Random Access
  - Samples can be tested as they arrive to the lab.
  - No need to batch samples > holding time validation

## Ease of use

- Easy technician qualification/training
- One error in standard curve preparation for traditional kinetic during a qualification means resampling & retesting of the whole water system



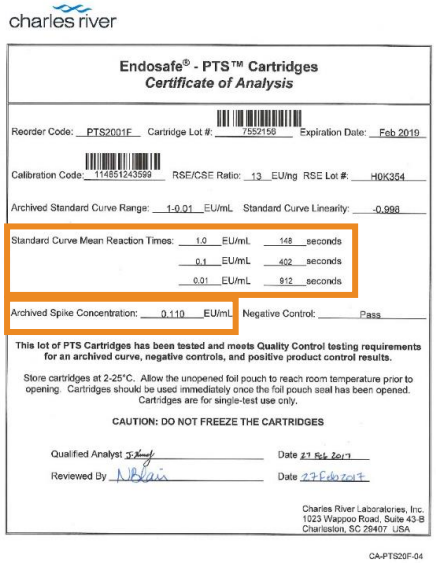
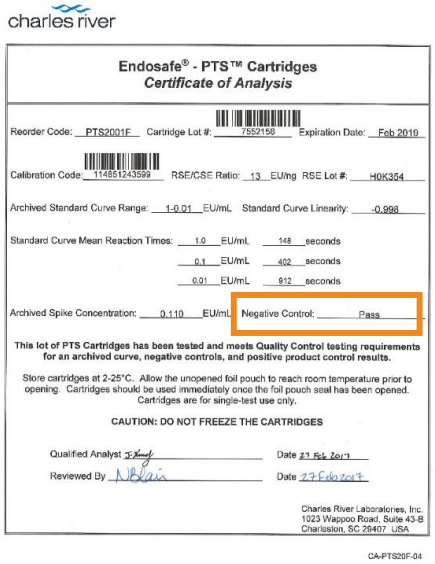

## At-line testing

- Remove/reduce sample transportation issues
- Testing next to point of use and data uploaded to Database to be analysed

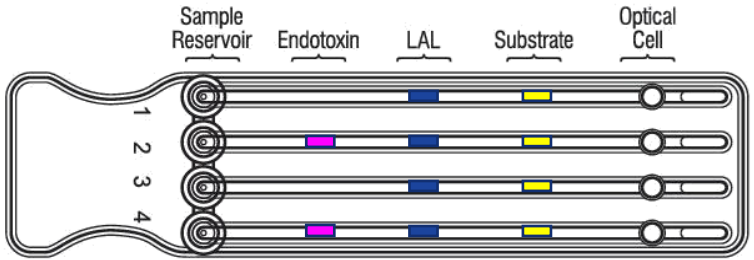
## Sustainability & Compliance

- Method D harmonized method for US and EU
- Uses up to 95% to 100% less horseshoe crab blood derived reagent than traditional kinetic methods

# Cartridge technology – How it works

| Standard Curve (SC) / Spike Concentration | Negative Product Control (NPC) | Positive Product Control (PPC) | Negative Control (NC) |
|---|---|---|---|
|  | Duplicate Sample (Well 1 & 3) | Duplicate Sample + endotoxin spike as PPC (Well 2 & 4) |  |





# Advancing Responsible Science through Sustainable Endotoxin Testing Solutions

## LAL Cartridge Technology

- **95% Reduction** in horseshoe crab blood from Gel-Clot.
- FDA-Licensed and compliant with harmonized Pharmacopeia chapters.
- Robust and scalable technology, from single test to full automation.

## rCR Cartridge Technology

- **100% Replacement** in horseshoe crab blood.
- Optimized kinetic chromogenic reagent formulated to simulate the natural LAL reaction.
- Seamless integration with existing suite of scalable, automated, cartridge technology

# Regulatory compliance

Complies with USP (<85> <86>) / EP(2.6.14) requirements:

