

Neutralizing and General Dilution Buffers

By: Douglas Wright, President

Neutralizing and General Dilution Buffers have 2 main uses:

- a. As part of a swab or sponge for environmental swabbing/sponging to collect environmental microbial samples. These in turn have 2 main uses: **monitoring potential sources of contamination** and verifying the **effectiveness of the cleaning /sanitation process**.

Microbiological analysis

- b. Microbiological analysis: for microbial indicators and pathogen-specific tests.

Indicator tests monitor the level of general hygiene through indicating residual level of ATP, Proteins and or Microbial tests (generally the total plate count, Yeast and mold or coliforms).

Specific tests detect pathogenic microorganisms such as E.coli, Salmonella, *Listeria etc.*

For information on cleaning and sanitizing measures, it is recommended that swabs be taken after cleaning and rinsing but prior to sanitizing or long after sanitizing at set time. This will allow for cleaning efficacy validation as well as effectiveness of residual sanitizers.

To get information on microbiological environmental conditions during production, it is recommended that samples be taken before and during production of surfaces and products and if after sanitizing using the recommended buffers.

It is important to ensure that the neutralizer/buffer chosen is effective against the disinfectants used.

When sampling on a surface previously exposed to chemicals (cleaners or sanitizers), it is necessary to incorporate a neutralizing agent suitable for the medium to preserve the viability of the microbial cells.

The recommended neutralizing solutions for microbiological quality monitoring in food plants are:

- Dey-Engley Neutralizing Broth (DE)
- Neutralizing buffer (NE)
- Buffered peptone water (BPW)
- Lethen broth (LT)
- Butterfield’s Phosphate buffer (BPB)

Guide for selecting a neutralizing solution

The following table serves as a guide for selecting the most efficient buffer/neutralizing solution for the most common cleaning products. For quantitative analysis, it is also important to use a neutralizing agent that will not promote bacterial growth, such as a neutralizing buffer.

In addition, microbiological analysis should be carried out as soon as possible after sampling to avoid losses due to the death of viable micro-organisms.

SANITIZER CLASS	BUFFER TYPE				
	D/E NEUTRALIZING BROTH	LETHEEN BROTH	NEUTRALIZING BUFFER	BUFFERED PEPTONE WATER	Butterfield’s Phosphate buffer
Glutaraldehyde’s	•		•		
Formaldehydes	•	•	•		
Chlorine, bromine and iodine	•		•		
Quaternary ammonium compounds (Quats/QACs)	•	•	•		
Chlorhexidine	•	•			
Phenols (LpH)	•	•			
Alcohol	•	•			
Peroxides	•				
Acetic and lactic acids	•			•	
Sample dilutions laboratory procedures					



[Scigene Sterile Cellulose Sponge all-in-one w/ DE neutralizing buffer](#)

D/E Neutralizing Buffer was developed by Dey and Engley and contains additional compounds to the neutralizing buffer. It is used to neutralize a broad spectrum of disinfectants and preservative antimicrobial chemicals, including chlorine and quaternary ammonium compounds, phenolics, iodine, mercurials, peracetic acid, hydrogen peroxide, formaldehyde, and glutaraldehyde. D/E Neutralizing media neutralize higher concentrations of residual antimicrobials when compared with other standard neutralizing formulations, such as Neutralizing Buffer, Lethen media etc. *Recommended use: D/E Neutralizing Broth is recommended for use in disinfectant evaluations, environmental sampling (swab and contact plate methods), and testing of water-miscible cosmetics/antiseptics. -As per CFIA, used for Listeria sample collection.

Lethen Broth is a liquid medium recommended for use in qualitative procedures for testing chlorine, fluoride and quaternary ammonium compounds for antimicrobial activity. It was originally developed as a subculture medium for the neutralization of quats.

*Recommended use: Recovering bacteria from the solutions containing residues of

sanitizers from food utensils and equipment.



[Scigene Sterile Cellulose Sponge w/handle and neutralizing buffer](#)

Neutralizing Buffer contains nutritive components which aid in the recovery and growth of microorganisms in samples taken from surfaces exposed to sanitizing agents. It has the ability to inactivate the bactericidal and bacteriostatic effects of sanitizing agents such as chlorine and quaternary ammonium compounds (quats).

*Recommended use: Neutralizing Buffer is recommended for detection of microorganisms found on dairy and food equipment disinfected with chlorine or quats. It is also recommended for the digestion and decontamination process of mycobacteria during TB diagnoses¹. Decontamination and digestion of the mucous components kills contaminating normal flora and allows slower growing mycobacteria to grow.

