Nasco SAMPLING
901 Janesville Avenue, Fort Atkinson, WI 53538-0901

Hi Capacity Broth Formulation Features

Used in Whirl-Pak® bags B01590WA, B01591WA, & B01592WA

- Has high neutralizing capacity for all sanitizers commonly used by food industry (including quats, peroxide/peroxyacetic, chlorine and chlorine compounds, phenolics, alcohols, and acid sanitizers)
- Has sufficient buffering capacity to handle low pH (high acid) sanitizers
- Only includes components that are found on U.S. Food and Drug Administration's EAFUS (Everything Added to Food in the United States) list, as materials acceptable for inclusion in food
- Only includes components that are considered to be non-allergenic
- Does not contain agents (citrate, bisulfite and thiosulfate) that are reported to interfere with 3M™ Petrifilm™
- Includes vegetable peptones (not digested with animal-derived enzymes) and yeast extract, instead of meat peptones or meat extracts
- Does not interfere with established immunoassays or newer generation molecular diagnostic tests with genetic amplification
- Can be stored at a temperature range of 2 to 30˚C
- 2-year shelf life

Nasco SAMPLING
901 Janesville Avenue, Fort Atkinson, WI 53538-0901

SDS for Whirl-Pak® PolySponge™ and PolyProbe™

B01590WA, B01591WA, & B01592WA

1 Identification

1.1 Product identifier:

Product name: Whirl-Pak® PolySponge™ and Whirl-Pak® PolyProbe™ with High Density Polyurethane Sponge, Sample Bag, and HiCap Neutralizing Broth
Item numbers: B01590WA, B01591WA, & B01592WA
Recommended use(s) of the product: Sampling of surfaces for microorganisms
Uses of the product not advised:

1.2 Details of the supplier of the safety data sheet:

Company information:
Nasco Sampling
901 Janesville Ave.
Fort Atkinson, WI 53538
Telephone 920 563-2446
FAX 920 563-8296
Email info@whirl-pak.com

2 Hazards Identification

2.1 Classification of the substance or mixture:

The substance is not classified according to the Globally Harmonized System (GHS)

2.2 Classification according to 67/548/EEC or 1999/45/EC:

The product contains no hazardous materials, or concentrations of materials of all constituents in this product are below regulatory limits, as described by OSHA 29 CFR 1910.1200, Canada's WHMIS, and EC directives 67/548/EEC and 1999/45/EC

2.3 GHS label elements, including precautionary statements:

GHS label: Void
Hazard Pictogram/Symbols: Void
Hazard statements: Void
Precautionary statements: Void

2.4 NFPA ratings (scale 0-4)

Health = 0
Flammability = 0
Reactivity = 0

2.5 HMIS ratings (scale 0-4)

HEALTH 0	*Health = 0*
FIRE 0	*Flammability = 0*
REACTIVITY 0	*Reactivity = 0*

2.6 Other hazards:
Results of PBT and vPvB assessment:
PBT: Not applicable
vPvB: Not applicable

3 Composition/Information on Ingredients

3.1 Chemical Characterization: Mixture
This product contains no hazardous constituents, or the concentration of all chemical constituents are below the regulatory limit threshold limits according to OSHA 29 CFR 1910.1200, Canada's WHMIS, and EC directives 67/548/EEC and 1999/45/EC. No components need to be disclosed according to applicable regulations.

3.2 Form:
Sampling device is comprised of a solution to hydrate a hydrophilic polyurethane sponge, a sample bag made with a polyethylene/nylon laminated film and a galvanized steel wire covered by a pressure sensitive tape. With proper aseptic sampling technique, the solution in this device will not come in contact with user.

4 First Aid Measures

4.1 General information:
No special measures expected

4.2 In case of skin contact:
Immediately wash with plenty water and soap; rinse thoroughly with water
If rash or irritation appear, get medical attention

4.2 In case of ingestion:
Rinse mouth with water
Never give anything by mouth to an unconscious person

4.3 In case of inhalation:
Move the victim to fresh air and consult doctor with any breathing difficulty
In case of irregular breathing or respiratory arrest, provide artificial respiration

4.4 In case of eye contact:
Immediately wash eye with plenty of water for at least 15 minutes lifting eyelids

4.5 Most important symptoms or effects, both acute and delayed: May cause irritation

4.6 Indication of any immediate medical attention and special treatment needed: no data available.

5 Fire-Fighting Measures

5.1 Extinguishing media:

Use water spray, ABC multipurpose dry chemical, carbon dioxide, or alcohol resistant foam

5.2 Special hazards arising from the product:

Burning plastic (polyethylene and nylon) in this product will give rise to toxic fumes and smoke. Decomposition products may include carbon oxides and toxic organic vapors.

5.3 Advice for firefighters:

Keep product exposed to fire cool by spraying with water
Use positive-pressure breathing apparatus
Wear chemical protection suit

6 Accidental Release Measures

6.1 Personal precautions, protective equipment and emergency procedures:

Wear protective clothing as recommended in section 8

6.2 Environmental precautions:

None. Wipe up with a damp sponge or mop

6.3 Reference to other sections:

See Section 7

7 Handling and Storage

7.1 Handling:

Handle according to Good Laboratory Practices
Do not eat, drink or smoke when using this product
Avoid contact with skin and eyes
Eyewash bottles should be available
Wash hands after using this product

7.2 Storage:

Keep individual sampling devices sealed in zip lock pouch. Store boxes in a dry environment at ambient temperatures

