

11. ISOLATION AND IDENTIFICATION OF PATHOGENIC VIBRIOS FROM SEAFOOD

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Introduction

Seafood is a nutritious food that constitutes one of the desirable components of a healthy diet. Nevertheless, there is health risks associated with the consumption of seafood. One of the major risks involves the consumption of raw or undercooked seafood that may be naturally contaminated by foodborne pathogens present in the marine environment. Such risk is further increased if the food is mishandled during processing where pathogens could multiply exponentially under favorable conditions. In contrast to most other foodborne pathogens, *Vibrio* spp. has the aquatic habitat as their natural niche. As a result, vibrios are most commonly associated with seafood as natural contaminants. Foodborne infections with *Vibrio* spp. are common in Asia. Most of these foodborne infections are caused by *V. parahaemolyticus* and *V. cholerae*, and to a lesser extent by *V. vulnificus* and *V. mimicus*.

Vibrio mimicus

Vibrio mimicus is a *Vibrio* species that mimics *V. cholerae*. *V. mimicus* has been recognized as a cause of gastroenteritis transmitted by raw oysters, fish, turtle eggs, prawns, squid, and crayfish. *V. mimicus*, when carrying genes that encode cholera toxin, can cause severe watery diarrhea. Consumers and physicians should be aware that improperly handled marine and aquatic animal products can be a source of *V. mimicus* infections. Consumers should avoid cross-contamination of cooked seafood and other foods with raw seafood and juices from raw seafood and should follow FDA recommendations for selecting seafood and preparing it safely.

Vibrio cholerae

V. cholerae, a Gram-negative motile rod causes massive cholera outbreaks. Cholera is a global threat to public health and it was estimated that between 2008 and 2012 cholera caused an annual average of 2.9 million cases, and 95,000 deaths, worldwide Particular serogroups (O1 and O139) of this bacterium are responsible for cholera epidemics and pandemics. Human infection with *V. cholerae* begins with ingestion of contaminated food or water containing the bacterium.

V. cholerae colonizes the small intestine and secretes cholera enterotoxin (CT) into the host cells resulting in rapid efflux of chloride ions and water into the lumen of the intestine, leading to profuse diarrhea and severe dehydration. *V. cholerae* is commonly associated with

chitin-containing zooplankton, particularly copepods and chironomids. Recent evidence supports the hypothesis that fish and water birds may also be intermediate reservoirs and vectors of *V. cholerae*.

