IRAQI
STANDARD OPERATING PROCEDURES (SOP)

LABORATORY IDENTIFICATION OF VIBRIO CHOLERA

DECEMBER 2014

SOP: NCL – BE 001
National Cholera Laboratory
IRAQ

DISCLAIMER
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ACRONYMS

Api 20 E  Analysis Profile Index 20 Enterobacteraceae
APW    Alkaline Peptone Water
CPHL   Central Public Health Laboratory
BE     Bacteriology Epidemiology
Dept.  Department
D.W.   Distal Water
KIA    Kligler Iron Agar
LIA    Lysine Iron Agar
NCL    National Cholera Laboratory
O1     V.cholerae  Serotype O1
O 139  V.cholerae  Serotype O139
O antigen Somatic antigen
TCBS   Thiosulfate Citrate Bile Salts Sucrose Agar
TSI    Triple Sugar Iron
WHO    World Health Organization
I. Preface

The United States Agency for International Development (USAID) has funded the Primary Health Care Project in Iraq (PHCPI) to assist the Iraqi Ministry of Health (MOH) achieve its strategic goal of quality primary health care (PHC) services in the country. PHCPI supports the MOH in three key components: 1) strengthening health management systems, 2) improving the quality of clinical services, and 3) encouraging community involvement to increase the demand for and use of PHC services. Since March 2011, PHCPI has assisted the MOH to put in place key building blocks to strengthen the health management systems and to support the delivery of quality PHC services at the community and facility levels. PHCPI has worked to build the capacity of the MOH and primary health care centers (PHCCs) to respond to the needs of the Iraqi population and to assist the MOH in achieving its country-level health priorities, such as meeting its Millennium Development Goals (MDGs) by 2015. Interventions have included updating clinical guidelines, training health providers at the PHC level, implementing supportive supervision and quality improvement models and referral systems to improve the quality of primary care services, particularly for maternal and child health.

In October 2013, a modification to PHCPI’s technical scope of work had the project re-focus its efforts to further help the MOH accelerate the achievement of MDGs 4 and 5, reduce child mortality and improving maternal health. Large numbers of displaced Iraqis has caused a rise in cholera cases, especially vulnerable groups. To respond, PHCPI, along with the MOH, has developed a standard operating procedure (SOP) to assist in the upgrading of laboratory diagnosis of cholera for the collection and transportation of stool samples patient isolation, identification on different media and susceptibility tests of Vibrio cholera. An improved and more streamlined process for transportation and diagnosis will allow the MOH to better and more rapidly respond to detected cholera cases, stemming or preventing potential epidemics or outbreaks.
II. Acknowledgement

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III. Introduction

The United States Agency for International Development (USAID) has funded the Primary Health Care Project in Iraq (PHCPI) to assist the Iraqi Ministry of Health (MOH) achieve its strategic goal of quality primary health care (PHC) services in the country. PHCPI supports the MOH in three key components: 1) strengthening health management systems, 2) improving the quality of clinical services, and 3) encouraging community involvement to increase the demand for and use of PHC services. Since March 2011, PHCPI has assisted the MOH to put in place key building blocks to strengthen the health management systems and to support the delivery of quality PHC services at the community and facility levels. PHCPI has worked to build the capacity of the MOH and primary health care centers (PHCCs) to respond to the needs of the Iraqi population and to assist the MOH in achieving its country-level health priorities, such as meeting its Millennium Development Goals (MDGs) by 2015. Interventions have included updating clinical guidelines, training health providers at the PHC level, implementing supportive supervision and quality improvement models and referral systems to improve the quality of primary care services, particularly for maternal and child health.

In October 2013, a modification to PHCPI’s technical scope of work had the project re-focus its efforts to further help the MOH accelerate the achievement of MDGs 4 and 5, reduce child mortality and improving maternal health. Given the rise in Cholera among Iraqis, PHCPI, along with the Ministry of Health (MOH), has developed a standard operating procedure (SOP) to assist in the upgrading of laboratory diagnosis of cholera for the collection and transportation of stool samples patient isolation, identification on different media and susceptibility tests of Vibrio cholera.

Cholera is an infection of the small intestine caused by the bacterium Vibrio cholerae. The main symptoms are watery diarrhea and vomiting. This may result in dehydration and in severe cases grayish-bluish skin. Transmission occurs primarily by drinking water or eating food that has been contaminated by the feces (waste product) of an infected person, including one with no apparent symptoms. The severity of the diarrhea and vomiting can lead to rapid dehydration and electrolyte imbalance, and death in some cases. The primary treatment is oral rehydration therapy, typically with oral rehydration solution, to replace water and electrolytes. If this is not tolerated or does not provide improvement fast enough, intravenous fluids can also be used. Antibacterial drugs are beneficial in those with severe disease to shorten its duration and severity.

Before 1992, of the more than 150 serogroups of Vibrio cholera that have been reported, only the O1 serogroup was associated with epidemic and pandemic cholera. However in late 1992 and early 1993, large outbreaks of cholera due to a newly described serogroup, O139, were reported in India and Bangladesh. This strain, like serogroup O1 V. cholera, produces cholera enterotoxin.

Worldwide, it affects 3–5 million people and causes 100,000–130,000 deaths a year as of 2010. Cholera was one of the earliest infections to be studied by epidemiological methods.

Outbreaks in Iraq

1. In 2007, 4,646 isolates were detected and confirmed for V. cholera O1 serotype (Inaba). There is resistance to antibiotics: methoprem, nalidixic acid and Vibriostatic disc O139 and sensitivity to: Tetracycline, chloramphenicol, Ampicillin, and Ciprofloxacin.
2. In 2012, 363 isolates were detected and confirmed for V. cholera O1 serotype (Ogawa). There is resistance to antibiotics: methoprem, nalidixic acid, chloramphenicol Ciprofloxacin, and Vibriostatic disc O139) and sensitivity to: Tetracycline, Ampicillin, and Azithromycin.

National Cholera Laboratory (NCL)

As a part of the Epidemiology Unit within the Bacteriology Department at the Central Public Health Laboratory, the National Cholera Laboratory (NCL) is responsible for testing suspected specimens (stools and rectal swabs) that are forwarded to them from all of the Iraqi districts (hospitals, central public health facilities) to diagnose the presence of Vibrio cholera.

The NCL is the only reference laboratory in Iraq that is integrated in terms of availability of staff and specialized laboratory equipment and supplies required for cholera diagnosis.

In order to instill proper laboratory knowledge and actions, including diagnosis of cholera bacteria (Vibrio cholera) this Standard Operating Procedure (SOP) has been developed for reference and to be integrated into training programs for lab workers.

IV. Objective

The objective of this SOP is to upgrade the skills of health workers in laboratory diagnosis of cholera through the adherence of protocol for the collection and transportation of stool samples patient isolation, identification on different media and susceptibility tests of Vibrio cholera.

