Summary of Validation and Sample Recovery using Hygiena® Polyurethane Sponges compared to Cellulose Sponges

Contents

Polyurethane Sponge Sampler

1. Objective:

To validate new Stick Sponges (Sponges pre-moistened with Buffered Peptone Water (BPW), Neutralizing Buffer (NB) and Letheen Broth (LET)) and compare the performance of the new polyurethane products with the previous cellulose sponge-based products in a growth and recovery study.

2. Materials

2.1 Materials and Equipment

- Sterile inoculating loops
- Sterile pipettes and tips
- Incubators capable of 35 ± 2 °C
- Alcohol wipes
- Buffered Peptone Water, BBL™
- Neutralizing Buffer, Difco™
- Letheen Broth, BD™
- Tryptic Soy Broth, Remel™
- Tryptic Soy Agar Plates, Manufactured at Hygiena LLC BD REF#236950
- DPBS (1X) – Dulbecco's Phosphate Buffered Saline REF# 14190-144

2.2 Microorganisms Tested

Microorganism	ATCC Number
Escherichia coli	8739
Listeria monocytogenes	33090
Staphylococcus aureus	6538
Salmonella Typhimurium	14028

2.3 Products Tested

Polyurethane Sponge		Cellulose Sponge	
Broth/Buffer	**Name**	**Broth/Buffer**	**Name**
Buffered Peptone Water (BPW)	SS100BPWP	Buffered Peptone Water (BPW)	SS100BPW
Letheen Broth (LET)	SS100LETP	Letheen Broth (LET)	SS100LET
Neutralizing Buffer (NB)	SS100NBP	Neutralizing Buffer (NB)	HSB100NB

3. Methods

3.1 Sample Preparation

Broths and buffers (TSB, BPW, LET, and NB) were manufactured in-house to run as controls. They were then placed into 10 mL vials and inoculated with bacteria as a positive control to verify growth. These were run in parallel with the vials containing sponges.

3.2 Inoculum preparation

Escherichia coli ATCC 8739, *Listeria monocytogenes* ATCC 33090, *Staphylococcus aureus* ATCC 6538, and *Salmonella typhimurium* 14028 were all grown in TSB broth and incubated at 37 °C overnight.

The actual inoculum for all microorganisms used during sample spiking was determined by plating each organism in duplicate onto TSA plates. The plates were incubated at 33 ± 2 ˚C and counted after 24 hours.

3.3 Test Methods

1. Overnight cultures were serially diluted in PBS from neat to 10^{-9}.
2. Dilutions of 10^{-5} to 10^{-7} were plated to determine the CFU/mL of the overnight culture.
3. For each sponge type, 100 µL of each of the selected dilutions (10^{-6} to 10^{-9}) was directly added onto the Stick Sponges of the three formats (BPW, LET, and NB).
4. The testing was performed in triplicate; inoculated sponges were incubated at 30 °C and assessed for growth after 24 and 48 hours by plating 100 µl of sample on agar plates and looking for growth the following day.
5. Controls were performed in triplicate. (10 mL of TSB, BPW, LET, and NB in sterile containers as negative controls).

3.4 Confirmation Plates

For each day of testing, 10 µL of each product sample was streaked onto Tryptic Soy Agar and incubated at 30 ± 2 °C for up to 48 hours.
Growth on confirmation plates was compared to the morphology of the microorganism recovered from the spiked sponges.

4. Results

4.1 Tryptic Soy Broth (TSB)

Micro-organism	Dilution Level	Control TSB					
		TSA Growth Confirmation					
		24 Hours			48 Hours		
		1	2	3	1	2	3
S. aureus	-6	+	+	+	+	+	+
	-7	+	+	+	+	+	+
	-8	-	-	-	-	-	-
	-9	-	-	-	-	-	-
E. coli	-6	+	+	+	+	+	+
	-7	+	+	+	+	+	+
	-8	-	-	+	-	-	+
	-9	+	-	-	+	-	-
L. mono	-6	+	+	+	+	+	+
	-7	+	+	+	+	+	+
	-8	-	-	-	+	-	-
	-9		-	-		-	-
S. Typhi	-6	+	+	+	+	+	+
	-7	+	+	+	+	+	+
	-8	-	-	+	-	-	+
	-9	-	-	-	-	-	-

