Case study –
Microbiological excursion during a phase I PQ of a WFI system

Walid El Azab
Technical Service Manager
STERIS Life Science
Case study – Microbiological excursion

- Description of the change and validation strategy
- Output of first investigation
- Benefits of a cross-functional investigation team

Return from experience
Water for injection production system – before the change

Microbial specification

- Alert: 2 CFU/200mL
- Action: 20 CFU/200mL

Technical specification

- 2.5 bars in the loop
- Water $t^\circ > 80^\circ$ C
- 1 m/s sub-loop
- 23m3/h return loop
Qualification and validation of a new sub-loop with three points of use (PU)

<table>
<thead>
<tr>
<th></th>
<th>Phase 1 PQ</th>
<th>Phase 2 PQ</th>
<th>Phase 3 PQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>IQ/OQ</td>
<td>Q production</td>
<td>Routine production</td>
<td></td>
</tr>
</tbody>
</table>
Validation strategy and planning

<table>
<thead>
<tr>
<th>Activity</th>
<th>Dec</th>
<th>Jan</th>
<th>Feb</th>
</tr>
</thead>
<tbody>
<tr>
<td>End construction</td>
<td>25/12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>20/12 – 27/12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OQ</td>
<td>26/12 – 01/01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase I PQ</td>
<td></td>
<td>02/01 - 15/01</td>
<td></td>
</tr>
<tr>
<td>Phase II PQ</td>
<td></td>
<td>21/01 – 03/02</td>
<td>11/02</td>
</tr>
<tr>
<td>Media fill start</td>
<td>23/01</td>
<td>05/02</td>
<td></td>
</tr>
<tr>
<td>Q. production</td>
<td>23/01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Release Q. status</td>
<td></td>
<td></td>
<td>12/02</td>
</tr>
</tbody>
</table>

Start production: 23/01
Start Release: 12/02
Agenda

- Case study – Microbiological excursion
  - Description of the change and validation strategy
  - Output of first investigation
  - Benefits of a cross-functional investigation team
- Return from experience
It seems to be P. aeruginosa – do not survive at 80°C water

Other non-conformity? Assess the impact on the PQI.

Ok let’s do it and continue to sample

What is the impact on the production planning?

06/01 (Day 4 – sampling):
- PU8 : 50 CFU/200mL
- PU1 : 20 CFU/200mL

What is the solution? When can we restart?

It seems to be P. aeruginosa – do not survive at 80°C water

Assess the impact on the PQI.

Ok let’s do it and continue to sample

Then sanitize the system overnight

What is the impact on the production planning?

It seems to be P. aeruginosa – do not survive at 80°C water

Assess the impact on the PQI.

Ok let’s do it and continue to sample

Then sanitize the system overnight

What is the impact on the production planning?
Restart phase I PQ – n° 2: microbial results after hot water (80°) sanitization

Position of the non-conformity

Samples at start or return loop were conform

Results per point of use (2nd phase I PQ)

- PU2: Day 2, 10
- PU9: Day 2, 70
Agenda

- **Case study – Microbiological excursion**
  - Description of the change and validation strategy
  - Output of first investigation
  - **Benefits of a cross-functional investigation team**
- **Return from experience**
Cross functional investigation team

1. Daily follow-up
2. Weekly steering
3. Shopfloor investigation

Investigation tools: fishbone

A. Material
B. Method
C. Man
D. Equipment
E. Environment
F. Measure

WFI Gram - contamination
Phase I PQ – n° 2: microbial results after hot water (80°) sanitization

- Samples at start and return loop were conform

Position of the non-conformity

Results per point of use (per day)

Day 6 Day 5 Day 4 Day 3 Day 2

PU2 10 30 70 15 25

PU3 70 24 19 15 15

PU9 80 10 70 15

PU13 15

PU15 120

Addition of sub-loop with 2 points of use (PU)
Is it a "real" biofilm or not?