V. Responsible positions

1. PhD. Clinical Microbiology/ Director of Bacteriology Department.
2. M.Sc. or High Diploma Clinical Microbiology / Director of epidemiological unit (NCL).
3. B.Sc. and Diploma of Microbiology (Staff of NCL).

VI. Functions

A. Collection and transportation of a stool sample.
B. Isolation of suspected Vibrio cholera on different media.
C. Identification of Vibrio cholera by classical and advance methods.
D. Susceptibility tests of Vibrio cholera.
E. Shipment of isolate to a reference lab for confirmation.
1. **Diagnosis of Vibrio cholera**

1.1. **Sampling**

* Materials Required (See Appendix 1)

1.1.1. **Stool**

Place a small quantity of stool in a wide mouth clean bottle. If mucus or flakes are present in the stool, they must be included in the provided stool sample.

1.1.2. **Rectal swab**

Rectal swabs - only if stool is not possible.

1.2. **Collection and Transportation**

1.2.1. **Collection and transport of stool specimens**

A. Fecal (stool) specimens should be collected in the early stages of the diarrheal disease, when pathogens are present in their highest number, and preferably before antimicrobial treatment is started.

B. The specimen should be collected in the morning to reach the laboratory before noon, so that it can be processed the same day. Formed stools should be rejected.

C. The patient should be asked to deliver the specimen to the clinic immediately after collection. If it is not possible for the specimen to be delivered to the laboratory within 2 hours of its collection, a small amount of the fecal specimen (together with mucus, blood and epithelial threads, if present) should be collected on two or three swabs and placed in a container with transport medium (Cary–Blair, Stuart or Amies).

**Note:** For cholera and other Vibrio spp., alkaline peptone water is an excellent transport (and enrichment) medium. Pathogens may survive in such media for up to 1 week, even at room temperature, although refrigeration is preferable.

1.2.2. **Collecting rectal swabs**

A. Moisten a cotton-tipped swab with sterile water. Insert the swab through the rectal sphincter, rotate, and withdraw.

B. Examine the swab for fecal staining and repeat the procedure until sufficient staining is evident.

C. The number of swabs to be collected will depend on the number and types of investigation required.

D. Place the swab in an empty sterile tube with a cotton plug or screw-cap, if it is to be processed within 1–2 hours. If the swab must be kept for longer than 2 hours, place it in transport medium.
2. Isolation and identification of Vibrio cholera

* Materials Required (See Appendix 2)

Isolation and identification of V. cholera can be greatly enhanced when optimal laboratory media and techniques are employed. The methods presented here are intended to be economical and to offer laboratory workers some flexibility in choice of protocol and media. Laboratories that do not have sufficient resources to adopt the methods should consider sending the specimens or isolates to other laboratory facilities that routinely perform these procedures.

2.1. Isolation Methods

Because the cultural and biochemical characteristics of these two serogroups are identical, the isolation and identification methods described below apply to both O1 and O139. Although V. cholera will grow on a variety of commonly used agar media, isolation from fecal specimens is more easily accomplished with specialized media as follows:

2.1.1. Enrichment in alkaline peptone water (APW)

APW is recommended as an enrichment broth it can enhance the isolation of V. cholera when few organisms are present.

A. Take specimens from convalescent patients and asymptomatic carriers. Vibrio spp. grows rapidly in APW. And at 6 to 8 hours will be present in greater numbers than non-Vibrio organisms.
B. APW can be inoculated with liquid stool, fecal suspension, or a rectal swab. The stool inoculums should not exceed 10% of the volume of the broth.
C. Incubate the tube with the cap loosened at (35° to 37°C) for (6 to 8 hours).

2.1.2. Selective and non-selective media

After incubation, subculture on TCBS (thiosulfate citrate bile salts sucrose agar) is the selective agar medium of choice and on MacConkey agar and blood agar.

A. One to two loopfuls of APW from the surface and topmost portion of the broth, since vibrio's preferentially grow in this area. Do not shake or mix the tube before subculturing.
B. Incubate the agar plates for the isolation V. cholera at (35° to 37°C) for (18-24 hours). Vibrio. Strains grow as pale, non-lactose-fermenting colonies on MacConkey agar. On TCBS agar V. cholera grows as medium-sized convex, smooth, yellow colonies, and on Blood agar the colonies show Beta heamolysis due to heamolsin enzyme (Figure -1)
2.2. Gram stain
Examining overnight growth from any media by Gram stain will demonstrate typical small, curved gram-negative rods (depends on leaflet of kits).

Note: Staining with crystal violet only is a more rapid technique and will still demonstrate the cell morphology typical of Vibrio spp.

2.3. Wet mount
Dark-field and phase-contrast microscopy have been used for screening suspected isolates of V. cholera. With these techniques:

Saline suspensions are microscopically examined for the presence of organisms with typical small, curved rods (comma shape) and darting (“shooting star”) motility.

2.4. Procedure for oxidase test
A. Place 2–3 drops of the oxidase reagent (1% tetramethyl- raphenylenediamine) on a piece of filter paper in a Petri dish.
B. Pick up a small amount of fresh growth from the MacConkey or nutrient agar (Not from TCBS) with a platinum (not Nichrome) loop or a clean wooden stick or toothpick. Smear the growth across the moistened part of the filter paper.
C. A positive reaction is indicated by the appearance of a dark purple color on the paper within 10 seconds (figure-2).
Among the Gram-negative rods Vibrio, Aeromonas, Plesiomonas, Pseudomonas, and Alcaligenes are oxidase-positive; all Enterobacteriaceae are oxidase-negative. The oxidase reagent should be tested regularly with positive and negative control strains.

(Figure - 2) vibrio cholera is positive of oxidase test

2.5. Procedure for string test
A. Place a drop of 0.5% aqueous solution of sodium deoxycholate on a slide and mix a small amount of growth from the MacConkey agar into the drop.
B. A positive reaction is indicated by the suspension within 60 seconds: it loses its turbidity and becomes mucoid; a “mucoid string” can be drawn when the loop is slowly lifted away from the drop (figure-3).

(Figure - 3) Vibrio cholera is positive of string test
2.6. Additional biochemical screening tests

The growth inoculum on:

A. Kligler iron agar (KIA) or triple sugar iron agar (TSI), semisolid manitol, peptone water for indol, simmon citrate, urea agar lysine iron agar (LIA), Ornithine, aesculin hydrolysis, Voges–Proskauer, and fermentation of Sucrose, mannitol, arabinose.
B. Incubate overnight at (35° to 37°C) for (18-24 hours).
C. Examine the reactions after overnight incubation. (Figure -4) and (table-1).