7.3 Specific end use(s)

For the sampling of surfaces for microorganisms

8 Exposure Controls/Personal Protection

8.1 Control parameters:

Contains no substances with occupational exposure limit values

8.2 Exposure controls:

Handle according to Good Laboratory Practices

8.3 Protection of the hands:
Wear chemical resistant gloves (e.g. nitrile, or equivalent)

8.4 Protection of the eyes:
Wear safety glasses approved to NIOSH or EN 166 standards

8.5 Protection of the body:
Wear a laboratory coat

9 Physical and Chemical Properties

9.1 Appearance/odor:
Form: Sample bag with wire tie containing a polyurethane sponge hydrated with HiCap Neutralizing Broth solution.
Color: Solution when squeezed from sponge has a tan color and is cloudy
Odor: Characteristic of a protein containing culture medium

9.2 Properties:
pH: Neutral
Melting point/range: No information available
Boiling point/range: No information available
Boiling point/boiling range: No information available
Flash point: No information available
Evaporation rate: No information available
Flammability: No information available
Vapor pressure: No information available
Vapor density: No information available
Auto igniting: Product is not self-igniting
Danger of explosion: Product does not present an explosion hazard
Density: No information available
Solubility in water: Soluble
Oxidizing properties: No information available
Viscosity: No information available

10 Stability and Reactivity

Reactivity: No information available
Chemical stability: Considered stable under normal conditions
Possibility of hazardous reactions: No hazardous reactions known if used for its intended purpose
Conditions to be avoided: No further relevant information available
Incompatible materials: No information is available
Hazardous decomposition products: Burning plastic (polyethylene and nylon) in this product will give rise to toxic fumes and smoke. Decomposition products may include carbon oxides and toxic organic vapors.

11 Toxicological Information

11.1 Information on toxicological effects:

Acute toxicity: No experimental data available

Primary irritant effect: May cause irritation of skin or redness. May cause irritation of eyes or redness

No evidence of carcinogenic effects (No component of this product is present at levels greater than or equal to 0.1% is identified as a carcinogen or anticipated to be a carcinogen by OSHA or NTP.)

No evidence of mutagenic effects

No information of teratogenic effects

Hazardous properties are unlikely when handled according to intended use.

12 Ecological Information

12.1 Toxicity:

Aquatic toxicity: No information available

Persistence and degradability: No information available

12.2 Behavior in environmental systems:

Bioaccumulative potential: No information available

Mobility in soil: No information available

12.3 Results of PBT and vPvB Assessment

PBT: Not applicable

vPvB: Not applicable

12.4 Other information:

To the best of our knowledge, the properties of this material have not been fully evaluated. No environmental hazard is anticipated when the material is used as intended and disposed of properly.

13 Disposal considerations

13.1 Waste treatment methods:

Disposal should be in accordance with local, state or national statutes

The device itself is not biohazardous. High levels of microorganisms may be obtained with incubation of the device and/or if additional nutritive solutions are added to the device followed by incubation. If this is the case, follow Good Laboratory Practices for effective decontamination before disposal.

Refer to local authorities for information on recycling.

14 Transport Information

UN number: Not applicable
UN proper shipping name: Not applicable
Transport hazard class: Not applicable
Packing group: Not applicable
Environmental hazards: Not applicable
Special precautions for user: Not applicable
DOT (US): Not dangerous goods
IMDG: Not dangerous goods
IATA: Not dangerous goods

15 Regulatory Information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

Chemical safety assessment: No information is available
SARA Section 355 (extremely hazardous substances): None of the ingredients is listed
SARA Section 313 (specific toxic chemical listings): None of the ingredients is listed
California Prop. 65 Components: The product does not contain any chemicals identified by the State of California to cause cancer, birth defects, or any other reproductive harm.

16 Other Information

The information provided herein is correct to the best of our knowledge, information, and belief at the date of this publication. The information given is designed only as guidance for safe handling, use, processing, storage, transportation, disposal, and release and is not considered a warranty or quality specification. Nasco Sampling assumes no liability whatsoever for the accuracy or completeness of the information stated in this Safety Data Sheet. Final determination of the suitability and purpose of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

Abbreviations and acronyms:

DOT	Department of Transportation (U.S.)
GHS	Globally Harmonized System
HMIS	Hazardous Materials Identification System (U.S.)
IATA	International Air Transport Association
IMDG	International Maritime Code for Dangerous Goods
NFPA	National Fire Protection Association (U.S.)
NIOSH	National Institute for Occupational Safety and Health
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration (U.S.)
PBT	Persistent Bioaccumulative and Toxic (PBT) Chemical Program (U.S.)
SARA	Superfund Amendments and Reauthorization Act (U.S.)
vPvB	very Persistent and very Bioaccumulative (U.S.)
WHMIS	Workplace Hazardous Materials Information System (Canada)

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White Paper

Collection Broths for Environmental Monitoring Programs

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Collection Broths for Environmental Monitoring Programs

1 EXECUTIVE SUMMARY

Collection broths play a critical role in the success of an environmental monitoring program. To avoid false negative results, a collection broth needs to have (i) the capacity to neutralize any sanitizer that may be present on a surface to be sampled and (ii) the ability to maintain the viability of all organisms collected by the sampling device until the sample is processed by the laboratory.

Traditionally three collection broths (neutralizing buffer, letheen broth and D/E neutralizing broth) have been used to collect surface samples. Each of these suffers from some deficiency.

Neutralizing buffer causes interference problems with several new generation diagnostic tests and is not recommended for use with 3M™ Petrifilm™. Also, we have learned that neutralizing buffer has little capacity to neutralize peroxide-based or acid sanitizers. Further, neutralizing buffer does not have sufficient buffering capacity to overcome high acid sanitizers, which could result in an unfavorably low pH environment for preservation of injured cells.