Testing confirmed:

- Gram coloration: Gram –
- Identification:
  - Pseudomonas aeruginosa
  - Pseudomonas picketti

Consider as objectionable organism
Investigation on material

- QC material: contamination not from the QC laboratory
- Sampling material: need to be sterile for each sampling PU
  - Manual stainless steel valve
  - “O” rings
  - Stainless steel connector
  - Silicone tube
- Valve visual check on shopflor!
  - Valve 15 was closed with a stainless steel cap
  - Valve 2 and valve 9 - presence of residual water after output valves

DO NOT STOP THE INVESTIGATION AT THE FIRST FINDING!
Investigation on method (1/2)

- QC method: contamination not from the QC laboratory

- Interviews of sampler operators:
  - Understand their way of working
    - Some operators were using the same sampling kits for different PU
  - Read the SOP requirement
    - Instructions were leading to interpretation
  - Shopflor visualization
    - Some PU are very difficult to access
    - Draining time and volume were not align between operators
Investigation on method (2/2)

☑ Protocol training:

☑ Knowledge transfer and time between training- phase I PQ starting were inadequate:
  ☑ Training time varies from 2 month to 0.5 day prior the PQ phase I
  ☑ Training in emergency!

☑ SOP for operator qualification:
  ☑ There were no guidance for new operator qualification
Investigation on manpower

- Human error is not the main root cause:
  - "poorly design processes will ultimately lead to errors"

Some readings:
Human errors models and managements; James Reason
People are people; Rony Lardner and Dave Nicholls
Investigation on equipment

- Review of the logbook and technical intervention
- On field investigation:
  - Presence of standing water in some output PU valves
  - PU not easily accessible
- Swab of the contaminated valves:
  - Presence of the same micro-organism identified
Cross-functional investigation team avoid false root cause identification

Summary of findings

A. Material
   - Valves design
   - Materials availability
     - Non-sterile materials used

B. Method
   - Unclear SOPs
     - Draining time
     - Requalification req.
   - SOP req.
   - Protocol training
     - Different WOW

C. Man
   - Knowledge transfer
     - PU access
     - Dead leg
     - Caps closing

D. Equipment
   - Environment

WFI Gram - contamination
# Short and long term corrective/preventive actions

<table>
<thead>
<tr>
<th>Actions list</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong> Material</td>
</tr>
<tr>
<td>• Purchase more material and develop visual lean tools for material management</td>
</tr>
<tr>
<td><strong>B</strong> Method</td>
</tr>
</tbody>
</table>
| • Design sampling instruction methods and check list  
• Knowledge transfer task force  
• Qualification and requalification requirement |
| **C** Man |
| • SOP/protocol qualification and re-training |
| **D** Equipment |
| • Purchase new valves |
| **E** Environment |
| **F** Measure |


Validation planning and production starting - impact and rational

<table>
<thead>
<tr>
<th>Activity</th>
<th>Dec</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
</tr>
</thead>
<tbody>
<tr>
<td>End construction</td>
<td>25/12</td>
<td>20/12 – 27/12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>25/12</td>
<td>20/12 – 27/12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OQ</td>
<td>25/12</td>
<td>20/12 – 27/12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase I PQ</td>
<td>02/01 – 15/01</td>
<td>22/01 – 04/02</td>
<td>11/02</td>
<td></td>
</tr>
<tr>
<td>Phase II PQ</td>
<td>02/01 – 15/01</td>
<td>22/01 – 04/02</td>
<td>11/02</td>
<td>13/02 – 26/02</td>
</tr>
<tr>
<td>Media fill start</td>
<td>23/01</td>
<td>05/02</td>
<td>14/02</td>
<td>27/02</td>
</tr>
<tr>
<td>Q. production</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Release Q. status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Initial planning
Impacted planning

Demonstrate sufficient data for product/ patient impact analysis

- Start of MFT
- Start of commercial batches (Final sterilization or not)
- Sufficient data and control for market product release
Effectiveness checks should be used to confirm the efficacy of corrective or preventive actions put in place.