(Figure - 4)
- Kligler iron agar (yellow butt) Acid / (alkaline slant) Alk no gas no H2S.
- Peptone water for indol: positive.
- Semi solid manitol: motile / manitol ferment.
- Simmon citrate: positive.
- Urea: negative.
(Table-1) Biochemical reactions of *vibrio cholera*

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Oxidase</th>
<th>KIA butt/slant</th>
<th>MIL</th>
<th>Ornithine</th>
<th>Citrate</th>
<th>Sucrose</th>
<th>Mannitol</th>
<th>Arabinose</th>
<th>Aesculin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vibrio cholera</td>
<td>+</td>
<td>K/A</td>
<td>+/+/+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

KIA: Kligler’s iron agar; MIL: motility–indole–lysine medium; Ornithine; aesculin hydrolysis; K: alkaline; A: acid; G: gas; fermentation of sugar (Sucrose, Mannitol, Arabinose).

2.7. API 20 E

Procedure depended on leaflet of kits (BioMerioux Company), See (Figure-5) to show positive or Negative reaction.

(Figure - 5) API 20 E for Vibrio cholera
3. Serologic Identification of V. cholera O1 and O139

* Materials Required (See Appendix 3)

3.1. Presumptive identification using O1 and O139 antisera

For slide agglutination testing with polyvalent O1 or O139 antisera.

A. Fresh growth of suspected V. cholera from a nonselective agar medium should be used. Using growth from TCBS agar may result in false-negative reactions.
B. Usually after 5 to 6 hours of incubation, growth on the surface of the slant is sufficient to perform slide serology with antisera; if not, incubate for a longer period.
C. If the isolate does not agglutinate in O1 antiserum, test in O139 antiserum.
D. If it is positive in the polyvalent O1 or in the O139 antiserum, it may be reported as presumptive V. cholera O1 or O139.
E. Presumptive V. cholera O1 isolates should be tested in monovalent Ogawa and Inaba antisera.

3.2. Confirmation of V. cholera O1 using Inaba and Ogawa antisera

Important notes:

A. The O1 serogroup of V. cholera has been further divided into three serotypes, Inaba, Ogawa, and Hikojima (very rare).
B. Serotype identification is based on agglutination in monovalent antisera to type-specific O antigens.
C. A positive reaction in either Inaba or Ogawa antiserum is sufficient to confirm the identification of a V. cholera O1 isolate.
D. Isolates that agglutinate weakly or slowly with serogroup O1 antiserum but do not agglutinate with either Inaba or Ogawa antiserum are not considered to be serogroup O1.

3.3. Slide agglutination procedures

A. Agglutination tests for V. cholera somatic O antigens may be carried out in a petri dish or on a clean glass slide.
B. Use an inoculating loop or needle, sterile applicator stick, or toothpick to remove a portion of the growth from the surface of KIA, TSI, or other nonselective agar medium.
C. Emulsify the growth in two small drops of physiological saline and mix thoroughly.
D. Add a small drop of antiserum to one of the suspensions. Usually approximately equal volumes of antiserum and growth suspension are mixed.
E. Mix the suspension and antiserum well and then tilt the slide back and forth to observe for agglutination.
F. If the reaction is positive, clumping will appear within 30 seconds to 1 minute. Examine the saline suspension carefully to ensure that it does not show clumping due to autoagglutination.
G. If autoagglutination occurs, the culture is termed “rough” and cannot be serotyped.
3.4. Confirmation of V. cholera O139

A. A suspected V. cholera isolate that reacts in O139 antiserum but not in polyvalent O1 antiserum should be sent to a reference laboratory.
B. Confirmation of V. cholera O139 includes testing for production of cholera enterotoxin and verification of the O139 antigen.

Note: No serotypes have been identified in the O139 serogroup.

4. Bio typing

Materials Required (See Appendix 4)
There are two biotype of V. cholera O1 into classical and El Tor.

Differentiation between of V. cholera O1, classical and El Tor biotypes is not necessary for treatment or control, but for some of the isolates it should be done by one of the following tests (See below).

<table>
<thead>
<tr>
<th></th>
<th>Classical</th>
<th>El Tor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Haemagglutination</td>
<td>Positive reaction, growth</td>
</tr>
<tr>
<td>2</td>
<td>Polymyxin B (50 units)</td>
<td>Resistant</td>
</tr>
<tr>
<td>3</td>
<td>Voges–Proskauer</td>
<td>Positive reaction, growth</td>
</tr>
<tr>
<td>4</td>
<td>Haemolysis</td>
<td>Variable</td>
</tr>
</tbody>
</table>

4.1. The indirect haemagglutination test

A. Prepare a 2.5% suspension of chicken or sheep red blood cells by repeated centrifugation and dilution in saline.
B. Divide a clean glass slide into several squares with a pencil and place a loopful (3 mm) of the red cell suspension in each square.
C. Place a small portion of the growth from an agar or KIA slant in each red cell suspension and mix well. Clumping of the red cells occurs within 30–60 seconds with strains of the El Tor biotype.

Known haemagglutinating (El Tor) and non-haemagglutinating strains should be used as controls for each new suspension of red cells. Newly isolated strains of classical biotypes are usually negative in the test.
4.2. Polymyxin B susceptibility test

A. Spread a loopful of overnight peptone water culture of the isolate on a Mueller–Hinton agar.
B. Place a susceptibility disk containing 50 units of polymyxin B in the middle of the culture.
C. Place the plate in the refrigerator for 1 hour.
D. Incubate the plate overnight at (35° to 37°C) for (18-24 hours).

Known strains of classical and El Tor biotypes should always be included as controls. Classical strains are sensitive to polymyxin B and a clear inhibitory zone is observed around the disk. The El Tor strains are resistant and no inhibitory zone is formed.

4.3. Voges–Proskauer

This test is done by API 20 E kits.

4.4. Haemolysis

These test shows on Blood agar (overnight growth) see (Figure - 1).

5. Susceptibility tests

* Materials Required (See Appendix 5)

5.1. Kirby-Bauer method

The disc-diffusion method, originally described in 1966, is well standardized and has been widely evaluated. Official agencies have recommended it, with minor modifications, as a reference method which could be used as a routine technique in the clinical laboratory.

5.2. Mueller–Hinton agar

A. Mueller–Hinton agar should be prepared from a dehydrated base according to the manufacturer’s instructions. The medium should be such that control zone sizes within the published limits are produced. It is important not to overheat the medium.
B. Cool the medium to 45–50 C0 and pour into the plates. Allow to set on a level surface, to a depth of approximately 4mm. A 9-cm plate requires approximately 25 ml of medium.
C. When the agar has solidified, dry the plates for immediate use for 10–30 minutes at 35 C0 by placing them in the upright position in the incubator with the lids tilted.
D. Any unused plates may be stored in a plastic bag, which should be sealed and placed in the refrigerator. Plates stored in this way will keep for 2weeks.
5.3. **Antimicrobial discs**
A. Any commercially available discs with the proper diameter and potency can be used.
B. Stocks of antimicrobial discs should preferably be kept at -20 degrees Celsius; the freezer compartment of a home refrigerator is convenient.
C. A small working supply of discs can be kept in the refrigerator for up to 1 month.
D. On removal from the refrigerator, the containers should be left at room temperature for about 1 hour to allow the temperature to equilibrate.