4.2 Buffered Peptone Water (BPW)

Micro-organism	Dilution Level	Control BPW						Cellulose BPW						Polyurethane BPW					
		TSA Growth Confirmation						TSA Growth Confirmation						TSA Growth Confirmation					
		24 Hours			48 Hours			24 Hours			48 Hours			24 Hours			48 Hours		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
S. aureus	-6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	-7	+	*+	-	+	*+	+	+	+	+	+	+	+	+	+	+	+	+	+
	-8	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	-9	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-
E. coli	-6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	-7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	-8	-	-	-	+	+	+	-	+	+	-	+	+	+	-	+	+	-	+
	-9	-	-	+	*+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
L. mono	-6	+	+	+	+	+	+	*+	+	+	*+	+	+	+	+	+	+	+	+
	-7	+	+	+	+	+	+	*+	+	+	*+	+	+	+	+	+	+	+	+
	-8	+	-	+	+	-	+	+	-	-	+	-	-	-	-	+	+	+	+
	-9	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-
S. Typhi	-6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	-7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	-8	*+	+	+	*+	+	+	-	+	-	-	+	-	+	+	+	+	+	+
	-9	-	-	*+	-	+	*+	-	-	+	-	-	+	+	-	-	+	-	-

Note: "+" confluent growth, "*+" <5 colonies, "-" negative

4.3 Letheen Broth (LET)

Micro-organism	Dilution Level	Control LET TSA Growth Confirmation						Cellulose LET TSA Growth Confirmation						Polyurethane LET TSA Growth Confirmation					
		24 Hours			48 Hours			24 Hours			48 Hours			24 Hours			48 Hours		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
S. aureus	-6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	-7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	-8	-	+	-	-	+	-	-	-	+	-	-	+	-	+	-	-	+	-
	-9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. coli	-6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	-7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	-8	+	+	+	+	+	+	-	+	+	-	+	+	+	*+	-	+	*+	-
	-9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L. mono	-6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	-7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	-8	-	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+
	-9	-	*+	-	-	*+	-	-	-	-	-	-	-	-	-	-	+	-	-
S. Typhi	-6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	-7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	-8	-	-	+	+	-	+	+	+	+	+	+	+	-	-	+	+	-	+
	-9	*+	-	+	*+	-	+	-	*+	+	-	*+	+	+	-	-	+	-	-

Note: "+" confluent growth, "*+" <5 colonies, "-" negative

4.4 Neutralizing Buffer (NB)

Micro-organism	Dilution Level	Control NB TSA Growth Confirmation						Cellulose NB TSA Growth Confirmation						Polyurethane NB TSA Growth Confirmation					
		24 Hours			48 Hours			24 Hours			48 Hours			24 Hours			48 Hours		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
S. aureus	-6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-8	-	-	-	ND	ND	ND	-	-	-	-	-	-	-	-	-	-	-	-
	-9	-	-	-	ND	ND	ND	-	-	-	-	-	-	-	-	-	-	-	-
E. coli	-6	*+	*+	*+	*+	*+	*+	+	+	+	+	+	+	+	+	+	+	+	+
	-7	*+	-	-	*+	+	+	+	+	-	+	+	-	+	+	+	+	+	+
	-8	-	-	-	-	+	+	-	-	-	-	+	-	+	+	+	+	+	+
	-9	-	-	-	-	*+	-	-	-	-	-	-	-	-	-	-	-	-	-
L. mono	-6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-8	-	-	-	ND	ND	ND	-	-	-	-	-	-	-	-	-	-	-	-
	-9	-	-	-	ND	ND	ND	-	-	-	-	-	-	-	-	-	-	-	-
S. Typhi	-6	-	-	*+	+	+	*+	+	-	-	+	+	+	+	+	+	+	+	+
	-7	-	-	-	+	-	-	+	-	+	+	+	+	+	-	+	+	+	+
	-8	-	-	-	ND	ND	ND	+	-	-	+	+	-	-	-	-	+	-	-
	-9	-	-	-	ND	ND	ND	-	-	-	-	-	-	-	-	-	-	-	-

Note: "+" confluent growth, "*+" <5 colonies, "-" negative, ND - not done

5. Summary of Results

The polyurethane sponges with BPW were able to demonstrate equivalent growth and recovery for *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Listeria monocytogenes* ATCC 33090 and *Salmonella* Typhimurium 14028 at dilutions of 10^{-6}, 10^{-7} and 10^{-8} within 24 hours. The collection of the organisms followed by addition of 100 mL of any growth media increase recovery from the sponge.

