Eyes on the Prize: How Many Eyes Are Too Many for Microbiological Data Integrity?

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Background

Cambrex is a contract development and manufacturing organization that delivers drug substance, drug product, and analytical services across the entire drug life cycle. The microbiology laboratory at Agawam, MA is a cGMP laboratory that tests non-sterile product per USP <60>, <61>, <62> /EP/ JP and performs bioburden testing per ANSI/AAMI/ISO methods. Both tests require visual observations and plate enumeration.

- Visual observations are essential to microbial assays. Microbial enumeration, colony morphology, growth vs no growth and turbidity all can be subjective to interpretation.
- ANSI/AAMI/ISO bioburden and USP <60>/<61>/<62> both involve critical decision points that can affect patient safety.
- It is up to laboratories to evaluate the risks and determine if second analyst verifications are required for microbial observations.

The risk question was defined as follows: What is the risk to patient safety and to cGMP decisions for not having a second analyst verification on manual microbial observations?

Method/Process Overview

The Agawam site assessed the potential risks associated with manual observations for bioburden testing per ANSI/AAMI/ISO and testing of non-sterile per USP <60>/<61>/<62>.

- A cross functional team was assembled.
- Process mapping of observations points within ANSI/AAMI/ISO bioburden and USP <60>/<61>/<62> testing workflows were determined.
- The failure modes and effects analysis (FMEA) tool was used as a basis to evaluate potential modes of failure for this process.
- Manual microbial observations were broken into process steps or component parts as it pertained to the microbial examination of non-sterile products.
- For each step or part, possible failure modes were identified. Each failure mode was then examined to determine possible cause(s) and effect(s), and all existing controls.

Failure Modes and Current Controls

ANSI/ AAMI/ ISO bioburden		USP <60>, <61>, and <62>	
Prevention	Detection	Prevention	Detection
• Procedure for plate counting methodology	• Identification of growth for invalids/ out-of-specification results	• Procedure for plate counting methodology	• Identification of growth for invalids/ out-of-specification results
• Procedure for the performance of morphological assessments	• Downstream testing/ results (i.e., sterility testing)	• Procedure for the performance of morphological assessments	• Gram stain analysis of growth recovered for specified microorganisms
• Training module for the submission of samples for identification	• Technical review	• Training module for the submission of samples for identification	• Technical review
• Skills assessment qualification for Gram staining		• Skills assessment qualification for Gram staining	
• Skills assessment qualification for bioburden testing		• Skills assessment qualification for USP <60>, <61>, and <62> testing	

Risk Matrix and Scoring

There are three components that help define the priority of a failure:

- **Occurrence (O):** Likelihood of an observation error
- **Severity (S):** Potential impact on product quality/ patient safety if observation was wrong
- **Detection (D):** Likelihood the error will be caught without second verification

Table 1: Risk Class Matrix (S x O)

Occurrence \ Severity	1	2	3	4
4	4 (Low)	8 (Medium)	12 (High)	16 (High)
3	3 (Low)	6 (Medium)	9 (Medium)	12 (High)
2	2 (Low)	4 (Low)	6 (Medium)	8 (Medium)
1	1 (Low)	2 (Low)	3 (Low)	4 (Low)

Table 2: Failure Mode Assessment and Mitigation for ANSI/AAMI Bioburden Testing

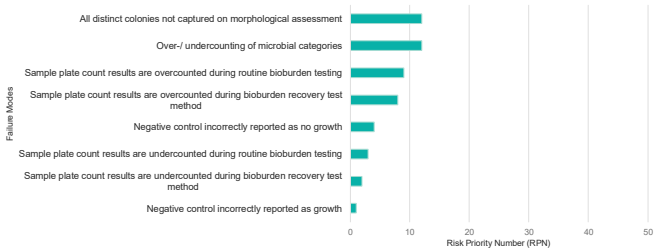
#	Risk Class Determination (Level of Concern)					Risk Priority				Mitigation		Residual Risk Priority			
Risk No.	Potential Failure Mode	Potential Failure Effect	Severity (S)	Potential Cause of the Failure mode	Occurrence (O)	S x O (Risk Class)	Current Controls: (P) Prevention (D) Detection	Detection (D)	Risk Priority Number (RPN) S x O x D (Dmax)	Mitigation	Severity (S)	Occurrence (O)	Detection (D)	Residual Risk (RPN) S x O x D
1	Sample plate count results are overcounted during bioburden recovery test method	Bioburden correction factor is not accurate	1	Product is mistaken for growth on post plate/ test method	2	Low	None	4	8	N/A	1	2	4	8
			1	Colonies are undistinct or clustered on membrane filtration method	2	Low	(P) Target inoculum to within the compendial range (>30 CFU > 80)	3	6		1	2	3	6
		Method that would normally fail, passes	3	Product is mistaken for growth on post plate/ test method	3	Medium	(D) Positive control	3	27		3	3	3	27
			2	Colonies are undistinct or clustered on membrane filtration method	2	Low	(D) Positive control	3	12		2	2	3	12
2	Sample plate count results are overcounted during routine bioburden testing	Incorrect determination of OOS results	3	Product is mistaken for growth on post plate/ test method	3	Medium	(D) Sterility test results	2	18	N/A	3	3	2	18
			4	Colonies are undistinct or clustered on membrane filtration method	4	High	(D) Sterility test results	2	32		3	4	2	24
			3	Debris on the filter or embedded in the agar	3	Medium	(P) Training on SOP00138	3	27		3	3	3	27
3	Sample plate count results are undercounted during routine bioburden testing	Missed alert/ action	2	Product breaks down and mimics growth	2	Low	None	4	16	N/A	1	2	4	8
			3	Spreader colonies	3	Medium	(P) Training on SOP00138 (procedure (SPR) are reported differently	2	24		3	3	2	18
			1	Pinpoint colonies are missed	2	Low	(P) Training on SOP00138	4	16		1	2	4	8
		Incorrect determination of OOS results	1	Overgrowth of mold	2	Low	None	4	16		1	2	4	8