This procedure reduces the amount of condensation that occurs when warm air reaches the cold container.

5.4. **Turbidity standard**
A. Prepare the turbidity standard by pouring 0.6 ml of a 1% (10 g/l) solution of barium chloride dihydrate into a 100-ml graduated cylinder.
B. Filling to 100 ml with 1% (10 ml/l) sulfuric acid.
C. The turbidity standard solution should be placed in a tube identical to the one used for the broth sample.

It can be stored in the dark at room temperature for 6 months, provided it is sealed to prevent evaporation.

5.5. **Swabs**
A. A supply of cotton wool swabs on wooden applicator sticks should be prepared.
B. They can be sterilized in tins, culture tubes, or on paper, either in the autoclave or by dry heat.

5.6. **Procedure**
A. To prepare the inoculum from the primary culture plate.
B. Touch with a loop the tops of each of 3–5 colonies, of similar appearance, of the organism to be tested (figure - 6).

(Figure - 6) chooses of colonies
C. Transfer this growth to a tube of saline or D.W. (figure - 7)

(Figure - 7)

D. When the inoculum has to be made from a pure culture, a loopful of the Confluent growth is similarly suspended in saline.
E. Compare the tube with the turbidity standard and adjust the density of the test suspension to that of the standard by adding more bacteria or more sterile saline.
F. Proper adjustment of the turbidity of the inoculum is essential to ensure that the resulting lawn of growth is confluent or almost confluent.
G. Inoculate the plates by dipping a sterile swab into the inoculum.
H. Remove excess inoculum by pressing and rotating the swab firmly against the side of the tube above the level of the liquid.
I. Reach the swab all over the surface of the medium three times.
J. Rotating the plate through an angle of 60 C0 after each application.
K. Finally, pass the swab Round the edge of the agar surface.
L. Leave the inoculum to dry for a few Minutes at room temperature with the lid closed (Figure 8).

(Figure - 8)
M. The antimicrobial discs may be placed on the inoculated plates using a pair of sterile forceps. It is convenient to use a template (Figure - 9) to place the disc uniformly.

(Figure - 9)

Notes:
- A sterile needle tip may also be used to place the antimicrobial discs on the inoculated plate.
- A maximum of seven discs can be placed on a 9–10 cm plate. Six discs may be spaced evenly, approximately 15 mm from the edge of the plate, and 1 disc placed in the center of the plate.

N. Each disc should be gently pressed down to ensure even contact with the medium.
O. The plates should be placed in an incubator at 35 °C within 30 minutes of preparation.
P. Incubate at (35°C to 37°C) for (18-24 hours).
Q. After overnight incubation, the diameter of each zone (including the diameter of the disc) should be measured and recorded in mm. The results should then be interpreted according to the critical diameters shown in (Appendix 6). The measurements can be made with a ruler on the under-surface of the plate without opening the lid (Figure - 10).

(Figure - 10) measurement of inhibition zone
5.7. Drugs of choice for *Vibrio cholera*

<table>
<thead>
<tr>
<th>#</th>
<th>Antibiotic</th>
<th>Concentration</th>
<th>Inhibition Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tetracycline</td>
<td>30µg</td>
<td>18-25 mm</td>
</tr>
<tr>
<td>2</td>
<td>Ampicillin</td>
<td>10mg</td>
<td>16-22 mm</td>
</tr>
<tr>
<td>3</td>
<td>Chloramphenicol</td>
<td>30 µg</td>
<td>21-27 mm</td>
</tr>
<tr>
<td>4</td>
<td>Trimethoprim –Sulfamethoxazole</td>
<td>1.25/ 23.75 µg</td>
<td>23-29 mm</td>
</tr>
<tr>
<td>5</td>
<td>Erythromycin</td>
<td>15 mg</td>
<td>22-30 mm</td>
</tr>
<tr>
<td>6</td>
<td>Doxycycline</td>
<td>30 mg</td>
<td>18-24 mm</td>
</tr>
<tr>
<td>7</td>
<td>Ciprofloxacin</td>
<td>5 µg</td>
<td>30-40 mm</td>
</tr>
<tr>
<td>8</td>
<td>Nalidixic acid</td>
<td>30 µg</td>
<td>22-28 mm</td>
</tr>
</tbody>
</table>

*Muller Hinton Agar, 35°C ± 16-18 hr., disc diffusion zone 0.5 McFarland Standard*

6. Biosafety in Medical Laboratories

* Materials Required (See Appendix 7)

6.1. Introduction

Throughout this manual, references are made to the relative hazards of infective microorganisms by risk group (WHO Risk Groups 1, 2, 3 and 4). This risk group classification is to be used for laboratory work only.

*Vibrio cholera* is human pathogens and can cause serious disease. Always use Biosafety Level 2 practices and extreme caution when transferring and handling strains of these species. Work in a biological safety cabinet when handling large amounts of cells. Disinfect or dispose of all plastic ware and glassware that come in contact with the cultures in a safe manner.

6.2. Facts:

Cholera is an acute intestinal infection caused by the bacterium *Vibrio cholera*. Cholera is spread through:

A. Contaminated food and water, including (undercooked or raw shellfish and fish).
B. Eating or drinking food or water contaminated by infected person’s exposure to feces of an infected person.
C. Cholera can spread very quickly in areas where sewage and drinking water are poorly treated.
D. Humans are a reservoir for the disease as are animals around aquatic environment.
E. The bacterium has been found in birds and herbivores surrounding freshwater lakes and rivers as well as in algae, copepods (zooplankton), crustaceans and insects.
6.3. Risk Group Classification:

A. Risk group 2. Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials, animals, or cultures.
B. Lab coat. Gloves when direct skin contact with infected materials or animals is unavoidable.
C. Eye protection must be used where there is a known or potential risk to splashes.
D. All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
E. The use of needles, syringes, and other sharp objects should be strictly limited.
F. Additional precautions should be considered with work involving animals or large scale activities.

6.4. Susceptibility to Disinfectants:

A. Susceptible to 2-5% phenol, 1% sodium hypochlorite, %4 formaldehyde, 2% glutaraldehyde, 70% ethanol, 70% propanol, 2% peracetic acid, 3-6% hydrogen peroxide, and 0.16% iodine.
B. Vibrio cholera is sensitive to cold (loss of viability after a cold shock at 0ºC). C-Cholera can survive in well water for 7.5 ± 1.9 days and the El Tor biotype can survive 19.3 ± 5.1 days. It has been found on fomites at room temperature for 1-7 days.

