GLOBAL TASK FORCE ON CHOLERA CONTROL

Isolation and Presumptive Identification of *Vibrio Cholerae* O1/O139 from fecal specimens

Quality Control of Media and Reagents

Laboratories must ensure adequate quality control of the media and reagents in use. Each batch of media prepared from individual ingredients should be tested for one or more of the following characteristics:

- Sterility/contamination check
- Ability to support growth
- Ability to produce appropriate biochemical reaction (if applicable)

Enrichment in Alkaline Peptone Water (APW):

APW is the recommended enrichment broth for *V. cholerae*. APW will improve the isolation of *V. cholerae* when few organisms are present (e.g., convalescent patients or suspected asymptomatic carriers), or when high numbers of competing organisms are present. Vibrio spp. grow rapidly in APW due to the alkaline pH and salinity of the enrichment, and at 6 to 8 hours will be present in greater numbers than non-*Vibrio* organisms.

The inoculum should be abundant but not exceed 10% of the broth volume. If possible, the medium can be distributed in small tubes containing 2 to 5 mL of APW to more rapidly observe the bacterial growth.

After 6 to 8 hours' incubation, subcultures to a selective and none selective solid medium, with one to two loopfuls of APW from the surface and topmost portion of the broth, since *vibrios* preferentially grow in this area. Do not shake or mix the tube before subculturing. If the broth cannot be subcultured after 6 to 8 hours of incubation, leave to grow overnight and subculture a 10µL loopful to a fresh tube of APW. This second tube should be subcultured to a solid medium after 6 to 8 hours incubation.

Selective plating media:

Thiosulfate citrate bile salts sucrose agar (TCBS) is the selective agar medium of choice for isolating *V. cholerae*. from stool specimens. It differentiates *V. cholerae* from other bacteria based on its capacity to grow on this medium and on the fermentation of sucrose, leading to change in the initially green color of the medium to yellow colonies after 18 to 24 hours growth at 35±2°C. Suspect colonies are large (2 to 4 mm in diameter), shiny, slightly flattened, and yellow.

Ensure each new batch of TCBS is tested with positive and negative controls (i.e. with sucrose positive and negative bacterial species) before use.

Inoculate selective media with a heavy inoculum from liquid stool, fecal suspension or rectal swab.

Yellow colonies on TCBS can turn green at ambient temperature if the inoculated plates are stored for more than 24 hours. **Check for growth less than 24h after plating.**

Growth on this medium is not suitable for direct oxidase testing nor testing with *V. cholerae* O1 or O139 antisera. Suspect colonies must be sub-cultured on non-selective agar before performing additional tests.

Non-selective agar (NSA):

These media include Mueller Hinton (recommended), Brain Heart Infusion Agar and Trypticase Soy Agar. NSA have low selectivity and must be lightly inoculated. When inoculated directly from a specimen, growth on NSA is likely to lead to mixed cultures, so it is often necessary to re-isolate suspect colonies on a new plate. However when suspect colonies are well isolated on the initial plate it is possible to proceed directly to the oxidase test as described below. Suspect colonies isolated on TCBS should be cultured on NSA prior to further testing.

Oxidase testing:

The oxidase reaction must be performed with a fresh (18-24h) **culture grown on non-selective agar**; it should not be performed from culture on TCBS since the composition of the medium can produce false results. The use of a metal handle can lead to a false positive reaction for the oxidase test. Use a glass or a disposable plastic inoculation loop. ***V. cholerae* are oxidase positive** while *Enterobacteriaceae* are oxidase negative.

Agglutination:

This is a key step for presumptive identification of cholera vibrios. Both O1 and O139 serogroups must be identified using O-group-specific antisera. Agglutination must be performed with a fresh (18-24h) culture from non-selective agar. Cultures on TCBS medium can give self-agglutination reactions in saline, preventing the interpretation of the reaction. Agglutination with saline should not occur. If agglutination does occur, it is a self-agglutinating strain, and therefore it is not necessary to carry out further agglutination testing with the anti-O1 or anti-O139 serum. Send the strain to a reference laboratory.