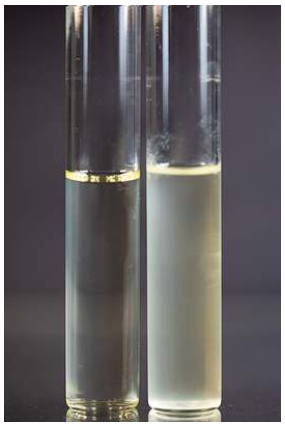
ALKALINE PEPTONE WATER

Dehydrated culture medium



Alkaline Peptone Water; from left: uninoculated tube and Vibrio *parahaemolyticus*

1 - INTENDED USE

Medium for enrichment of *Vibrio* spp. in food chain.

2 - COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	20 g
Sodium chloride	20 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Alkaline Peptone Water in the above formulation corresponds to the medium recommended by ISO 21872-1.[1] The preparation of the medium at half the concentration (20 g/L) corresponds to the FDA-BAM recommended formulation.[2] The medium is suitable for enrichment of *Vibrio* spp. in foods, animal feeding stuffs, environmental samples in the area of food production and food handling.[1,2]
The WHO[3] has identified that *V. parahaemolyticus*, *V. cholerae* and *V.vulnificus* are the major food-borne *Vibrio* spp. However, the method reported by ISO Standard can also be appropriate for the identification of other *Vibrio* spp. causing illness in humans. According to ISO 21872-1, the determination of potentially enteropathogenic species requires an analytical procedure divided into four steps: pre-enrichment and enrichment in Alkaline Peptone Water with differentiated incubation temperature at 37°C and 41.5°C, isolation on TCBS Agar and on a second medium of the user's choice, confirmation tests.
Recovery of *V. parahaemolyticus* and *V. cholerae* from fresh food is enhanced by incubation at 41.5°C, while recovery of *V. vulnificus, V.parahaemolyticus* and *V. cholerae* from frozen, dried or salted food is enhanced by 37°C. Peptone provides nitrogen, carbon, amino acids for bacterial growth; the alkaline pH and high concentration of sodium chloride favour the growth of halophilic vibrios.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 40 g in 1000 mL of cold purified water. If a medium according to FDA-BAM is required, weigh 20 g of powder in 1000 mL of cold purified water. Heat to complete solution, dispense into screw-capped tubes or bottles and autoclave at 121°C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	amber, fine, homogeneous, free-flowing powder
Prepared tubes appearance	colourless or pale yellow, limpid
Final pH at 20-25 °C	8.6 ± 0.1

6 - MATERIALS PROVIDED

Product	Type	REF	Pack
Alkaline Peptone Water	Dehydrated medium	4010322	500 g (12.5 / 25 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, sterile loops, swabs and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, tubes, ancillary culture media and reagents for the complete identification of the isolated strains.

8 – SPECIMENS

Foods, animal feeding stuffs, environmental samples in the area of food production and food handling. Refer to applicable international standards and regulations[1,2] for the collection, transport, storage of samples and operate in accordance with good laboratory practice.

9 - TEST PROCEDURE

Prepare the initial suspension by suspending 25 g or mL of sample into 225 mL of Alkaline Peptone Water.
Incubate the initial suspension at 37 °C ± 1 °C for 6 h ± 1h (for detection of *V.parahaemolyticus* and *V. cholerae* in deep frozen, dried or salted products and *V.vulnificus* in all samples).
Incubate the initial suspension at 41.5 °C ± 1 °C for 6 h ± 1h (for detection of *V. parahaemolyticus* and *V. cholerae* in fresh products).
Transfer 1 mL of the pre-enrichment culture into tubes containing 10 mL of Alkaline Peptone Water and incubate at 41.5 °C for 18 h ± 1h for the detection of *V.parahaemolyticus* and *V. cholerae* and at 37 °C ± 1 °C for 18 h ± 1h for *V.vulnificus*.
Inoculate with a 1 µl sampling loop the surface of a TCBS agar plate so as to permit the development of well-isolated colonies. Proceed likewise with the chosen second selective isolation medium.
Incubate the TCBS Agar at 37°C for 24h ± 3h. Incubate the second medium according to the Instructions for Use.