6.5. Laboratory-Acquired Infections

12 cases of infection with 4 deaths were reported up to 1979. The deaths were associated with mouth pipetting.

A. Contact with infectious feces and contaminated laboratory laundry.
B. Feces and naturally or experimentally infected animals are the main specimens which contain the infectious agent.
C. The primary hazards when working with this agent are ingestion and accidental parenteral inoculation. The risk of aerosol exposure is not known.
D. The risk of infection is higher in people who don't have gastric acid (i.e. due to gastrectomy or achlorhydria).

7. Shipment / Packaging, labelling and documentation / requirements for infectious substances in Category B

* Materials Required (See Appendix 8)

7.1. Packaging

A. The triple packaging system continues to apply, including for local surface transport.
B. Testing documents are not required, however. It may be possible to source packaging's locally rather than finding an authorized supplier.
C. Provided that the packaging manufacturer and the shipper can comply fully with the requirements.
D. As for P620, there is no comprehensive list of suppliers of packaging's that comply with Packing Instruction P650. However, an Internet search using a suitable international or national search engine usually provides appropriate information, as well as access to national regulations. Search phrases such as “UN packaging” and “UN infectious substance packaging” produce extensive results. Carriers and forwarding agents should also be able to supply details of local suppliers or local companies that can provide such information.

To ensure correct preparation for transport, packaging manufacturers and subsequent distributors shall provide clear instructions to the consignor or persons preparing packages (e.g. patients) on how the packaging should be filled and closed.

- No primary receptacle shall exceed 1L and the outer packaging must not contain more than 4 L (for liquids)
- Except for packages containing body parts, organs or whole bodies, the outer packaging must not contain more than 4 kg (for solids).

For surface transport there is no maximum quantity per package. For air transport:

(Figure - 11) Example of the triple packaging system for the packing and labelling of Category infectious substances (Figure kindly provided by IATA, Montreal, Canada).

Provided all the requirements of P650 are met, there are no other transport requirements. P650 incorporates all that is needed to make a shipment for Category B infectious substances.
7.2. Marking

Each package shall display the following information for air:
A. the shipper’s (sender’s, consignor’s) name, address and telephone number for air: the telephone number of a responsible person, knowledgeable about the shipment the receiver’s (consignee’s) name, address and telephone number.
B. the proper shipping name (“BIOLOGICAL SUBSTANCE, CATEGORY B”) adjacent to the diamond-shaped mark shown in Figure 10 temperature storage requirements (optional).
C. The marking shown in Figure 10 is used for shipments of Category B infectious substances.
D. Minimum dimension: the width of the line forming the square shall be at least 2 mm, and the letters and numbers shall be at least 6 mm high.
E. For air transport, each side of the square shall have a length of at least 50 mm.
F. Colour: none specified, provided the mark is displayed on the external surface of the outer packaging on a background of contrasting colour and that it is clearly visible and legible The words “BIOLOGICAL SUBSTANCE, CATEGORY B” in letters at least 6 mm high shall be displayed adjacent to the mark.

(Figure – 12) Marking for infectious substances of Category B and for genetically modified microorganisms or organisms that meet the definition of an infectious substance, Category B

Note: For air transport:
When dry ice (solid carbon dioxide) is used, the label shown below shall be applied for cryogenic liquids the labels shown in Figures 13 and 14 shall also be affixed.

Label name: Miscellaneous dangerous substances
Minimum dimensions: 100 × 100 mm
(for small packages: 50 × 50 mm)
No. of labels per package: 1
Color: Black and white
Figure 13: Hazard label for certain noninfectious genetically modified microorganisms and organisms (UN 3245) and for carbon dioxide, solid (dry ice) (UN 1845); substances packed in dry ice shall bear this label in addition to the primary risk label (Figure 12).

Label name: Non flammable, non-toxic gas
Minimum dimensions: 100 × 100 mm
(for small packages: 50 × 50 mm)
# of labels per package: 1
Color: Green and white or green and black

Figure 14: Hazard label for liquid nitrogen; substances packed using liquid nitrogen shall bear this label in addition to the primary risk label (Figure 12).

Label name: Cryogenic liquid
Minimum dimensions: Standard A7: 74 × 105 mm
# of labels per package: 1
Color: Green and white

7.3. Documentation

Dangerous goods documentation (including a shipper’s declaration) is not required for Category B infectious substances. The following shipping documents are required. To be prepared and signed by the shipper (sender, consignor):

A. for international shipments: a packing list/proforma invoice that includes the shipper's and the receiver's address, the number of packages, detail of contents, weight, value (Note: the statement “no commercial value” shall appear if the items are supplied free of charge) an import and/or export
B. To be prepared by the shipper or the shipper’s agent:
   An air waybill for air transport or equivalent documents for road, rail and sea journeys.

Appendices

Appendix 1
1. Clean Cups
2. Rectal Swabs
3. Cary-Blair Transport Media Swabs
4. Refrigerator
5. Screw Cups

Appendix 2

1. Alkaline Peptone Water Enrichment Media (APW)
2. TCBS Selective Agar Media
3. Incubator
4. Loop
5. MacConkey Agar
6. Blood Agar
7. Gram Stain Kit
8. Microscope
9. Oxidase Reagent
10. Wooden Stick
11. Filter Paper
12. Sodium Deoxycholate

Appendix 3

1. Api 20 E Kit
2. Ogawa, Inaba, Polyvalent (O1) Antisera

Appendix 4

1. 2.5% Suspension of Chicken or Sheep Red Blood Cells
2. Muller – Hinton Agar

Appendix 5

1- Antimicrobial Disc
2- 1% Solution of Barium Chloride Dihydrate
3- Sterile Swabs
4- 1% Sulphuric acid
5- Muller-Hinton Agar
## Appendix 6