Letheen broth shows the weakest capacity of the three to neutralize quaternary ammonium compound ("quat") sanitizers. Like neutralizing buffer, letheen broth also shows minimal neutralizing activity against peroxide-based sanitizers and limited buffering capacity to handle low pH sanitizers.

Of the three traditional collection broths, D/E neutralizing broth shows the strongest neutralizing activities against the commonly used sanitizers. However, D/E neutralizing broth is unstable, showing considerable pH degradation if stored above refrigerated temperatures. Also, D/E neutralizing broth is incompatible with 3M Petrifilm.

Other considerations concerning selecting a collection broth for an environmental monitoring program include the potential presence of allergenic components or animal derived materials in the collection broth, acceptability in contact with food, and compatibility with requirements by manufacturers producing Kosher and Halal certified products.

World Bioproducts recently introduced HiCap™ neutralizing broth as an alternative to these 3 collection broths. HiCap neutralizing broth has excellent neutralizing capability for commonly used sanitizers, is formulated using only components that are accepted for use in foods, uses only components considered to be non-allergenic, does not include any of the materials that are of animal origin or are incompatible with 3M Petrifilm, and can be stored at temperatures between 2 and 30˚C.

2 THE PURPOSES OF COLLECTION BROTHS FOR ENVIRONMENTAL SAMPLING

The sampling of surfaces in food production areas typically involves the use of sponges or fiber-tipped swabs that are hydrated with a collection broth or solution (also referred to in the technical literature as "transport", "rinse" or "wetting" broth or solution). Collection broths play a critical role in the success of a surface sampling program. In the U.S., commonly used collection broths are letheen broth, neutralizing buffer, and D/E neutralizing broth.

A collection broth has two primary purposes. The first is to neutralize sanitizers that may be present on the surface that is being sampled. If a sanitizer is picked up by the sponge or swab during sample collection and is not neutralized, false negative results could occur as the collected microorganisms die from exposure to the sanitizer before the sample is processed in the laboratory.

The second purpose of the collection broth is to maintain the viability of the microorganisms after a sample is collected and until the sample is processed by the laboratory. It should be expected that the organisms that are collected on the sponge or swab are stressed due to prior exposure to a sanitizer or other injurious environmental conditions such as drying, extremes in pH, or high or freezing temperature. In order to maintain the viability of these collected organisms, it is critical that the microorganisms be placed into a favorable and stabilizing environment that is carefully controlled for isotonicity and pH.

Sometimes the collection solution is formulated with growth promoting nutrients. Some investigators have found that low nutrient levels are helpful in the resuscitation of injured bacteria (5,9). The presence of nutrients could complicate quantitative testing if the sample is not maintained at growth restrictive (typically 2-8˚C) temperatures after the sample is collected and there is growth of the collected microorganisms before the sample is processed in the laboratory.

The constituents of the collection broth may also aid in the collection of the sample by helping to loosen and disrupt biofilms that have formed on a surface. For example, Tween 80 (polysorbate 80), which is present in letheen and D/E neutralizing broths, may aid in release of organisms from a surface (7).

3 COMPATIBILITY WITH LABORATORY PROCEDURES

In addition to the important roles that the collection broths play in sample collection and cell preservation, the ideal collection broth should not interfere with the laboratory test(s) performed on the swab or sponge. However, this is not always the case. For example, sodium thiosulfate, bisulfite and citrate are reported to interfere with 3M Petrifilm (1). Sodium thiosulfate is present in neutralizing buffer and sodium thiosulfate and sodium bisulfite are present in D/E neutralizing broth. For many users of 3M Petrifilm, the default collection broth is letheen broth because it does not contain any of the abovementioned interfering compounds.

Developers of new generation molecular detection tests have encountered interference problems with neutralizing buffer (World Bioproducts' communications with manufacturers of diagnostic products and with customers). When interference is seen, the recommendation by the companies supporting the diagnostic product is to use an alternative collection broth (frequently D/E neutralizing broth is designated) or to dilute the neutralizing buffer sufficiently with enrichment broth to eliminate the interference.

4 APPROPRIATENESS FOR USE

Another consideration for the selection of a collection broth relates to the appropriateness for use in a manufacturing facility. Here are some examples:

a. Some collection broths may contain allergenic materials that could be left on a food contact surface after sampling. Letheen broth and D/E neutralizing broth both contain lecithin, which is typically derived from soy or egg sources. D/E neutralizing broth also contains casein, which is a milk protein. While it seems unlikely that the very low concentrations of allergenic materials in a collection broth would represent a public health hazard if these materials came into contact with food, food producers may prefer not to use letheen or D/E neutralizing broth in their facility because of allergen concerns or declarations that they make to their customers about the absence of allergenic materials in their production facility.

b. Some pharmaceutical and biotechnology manufacturers are concerned with the use of collection broths containing animal-derived materials, because of the possibility that these animal derived materials may harbor prions responsible for Bovine Spongiform Encephalopathy (BSE) and other Transmissable Spongiform Encephalopathies (TSE) (2). Both D/E neutralizing broth and letheen broth contain animal-derived peptones.

c. Food manufacturing facilities that follow Kosher or Halal practices may be concerned with the use of collection broths that contain components in conflict with their traditions. Jewish dietary laws specify the separation of dairy and meat (10). D/E neutralizing broth, which contains casein (dairy-based) peptone, would likely be prohibited in facilities handling beef products. Halal laws forbid Muslims from eating any pork or pork-derived products (4). Some peptones are manufactured using porcine-derived enzymes (4). Consequently, even if the peptones are beef or vegetable based, they could contain some pork materials. Letheen broth contains Proteose Peptone No. 3 and D/E neutralizing broth includes a pancreatic digest of casein. For a manufacturer producing Halal certified foods, it would be important to confirm that the peptones in their collection broth do not include porcine materials.