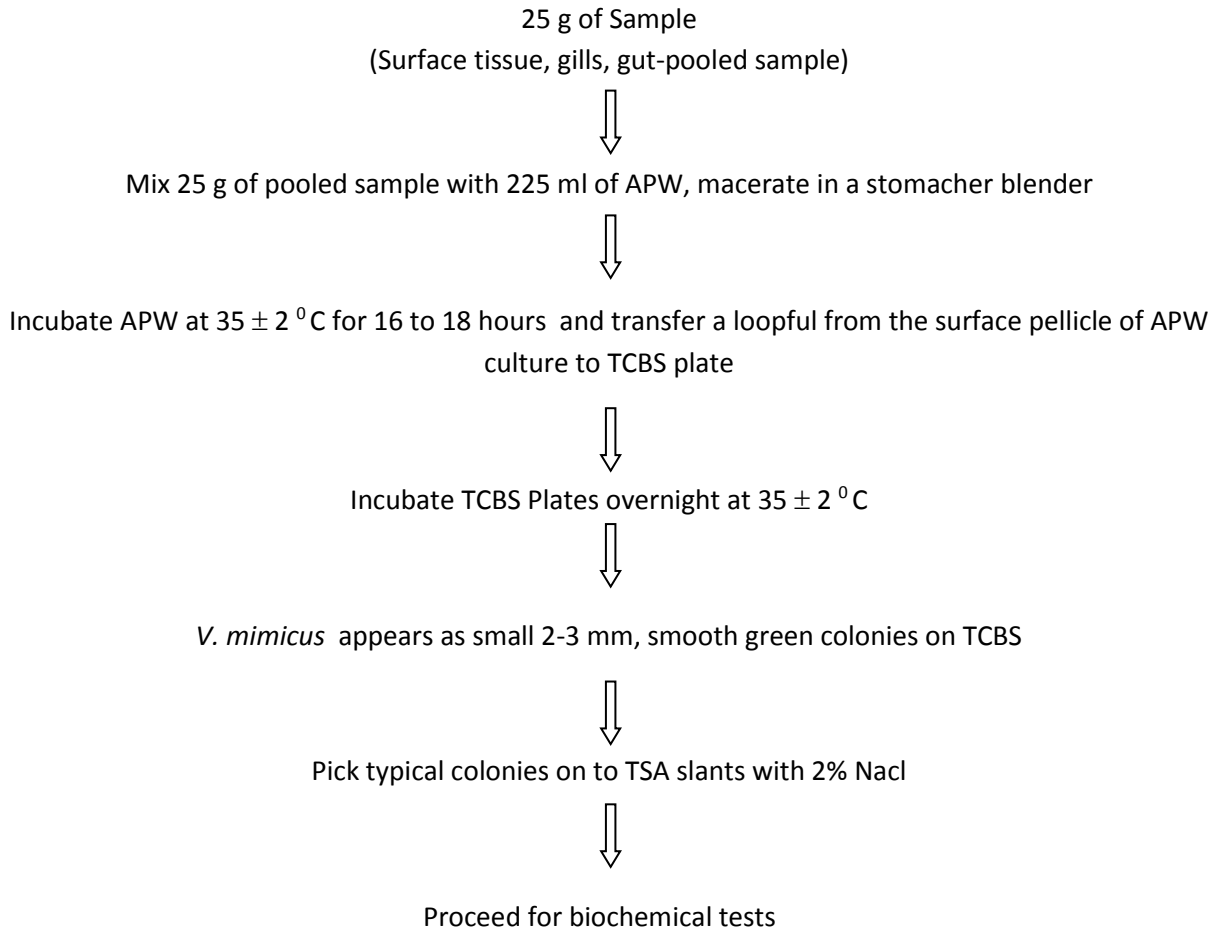
Vibrio parahaemolyticus

Vibrio parahaemolyticus was first discovered by Tsunesaburo Fujino in 1950 as a causative agent of food borne disease following a large outbreak in Japan which recorded 272 illnesses with 20 deaths after consumption of *shirasu*. Virulent *V. parahaemolyticus* strains are transmitted by consumption of raw or undercooked seafood causing acute gastroenteritis. Since its discovery, *V. parahaemolyticus* has been found to be responsible for 20–30% of food poisoning cases in Japan and seafood borne diseases in many Asian countries. *V. parahaemolyticus* was also recognized as the leading cause of human gastroenteritis associated with seafood consumption in the United States. The worldwide prevalence of *V. parahaemolyticus* gastroenteritis cases stresses the need for understanding of the virulence factors involved and their effects on humans.

Vibrio vulnificus

V. vulnificus the leading cause of death in the US related to seafood consumption and nearly always associated with raw Gulf Coast oysters resembles *V. parahaemolyticus* on TCBS agar, but can be differentiated by several biochemical reactions, including β -galactosidase activity . Epidemiological and clinical investigations have shown that *V. vulnificus* causes septicemia and death following ingestion of seafood or after wound infections originating from the marine environment . Recent gene probe assays, PCR procedures , fatty acid profiles and enzyme immunoassay have been developed to detect and identify this pathogen.