**Disk Diffusion: Quality Control Ranges for Nonfastidious Organisms (Unsupplemented Mueller-Hinton Medium)**

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Disk Content</th>
<th>Escherichia coli ATCC® 25922</th>
<th>Staphylococcus aureus ATCC® 29233</th>
<th>Pseudomonas aeruginosa ATCC® 27853</th>
<th>Escherichia coli ATCC® 35218&lt;sup&gt;ab&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loracarbef</td>
<td>30 μg</td>
<td>23-29</td>
<td>23-31</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mecillinam</td>
<td>10 μg</td>
<td>24-30</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Meropenem</td>
<td>10 μg</td>
<td>28-34</td>
<td>29-37</td>
<td>27-33</td>
<td>–</td>
</tr>
<tr>
<td>Methicillin</td>
<td>5 μg</td>
<td>–</td>
<td>17-22</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mezlocillin</td>
<td>75 μg</td>
<td>23-29</td>
<td>–</td>
<td>–</td>
<td>19-25</td>
</tr>
<tr>
<td>Minocycline</td>
<td>30 μg</td>
<td>19-25</td>
<td>25-30</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Moxalactam</td>
<td>30 μg</td>
<td>28-35</td>
<td>18-24</td>
<td>17-25</td>
<td>–</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>5 μg</td>
<td>28-35</td>
<td>17-28</td>
<td>17-25</td>
<td>–</td>
</tr>
<tr>
<td>Nafcillin</td>
<td>1 μg</td>
<td>–</td>
<td>16-22</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>30 μg</td>
<td>22-28</td>
<td>22-30</td>
<td>17-23</td>
<td>–</td>
</tr>
<tr>
<td>Neomycin</td>
<td>30 μg</td>
<td>22-30</td>
<td>22-31</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>300 μg</td>
<td>20-25</td>
<td>18-22</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>10 μg</td>
<td>28-35</td>
<td>17-28</td>
<td>22-29</td>
<td>–</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>5 μg</td>
<td>29-33</td>
<td>24-28</td>
<td>17-21</td>
<td>–</td>
</tr>
<tr>
<td>Omadacycline</td>
<td>30 μg</td>
<td>22-28</td>
<td>22-30</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>1 μg</td>
<td>–</td>
<td>18-24</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Penicillin</td>
<td>10 units</td>
<td>–</td>
<td>26-37</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>100 μg</td>
<td>24-30</td>
<td>–</td>
<td>25-33</td>
<td>12-18</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>100/10 μg</td>
<td>24-30</td>
<td>27-36</td>
<td>25-33</td>
<td>24-30</td>
</tr>
<tr>
<td>Plazomycin</td>
<td>30 μg</td>
<td>21-27</td>
<td>19-25</td>
<td>15-21</td>
<td>–</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>300 units</td>
<td>13-19</td>
<td>–</td>
<td>14-18</td>
<td>–</td>
</tr>
<tr>
<td>Quinupristin-dalfopristin</td>
<td>15 μg</td>
<td>–</td>
<td>21-28</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Razupenem</td>
<td>10 μg</td>
<td>21-26</td>
<td>–&lt;sup&gt;a&lt;/sup&gt;</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Rifampin</td>
<td>5 μg</td>
<td>8-10</td>
<td>26-34</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Solithromycin</td>
<td>15 μg</td>
<td>–</td>
<td>22-30</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>5 μg</td>
<td>30-38</td>
<td>27-33</td>
<td>21-29</td>
<td>–</td>
</tr>
<tr>
<td>Streptomycin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10 μg</td>
<td>12-20</td>
<td>14-22</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sulfoxazole&lt;sup&gt;c&lt;/sup&gt;</td>
<td>250 μg or 300 μg</td>
<td>15-23</td>
<td>24-34</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tedizolid</td>
<td>20 μg</td>
<td>–</td>
<td>22-29</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>30 μg</td>
<td>–</td>
<td>15-21</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Televancin</td>
<td>30 μg</td>
<td>–</td>
<td>16-20</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Telithromycin</td>
<td>15 μg</td>
<td>–</td>
<td>24-30</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30 μg</td>
<td>18-25</td>
<td>24-30</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>75 μg</td>
<td>24-30</td>
<td>–</td>
<td>21-27</td>
<td>6</td>
</tr>
<tr>
<td>Ticarcillin-clavulanate</td>
<td>75/10 μg</td>
<td>24-30</td>
<td>29-37</td>
<td>20-28</td>
<td>21-25</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>15 μg</td>
<td>20-27</td>
<td>20-25</td>
<td>9-13</td>
<td>–</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>10 μg</td>
<td>18-26</td>
<td>19-29</td>
<td>20-26</td>
<td>–</td>
</tr>
<tr>
<td>Trimethoprim&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5 μg</td>
<td>21-28</td>
<td>19-26</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.25/23.75 μg</td>
<td>23-29</td>
<td>24-32</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Trospectomycin</td>
<td>30 μg</td>
<td>10-16</td>
<td>15-20</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Trovafloxacin</td>
<td>10 μg</td>
<td>29-36</td>
<td>29-35</td>
<td>21-27</td>
<td>–</td>
</tr>
<tr>
<td>Ullitoxacin</td>
<td>5 μg</td>
<td>32-38</td>
<td>20-26</td>
<td>27-33</td>
<td>–</td>
</tr>
<tr>
<td>(prulifloxacin)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5 μg</td>
<td>–</td>
<td>17-21</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>30 μg</td>
<td>–</td>
<td>17-21</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Antimicrobial Agent</td>
<td>Disk Content</td>
<td>Escherichia coli ATCC® 25922</td>
<td>Staphylococcus aureus ATCC® 29219</td>
<td>Pseudomonas aeruginosa ATCC® 27853</td>
<td>Escherichia coli ATCC® 25219&lt;sup&gt;10&lt;/sup&gt;</td>
</tr>
<tr>
<td>---------------------</td>
<td>-------------</td>
<td>------------------------------</td>
<td>----------------------------------</td>
<td>----------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>Amikacin</td>
<td>30 µg</td>
<td>19–26</td>
<td>20–26</td>
<td>18–26</td>
<td>17–22</td>
</tr>
<tr>
<td>Amoxicillin-clavulanate</td>
<td>20/10 µg</td>
<td>18–24</td>
<td>28–36</td>
<td>–</td>
<td>6</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>10 µg</td>
<td>16–22</td>
<td>27–35</td>
<td>–</td>
<td>13–19</td>
</tr>
<tr>
<td>Ampicillin-sulbactam</td>
<td>10/10 µg</td>
<td>19–24</td>
<td>29–37</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>15 µg</td>
<td>–</td>
<td>21–26</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Azlocillin</td>
<td>75 µg</td>
<td>–</td>
<td>20–28</td>
<td>24–30</td>
<td>–</td>
</tr>
<tr>
<td>Aztreomycin</td>
<td>30 µg</td>
<td>28–36</td>
<td>–</td>
