



## ALKALINE SALINE PEPTONE WATER (ISO)

**Code:** CM1117

a broth medium for the enrichment of *Vibrio* species from food and water samples; this formulation conform to ISO/TS 21872-1&2:2007<sup>1,2</sup>

<b>Typical Formula*</b>	g/litre
Peptone	20.0
Sodium chloride	20.0
pH	8.6 ± 0.2 @ 25°C

\* Adjusted as required to meet performance standards

### Directions

Dissolve 40g in 1 litre of distilled water. Mix well and distribute into final containers. Sterilize by autoclaving at 121°C for 15 minutes.

### Description

Alkaline Saline Peptone Water (ASPW) was first formulated by Shread, Donovan and Lee<sup>3</sup> to be used as a non-selective enrichment broth for the cultivation of *Aeromonas* species. Cruickshank reported that the raised pH of the medium could be used to effectively cultivate *Vibrio* species<sup>4</sup>. The medium exploits the ability of *Vibrio* species to tolerate alkaline conditions and high sodium chloride levels.

### Technique

For the detection of *Vibrio parahaemolyticus* and *Vibrio cholerae*

The following is an overview of ISO/TS 21872-1:2007<sup>1</sup> – Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. Part 1: Detection of *Vibrio parahaemolyticus* and *Vibrio cholerae*. Prepare samples in accordance with ISO 6887 (appropriate part)<sup>5</sup>, ISO 7218<sup>6</sup> and ISO 8261<sup>7</sup>.

1. Add xg of sample to 9xml or 9xg of ASPW (ISO) at ambient temperature.
2. Incubate for 6 ± 1 hour, at 41.5 ± 1°C for fresh products or 37 ± 1°C for deep-frozen/salted products.
3. Remove 1ml of incubated medium and inoculate into a fresh 10ml of Alkaline Saline Peptone Water (ISO) and incubate for 18 ± 1 hour at 41.5°C.
4. From the cultures obtained in the ASPW (ISO), inoculate with a microbiological loop the surface of a TCBS Agar plate (CM0333) and second optional selective isolation medium in order to obtain single colonies.
5. Incubate the plates at 37°C for 24 ± 3 hours (or as appropriate for the second optional medium).

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6. Examine the plates for the presence of typical colonies\* of presumptive pathogenic *Vibrio* spp. Select 5 typical colonies for subculture onto Saline Nutrient Agar followed by biochemical confirmation.

For the detection of species other than *Vibrio parahaemolyticus* and *Vibrio cholerae*  
The following is an overview of ISO/TS 21872-2:2007<sup>2</sup> – Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. Part 2: Detection of species other than *Vibrio parahaemolyticus* and *Vibrio cholerae*. Prepare samples in accordance with ISO 6887 (appropriate part)<sup>5</sup>, ISO 7218<sup>6</sup> and ISO 8261<sup>7</sup>.

1. Add xg of sample to 9xml or 9xg of ASPW (ISO) at ambient temperature.
2. Incubate for  $6 \pm 1$  hour, at  $41.5 \pm 1^\circ\text{C}$  for fresh products or  $37 \pm 1^\circ\text{C}$  for deep-frozen/salted products.
3. Remove 1 ml of incubated medium and inoculate into a fresh 10ml of Alkaline Saline Peptone Water (ISO) and incubate for  $18 \pm 1$  hour at  $37 \pm 1^\circ\text{C}$ .
4. From the cultures obtained in the ASPW (ISO), inoculate with a microbiological loop the surface of a TCBS Agar plate (CM0333) and second optional selective isolation medium in order to obtain single colonies.
5. Incubate the plates at  $37^\circ\text{C}$  for  $24 \pm 3$  hours (or as appropriate for the second optional medium).
6. Examine the plates for the presence of typical colonies<sup>†</sup> of presumptive pathogenic *Vibrio* spp. Select 5 typical colonies for subculture onto Saline Nutrient Agar followed by biochemical confirmation.

<sup>†</sup>Refer to ISO/TS 21872-1&2:2007<sup>1</sup> or Oxoid manual entries for CM0333 (and entries for other optional second media as appropriate) for interpretation of colony type.

## Quality control

### Positive controls:

*Vibrio parahaemolyticus* NCTC 10885

*Vibrio furnissii* NCTC 11218

*Vibrio vulnificus* ATCC® 29307

*Vibrio cholerae* non-O1/non-O139 ATCC® 14733\*

### Negative control:

Uninoculated medium

### Expected results

Turbid growth

Turbid growth

Turbid growth

Turbid growth

No change

\* This organism is available as a Culti-Loop®

## References

1. ISO/TS 21872-1:20071, Microbiology of food and animal feeding stuffs – Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. Part 1: Detection of *Vibrio parahaemolyticus* and *Vibrio cholerae*.
2. ISO/TS 21872-2:20072, Microbiology of food and animal feeding stuffs – Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. Part 2: Detection of

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species other than *Vibrio parahaemolyticus* and *Vibrio cholerae*.

3. Shread P., Donovan T. J., and Lee J. V. (1991) *Soc. Gen. Microbiol. Q.* 8:184.

4. Cruickshank R. (1968) *Medical Microbiology*. 11th ed. Livingstone Ltd, London, UK.

5. ISO 6887 (all parts), *Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*.

6. ISO 7218 *Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations*.

7. ISO 8261 *Microbiology of food and animal feeding stuffs – General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*.