5 FORMULAS AND CHARACTERISTICS OF COMMONLY USED COLLECTION BROTHS

Table 1 below shows the formulas and characteristics for the 3 commonly used collection broths by food industry for environmental sampling.

Table 1. Commonly used collection broths for environmental sampling

Ingredients	Grams/Liter	Purpose in the Medium (from The Difco Manual (3))	Listed in EAFUS as Acceptable in Food?	Identified as Incompatible with 3M Petrifilm? (1)	Possible Allergen Associated with Ingredient in the Solution?
Neutralizing Buffer					
Monopotassium Phosphate	42.5 mg	Buffering	Yes	No	No
Sodium Thiosulfate	0.16	Neutralizes iodine and chlorine compounds	Yes	Yes	No
Aryl Sulfonate Complex	5	Neutralizes quaternary ammonium compounds	No	No	No
Letheen Broth					
Beef Extract	5.0	Nutrients for Growth of Microorganisms	Beef extract is not specifically listed but meat extract has historically been used in food products	No	No
Proteose Peptone No. 3	10.0		Hydrolyzed animal protein is listed	No	No
Polysorbate 80	5.0	Neutralizes phenols, hexachlorophene, formalin	Yes	No	No
Lecithin	0.7	Neutralizes quaternary ammonium compounds; lecithin with polysorbate 80, neutralizes ethanol	Yes	No	Yes, if source is soy or egg
Sodium Chloride	5.0	Maintains osmotic balance	Yes	No	No
D/E Neutralizing Broth					
Pancreatic Digest of Casein	5	Nutrients for Growth of Microorganisms	Casein and hydrolyzed milk protein are listed	No	Yes, casein is a milk based protein
Yeast Extract	2.5		Baker's Yeast Extract is listed	No	No
Dextrose (glucose)	10		Yes	No	No
Sodium Thioglycollate	1	Neutralizes mercurials	No	No	No
Sodium Thiosulfate	6	Neutralizes iodine and chlorine	Yes	Yes	No
Sodium Bisulfite	2.5	Neutralizes formaldehyde and gluteraldehyde	Yes	Yes	No
Polysorbate 80	5	Neutralizes phenols, hexachlorophene, formalin	Yes	No	No
Lecithin	7	Neutralizes quaternary ammonium compounds; lecithin with polysorbate 80, neutralizes ethanol	Yes	No	Yes, if source is soy or egg
Bromcresol Purple	0.02	pH indicator for acid production from microbial glucose fermentation	No	No	No

6 SELECTING A COLLECTION BROTH FOR AN ENVIRONMENTAL MONITORING PROGRAM

One of the challenges when developing an environmental sampling program is determining whether the collection broth used with the sampling device has sufficient capacity to neutralize the sanitizer(s) employed in all situations within a production facility. For example, a sanitizer used in the facility might be employed at a low concentration on food contact surfaces, but at far higher concentrations in locations away for the food production line (for example, an application of dry quat crystals to a zone 3 floor). Table 2 is taken from a good practices guide authored by Parker (8) that identifies the wide range of quat sanitizer concentrations that may be encountered within an aquaculture facility.

Table 2. Range of quat sanitizer concentrations that may be encountered in a food production facility

Walls and ceilings for mold	2,000 to 5,000 ppm
Equipment sanitizing	200 ppm
Floors and drains	800 ppm
Floor mats	1,800 ppm
Foot baths	2,400 ppm

While there is general information available on types of sanitizers neutralized by collection broth (as shown in Table 1), there is little to no information in the technical literature on the amount of sanitizer that each can neutralize. Furthermore, there is need for information on the ability of collection broths to neutralize newer generation sanitizers, such as peroxyacetic acid-based or fatty acid-based sanitizers. This information is generally lacking.

The second challenge in deciding about the best collection broth is to verify that the collection broth has the ability to maintain the viability of the microorganisms until sample processing, typically for 1 to 2 days, but in some cases up to 5 days. Microorganisms that have experienced significant injury may not remain viable during the period between collection and detection if the collection broth is not properly formulated.

Following discussions with many managers responsible for developing and supervising their company's environmental sampling program, we have found that the use of a sampling device with a particular collection broth is often based upon past practices. With the fact that sanitization programs continue to evolve with the use of new sanitizing agents, new application approaches, and new sanitizer rotation schemes, it seems reasonable that the following questions be addressed when assessing the adequacy of the surface sampling program:

- Does the collection broth have the capability to neutralize the sanitizer(s) that are used in this production facility?
- Do we know the maximum amount of sanitizer that might be present on all of the surfaces that are sampled in this production facility? Further, if we have defined a standard sampling area (for example, 1 ft^2), how much sanitizer would likely be picked up when a sample is collected from this area? Does my sampling device have the ability to handle this amount of sanitizer?

Next, it is important to verify that the collection broth is appropriate for all of the laboratory tests that will be performed on the environmental sample. As mentioned earlier, certain pathogen detection tests are incompatible with neutralizing buffer. 3M Petrifilm should not be used with collection broths containing sodium thiosulfate, bisulfite and citrate (1).

Finally, a practical consideration is the temperature storage requirements for the collection broth. Neutralizing buffer shows good stability over a temperature range of 2 to 30˚C. While most manufacturers of sampling devices with letheen broth recommend refrigerated storage (4-8˚C), we have completed studies that show that letheen broth is stable with storage at 2 to 30˚C. (Note. We will be modifying our recommended storage temperatures for our sampling devices with letheen broth to specify 2 to 30˚C.) D/E neutralizing broth shows considerable pH **instability** and should be refrigerated (2 to 8˚C) during storage. This pH degradation results in a change in color of the broth from a typical purple color (with pH above 7.2), to a reddish color at pH 6.6 to 6.8 and a yellow color below pH 6.2. (Note: the acceptable pH range for D/E neutralizing broth is 7.4 to 7.8. D/E neutralizing broth at this pH is purple due to the presence of the pH indicator brom cresol purple.)