Timely neutralization prevents potential loss of mycobacteria caused by high pH levels of decontaminants, resulting in the preservation of more viable organisms for diagnostic protocols.



[Q-Swab - Environment Swab Collection System with Lethen Broth](#)

Buffered Peptone Water (BPW) is a pre-enrichment medium designed to help recovery of sub-lethally damaged Salmonella before transfer to a selective medium. This pre-enrichment medium is free from inhibitors and is well buffered and provides conditions for resuscitation of the cells that have been injured by processes of food preservation (heat, desiccation, low pH, food preservatives etc.)³ *Recommended use: Buffered peptone water is highly recommended for vegetable specimens and fermented products which have low buffering capacity. This medium can be used for testing dry poultry feed.

Butterfield's Phosphate Buffer provides a standardized medium for the preparation of sample dilutions during plate count and other laboratory procedures. It eliminates the variations in pH associated with the use of distilled water. BPB is also known as Butterfield's phosphate buffered dilution water or Butterfield's Phosphate Diluent. *Recommended use: It is recommended as a general diluent in laboratory procedures. In addition, BPB is described in Standard Methods for the Examination of Water and Wastewater for use in water testing. The buffer is also recommended for use in microbial limit testing for pharmaceutical products.



[Sterile Phosphate Buffer Bottles with Butterfields or Magnesium Chloride](#)

References: 1. Cernoch, Enns, Saubolle and Wallace. 1994. Cumitech 16A, Laboratory diagnosis of the mycobacterioses. Coord. ed., Weissfeld. American Society for Microbiology, Washington, D.C 2. Engley, F. B., Jr. and B. P. Dey. 1970. A universal neutralizing medium for antimicrobial chemicals. Presented at the Chemical Specialties Manufacturing Association (CSMA) Proceedings. 56th Mid-Year Meeting. 3. Sadovski A.Y. (1977) J. Food Technol. 12. 85-91. 4. The United States Pharmacopeia. 2009. 32nd ed. United states Pharmacopeial Convention, Rockville, MD

The table below describes each of the neutralizing solutions offered with our products

		RECOMMENDED USE	DESCRIPTION	
SH10NB	Sterile Cellulose Sponge w/ handle- Neutralizing Buffer, 100/cs	Non-food and food contact surfaces	Neutralizing buffer is used for inactivation of the bactericidal and bacteriostatic effect of disinfectants used on food and dairy equipment. This buffer is used to preserve microorganisms without promoting their growth.	https://www.scigiene.com/Scigiene-Sterile-Cellulose-Sponge-with-quick-release-handle-and-Neutralizing-Buffer
SH10DE	Sterile Cellulose Sponge w/handle - DE neutralizing buffer, 100/cs	Non-food and food contact surfaces	Dey-Engley Neutralizing broth is a solution inhibiting the activity of a wide range of disinfectants. It contains glucose, tryptone and yeast extract components which allow easy bacterial growth for microorganisms detection.	https://www.scigiene.com/Sterile-Cellulose-Sponge-with-handle-DE-neutralizing-buffer
SH10BPW	Sterile Cellulose Sponge w/handle - Buffered Peptone Water, 100/cs	Carcasses, Non-food and food contact surfaces	Buffered Peptone Water is a non-selective, pre-enrichment medium used to help bacteria recover from food processing injuries. Its enzymatic digest and stable pH allow bacterial growth.	https://www.scigiene.com/Sterile-Cellulose-Sponge-with-handle-Buffered-Peptone-Water
SH10LB	Sterile Cellulose Sponge w/handle - Lethen Broth, 100/cs	Non-food and food contact surfaces	Lethen broth was developed as a medium meant for neutralization of multiple compounds found in sanitizers. Its enzymatic digest and beef extract components allow easy bacterial growth for microorganisms detection.	https://www.scigiene.com/Sterile-Cellulose-Sponge-with-handle-Lethen-Broth
SS10DE-PB-2	All-in-One SurfACE Sponge-Stick with DE Neutralizing Broth, 100/cs	Non-food and food contact surfaces	Dey-Engley Neutralizing broth is a solution inhibiting the activity of a wide range of disinfectants. It contains glucose, tryptone and yeast extract components which allow easy bacterial growth for microorganisms detection.	https://www.scigiene.com/SS10DE-PB-2
SS10DR	All-in-One SurfACE Sponge-Stick - Dry, 100/cs			
BS10NB	Sterile Cellulose Sponge - Neutralizing Buffer, 100/cs	Non-food and food contact surfaces	Neutralizing buffer is used for inactivation of the bactericidal and bacteriostatic effect of disinfectants used on food and dairy equipment. This buffer is used to preserve microorganisms without promoting their growth.	https://www.scigiene.com/Scigiene-Sterile-Cellulose-Sponges-Neutralizing-Buffer
BS10DE	Sterile Cellulose Sponge - DE neutralizing buffer, 100/cs	Non-food and food contact surfaces	Dey-Engley Neutralizing broth is a solution inhibiting the activity of a wide range of disinfectants. It contains glucose, tryptone and yeast extract components which allow easy bacterial growth for microorganisms detection.	https://www.scigiene.com/Scigiene-Sterile-Cellulose-Sponges-DE-Neutralizing-Buffer
BS10BPW	Sterile Cellulose Sponge -Buffered Peptone Water, 100/cs	Carcasses, Non-food and food contact surfaces	Buffered Peptone Water is a non-selective, pre-enrichment medium used to help bacteria recover from food processing injuries. Its enzymatic digest and stable pH allow bacterial growth.	https://www.scigiene.com/Scigiene-Sterile-Cellulose-Sponges-Buffered-Peptone-Water