10 - READING AND INTERPRETATION

Bacterial growth in Alkaline Peptone Water is evidenced by the development of turbidity in the broth.
On TCBS Agar *V.cholerae* develops smooth, yellow (sucrose positive) colonies with a diameter of 1-2 mm, *V.parahaemolyticus* and *V.vulnificus* develop smooth, green (sucrose negative) colonies with a diameter of 2-3 mm.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
V. furnissii ATCC 11128	37°C / 18 h / A	good growth (turbidity)
V.parahaemolyticus ATCC 17802	37°C / 18 h / A	good growth (turbidity)

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated Alkaline Peptone Water, is tested for productivity and selectivity by comparing the results with previously approved Reference Batches.
Productivity and selectivity are tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of target and non-target organisms in test tubes, incubating at 35-37°C for 18-24 hours and recording the highest dilution showing growth in Reference Batch (GrRB) and in Test Batch (GrTB). Productivity is tested with the following target strains: *V.furnissii* ATCC 11128, V. *parahaemolyticus* ATCC 17802; selectivity is tested with the following non-target strains: *E.coli* ATCC 25922, *E.aerogenes* ATCC 13048, *E.faecalis* ATCC 19433, *S.aureus* ATCC 25923, *P.mirabilis* ATCC 25933. Target strains grow very well and the productivity index GrRB-GrTB for each test strain shall be ≤ 1. Since the selectivity of Alkaline Peptone Water is poor the non-target strains exhibit a growth after incubation.

13 – LIMITATIONS OF THE METHOD

- For enhanced recovery of *V. vulnificus*, medium containing derivatives of cellobiose-polymyxin B-colistin and cellobiose-colistin has been shown to be effective.[2]
- *V. parahaemolyticus, V. cholerae* and *V. vulnificus* may be present in small numbers in the samples and are often accompanied by a much larger number of other microorganisms belonging to the *Vibrionaceae* family or to other families.[2]
- Although the intended use and method of use of the medium described here refers to the detection of *Vibrio* spp. in food and other materials and therefore the product should not be regarded as an *in vitro* diagnostic, the literature reports the use of Alkaline Peptone Water for the examination of human clinical specimens.[4] Use simple concentration medium, incubate at 35°C for 18 hours and subculture onto TCBS Agar plate; occasionally vibrios grow with a shorter incubation (6 hours) and for these samples longer incubations cause overgrowth of contaminants masking the presence of *Vibrio* spp.[5] Clinical applications must be validated by the user.

14- PRECAUTIONS AND WARNINGS

- This product is for microbiological control only and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The ante and post mortem controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized inoculated medium with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps). The user is responsible for the manufacturing and quality control processes.
The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (tubes/flasks) and the applied storage conditions (temperature and packaging).

16 – REFERENCES

1. FDA (1995) Bacteriological Analytical Manual, Chapter 9, Vibrio, May 2004. Content current as of:10/31/2017
2. ISO 21872 (2017) Microbiology of the food chain — Horizontal method for the determination of Vibrio spp. —Part 1: Detection of potentially enteropathogenic Vibrio parahaemolyticus, Vibrio cholerae and Vibrio vulnificus.
3. FAO/WHO. 2001, Hazard identification, exposure assessment and hazard characterization of Campylobacter spp. in broiler chickens and Vibrio spp. in seafood, a joint FAO/WHO expert consultation, Geneva, Switzerland, 23–27 July 2001.
4. M.Lesmana et al. (1985) An evaluation of Alkaline Peptone Water for enrichment of Vibrio cholera in feces. Southeast Asian J. Trop. Med Pub. Healt. 16, 265.
5. Tarr C.L., Bopp C.A., Farmer III J.J. Vibrios and Related Organisms. In Manual of Clinical Microbiology, 11th ed. 2015, ASM Press.

TABLE OF APPLICABLE SYMBOLS

REF Or **REF** Catalogue number	**LOT** Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/04

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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