7 HICAP™ NEUTRALIZING BROTH

We embarked upon the development of a new broth that we call HiCap (for High Capacity) neutralizing broth in order to offer our customers an alternative to neutralizing buffer, letheen broth and D/E neutralizing broth. HiCap™ neutralizing broth was formulated to meet the following specifications:

a. Has high neutralizing capacity for all sanitizers commonly used by food industry (including quats, peroxide/peroxyacetic, chlorine and chlorine compounds, phenolics, alcohols, and acid sanitizers);

b. Has sufficient buffering capacity to handle low pH (high acid) sanitizers;

c. Only Includes components that are found on U.S. Food and Drug Administration's EAFUS (Everything Added to Food in the United States) list, as materials acceptable for inclusion in food;

d. Only includes components that are considered to be non-allergenic;

e. Does not contain agents (citrate, bisulfite and thiosulfate) that are reported to interfere with 3M Petrifilm (1);

f. Includes vegetable peptones (not digested with animal-derived enzymes) and yeast extract, instead of meat peptones or meat extracts;

g. Does not interfere with established immunoassays or newer generation molecular diagnostic tests with genetic amplification;

h. Can be stored at a temperature range of 2 to 30˚C.

8 LABORATORY FINDINGS WITH HICAP NEUTRALIZING BROTH

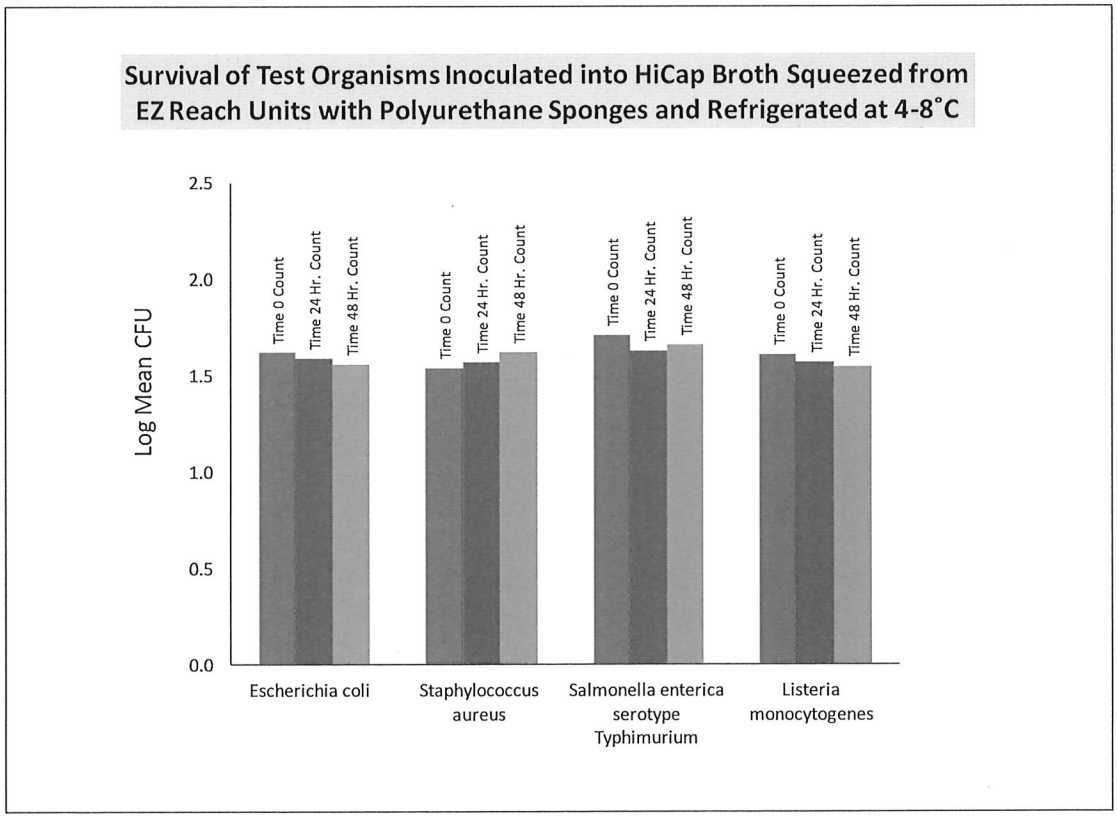
a. 48 hour survival experiments

These experiments were designed to assess whether HiCap neutralizing broth can maintain the viability of cells over a 48 hour period at refrigerated temperatures.

Method: These experiments were conducted by squeezing HiCap neutralizing broth from sterile EZ Reach™ Sponge Sampling units (with polyurethane sponges) and dispensing into sterile polypropylene vials. Low levels of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica* serotype Typhimurium, and *Listeria monocytogenes* were inoculated into containers with the HiCap neutralizing broth. Counts, using a pour plate method with Standard Methods Agar (SMA), were made at time of inoculation (Time=0) and after 24 hours (Time=24 hr) and 48 hours (Time=48 hr) of holding at refrigerated temperatures (4-8˚C).

Results: The chart below shows that these test organisms maintained their viability over 48 hours of refrigerated storage.