The polyurethane sponges with Letheen were able to demonstrate equivalent growth and recovery for *Escherichia coli* ATCC 8739, *Listeria monocytogenes* ATCC 33090, *Salmonella* Typhimurium 14028 and *Staphylococcus aureus* ATCC 6538 at dilution 10^{-6} and 10^{-7} within 24 hours. The comparison testing of Hygiena polyurethane vs. cellulose sponge products showed that the growth and recovery of these organisms from both products were similar.

For Stick Sponges with Neutralizing Buffer, Hygiena polyurethane sponges demonstrated equivalent growth and recovery for *Escherichia coli* ATCC 8739 at dilutions of 10^{-6}, 10^{-7} and 10^{-8} and for *Salmonella* Typhimurium 14028 at dilutions of 10^{-6} and 10^{-7} within 24 hours when compared to cellulose sponges. *Listeria monocytogenes* ATCC 33090 and *Staphylococcus aureus* ATCC 6538 failed to grow in Neutralizing Buffer. The comparison testing of Hygiena polyurethane with cellulose sponge products showed that the growth and recovery of these organisms in both products were similar.

6. Final Conclusion

Hygiena polyurethane sponges demonstrated equivalent growth and recovery when compared to cellulose sponges when tested using standard microorganisms (*Escherichia coli* ATCC 8739, *Listeria monocytogenes* ATCC 33090, *Salmonella* Typhimurium 14028 and *Staphylococcus aureus* ATCC 6538).

Therefore, polyurethane sponges are acceptable for environmental monitoring and do not interfere with microorganism recovery.

Appendix:

Independent Vendor Report on Equivalence between cellulose and polyurethane sponge

1. Context

A polyurethane foam sponge (PFS) is a synthetic sponge produced in batches of open-topped molds.

A great advantage of a PFS is its flexibility and its high tensile strength, making it less susceptible to flaking and tearing during environmental sample collection. Therefore, surfaces, including those that are rough and irregular, can be rigorously scrubbed during sample collection without concern that the sponge could deteriorate.

In addition, the polyurethane industry is one of the few industries with virtually no production waste, thus decreasing the ecological footprint. Indeed, trims (or waste foam from cutting operations) are re-used to produce new products, such as carpet underlay or bonded foam.

Considering such advantages, a synthetic sponge that has been thoroughly validated represents a superior alternative to a cellulose sponge.

2. Objective

To confirm that polyurethane and cellulose sponges offer similar performance.

3. Methodology

Both polyurethane and cellulose sponges are evaluated upon reception to ensure that they meet or exceed the industry standard. The evaluated characteristics are the following:

3.1. Visual inspection

Statistically representative sampling is performed for each new batch. A visual inspection for minor, major and critical defects is carried out. Destructive tests such as surface scrubbing and water immersion are conducted to validate the sponge's ability to withstand laboratory work.

3.2. pH

Ten sponges are hydrated with 10 mL of tap water and are kept at room temperature. Ten milliliters of collected tap water is also kept at room temperature and used as a control. After 30 minutes, the pH of the samples and control are measured and compared.

Interpretation of results: the pH ratio of each sponge and pH of water is calculated. The pH ratio for each sponge versus control must stay between 0.89 and 1.10.

If the ratio is outside this range, the batch is rejected.

3.3. Bioburden

For each batch, 10 samples are collected and the bioburden is determined. Batches cannot exceed the bioburden tolerance as high values could compromise sterilization effectiveness. Thus, any batch exceeding bioburden tolerance is rejected.

