VIBRIO PARAHAEMOLYTICUS

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INTRODUCTION

Food poisoning due to V. parahaemolyticus is a food-borne infection resulting from the ingestion of a large number of this organism (about 10^6 - 10^9 viable cells). The major symptoms are diarrhoea and abdominal pain with headache, fever and vomiting also occurring. The organisms are excreted during the acute stage of the illness after which they decrease rapidly.

The differentiation of V. parahaemolyticus from other pathogenic species of Vibrio is based mainly on salt tolerance, Voges-Proskauer reaction, fermentation of sucrose and growth at 43°C.

I CULTURE MEDIA*

Glucose salt teepol broth (GSTB)
Modified Wagatsuma agar
Thiosulphate citrate bile salts sucrose agar (TCBS)
MRVP medium
Triple sugar iron agar (TSI)
Andrade peptone water
Koser citrate medium
Simmons citrate agar
Phenylalanine agar (PPA)
Bacto-peptone (PW)
Decarboxylase medium base
Nutrient gelatin
Aesculin broth
SIM medium
Butterfield's buffered phosphate diluent
Nutrient agar (+3% NaCl)
Sodium chloride (NaCl)

* Refer to Appendix B for methods of media preparation.

a) 1% solution (w/v) of each of the following amino acids:

L-arginine HCl
L-lysine HCl
L-ornithine HCl

b) 1% solution (w/v) of the following sugars:

Arabinose	Mannitol	Salicin
Glucose	Mannose	Sucrose
Lactose	Melibiose	

II CHEMICAL REAGENTS**

Tetramethyl-p-phenylenediamine di-HCl aq. soln. (1% w/v)

a) Kovac's reagent

b) Methyl red solution

c) α-naphthol solution (5% w/v)

d) 0.1N HCl

e) KOH solution (40% w/v)

f) $FeCl_3$ aq. soln. (10% w/v)

** Refer to Appendix D for methods of reagent preparation.

III APPARATUS

'Waring' blender & flasks
Pipettes
Scissors & forceps
Alcohol lamps
Alcohol (70% v/v) swabs
Plating loops
Autoclave
Incubator
Water-bath
Weighing balance
Laminar flow chamber

IV SAMPLING PROCEDURE

Refer to "AEROBIC PLATE COUNT" (E-2) Section III.

V SAMPLE PREPARATION

Refer to "AEROBIC PLATE COUNT" (E-2) Section IV.

VI PROCEDURE

1. Select appropriate dilutions and for first dilution, transfer 10 ml aliquots into each of 3 tubes of double strength GSTB.

2. For each of the next 2 further dilutions, transfer 1 ml aliquots into each of 3 tubes of single strength GSTB.

3. Incubate the tubes at 35°C for not more than 18 hrs.

4. Transfer a loopful of suspension from the top 1 cm of a positive GSTB tube onto a TCBS plate and streak to obtain isolated colonies.

5. Incubate the plates at 35°C for 18 hrs.

6. Examine the plates for typical V. parahaemolyticus colonies which are large and blue-green with a dark centre.

7. Screen suspected isolates by inoculating the following media* and incubate at 35°C for 24 hrs.

TSI agar	K/Acid (no gas; no H_2S)
Indole (SIM)	+
Motility (SIM)	+
L-lysine HCl	+

8. Inoculate the TSI culture into peptone water (+3% NaCl) and nutrient agar (+3% NaCl) slant and incubate at 35°C for 24 hrs.

9. Perform the oxidase test from the nutrient agar slant and use the peptone water culture as inoculum for the following biochemical tests.*

Oxidase	+
Voges-Proskauer	–
Sucrose	–
Mannitol	+
Peptone water (PW) + 0% NaCl	–
Peptone water (PW) + 3% NACl	+
Peptone water (PW) + 7% NaCl	+
Peptone water (PW) + 9% NaCl	+
Peptone water (PW) + 11% NaCl	–

* Refer to Appendix C for biochemical tests procedures.

10. Carry out the following confirmatory biochemical tests*

Methyl Red (MR)	+
Citrate	+
L-arginine HCl	–
L-ornithine HCl	+
Phenylalanine (PPA)	–
Nutrient gelatin (5°C)	+
Gas from glucose	–
Lactose	–
Arabinose	+
Mannose	+
Mannitol	+
Salicin	–
Aesculin	+
Melibiose	–

* Refer to Appendix C for biochemical tests procedures.

11. Calculate the MPN of V. parahaemolyticus based on the proportion of positive GSTB tubes which are confirmed for the presence of V. parahaemolyticus. (See below)

VII CALCULATION OF MPN

$$\text{Most Probable Number (MPN)} = \frac{\text{Index}}{10} \times (450 + W) \times \frac{1}{W}$$