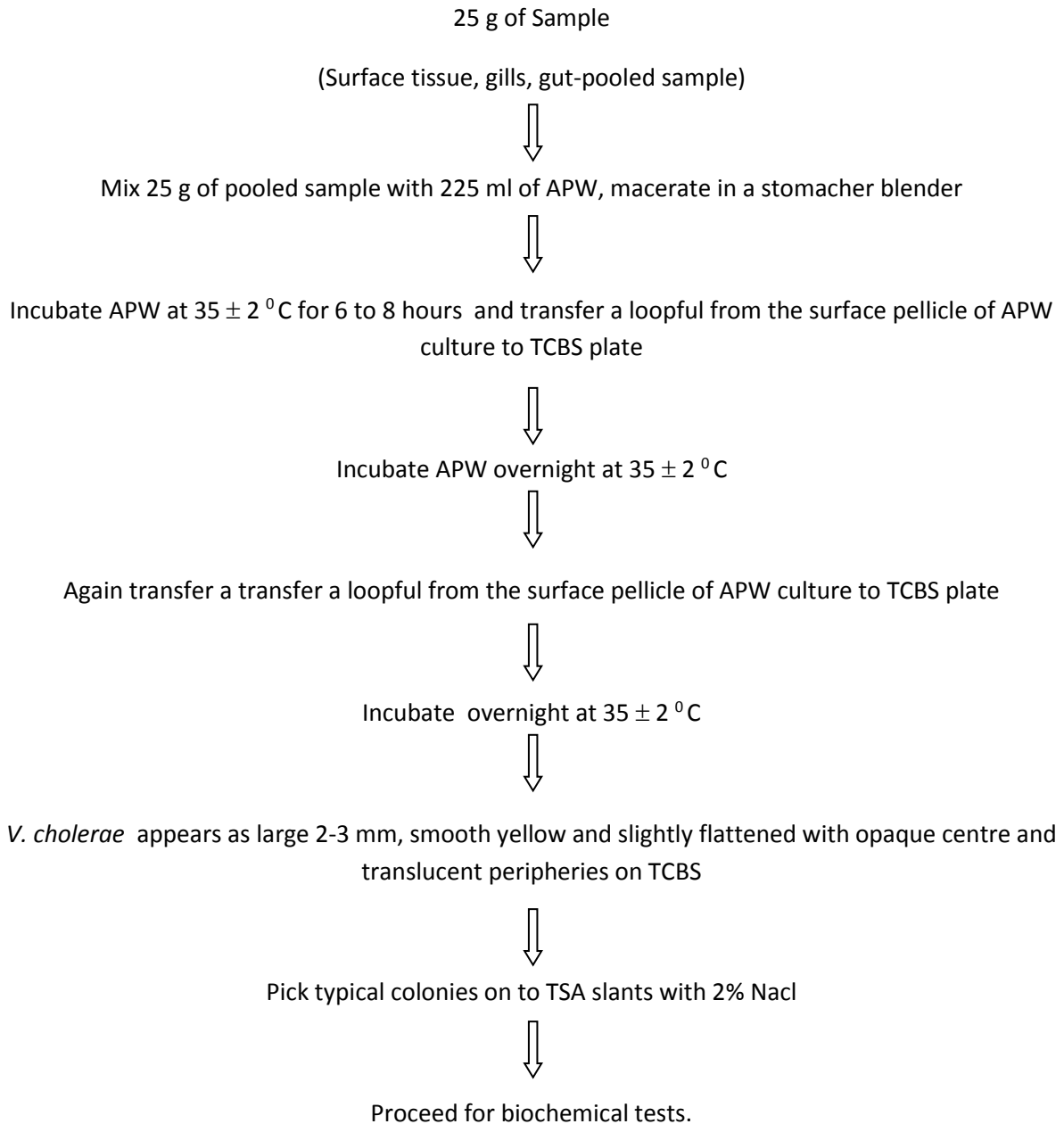
Protocol for the isolation of *V. mimicus* from fish



Biochemical confirmation:

- ❖ Oxidase positive
- ❖ Gram negative short rods
- ❖ String test positive
- ❖ Arginine decarboxylase-Negative
- ❖ Lysine, Ornithine decarboxylase- positive
- ❖ Sucrose Negative
- ❖ Growth in 0% salt, no growth in 6% salt