Table 3. Survival of test organisms in HiCap neutralizing broth held at refrigerated temperatures for 48 hours.



b. pH neutralization experiment

These experiments were designed to test whether neutralizing buffer, letheen broth, D/E neutralizing broth and HiCap neutralizing broth are able to establish a neutral pH when mixed with sanitizer.

Some sanitizers have very low pH. For example, two sanitizers from Ecolab Inc. (Vortexx™ and Mandate™ Plus) are defined as acid sanitizers. A 1% solution of Vortexx has a pH of 2.5 and whereas an undiluted preparation of Mandate Plus has a pH of 1. Tsunami® 200, also from Ecolab, is used for produce wash and has a pH of 2.5 (1% solution).

On the alkaline side of the pH spectrum, Whisper™ V, which is a 5th generation quat sanitizer from Ecolab, has a pH of 7.7 (100% solution). XY-12® is a sodium hypochlorite sanitizer with pH of 8.3 (25% solution).

It is important that a neutralizing solution has sufficient buffering capacity to neutralize low and high pH sanitizers. Microorganisms when collected during sampling of a surface may be sublethally injured due to exposure to a sanitizer, starvation, freezing, heating, high or low pH, osmotic shock, and desiccation. The viability of these injured cells may be better maintained in a collection broth that is sufficiently buffered to overcome an acid sanitizer and bring pH into an optimal range of 6-8 (12).

This experiment was conducted to compare the pH adjusting capacity of neutralizing buffer, letheen broth, D/E neutralizing broth and HiCap neutralizing broth when mixed with different types of sanitizers.

Method: Sterile solutions of neutralizing buffer, letheen broth, D/E neutralizing broth and HiCap neutralizing broth were dispensed in sterile polypropylene vials. Sanitizer was added to each tube to establish a final dilution of 1:400. The pH of each solution after addition of the sanitizer was determined.

Results: Table 4 shows results of pH measurements after mixing 4 collection broths with 5 different sanitizers. The data show that neutralizing buffer, letheen broth and D/E neutralizing broth have insufficient buffering capacity when mixed with acid sanitizers (Vortexx, Tsunami 200 and Mandate Plus) to adjust the pH above 6. HiCap neutralizing buffer, when mixed with any of the sanitizers tested, resulted in pH values between 6.5 and 7.2.

Table 4. pH of collection broths mixed with different types of sanitizers

Neutralizing Solution	1:400 Dilution of Vortexx™* (Hydrogen peroxide (6.9%); Peroxyacetic acid (4.4%); Octanoic Acid (3.3%)) - Use dilutions recommended by manufacturer are 1:200 to 1:1000 for non-food contact surfaces; Use dilutions are 1:500 to 1:1000 for food contact surfaces	1:400 Dilution of XY-12® (Sodium Hypochlorite 8.4%) - Use dilution of 1:416 for nonporous food contact surfaces is recommended by manufacturer if no test kit is available.	1:400 Dilution of Whisper™ V (mixed quaternary ammonium sanitizers) - Use dilutions are 1:63 to 1:256 are recommended by manufacturer.	1:400 Dilution of Tsunami® 200 (Peracetic acid (13%); Hydrogen peroxide (1-5%); Caprylic Acid (5-20%)) - Use dilution recommended by manufacturer is 1:3167 for produce wash	1:400 Diluton of Mandate™ Plus (Nonanoic (Pelargonic) Acid 6.30%; Decanoic (Capric) Acid 1.09%) - Use dilution recommended by manufacturer is 1:427 for coarse spray application
Neutralizing Buffer	4.3	7.0	7.5	3.8	3.6
Letheen Broth	4.7	6.8	6.8	4.4	4.6
D/E Neutralizing Broth	5.3	7.4	7.5	4.8	5.6
HiCap Neutralizing Broth	6.7	7.2	7.2	6.5	6.8
* Vortexx, XY-12, Whisper, Tsunami, and Mandate are trademarks of Ecolab Inc., St. Paul, MN					

c. Sanitizer neutralization experiments

These experiments were performed to assess the neutralization capacities of letheen broth, neutralizing buffer, D/E neutralizing broth and HiCap neutralizing broth with 4 types of sanitizers commonly used by food industry. One or more sanitizers representing the following sanitizer types were included in this study.

(1) Chlorine
(2) Quaternary ammonium compounds
(3) Peroxide and peroxyacetic acid mixtures
(4) Mixed acid

Method: One milliliter aliquots of sterile solutions of neutralizing buffer, letheen broth, D/E neutralizing broth and HiCap neutralizing broth were dispensed into sterile polypropylene vials. Each sanitizer was diluted in sterile water and 0.010 ml was added to the individual tubes to give final concentrations of 1:100, 1:200, 1:400, 1:800, 1:1600 and 1:3200. Ten microliters of a diluted culture of *Escherichia coli* ATCC 25922 (giving a final concentration of approximately 500 to 1000 cells per ml) was added to each tube. Controls were also performed. One control is a sanitizer activity control which was performed as above, with the exception that sterile purified water was used instead of a collection broth. This control verifies that the sanitizer at the test concentration is lethal to *E. coli* in the absence of the collection broth. A growth control was performed as above, with the exception that no sanitizer was added to the collection broth.

Each tube was incubated at refrigerated temperatures (4-8˚C) for 60 minutes. One ml of a sterile 2X solution of tryp soy broth supplemented with 1% yeast extract was then added to the neutralization tubes. All tubes were incubated for 24 +/- 2 hours at 35˚C and observed for

turbidity. The presence of turbidity indicated that a sanitizer was successfully neutralized by the collection broth.

Chlorine sanitizer neutralization studies:

XY-12 contains 8.4% sodium hypochlorite as the active ingredient. Table 5 shows the neutralization activity profiles of D/E neutralizing broth, letheen broth, neutralizing buffer, and HiCap neutralizing broth with XY-12.