- Kinetic Chromogenic method
- Calibrated to USP RSE, the primary standard
- 2-log ranges with mid-point spikes
- The required sample and spike in duplicates



edqm
European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

COUNCIL OF EUROPE
CONSEIL DE L'EUROPE

# Customer Case Study 1 – Biotech Efficiency

- During the manufacturing process, both production and QC were involved in the collection of samples for testing
- Often, in-process test results were not available to production within the short time frame required to proceed with downstream processing
- A rapid turnaround of results was required to determine whether endotoxin levels were within limits before proceeding to next step in the manufacturing process
- Six Sigma analysis carried out by objective third party consultant to analyse BET efficiency
- Study analysed sample process flow and turnaround time (TAT) for two sets of in-process buffer samples
- One set tested by central QC using conventional Kinetic Chromogenic  plate method
- The other tested using cartridge technology by manufacturing on production floor
- A benefit analysis was performed, taking productivity, quality and financial implications into account

# Customer Case Study 1

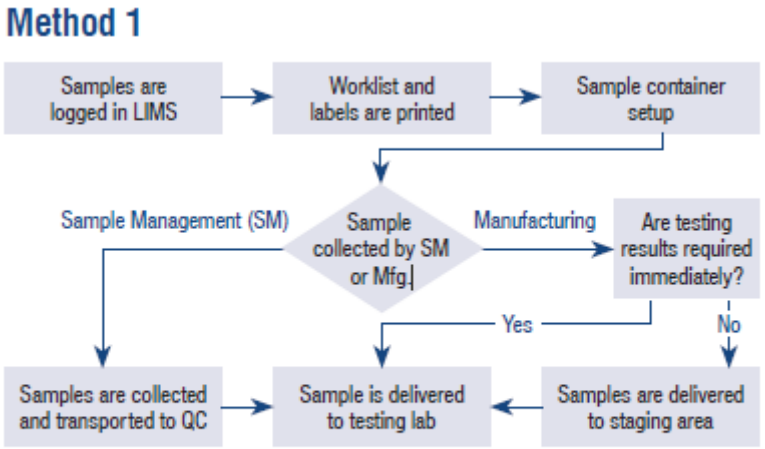



**Figure 1a:** BET flow chart for in-process buffer samples sent to QC lab using kinetic assays/microplate reader

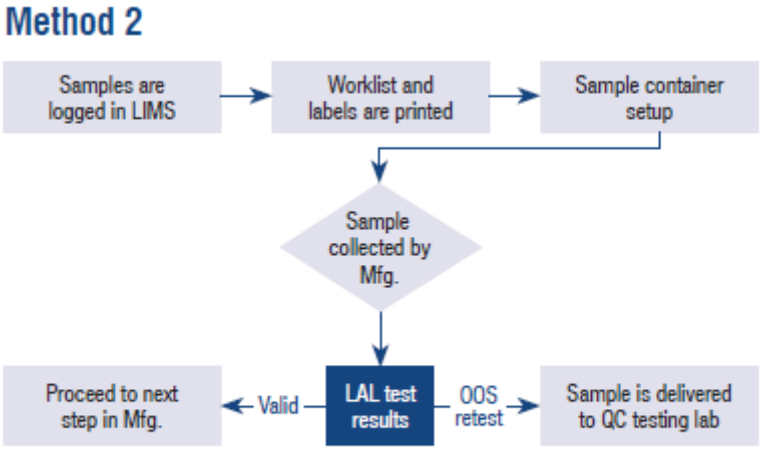



**Figure 2a:** BET flow chart for in-process buffer samples using the LAL Cartridge/PTS™ at point of use

# Customer Case Study 1

## Method 1

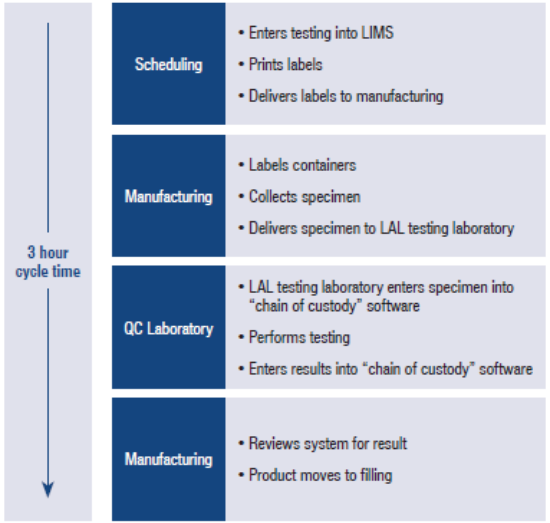



**Figure 1b:** TAT for BET using kinetic assay/microplate reader

## Method 2

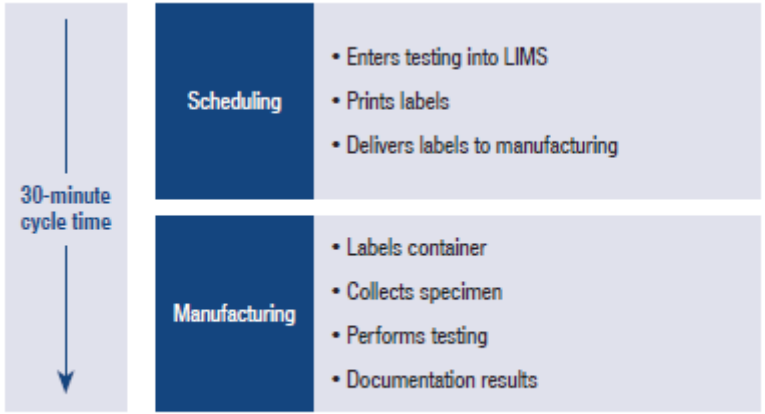



**Figure 2b:** TAT for BET using LAL cartridges/PTS™

# Customer Case Study 1 – Benefit Analysis

## Productivity

- Method 2 was shown to reduce total TAT by 1.25 hours per sample set.
- Efficiency was improved by testing at sample collection point.
- Testing performed by manufacturing technicians after a one-day training period.