Bioburden tolerance:

- Average of 2 fungi per sponge
- Average of 200 CFU per sponge

3.4. Performance

Thirty-two (32) sponges per batch are hydrated with 10 mL of D/E Neutralizing Broth and sterilized by gamma irradiation. The performance of sterilized sponges is verified following the environmental test described by Claveau, et al. (J. AOAC Int. 97, 1127-1136) with some modifications. This test's aim is to mimic industrial environmental testing. Thus, stainless steel surfaces (100 cm^2) are inoculated with *Listeria monocytogenes*. The strain is exposed to dehydration stress over 18-20 h. Sponges, humidified with D/E Neutralizing Broth, are used to recover the bacteria (*L. monocytogenes*). They are placed in a sterile bag with enrichment medium and are incubated for 20 ± 2 h. Finally, the enrichment is plated and bacterial counts are collected. Sponge performance is evaluated based on expected recovery results. Sponges with good performance, indicating they are biocide-free, must show a viable count higher than 1 x 10^6 CFU/mL after enrichment.

This test was developed using sponges with and without biocides as positive and negative controls.

4. Results and discussion

The goal of this evaluation is to guarantee the quality of our product, and guarantee to our customers that we are committed to protecting their brand.

4.1. Visual inspection

Both cellulose and polyurethane sponges meet all specifications.

4.2. pH

During the manufacture of sponges, many chemical products are employed. If they are not thoroughly removed, they can interfere with the neutralizing solution or broth used in the manufacturing of sampling tools, as they can specifically affect the pH. Thus, the pH of the water released from a wet sponge must be very close to the pH of the water used to hydrate it, with a ratio close to 1.

Both cellulose and polyurethane sponges present similar conforming results as can be observed in Table 1.

Table 1: Comparison of pH Ratio of Cellulose and Polyurethane Sponges

Cellulose Sponges		Polyurethane Sponges	
Lot	pH (ratio)	**Lot**	pH (ratio)
2019-009	0.97	**2019-030**	1.04
2018-107	0.89	**2018-134**	1.03
2018-100	0.94		

Acceptance criteria: 0.89 – 1.10

4.3. Bioburden

The international standard ISO 11137 was followed to guarantee a sterility assurance level of 10^{-3} for a product. This bioburden information is used to set the irradiation dose (Method 1). In consequence, the bioburden of the finished product as well as all its components must be controlled to avoid compromising the irradiation effectiveness. Values for cellulose and polyurethane sponges are shown in Table 2.

Table 2: Comparison of Bioburden in Cellulose and Polyurethane Sponges

Cellulose Sponges			Polyurethane Sponges		
Lot	Microorganism (Avg.)	Fungi (Avg.)	**Lot**	Microorganism (Avg.)	Fungi (Avg.)
2019-009	165	0	**2019-030**	40	0
2018-107	26	0.4	**2018-134**	18	0
2018-100	98	0.4			

Avg.: Average. • Bioburden acceptance criteria: average: 2 fungi per sponge and average: 200 C.F.U. per sponge.

The low bioburden of both the cellulose and polyurethane sponges guarantees that minimal interference will be observed after sterilization by gamma irradiation as shown in Table 2.

4.4. Performance

As explained before, during the manufacture of sponges, many chemical products are used. If they are not removed, residual chemicals can be liberated in the neutralizing solution or broth used in the composition of the sampling tool. These chemicals can affect the pH of the solution and they can also have a biocidal or bacteriostatic effect. For this reason, each batch of sponge is verified as suitable for its application using a method that mimics usage.

During this test, it was observed that polyurethane sponges soaked with D/E Neutralizing Broth generated a variable color change, in comparison with cellulose sponges which remained unchanged. However, both types performed appropriately as it is shown in Table 3.

Table 3: Comparison of Performance of Cellulose and Polyurethane Sponges

Cellulose Sponges				Polyurethane Sponges			
Lot	Exc. (%)	Sat. (%)	Fail (%)	**Lot**	Exc. (%)	Sat. (%)	Fail (%)
2019-009	73.3	26.7	0	**2019-030**	100	0	0
2018-107	100	0	0	**2018-134**	100	0	0
2018-100	100	0	0				

Exc.: Excellent; Sat: Satisfactory; Acceptance criteria: Not more than 3.12% of samples can fail.

5. Conclusion

All tests evaluating the functionality of both the cellulose and polyurethane sponges demonstrate satisfactory and similar results. Therefore, we can confirm that both types offer similar and adequate performance for microorganism collection and testing.