Laboratory diagnosis of *Vibrio cholerae* in clinical specimen

HKU SPACE Medical Laboratory Science (Applied Learning) 2015-17 Cohort

**Introduction**

- Cholera is an acute, diarrheal illness caused by an infection of the intestine with the bacterium *Vibrio cholerae*.
- *Vibrio cholerae* is a Gram-negative, comma-shaped bacterium and has many different types of serogroups. Serogroup O1 and serogroup O139 can cause epidemic cholera while non-O1 and non-O139 serogroups can cause a diarrheal disease.
- According to CDC information, an estimated 3-5 million cases and over 100,000 deaths occur each year over the world.
- People can be infected by eating the food (especially seafood like oysters, crabs and fishes) contaminated with *Vibrio cholera*, and drinking polluted water contaminated by patients’ stool. If being untreated, the patient will die within hours.
- The symptoms of people infected by *Vibrio cholerae*: diarrhea and vomiting, dehydration, circulatory collapse and shock.

**Objective**

To understand the nature of *Vibrio cholerae* and to study the laboratory diagnosis of *Vibrio cholerae* infection in clinical specimens.

**Detection Methods**

I. Isolation and Gram staining of positive yellow colonies in TCBS agar

TCBS agar is the medium for the isolation of *Vibrio cholerae* from the specimens. *Vibrio cholerae* will grow and produce yellow colonies. The yellow colonies are picked and stained by Gram staining.

Procedures of Gram staining:

- Add a drop of water containing the yellow colonies on a slide with an inoculation loop.
- Air-dry and fix it over a gentle flame.
- Add crystal violet stain and stain for 15s.
- Pour off the stain, rinse with a stream of water.
- Add iodine solution and stand for 60s, pour off solution and rinse the slide with running water.
- Add a few drops of decolorizer until no colour coming out from the slide.
- Rinse the slide with water after 5s.
- Counterstain the slide with basic fuchsin solution for 60s.
- Wash off the solution with water and blot dry.
- Add a drop of immersion oil onto the sample and observe under the microscope.

II. Rapid Dipstick (Crystal VC) test kit

This kit uses monoclonal antibodies specific for the *Vibrio cholerae* serogroups O1 and O139 in an immuno-chromatography dipstick to detect *Vibrio cholerae* infection.

Procedures of Rapid Dipstick (Crystal VC) test kit:

- Inoculate the specimen with a cotton swab into a test tube of alkaline peptone water and keep it at 35-37°C for 6 hrs.
- Add 2-4 drops of the alkaline peptone water into the kit test tube and put a Crystal VC dipstick into the kit test tube.
- Leave it for 15 mins and observe the dipstick.

Positive result is obtained when two red lines develop, one at control region and the other at the test region. This indicates the presence of *Vibrio cholerae* in the specimen.

III. Detection of *Vibrio cholera* DNA by polymerase chain reaction (PCR)

It is to detect the presence of *Vibrio cholera* DNA in the patient’s specimen.

Procedures of PCR:

- Clinical samples collection
- *Vibrio cholera* DNA extraction
- Polymerase chain reaction to amplify the target DNA
- Detection of the PCR products by gel electrophoresis

A 300 kb PCR positive band is observed if the specimen contains *Vibrio cholerae*.

**Summary & Conclusion**

These methods can be used for detection of *Vibrio cholerae* in clinical specimens. This can help to identify people infected with *Vibrio cholerae* for proper treatment and control the cholera epidemic.

**References**

4. [http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6117a1htm](http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6117a1htm)
5. [http://textbookofbacteriology.net/cholera.html](http://textbookofbacteriology.net/cholera.html)
**HKUSPACE Applied Learning - Medical Laboratory Science**  
Project report and Oral Presentation: 19 November 2016  
Assessment Form

| Student Name: | Student No.: | Class Code: | Group: 6 | Title: Laboratory diagnosis of *Vibrio cholerae* (霍亂弧菌) in clinical specimen |

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<th>Learning attitude (10%)</th>
<th>Scientific content (50%)</th>
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*You may give a score on the scale according to the suggested criteria (in bracket). For details, please refer to the assessment scheme (attached)*