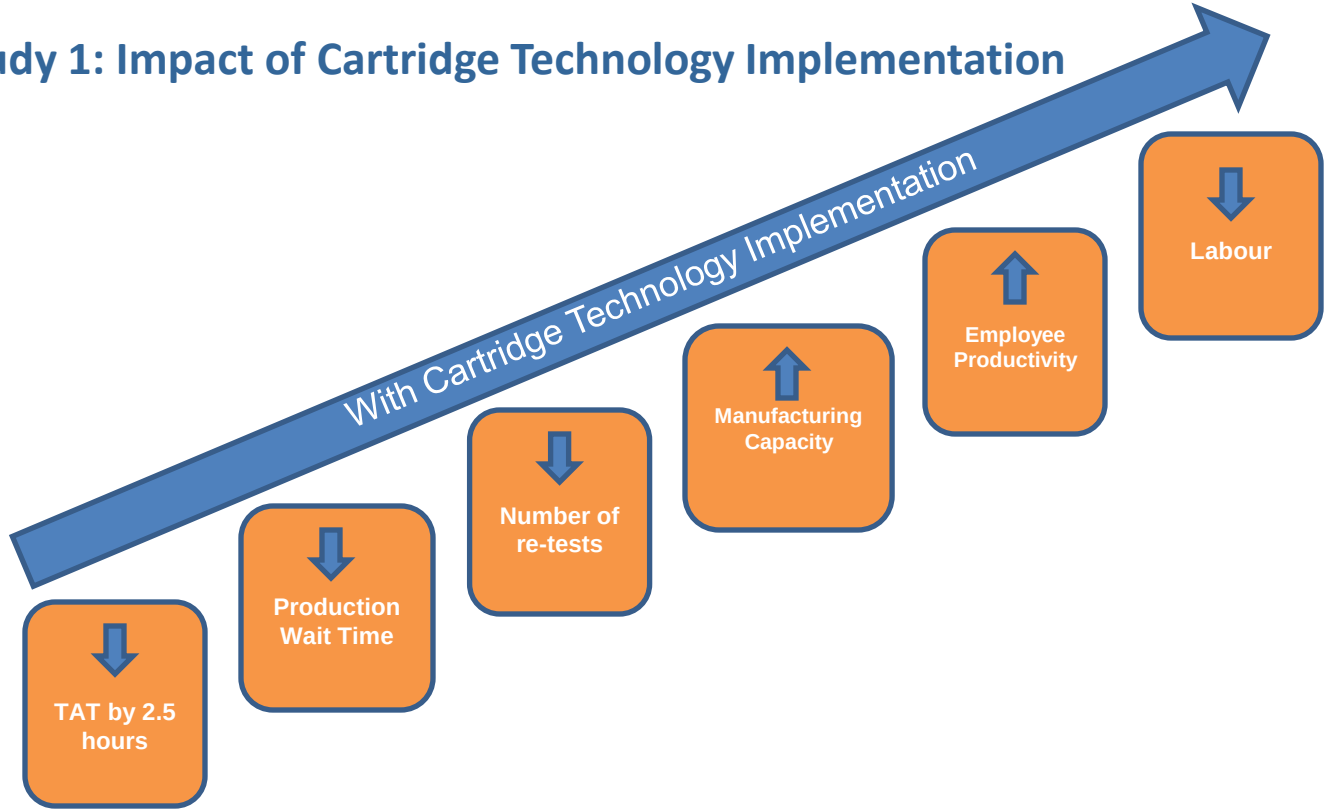
## Quality

- Analyst-to-analyst and lab-to-lab variation was reduced using Method 2 as results were not based on analyst prepared standard curve.
- Retest could be performed immediately eliminating 2.5-hour downtime.
- Simplified OOS investigations due to fewer reagents, steps and accessories required.

## Financials

- Employee efficiencies enabled two third-shift employees to transition to other roles.
- One hour reduction in manufacturing cycle time improved capacity and ability to product two additional batches per year.
- Overall efficiency reduced scrap and employee downtime, eliminated waste and decreased cycle time.

# Case Study 1: Impact of Cartridge Technology Implementation



# Customer Case Study 2

- API produced from Human Urine
- Microbiological “dirty” raw material
  - Exceeds $10^6$ cfu/mL
  - High endotoxin levels
- Production process consists of several microbiological and endotoxin reducing steps
- Efficiency of those steps dictates the production process
- Depending on the level of endotoxin contamination rework is needed
- The process continues as production does not have the time to wait for the lab

# Case Study 2 - Things to consider….

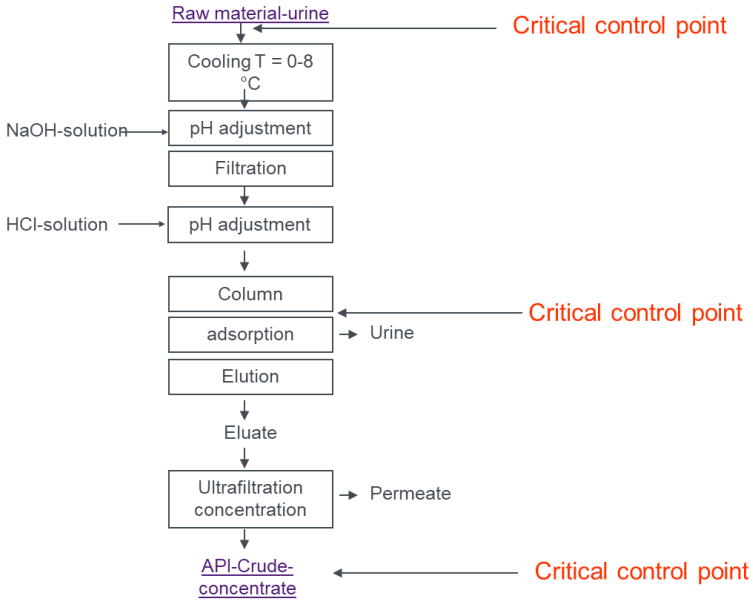
- *Time to report =*
  - *Time to ship the sample to the lab*
  - *Administrative time*
  - Sample prep time
  - Test incubation time
  - Result analysis time
  - *Verify time*
  - *Approval time*
  - *Time to report the result*

For endotoxin testing this is within one day

- Product release cycle times are protracted and are the sum of all these sequential activities. That means eight (8) items to work on to speed up the overall testing process

# Case Study 2 - Process flowchart

- Dedicated to a process & sample type
- Starting material: Human Urine
- Several biochemical column purification
- Samples: Purified protein in different buffers
- Endotoxin concentration must be determined



# Case Study 2 - Return on investment?

- RMM PAT Cartridge Technology was implemented
- Cost savings:
  - One batch: $2 Million
  - Rework: 10% loss in activity (happened in the past)
  - Loss of $200.000 per batch.
  - 10 batches per year were made.
  - With appropriate in-process analysis rework can be avoided
  - Also testing of the in-coming raw material will avoid bad quality batches
  - No lab needed – all testing performed on production floor

# Thank You!

Alan Hoffmeister – Senior Global Scientific Portfolio Specialist
alan.hoffmeister@crl.com