VIBRIO CHOLERA

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INTRODUCTION

Cholera is an acute specific infection caused by the organism, Vibrio cholera. Diagnosis may be confirmed by the presence of large numbers of the comma-shaped bacilli on direct microscopic examination of a faecal or vomitus smear, and by the isolation of the organism on culture.

Fish and shellfish have been identified as vehicles of cholera. Large numbers of V. cholera must usually be ingested to cause cholera, thus problems often occur when poor handling and inadequate refrigeration have allowed the organism to multiply.

I CULTURE MEDIA*

Alkaline peptone water (pH 8.6-9.0)
Andrade peptone water
Aesculin broth
Decarboxylase medium base
Koser citrate medium
MRVP medium
Nutrient agar (+ 3% NaCl)
Nutrient gelatin
Phenylalanine agar (PPA)
SIM medium
Simmons citrate agar
Thiosulphate citrate bile salts sucrose agar (TCBS)
Triple sugar iron agar (TSI)
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 each of the following sugars:-

Arabinose	Lactose	Melibiose
Glucose	Mannitol	Salicin
Inositol	Mannose	Sucrose

II CHEMICAL REAGENTS#

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

b) Kovac's reagent

c) 0.1N HCl

d) Methyl red solution

e) KOH solution (40% w/v)

f) α-naphthol solution (5% w/v)

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

\# Refer to Appendix D for methods of reagent preparation.

III APPARATUS

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

IV SAMPLING PROCEDURE

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

V PROCEDURE

1. Weigh about 50 g of the sample and add approximately 200 ml of alkaline peptone water in a 'Waring' blender flask. Blend for 1 min at low speed.

2. Incubate at 35°C for 6-8 hrs.

3. At the end of the incubation period, transfer a loopful obtained from the pellicle (surface growth) onto TCBS agar and streak to obtain isolated colonies.

4. Incubate the plates at 35°C for 18-24 hrs.

5. V. cholera colonies on TCBS agar appear as large, smooth and yellow.

6. Screen isolates with the following tests*:-

Tests	Results
TSI	acid slant/accid butt; no gas; no H_2S
Indole (SIM)	+
Motility (SIM)	+
Lysine decarboxylase	+
Peptone water (+3% NaCl)	growth

* Refer to Appendix C for biochemical tests procedures.

7. From the TSI slant, inoculate a nutrient agar (+3% NaCl) slant and incubate at 35°C for 24 hrs.

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

Tests	Results
Oxidase	+
Lysine	+
Ornithine	+
Arginine	−
Sucrose	+
Mannitol	+
Inositol	−
MR	$+^{w}$ (Reaction delayed & weak)
VP	+/− (Indefinite)
PW + 0% NaCl	+
PW + 3% NaCl	+
PW + 7% NaCl	d (16-84% strains positive)
PW + 9% NaCl	−
PW + 11% NaCl	−

* Refer to Appendix C for biochemical tests procedures.

9. Carry out the following confirmatory biochemical tests*:-

Tests	Results
Citrate	$+^{w}$ (Reaction delayed & weak)
Phenylalanine	−
Gelatin (5°C)	+
Gas from glucose	−
Lactose	−
Arabinose	−
Mannose	+
Salicin	−
Melibiose	−
Aesculin	−

* Refer to Appendix C for biochemical tests procedures.

10. Serological agglutination tests are performed on confirmed isolates using polyvalent O anti-serum and Ogawa and Inaba anti-sera.

VI BACTERIOLOGICAL LIMITS OF VIBRIO CHOLERA FOR FISH/FISHERY PRODUCTS (COOKED & RAW)

This organism should not be detected in 50 g sample.

