IFU

Instructions for Use

NEUTRALIZING BUFFERED PEPTONE WATER

Cat. no. U482	Neutralizing Buffered Peptone Water (nBPW), 500ml PET Bottle, 400mL	10 bottles/box

INTENDED USE

Hardy Diagnostics Neutralizing Buffered Peptone Water (nBPW) is recommended for use in the recovery of sub-lethally injured *Salmonella* species from industrial samples prior to selective enrichment and isolation.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Salmonella spp. may be present in foods, particularly poultry products, yet cells may be sub-lethally injured by food processing techniques. Consequently, it may be difficult to recover injured cells when plating direct to selective media; the organism may go undetected using traditional culture techniques. Beginning July 1, 2016, the United States Department of Agriculture, Food Safety and Inspection Service (USDA FSIS) instituted new guidelines for the development of Neutralizing Buffered Peptone Water (nBPW) to aid in the recovery of *Salmonella* spp. from domestic and imported poultry verification sampling, including chicken carcass rinses, poultry parts rinses and young turkey carcass sponge swabs.[6] The USDA FSIS also states nBPW is safe as a direct rinse or swab, and should be used as a non-selective pre-enrichment medium to promote the recovery of sub-lethally injured bacteria, particularly *Salmonella* spp.[6]

Campylobacter spp. have been characterized as among the top bacterial agents of human foodborne gastroenteritis. The organisms may be transmitted by contaminated food or water. Poultry is a primary reservoir of *Campylobacter* spp. and studies show that prevalence of this organism may be greater than 80% in commercial chicken carcasses. Ninety five percent of human illnesses are associated with *Campylobacter jejuni*, followed by *C. coli* at 4%. Other species are involved in only 1% of infections. Like *Salmonella*, *Campylobacter* spp. may be difficult to recover from poultry rinse solutions unless agents from processing techniques are sufficiently neutralized.

nBPW contains peptones that act as nitrogenous compounds to promote bacterial growth. Phosphate salts in the buffer help to maintain pH. Maintenance of pH is important when attempting to recover sub-lethally injured cells, because a low pH can be detrimental to the repair and growth of damaged microorganisms. In addition, nBPW contains neutralizing agents to reduce the inhibitory effects of carryover from microbial interventions.[6]

FORMULA

Ingredients per liter of deionized water:*

Buffered Peptone Water (BPW)	20.0g
Sodium Bicarbonate	12.5g
Lecithin	7.0g

Sodium Thiosulfate	1.0g

Final pH 7.7 +/- 0.5 at 25ºC.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt, store away from direct light at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Sample Collection: Consult reference methods for complete procedures on sample collection.[1-6]

Method of Use: Consult listed references for complete procedures for handling poultry carcasses or parts prior to rinsing and swabbing, and for information on the recovery of *Salmonella* of *Campylobacter* spp. from food or poultry samples.[1,2,5,6]

1. Inoculate 10g or 10mL of sample for every 50mL of Neutralizing Buffered Peptone Water (nBPW).

2. For *Salmonella* recovery, incubate broth aerobically at 35±2ºC. for 18 to 24 hours. For *Campylobacter* recovery, incubate broth tight capped, microaerobically, at 42±1ºC for 46-50 hours.

3. Transfer an aliquot of the sample to 100mL of Tetrathionate Broth (Cat. no. U165) for *Salmonella* enrichment and incubate at 35ºC. For *Campylobacter* spp., transfer an aliquot of the sample to an enrichment broth such as Modified Blood-Free Bolton Broth (Cat. no. U401) and incubate tight capped, microaerobically, at 42±1ºC for 46-50 hours. Other selective enrichments may also be used.[1,2,5,6]

4. For Salmonella spp. subculture from the enrichment broth to Brilliant Green Sulfa Agar (Cat. no. G87), Brilliant

Green Agar (Cat. no. G75), XLD Agar (Cat. no. G65) and/or HE Agar (Cat. no. G63) and incubate plates for 18 to 24 hours at 35±2ºC. NOTE: It is recommended that more than one selective agar be used in parallel, since no single medium is appropriate in all situations, to ensure recovery when salmonellae are present.[1,2,5,6] For *Campylobacter* spp., subculture from the enrichment broth to Campy Cefex Agar (Cat. no. A122) and incubate plates under microaerophilic conditions for 48-72 hours at 42±1ºC.

5. Examine respective plates for typical colonies of *Salmonella* or *Campylobacter* spp. and perform further testing for complete identification.

INTERPRETATION OF RESULTS

Consult listed references for appropriate interpretation of results.[1-6]

Following incubation, examine solid media for growth and typical colony morphology.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

nBPW is a non-selective medium. Overgrowth of competing flora in the test sample may affect recovery of salmonellae.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media such as Brilliant Green Agar (Cat. no. G75), XLD Agar (Cat. no. G65), HE Agar (Cat. no. G63), or Tetrathionate Broth (Cat. no. U165), Brilliant Green Sulfa Agar (Cat. no. G87), Modified Blood-Free Bolton Broth (Cat. no. U401), Campy Cefex Agar (Cat. no. A122), swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Salmonella enterica ATCC® 14028	A	18-24hr	35°C	Aerobic	Growth and typical colony morphology upon subculture to selective agar
Escherichia coli ATCC® 25922	A	18-24hr	35°C	Aerobic	Partial to complete inhibition upon subculture to selective agar
Campylobacter jejuni ATCC® 33291	0.5MF	18-24hr	35°C	Tight Cap	Growth and typical colony morphology upon subculture to selective agar

* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Neutralizing Buffered Peptone Water (nBPW) should appear opaque, cloudy, and light yellow in color.

REFERENCES

1. Juven, B.J., N. Cox, J.S. Bailey, J.E. Thomson, O.W. Charles, and J.V. Schutze. 1984. Recovery of *Salmonella* from artificially contaminated poultry feeds in non-selective and selective broth media. *Jour. of Food Prot.* ; 47:299-302.

2. Sadovski, A.Y. 1977. *J. Food Technology*; 12:85-91.

3. American Public Health Association. *Standard Methods for the Examination of Dairy Products,* APHA, Washington, D.C.

4. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods,* APHA, Washington, D.C.

5. U.S. Food and Drug Administration. *Bacteriological Analytical Manual.* Arlington, VA http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm

6. USDA FSIS. June 8, 2016. FSIS Notice 41-16. Washington D.C

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HDQA 2207D [D]