Protocol for the isolation of *V. cholerae* from fish

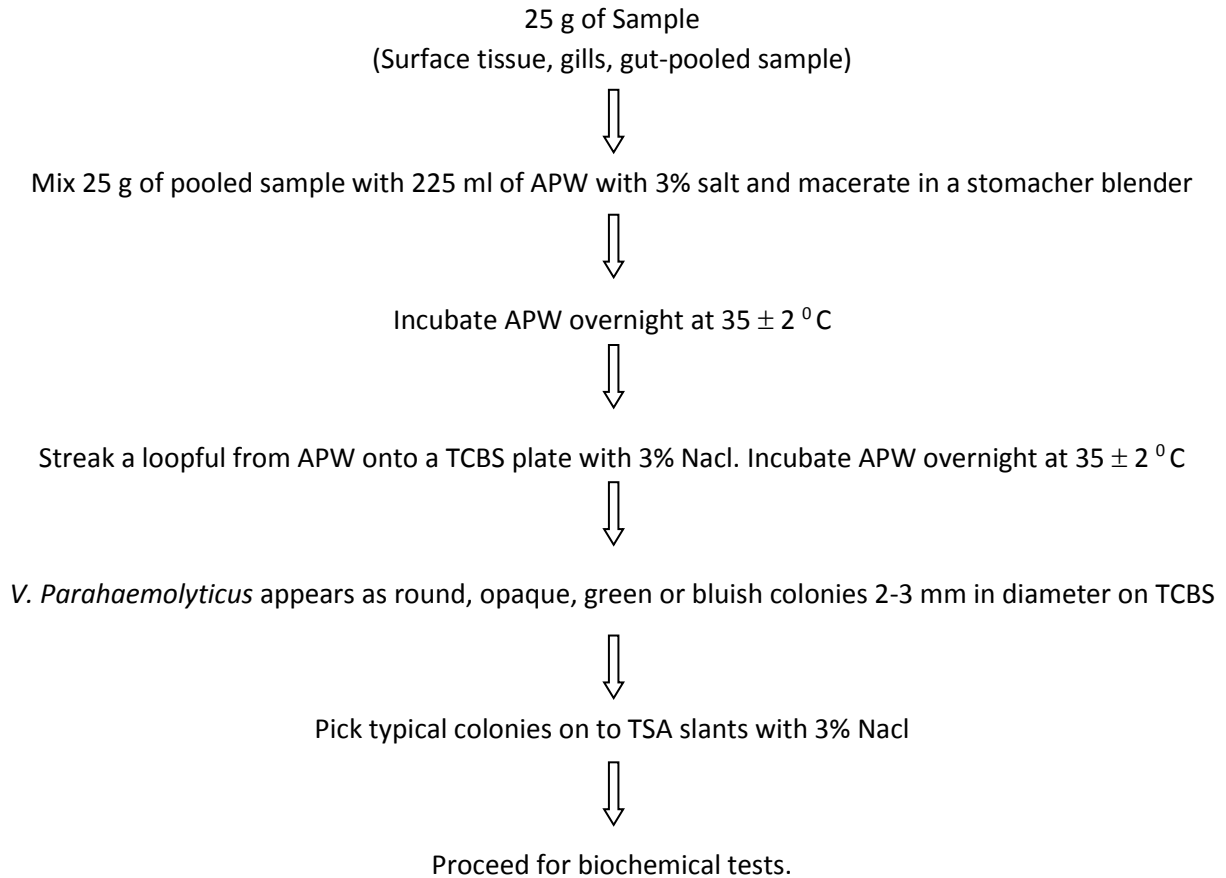


Biochemical confirmation:

- ❖ Oxidase positive
- ❖ String test positive
- ❖ Arginine decarboxylase-Negative
- ❖ Lysine, Ornithine Decarboxylase- positive
- ❖ Sucrose positive; Growth in 0% salt, no growth in 6% salt

Protocol for the isolation of *V. Parahaemolyticus* from seafood

All the media used for the biochemical identification of *Vibrio parahamolyticus* should contain 2 or 3% Nacl.