Table 5. Neutralization activity profiles of 4 collection broths with XY-12 chlorine sanitizer

			Dilution of Sanitizer Used in Assay					
Sanitizer Type	**Manufacturer**	**Collection Broth**	**1:100**	**1:200**	**1:400**	**1:800**	**1:1600**	**1:3200**
XY-12 (Sodium Hypochlorite 8.4%) - Use dilution is 1:833 for nonporous food contact equipment (Use dilution of 1:416 for nonporous food contact surfaces if no test kit is available)	Ecolabs	D/E Neutralizing Broth	+	+	+	+	+	+
		Letheen Broth	-	-	+	+	+	+
		Neutralizing Buffer	-	-	-	+	+	+
		HiCap Neutralizing Broth	+	+	+	+	+	+

Note 1. The blue highlighted area gives an approximation of the highest concentration of sanitizer (lowest use dilution) recommended by the manufacturer.

Note 2. + is positive for neutralization in the bioassay. Without neutralization, this concentration is lethal to the test organism.

Note 3. HiCap is a trademark of World Bioproducts

The greatest neutralization capacity against XY-12 was seen with D/E neutralizing broth and HiCap neutralizing broth. The weakest neutralization activity was seen with neutralizing buffer, which showed neutralizing activity only when the XY-12 was diluted 1:800.

Quat sanitizer neutralization studies:

Whisper V and Virex® 256, both fifth generation quat sanitizers, were analyzed in this neutralization study. Table 6 shows the neutralization activity profiles of D/E neutralizing broth, letheen broth, neutralizing buffer, and HiCap neutralizing broth with these mixed quat sanitizers.

Table 6. Neutralization activity profiles of 4 collection broths with two quat preparations

			Dilution of Sanitizer Used in Assay					
Sanitizer Type	**Manufacturer**	**Collection Broth**	**1:100**	**1:200**	**1:400**	**1:800**	**1:1600**	**1:3200**
Whisper V (mixed quaternary ammonium sanitizers Alkyl dimethyl benzyl ammonium chloride 3.00%; Octyl decyl dimethyl ammonium chloride 2.25%; Didecyl dimethyl ammonium chloride (1.35%); Dioctyl dimethyl ammonium chloride 0.9%) - Use dilutions are 1:63 to 1:256	Ecolabs	D/E Neutralizing Broth	+	+	+	+	+	+
		Letheen Broth	-	-	+	+	+	+
		Neutralizing Buffer	+	+	+	+	+	+
		HiCap Neutralizing Broth	+	+	+	+	+	+
Virex 256 (Didecyl dimethyl ammonium chloride (8.704%); n-alkyl dimethyl benzyl ammonium chloride (8.190%)) - Use dilution is 1:256	Diversey (Virex is a registered trademark of Diversey, Inc., Sturtevant, WI)	D/E Neutralizing Broth	+	+	+	+	+	
		Letheen Broth	-	-	-	+	+	
		Neutralizing Buffer	-	+	+	+	+	
		HiCap Neutralizing Broth	+	+	+	+	+	

Letheen broth showed a lower ability to neutralize both quat sanitizers when compared to the other three collection broths. With the Virex 256 preparation, D/E neutralizing broth and HiCap neutralizing broth showed a higher neutralization capability than neutralizing buffer.

Peroxide-peroxyacetic acid sanitizer neutralization studies:

Two peroxide-based sanitizers, Vortexx and Tsunami 200, were studied in these neutralization experiments. Table 7 shows the neutralization profiles of the 4 collection broths with these sanitizers.

Table 7. Neutralization activity profiles of 4 collection broths with peroxide-based sanitizers

			Dilution of Sanitizer Used in Assay					
Sanitizer Type	**Manufacturer**	**Collection Broth**	**1:100**	**1:200**	**1:400**	**1:800**	**1:1600**	**1:3200**
Vortexx (Hydrogen peroxide (6.9%); Peroxyacetic acid (4.4%); Octanoic Acid (3.3%)) - Use dilutions are 1:200 to 1:1000 for non-food contact surfaces; Use dilutions are 1:500 to 1:1000 for food contact surfaces	Ecolabs	D/E Neutralizing Broth	-	+	+	+	+	+
		Letheen Broth	-	-	-	-	-	+
		Neutralizing Buffer	-	-	-	-	+	+
		HiCap Neutralizing Broth	+	+	+	+	+	+
Tsunami 200 (Peracetic acid (13%); Hydrogen peroxide (1-5%); Caprylic Acid (5-20%)) - Use dilution is 1:3167	Ecolabs	D/E Neutralizing Broth	-	+	+	+	+	+
		Letheen Broth	-	-	-	-	+	+
		Neutralizing Buffer	-	-	-	-	+	+
		HiCap Neutralizing Broth	-	+	+	+	+	+

Both letheen broth and neutralizing buffer showed little neutralization capability with the Vortexx and Tsunami 200 preparations. In contrast, both D/E neutralizing broth and HiCap neutralizing broth showed a high capacity to neutralize these peroxide-based sanitizers.

Mixed acid sanitizer neutralization studies:

Mandate Plus is an acid sanitizer with two active ingredients (nonanoic (pelargonic) acid and decanoic (capric) acid). Mandate Plus also contains acetic acid, nitric acid, phosphoric acid and octanesulfonic acid.

Table 8 shows the neutralization profiles of the 4 collection broths with this acid sanitizer.