<td>23–29</td>
<td>–</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>100 µg</td>
<td>23–29</td>
<td>–</td>
<td>18–24</td>
<td>–</td>
</tr>
<tr>
<td>Cefadroxin</td>
<td>30 µg</td>
<td>23–27</td>
<td>27–31</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cefamandole</td>
<td>30 µg</td>
<td>26–32</td>
<td>26–34</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>30 µg</td>
<td>21–27</td>
<td>29–35</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cefdinir</td>
<td>5 µg</td>
<td>24–28</td>
<td>25–32</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cefditoren</td>
<td>5 µg</td>
<td>22–28</td>
<td>20–28</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cefepime</td>
<td>30 µg</td>
<td>31–37</td>
<td>23–29</td>
<td>24–30</td>
<td>–</td>
</tr>
<tr>
<td>Cefetamet</td>
<td>10 µg</td>
<td>24–29</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cefixime</td>
<td>5 µg</td>
<td>23–27</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cefmetazole</td>
<td>30 µg</td>
<td>26–32</td>
<td>25–34</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cefonicid</td>
<td>30 µg</td>
<td>25–29</td>
<td>22–29</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>75 µg</td>
<td>28–34</td>
<td>24–33</td>
<td>23–29</td>
<td>–</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>30 µg</td>
<td>29–35</td>
<td>25–31</td>
<td>18–22</td>
<td>–</td>
</tr>
<tr>
<td>Cefotetan</td>
<td>30 µg</td>
<td>28–34</td>
<td>17–23</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>30 µg</td>
<td>23–29</td>
<td>23–29</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cefpodoxime</td>
<td>10 µg</td>
<td>23–28</td>
<td>19–25</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cefprozil</td>
<td>30 µg</td>
<td>21–27</td>
<td>27–33</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>30 µg</td>
<td>26–34</td>
<td>26–35</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cefuroxime-avibactam&lt;sup&gt;f&lt;/sup&gt;</td>
<td>30/15 µg</td>
<td>27–34</td>
<td>25–34</td>
<td>17–26</td>
<td>27–35</td>
</tr>
<tr>
<td>Cefazidime-avibactam&lt;sup&gt;g&lt;/sup&gt;</td>
<td>30/20 µg</td>
<td>27–35</td>
<td>16–22</td>
<td>25–31</td>
<td>28–35</td>
</tr>
<tr>
<td>Cefibutin</td>
<td>30 µg</td>
<td>27–35</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cefloxicime</td>
<td>30 µg</td>
<td>30–36</td>
<td>27–35</td>
<td>12–17</td>
<td>–</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>30 µg</td>
<td>30–36</td>
<td>26–34</td>
<td>24–30</td>
<td>–</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>30 µg</td>
<td>29–35</td>
<td>22–28</td>
<td>17–23</td>
<td>–</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>30 µg</td>
<td>20–26</td>
<td>27–35</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>30 µg</td>
<td>15–21</td>
<td>29–37</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30 µg</td>
<td>21–27</td>
<td>19–26</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cinocaxin</td>
<td>100 µg</td>
<td>26–32</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5 µg</td>
<td>30–40</td>
<td>22–30</td>
<td>25–33</td>
<td>–</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>15 µg</td>
<td>–</td>
<td>24–32</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Clindamycin&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5 µg</td>
<td>31–40</td>
<td>27–37</td>
<td>27–35</td>
<td>–</td>
</tr>
<tr>
<td>Clindamycin&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2 µg</td>
<td>–</td>
<td>24–30</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Colistin</td>
<td>10 µg</td>
<td>11–17</td>
<td>11–17</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dilithromycin</td>
<td>15 µg</td>
<td>–</td>
<td>18–26</td>
<td>–</td>
<td>–</td>
</tr>
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<td>Doripenem</td>
<td>10 µg</td>
<td>27–35</td>
<td>33–42</td>
<td>28–35</td>
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<tr>
<td>Doxycycline</td>
<td>30 µg</td>
<td>18–24</td>
<td>23–29</td>
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</tr>
<tr>
<td>Eravacycline</td>
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<td>16–23</td>
<td>19–26</td>
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</tr>
<tr>
<td>Ertapenem</td>
<td>10 µg</td>
<td>29–36</td>
<td>24–31</td>
<td>13–21</td>
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</tr>
<tr>
<td>Erythromycin&lt;sup&gt;2&lt;/sup&gt;</td>
<td>15 µg</td>
<td>–</td>
<td>22–30</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Faropenem</td>
<td>5 µg</td>
<td>20–26</td>
<td>27–34</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Fleroxacin</td>
<td>5 µg</td>
<td>26–34</td>
<td>21–27</td>
<td>12–20</td>
<td>–</td>
</tr>
<tr>
<td>Fosfomycin&lt;sup&gt;1&lt;/sup&gt;</td>
<td>200 µg</td>
<td>22–30</td>
<td>25–33</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>10 µg</td>
<td>–</td>
<td>24–32</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Garenoxacin</td>
<td>5 µg</td>
<td>28–35</td>
<td>30–36</td>
<td>19–25</td>
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</tr>
<tr>
<td>Gatofloxacin</td>
<td>5 µg</td>
<td>30–37</td>
<td>27–33</td>
<td>20–28</td>
<td>–</td>
</tr>
<tr>
<td>Gemifloxacin</td>
<td>5 µg</td>
<td>29–36</td>
<td>27–33</td>
<td>19–25</td>
<td>–</td>
</tr>
<tr>
<td>Gentamicin&lt;sup&gt;1&lt;/sup&gt;</td>
<td>10 µg</td>
<td>19–26</td>
<td>19–27</td>
<td>17–23</td>
<td>–</td>
</tr>
<tr>
<td>Greepafloxacin</td>
<td>5 µg</td>
<td>28–36</td>
<td>26–31</td>
<td>20–27</td>
<td>–</td>
</tr>
<tr>
<td>Iloprin</td>
<td>5 µg</td>
<td>14–22</td>
<td>25–33</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Imipenem</td>
<td>10 µg</td>
<td>26–32</td>
<td>20–28</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>30 µg</td>
<td>17–25</td>
<td>19–26</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>5 µg</td>
<td>29–37</td>
<td>25–30</td>
<td>19–26</td>
<td>–</td>
</tr>
<tr>
<td>Linezolid</td>
<td>30 µg</td>
<td>–</td>
<td>25–32</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lincofloxacin</td>
<td>30 µg</td>
<td>–</td>
<td>25–30</td>
<td>19–26</td>
<td>–</td>
</tr>
<tr>
<td>Lorimoxacin</td>
<td>10 µg</td>
<td>–</td>
<td>25–31</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lineofloxacin</td>
<td>10 µg</td>
<td>27–33</td>
<td>23–29</td>
<td>22–28</td>
<td>–</td>
</tr>
</tbody>
</table>
Appendix 7