<table>
<thead>
<tr>
<th>KPI identification</th>
<th>KPI (%) improvement after 6 month of the CAPA implementation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Quality performance:</strong></td>
<td></td>
</tr>
<tr>
<td>a. No deviation related to water system</td>
<td>100%</td>
</tr>
<tr>
<td>b. No deviation related to project</td>
<td>50%</td>
</tr>
<tr>
<td>a. Human hours (productivity not impacted by water related deviation)</td>
<td>70%</td>
</tr>
<tr>
<td><strong>1. Maintenance performance:</strong></td>
<td></td>
</tr>
<tr>
<td>a. Corrective maintenance frequency</td>
<td>60%</td>
</tr>
<tr>
<td>b. Maintenance intervention (numbers)</td>
<td>80%</td>
</tr>
<tr>
<td><strong>1. Production performance:</strong></td>
<td></td>
</tr>
<tr>
<td>a. Planning respect (no delay due to water deviation)</td>
<td>80%</td>
</tr>
<tr>
<td>b. Human hours (productivity not impacted by water related deviation)</td>
<td>70%</td>
</tr>
<tr>
<td>c. Deviation related to water</td>
<td>100%</td>
</tr>
</tbody>
</table>
Agenda

- Case study – Microbiological excursion
- Return from experience
Combination of cleaning approaches have shown effectiveness against biofilm

Biofilm remediation will always use a combine strategy:

1. Use of alkaline cleaning chemistry:
   ✓ Increase the chemical action
   ✓ Increase the mechanical action

2. Use of the sporicidal chemistry or thermal to sterilize the system

Fluorescently labeled P. aeruginosa exposed to 5% concentration at 60°C:

Before exposure

biocide (3min)

alkaline detergent (6min)

Source: STERIS Technical tip 410-200-3088
Good sampling practice

- Clear instruction sampling via check list
- Attention points – staying water, rouging, visual check…
- Through freshly autoclaved tubing
- Allowing outlet to be flush (volume or time)
- Take sampling via good aseptic manipulation
- Testing sampling time (~2h)
- Testing sample : temperature holding time
- Sampling process holding time (~24h)
Proactively reduce a biofilm generation is a continuous process

- Engineering Design
- Good sampling method and practice
- Optimal cleaning/sanitization frequency and procedure
- Optimal disinfection/sterilization frequency and procedure
- Correct chemistry and disinfectant choice
- Routine trend analysis is also important

Thank You
References

✓ European Pharmacopeia (EP) (5.1.4), Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.
✓ European Pharmacopeia (EP) (0008) Water, Purified monograph
✓ European Pharmacopeia (EP) (0169) Water For Injection monograph
✓ European Pharmacopeia (EP) (1729) highly Purified Water monograph
✓ United States Pharmacopeia (USP) <1231> WATER FOR PHARMACEUTICAL PURPOSES. The United states Pharmacopeial Convention/National Formulary, Rockville, MD.
✓ Japanese Pharmacopeia (JP) <G8> Water - Quality Control of Water for Pharmaceutical Use

✓ Note: This is not a complete listing, just a guidance to literature the speaker has found to be interesting/beneficial.


USP chap 1111

Note: This is not a complete listing, just a guidance to literature the speaker has found to be interesting/beneficial.
Biofilm is generally composed of multiple microorganism encased in matrix extracellular polymeric substance (EPS):

- Flow velocity: Laminar velocity increase the probability of microorganism to fix on the surface
- Deadleg: Increase the presence of wet environment and conducive to biofilm growth
- Rugosity: > 0.5 Ra for clean piping and product contact. Rouging is also a source increasing rugosity
- Residue: Poor cleaning procedure will increase residue on the surface, increasing rugosity
Micro-organism found in pharmaceutical water system

<table>
<thead>
<tr>
<th>Gram -</th>
<th>Gram +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ralstonia pickettii</td>
<td>Micrococcus Luteus</td>
</tr>
<tr>
<td>Pseudomonas spicies (spores)</td>
<td></td>
</tr>
<tr>
<td>Chryseobacterium indologenes</td>
<td></td>
</tr>
<tr>
<td>Burkholderia cepacia</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td></td>
</tr>
<tr>
<td>Maroxella species</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus maltophilia</td>
<td></td>
</tr>
<tr>
<td>Flavimonas oryzihabitans</td>
<td></td>
</tr>
<tr>
<td>Ochrobactrum anthropi</td>
<td></td>
</tr>
</tbody>
</table>

LIST NON EXHAUSTIVE

Source: Assessment of the suitability of R3A agar for the subculture of microorganism isolated from pharmaceutical water; EJPPS 2014; 19(3):85-93