Table 8. Neutralization activity profiles of 4 collection broths with Mandate Plus acid sanitizer

			Dilution of Sanitizer Used in Assay					
Sanitizer Type	**Manufacturer**	**Collection Broth**	**1:100**	**1:200**	**1:400**	**1:800**	**1:1600**	**1:3200**
Mandate Plus (Nonanoic (Pelargonic) Acid 6.30%; Decanoic (Capric) Acid 1.09%) - Use dilution is 1:427 for coarse spray application	Ecolabs	D/E Neutralizing Broth	-	+	+	+	+	+
		Letheen Broth	-	-	+	+	+	+
		Neutralizing Buffer	-	-	-	-	+	+
		HiCap Neutralizing Broth	-	+	+	+	+	+

Neutralizing buffer showed very little capacity to neutralize Mandate Plus. Letheen broth was able to neutralize this acid sanitizer when diluted 1:400. The greatest neutralization capability was seen with the D/E neutralizing broth and the HiCap neutralizing broth.

9 EVALUATION OF HICAP NEUTRALIZING BROTH WITH NEW GENERATION DIAGNOSTIC TESTS

HiCap Neutralization Broth was evaluated by customers with two new generation molecular-based diagnostic tests that experienced interference problems with neutralizing buffer. In both cases, no interference was observed with the HiCap Broth.

To date, no interferences have been reported by any users or evaluators of HiCap with any quantitative or qualitative laboratory procedures.

10 CONCLUSIONS AND DISCUSSION

The selection of a collection broth for an environmental sampling program should include an assessment of whether the collection broth can maintain the viability of the collected microorganisms until the sample is processed in the laboratory and whether the collection broth can effectively neutralize all sanitizer(s) used in all places in the production facility.

Our work indicates that HiCap neutralizing broth squeezed from EZ Reach Sponge Samplers with polyurethane sponges is able to maintain the viability of *E. coli*, *Salmonella*, *S. aureus* and *L. monocytogenes* over a 48 hour period at refrigerated temperatures. However, this conclusion needs to be tempered by the fact that these data were generated with "healthy" laboratory cultures. More work is needed to assess preservation of bacteria that have experienced injury due to stressors such as high temperature and low pH.

Maintenance of viability is likely enhanced when the collected microorganisms are immediately placed into a culture environment favorable to the resuscitation of these cells. There are many factors that come into play when considering the resuscitation of injured microorganisms. Papers of Stephens et al (11), Wesche et al (12) and McFeters et al (6) provide a starting point for more information on this expansive topic.

Maintaining a neutral pH may improve recovery of injured bacteria. In our studies, we found that when acid sanitizers were mixed with traditional collection broths that the pH remained low. For example, when neutralizing buffer was mixed with 3 sanitizers with high acidity (Vortexx, Tsunami 200 and Mandate Plus) that had been diluted 1:400, the pH remained below pH 4.5. Letheen broth and D/E neutralizing broth were only slightly better than neutralizing buffer for raising pH when mixed with these high acid sanitizers. HiCap neutralizing buffer was formulated with a sufficient buffering system to overcome the acidity of these low pH sanitizers.

In our studies, D/E and HiCap neutralizing broths show excellent neutralization capacities with all of the sanitizers tested. Letheen broth was found to have 25% (or less) of the neutralization capacity of the other 3 collection broths when tested with quat sanitizers. Both neutralizing buffer and letheen broth showed a poor ability to neutralize peroxide-based sanitizers. Finally, neutralizing buffer showed a reduced capacity to neutralize sodium hypochlorite (XY-12) and an acid sanitizer (Mandate Plus).

For companies that employ a rotation of sanitizers to minimize selection of resistant organisms, it is especially important to confirm that the collection broth employed in their environmental monitoring program has the ability to neutralize all of the sanitizers at all of the concentrations used in the production facility. This confirmation is only possible by knowing the amount of sanitizer likely to be present on the area to be sampled and an understanding of the neutralization capabilities of the collection device.

Here is an example to illustrate this point. Our data indicate that 1 ml of letheen broth has a neutralization limit of about 0.2 mg for Virex 256 (10 mls of letheen would have a neutralization limit of about 2 mg of Virex 256). When we have estimated the amount of Virex 256 that might be present on a non-porous surface (when diluted according to the manufacturer's recommendation, evenly spread and dried onto the non-porous surface), we find that a 100 cm^2 area may have up to 0.7 mg or a $1ft^2$ area may have up to 6.3 mg of Virex 256. What this suggests is that 10 mls of letheen broth would be adequate for sampling a smaller area (4 in^2 or 100 cm^2), but might be inadequate to fully neutralize the amount of sanitizer on a 1 ft^2 area. One ml of letheen broth may be inadequate for sampling an area greater than about 25 cm^2.

Concern for reaching a neutralization limit during sampling is substantially mitigated by employing a collection broth with sufficient capacity. For example, 1 ml of HiCap neutralizing

broth has the capacity to neutralize more than 1.7 mg of Virex 256, which should provide ample neutralization capacity to sample an area of 100 cm^2 when the quat sanitizer is applied to the surface. Ten ml of HiCap can neutralize more than 17 mg of Virex 256. The use of a higher volume of HiCap (5 to 10 ml) may merit consideration when sampling large areas such as 1 ft^2.

In addition to the critical requirements that a collection broth effectively neutralize residual sanitizer and maintain the viability of the collected microorganisms, other considerations may come into play when selecting a collection broth. These include compatibility with laboratory procedures, the potential presence of allergenic components or animal derived materials in the collection broth, acceptability of the collection broth in contact with food, temperature storage requirements, and compatibility with Kosher and Halal practices.

HiCap neutralizing broth was formulated to have universal application for surface sampling with features included to help assure that an environmental monitoring program produces meaningful information for the food company.

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