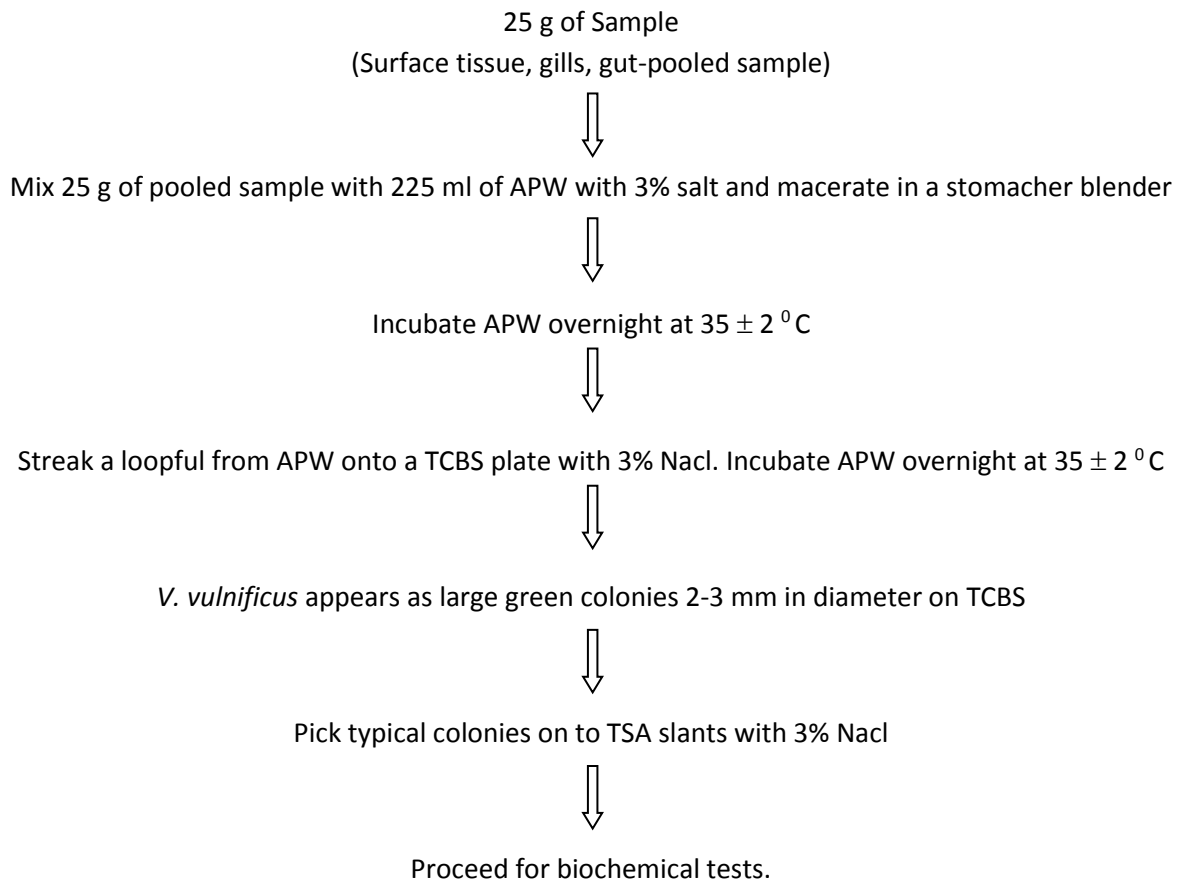


Biochemical confirmation:

- Oxidase positive
- Gram negative, straight/ curved rods
- Non H₂S producer
- Growth in 3 %, 6%, 8% Nacl, No growth in 0 % Nacl
- *V.Parahaemolyticus* can be differentiated from other Vibrios by ONPG, Salt tolerance and lactose reactions; Resistance to 10 µg of O/129, sensitive to 150 µg of O/129.

Protocol for the isolation of *V. vulnificus* from seafood

All the media used for the biochemical identification of *Vibrio vulnificus* should contain 3% NaCl



Biochemical confirmation:

- Oxidase positive
- Gram negative, straight/ curved rods
- Non H₂S producer
- lactose positive
- Growth in 3 %, 6% NaCl, No growth in 0 % NaCl
- Sensitive to 10 µg of O/129, 150 µg of O/129.