BS10BPB	Sterile Cellulose Sponge -Butterfields Phosphate Buffer, 100/cs	a general diluent in laboratory procedures	It eliminates the variations in pH associated with the use of distilled water. BPB is also known as Butterfield's phosphate buffered dilution water or Butterfield's Phosphate Diluent. In addition, BPB is described in Standard Methods for the Examination of Water and Wastewater for use in water testing. The buffer is also recommended for use in microbial limit testing for pharmaceutical products	https://www.scigiene.com/Scigiene-Sterile-Cellulose-Sponges-Butterfields-Phosphate-Buffer
BS10LB	Sterile Cellulose Sponge - Lethen Broth, 100/cs	Non-food and food contact surfaces	Lethen broth was developed as a medium meant for neutralization of multiple compounds found in sanitizers. Its enzymatic digest and beef extract components allow easy bacterial growth for microorganisms detection.	https://www.scigiene.com/Scigiene-Sterile-Cellulose-Sponges-Lethen%20Broth
BS02NB	Sterile Cellulose Sponge - Neutralizing Buffer, w/Gloves - 50/cs	Non-food and food contact surfaces	Neutralizing buffer is used for inactivation of the bactericidal and bacteriostatic effect of disinfectants used on food and dairy equipment. This buffer is used to preserve microorganisms without promoting their growth.	https://www.scigiene.com/Scigiene-Sterile-Cellulose-Sponges-Neutralizing-Buffer-With-Gloves
BS02DE	Sterile Cellulose Sponge - DE neutralizing buffer, w/Gloves - 50/cs	Non-food and food contact surfaces	Dey-Engley Neutralizing broth is a solution inhibiting the activity of a wide range of disinfectants. It contains glucose, tryptone and yeast extract components which allow easy bacterial growth for microorganisms detection.	https://www.scigiene.com/Scigiene-Sterile-Cellulose-Sponges-DE-Neutralizing-Buffer-With-Gloves
BS02BPW	Sterile Cellulose Sponge -Buffered Peptone Water, w/ Gloves - 50/cs	Carcasses, Non-food and food contact surfaces	Buffered Peptone Water is a non-selective, pre-enrichment medium used to help bacteria recover from food processing injuries. Its enzymatic digest and stable pH allow bacterial growth.	https://www.scigiene.com/Scigiene-Sterile-Cellulose-Sponges-Buffered-Peptone-Water-With-Gloves
BS02BPB	Sterile Cellulose Sponge -Butterfields Phosphate Buffer, w/ Gloves - 50/cs	a general diluent in laboratory procedures	It eliminates the variations in pH associated with the use of distilled water. BPB is also known as Butterfield's phosphate buffered dilution water or Butterfield's Phosphate Diluent. In addition, BPB is described in Standard Methods for the Examination of Water and Wastewater for use in water testing. The buffer is also recommended for use in microbial limit testing for pharmaceutical products	https://www.scigiene.com/Scigiene-Sterile-Cellulose-Sponges-Butterfields-Phosphate-Buffer-With-Gloves