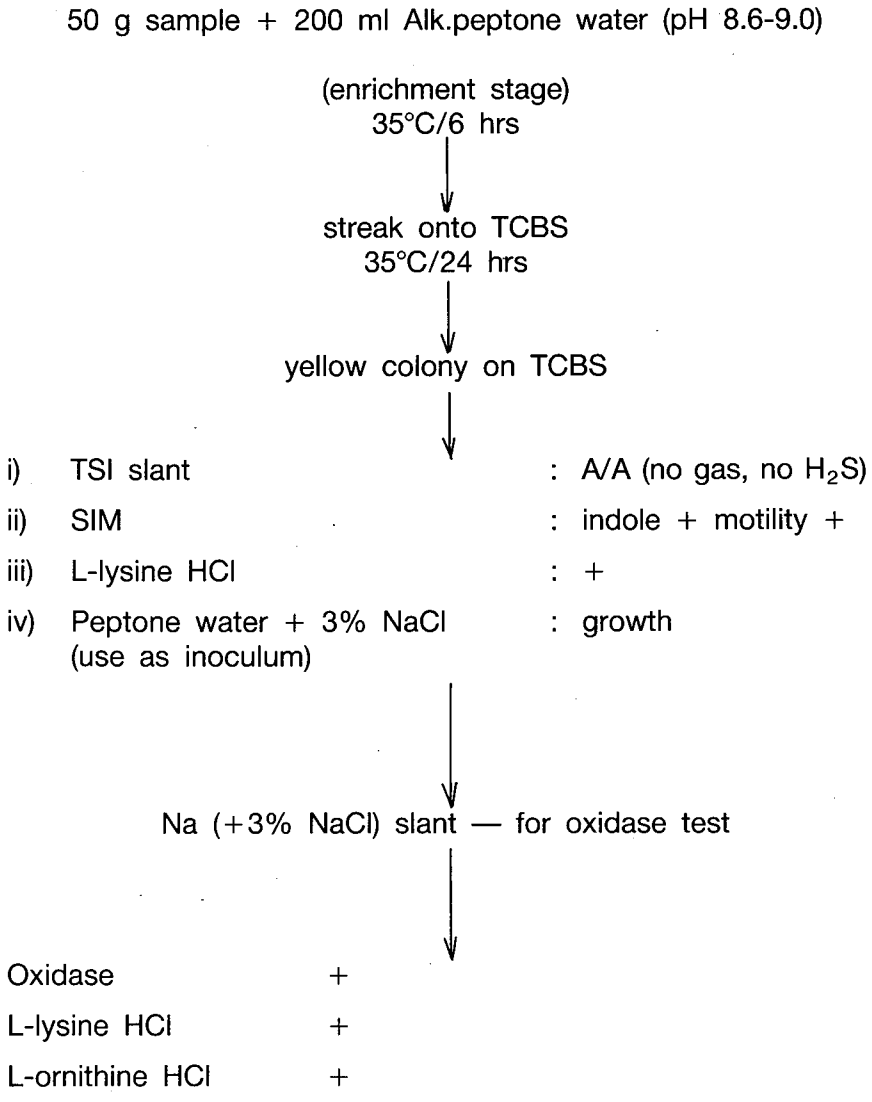
REFERENCES

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: 68 & 77.

See Reference 2 in E-2.

A flow diagram of the examination procedures for V. cholera is included as the following figure.

FLOW DIAGRAM OF EXAMINATION PROCEDURES FOR VIBRIO CHOLERA



L-arginine HCl	−	
Sucrose	+	
Mannitol	+	
Inositol	−	
MR	$+^{w}$	(Reaction delayed & weak)
VP	+/−	(Indefinite)
PW + 0% NaCl	+	
PW + 3% NaCl	+	
PW + 7% NaCl	d	(16-84% strains positive)
PW + 9% NaCl	−	
PW + 11% NaCl	−	

Confirmatory biochemical tests

Citrate	$+^{w}$	(Reaction delayed & weak)
Phenylalanine	−	
Gelatin (5°C)	+	
Gas from glucose	−	
Lactose	−	
Arabinose	−	
Mannose	+	
Salicin	−	
Aesculin	−	
Melibiose	−	

serology for V. cholera