Table 3: Failure Mode Assessment for USP <61>

#	Risk Class Determination (Level of Concern)					Risk Priority				
Risk No.	Potential Failure Mode	Potential Failure Effect	Severity (S)	Potential Cause of the Failure mode	Occurrence (O)	S x O (Risk Class)	Current Controls: (P) Prevention (D) Detection	Detection (D)	Risk Priority Number (RPN) S x O x D	
1	Sample plate counts are overcounted during routine USP <61> testing	False OOS	1	Product is mistaken for growth on post plate/ membrane filtration test method	2	Low	None	4	8	
			1	Colonies are undistinct or clustered on membrane filtration method	1	Low	(P) SOP00138	4	4	
		Reporting incorrect results	2	Product is mistaken for growth on post plate/ membrane filtration test method	2	Low	None	4	16	
			2	Colonies are undistinct or clustered on membrane filtration method	1	Low	(P) SOP00138	4	8	
2	Sample plate counts are undercounted during routine USP <61> testing	Heavy product routine lidtag growth	3	Inappropriate method	3	Medium	None	4	36	
			3	Analyst subjectivity with interpreting results	3	Medium	None	4	36	
		Missed OOS	3	Spreader colonies	1	Low	(P) SOP00138	4	12	
			3	Missed pinpoint colonies (pour plate/ membrane filtration)	2	Medium	(P) SOP00138	4	24	
3	Sample plate counts are overcounted during suitability for USP <61>	Method that would normally fail, passes	3	Product is mistaken for growth on pour plate test method	4	High	None	4	48	

Results

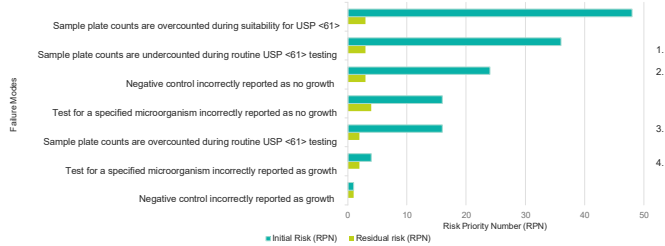
Figure 1: Top RPN Contributors for ANSI/AAMI/ISO Bioburden testing



ANSI/AAMI/ISO Bioburden Mitigations

Risk level is acceptable, no mitigations required.

Figure 2: Top RPN Contributors for USP <60>, <61>, and <62> Testing



USP <60>, <61>, and <62> Mitigations

1. Added second analyst verification.
2. Updated plate counting procedure to include instructions on how to enumerate pinpoint/translucent colonies.
3. Created a training module for plate counting assessment.
4. Developed an annual assessment for plate counting to ensure consistent monitoring and evaluation of the process.

Conclusion

ANSI/AAMI/ISO	USP <60>/<61>/<62>
The testing is early in the manufacturing process.	**Conducted on finished product or product contact components, results have direct impact on patient safety.**
Errors at this stage can often be caught during subsequent testing in release Sterility Testing USP <71> or in Environmental Monitoring.	**Errors directly impact patient safety if not detected.**
Had a reduced severity score due to multiple downstream controls which resulted in a lower RPN.	**Severity score higher due to direct contact with patient resulting in higher RPN.**
Second analyst verification is not required but was determined for critical quality decisions. For example, when issuing an Out of Specification result.	**Second analyst verification implemented for all microbial observations to help mitigate the risk.**

Bioburden testing, as defined by ANSI/AAMI/ISO standards, is not considered a release test. Plate enumeration associated with bioburden testing is regarded as an estimation rather than an exact count. Microbial observations obtained through bioburden testing carry a lower risk profile, as they are not directly linked to patient safety and are subject to multiple downstream controls.

In contrast, testing performed under USP <60>, <61>, and <62> is conducted on finished goods and has a direct impact on patient safety. To ensure the accuracy and reliability of microbial observations in this context, a second analyst verification has been implemented for all results.