where W : weight of sample in g

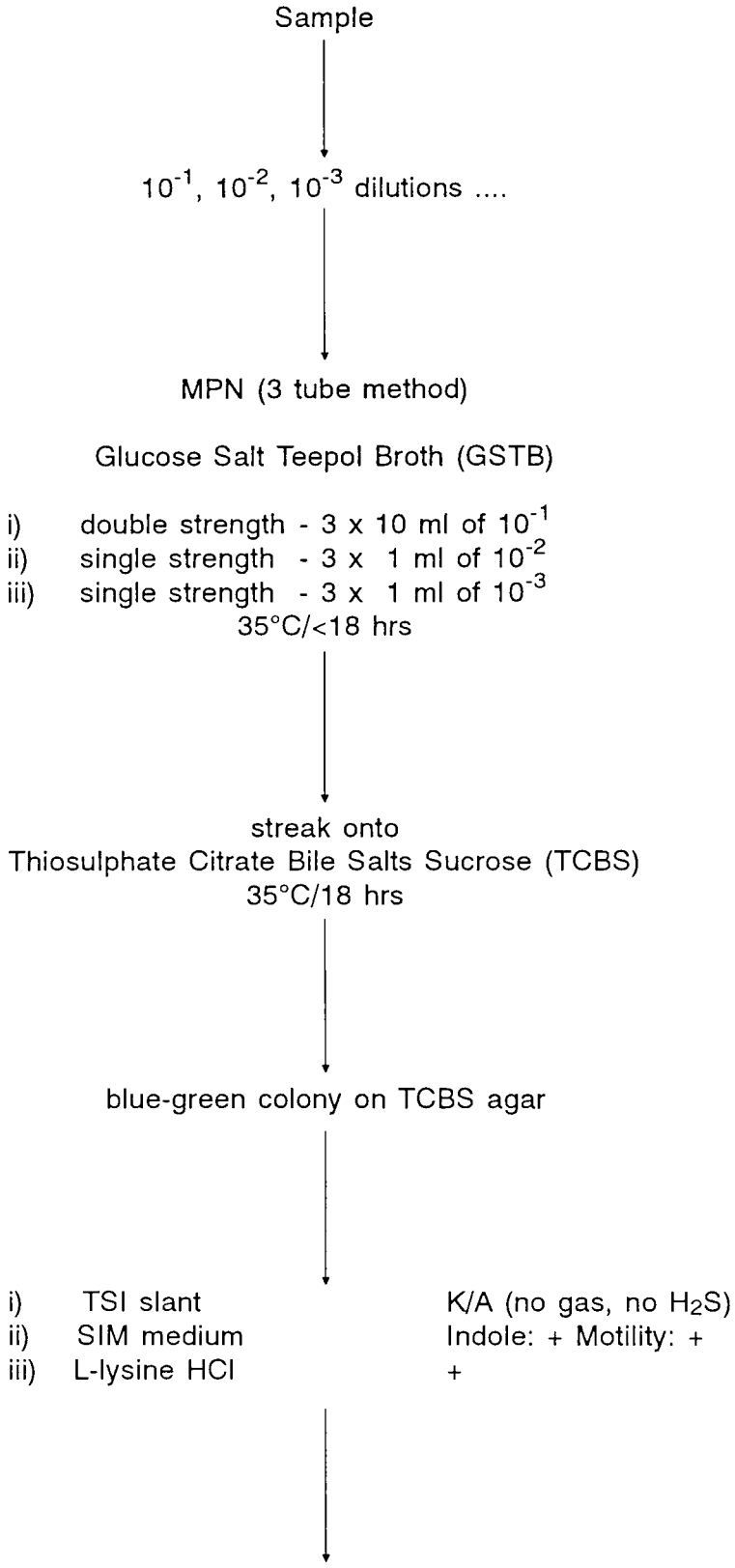
Index : from MPN Tables (Appendix A)

REFERENCES

A. Hazzard. (1985). ASEAN Training Course in Fish Quality Control. Training course organised by HAWKAID, Hawkesbury Agricultural College Research and Development Co., Ltd. Chapter: microbiology in seafood quality control.
Section 6 : 69-70

Isolation and identification of Vibrio parahaemolyticus. Bacteriological Analytical Manual. Jan. 1969.

FLOW DIAGRAM OF EXAMINATION PROCEDURES FOR V. PARAHAEMOLYTICUS



Inoculate i) Peptone water (+3% NaCl) (as inoculum)
ii) Nutrient agar (+3% NaCl) slant (for oxidase test)

i)	Oxidase	+
ii)	Voges-Proskauer	–
iii)	Sucrose	–
iv)	Mannitol	+
v)	PW + 0% NaCl	–
	PW + 3% NaCl	+
	PW + 7% NaCl	+
	PW + 9% NaCl	+
	PW + 11% NaCl	–

confirmatory biochemical tests

Methyl Red (MR)	+
Citrate	+
L-arginine HCl	–
L-ornithine HCl	+
Phenylalanine (PPA)	–
Nutrient gelatin (5°C)	+
Melibiose	–
Gas from glucose	–
Lactose	–
Arabinose	+
Mannose	+
Mannitol	+
Salicin	–
Aesculin	+

Kanagawa reaction test (if required) (see next page)

KANAGAWA REACTION OF V. PARAHAEMOLYTICUS

APPLICATION

The Kanagawa reaction tests for the presence of specific haemolysis on Watgatsuma agar.

A positive reaction has been found to correlate closely with the pathogenicity of V. parahaemolyticus isolates. The isolates that have caused illness in humans are almost always Kanagawa-positive, although isolates from seafood are almost always Kanagawa-negative.

PROCEDURE

1. Subculture the isolate into 3% NaCl peptone water and incubate at 35°C for 18 hrs.

2. Spot a loopful of this culture onto a freshly prepared, dried modified Wagatsuma agar plate. Several spottings may be made on the same plate.

3. Incubate at 35°C for 18 ± 2 hrs.

4. A positive test consists of β-haemolysis: a zone of transparent clearing of the blood cells around the colony.

5. It is very important to remember that only observations within 24 hrs is valid in this test.