1- Biological Safety Cabinet
2- Gloves
3- Eye Protection
4- Disinfectants

Appendix 8

1- Triple Packaging System

Appendix 9

For materials of *Vibrio cholerae* (source / calibration/ concentration)

<table>
<thead>
<tr>
<th>#</th>
<th>Item</th>
<th>concentration</th>
<th>Source</th>
<th>Calibration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TCBS</td>
<td>500g / Bottle</td>
<td>Mast or Oxoid (U.K)</td>
<td>CPHL/ Bacteriology dep.</td>
</tr>
<tr>
<td>2</td>
<td>Kligler Iron Agar</td>
<td>500g / Bottle</td>
<td>Mast or Oxoid (U.K)</td>
<td>CPHL/ Bacteriology dep.</td>
</tr>
<tr>
<td>3</td>
<td>Peptone</td>
<td>500g / Bottle</td>
<td>Mast or Oxoid (U.K)</td>
<td>CPHL/ Bacteriology dep.</td>
</tr>
<tr>
<td>4</td>
<td>Oxidase Reagent</td>
<td>2 ml vial / 100 vial / Box</td>
<td>Mast or Oxoid (U.K)</td>
<td>CPHL/ Bacteriology dep.</td>
</tr>
<tr>
<td>5</td>
<td>sodium Deoxycholate</td>
<td>2 ml vial / 100 vial / Box</td>
<td>Mast or Oxoid (U.K)</td>
<td>CPHL/ Bacteriology dep.</td>
</tr>
<tr>
<td>6</td>
<td>Api 20 E with Nacl 0.85% medium ( suspension medium ) and reagent kit for Api 20 E kit) TDA,IND,VP1,VP2,NIT1,NIT2,And OX)or individual reagents, Zn reagent , Mineral oil</td>
<td>100 test / kit</td>
<td>Biomerieux ( France)</td>
<td>CPHL/ Bacteriology dep.</td>
</tr>
<tr>
<td>7</td>
<td><em>Vibrio cholerae</em> anti sera Poly</td>
<td>2ml / Vial</td>
<td>BD (UK) or Difco (USA)</td>
<td>CPHL/ Bacteriology dep.</td>
</tr>
<tr>
<td></td>
<td>Description</td>
<td>Quantity/Container</td>
<td>Supplier</td>
<td>Department</td>
</tr>
<tr>
<td>---</td>
<td>--------------------------------------------------</td>
<td>--------------------</td>
<td>-----------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>8</td>
<td><em>Vibrio cholerae</em> anti sera Ogawa</td>
<td>2ml / Vial</td>
<td>BD (UK) or Difco (USA)</td>
<td>CPHL/Bacteriology dep.</td>
</tr>
<tr>
<td>9</td>
<td><em>Vibrio cholerae</em> anti sera Inaba</td>
<td>2ml / Vial</td>
<td>BD (UK) or Difco (USA)</td>
<td>CPHL/Bacteriology dep.</td>
</tr>
<tr>
<td>10</td>
<td><em>Vibrio cholerae</em> anti sera O 139</td>
<td>2ml / Vial</td>
<td>BD (UK) or Difco (USA)</td>
<td>CPHL/Bacteriology dep.</td>
</tr>
<tr>
<td>11</td>
<td>Cary &amp; Blair Medium</td>
<td>500 gm/ Bottle</td>
<td>Mast or Oxoid (U.K)</td>
<td>CPHL/Bacteriology dep.</td>
</tr>
<tr>
<td>12</td>
<td>VC Rapid test</td>
<td>50 test / kit</td>
<td>SPAN (India)</td>
<td>CPHL/Bacteriology dep.</td>
</tr>
<tr>
<td>13</td>
<td>Sterile transport swab with Cary &amp; Blair medium ready to use</td>
<td>2ml / Vial</td>
<td>Mast or Oxoid (U.K)</td>
<td>CPHL/Bacteriology dep.</td>
</tr>
<tr>
<td>14</td>
<td>Chloramphenicol</td>
<td>30 mcg / disc / 100 disc / vial</td>
<td>BD (UK) or Difco (USA)</td>
<td>CPHL/Bacteriology dep.</td>
</tr>
<tr>
<td>15</td>
<td>Ciprofloxacin</td>
<td>5 mcg / disc / 100 disc / vial</td>
<td>Any company</td>
<td>CPHL/Bacteriology dep.</td>
</tr>
<tr>
<td>16</td>
<td>Erythromycin</td>
<td>15 mcg / disc / 100 disc / vial</td>
<td>Any company</td>
<td>CPHL/Bacteriology dep.</td>
</tr>
<tr>
<td>17</td>
<td>Gentamycin</td>
<td>10 mcg / disc / 100 disc / vial</td>
<td>Any company</td>
<td>CPHL/Bacteriology dep.</td>
</tr>
<tr>
<td>18</td>
<td>Nalidixic acid</td>
<td>30 mcg / disc / 100 disc / vial</td>
<td>Any company</td>
<td>CPHL/Bacteriology dep.</td>
</tr>
<tr>
<td>19</td>
<td>Tetracyclin</td>
<td>30 mcg / disc / 100 disc / vial</td>
<td>BD (UK) or Difco (USA)</td>
<td>CPHL/Bacteriology dep.</td>
</tr>
<tr>
<td>20</td>
<td>Azithromycin</td>
<td>15 mcg / disc / 100 disc / vial</td>
<td>BD (UK) or Difco (USA)</td>
<td>CPHL/Bacteriology dep.</td>
</tr>
<tr>
<td>21</td>
<td>Trimethoprim</td>
<td>1.25 with sulphamethoxazole 23.75 mcg / 100 disc / vial</td>
<td>BD (UK) or Difco (USA)</td>
<td>CPHL/Bacteriology dep.</td>
</tr>
<tr>
<td>22</td>
<td>Ampicilline</td>
<td>30 mcg / 100 disc / vial</td>
<td>BD (UK) or Difco (USA)</td>
<td>CPHL/Bacteriology dep.</td>
</tr>
<tr>
<td>23</td>
<td>Disposable loop</td>
<td>500 pc / Box</td>
<td>Any company</td>
<td>CPHL/ Bacteriology dep.</td>
</tr>
<tr>
<td>----</td>
<td>----------------</td>
<td>--------------</td>
<td>-------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>24</td>
<td>Disposable plate</td>
<td>500 pc / Box</td>
<td>Any company</td>
<td>CPHL/ Bacteriology dep.</td>
</tr>
<tr>
<td>25</td>
<td>Disposable plane tube</td>
<td>Pack</td>
<td>Any company</td>
<td>CPHL/ Bacteriology dep.</td>
</tr>
<tr>
<td>26</td>
<td>Glass pasteure pipette</td>
<td>100 pc / Box</td>
<td>Any company</td>
<td>CPHL/ Bacteriology dep.</td>
</tr>
<tr>
<td>27</td>
<td>Filter paper</td>
<td>100 pc / Box</td>
<td>Any company</td>
<td>CPHL/ Bacteriology dep.</td>
</tr>
<tr>
<td>28</td>
<td>Sharp Objects Container</td>
<td>100 pc / Box</td>
<td>Any company</td>
<td>CPHL/ Bacteriology dep.</td>
</tr>
<tr>
<td>29</td>
<td>Gloves</td>
<td>100 pair / Box</td>
<td>Any company</td>
<td>CPHL/ Bacteriology dep.</td>
</tr>
<tr>
<td>30</td>
<td>Wood stick</td>
<td>100 pc / Box</td>
<td>Any company</td>
<td>CPHL/ Bacteriology dep.</td>
</tr>
</tbody>
</table>
References


7- Guidelines for Biosafety in Teaching Laboratories (American Society for Microbiology 2012).


( WHO/HSE/IHR/2010.8)