

Appendix (1)

Questionnaire:

1. Sample number.....
2. Patient name.....
3. Age.....
4. Gender.....
5. Address.....
6. Cause of renal failure.....
7. Do you had any antibiotic during this week? Yes() No()
8. Are you affected with urinary tract infection before(recurrent UTI): Yes()
No()
9. Mention the last time of the infection with UTI.....

Appendix (2)

Media and Reagents:

2.1. Alcohol fixative solution:

To make 200 ml:

Ethanol (ethyl alcohol), absolute. 180 ml

acetic acid, glacial 10 ml

Distilled water. 10 ml

1. Fill a cylinder (250 ml capacity) to the 180 ml mark with absolute ethanol. Add water to the 190 ml mark. *Caution:* Ethanol is highly flammable; therefore use it well away from an open flame.

2. Add 10 ml of glacial acetic acid, i.e. to the 200 ml mark. Transfer the solution to a screw-cap bottle, and mix well. *Caution:* Glacial acetic acid is a corrosive chemical with irritating vapors; therefore use it in a well ventilated room. Does not mouth pipette.

3. Label the bottle, and mark it *Flammable*. Store at room temperature in a safe place.

2.2. Catalase:

Required: Hydrogen peroxide, 3% H₂O₂.

2.3. Coagulase:

Required: EDTA anticoagulated human plasma (preferably pooled and previously HIV and hepatitis tested) or rabbit plasma. The plasma should be allowed to warm to room temperature before being used.

Plasma: Oxalate or heparin plasma can also be used. Do not use citrated plasma because citrate-utilizing bacteria e.g. *Enterococci*, *Pseudomonas Serratia* and may cause clotting of the plasma (in tube test). Occasionally, human plasma may

contain inhibitory substances which can interfere with coagulase testing. The plasma can be stored frozen in amounts ready for use.

2.4. Cystine lactose electrolyte deficient (CLED):

This medium is best prepared from ready to use dehydrated powder, available from most suppliers of culture media.

Contents: Peptone, *Lab-Lemco* powder, tryptone, lactose, *L*-cystine, bromothymol blue, agar. The medium is usually used at a concentration of 18.1 g in every 500 ml distilled water (concentration may vary depending on manufacturer).

1. Prepare as instructed by the manufacturer. Sterilize by autoclaving at 121°C for 15 minutes.
2. Mix well before pouring (avoid air bubbles forming). Dispense aseptically in 15 ml amounts in sterile Petri dishes.
3. Date the medium and give it a batch number.
4. Store the plates at 2–8°C, preferably sealed in plastic bags to prevent loss of moisture.

Shelf-life: Up to 4 weeks or longer provided there is no change in the appearance of the medium to suggest contamination or a change in PH.

PH of medium: This should be within the range pH 7.3–7.5 at room temperature.

2.5. Crystal violet:

To make 1 liter:

Crystal violet	20 g
Ammonium oxalate	9 g
Ethanol or methanol, absolute	95 ml
Distilled water	To 1 liter

1. Weigh the crystal violet on a piece of clean paper (preweighed). Transfer to a brown bottle premarked to hold 1 liter.

2. Add the absolute ethanol or methanol (technical grade is suitable) and mix until the dye is completely dissolved. *Caution:* Ethanol and methanol are highly flammable; therefore use these chemicals well away from an open flame.
3. Weigh the ammonium oxalate and dissolve in about 200 ml of distilled water. Add to the stain. Make up to the 1 liter, mark, and mix well. *Caution:* Ammonium oxalate is a toxic chemical, therefore handle it with care.
4. Label the bottle, and store it at room temperature. The stain is stable for several months.

For use: Filter a small amount of the stain into a dropper bottle or other stain dispensing container.

2.6. Christensen's urea broth:

To prepare about 33 bottles:

Urea broth base..... 95 ml

Sterile urea solution, 40% w/v 5 ml

1. Prepare and sterilize the urea broth base as instructed by the manufacturer.

Transfer to a 50–55°C water bath.

2. When the medium has cooled to 50–55°C, add aseptically the sterile urea solution, and mix well.

3. Dispense aseptically in 3 ml amounts in sterile Bijou bottles or screw-cap tubes.

Date the medium and give it a batch number.

4. Store in a cool dark place or at 2–8°C. *Shelf-life:* Up to 6 months providing there is no change in the volume or appearance of the medium to suggest contamination or alteration of PH.

PH of medium: This should be within the range 6.6–7.0 at room temperature.

Sterilization: in autoclave at 121°C for 15 minutes.

2.7. Citrate:

Using a Rosco citrate identification tablet. This is the most economical method when only a few tests are performed. The tablets have a long shelf-life and good stability in tropical climates. Using Simmon's citrate agar but the dehydrated medium is only available in 500 g pack size from manufacturers. After being opened the medium does not have good stability in tropical climates

2.8. DNase agar:

This medium is best prepared from ready to use dehydrated powder, available from most suppliers of culture media.

Contents: Tryptose, deoxyribonucleic acid, sodium chloride, agar. The medium is usually used at a concentration of 3.9 g in every 100 ml distilled water (concentration may vary depending on manufacturer).

1. Prepare and sterilize as instructed by the manufacturer.
2. When the medium has cooled to 50–55°C, mix well and dispense in sterile Petri dishes. Date the medium and give it a batch number.
3. Store the plates at 2–8°C in sealed plastic bags to prevent loss of moisture.

Shelf-life: 3–4 weeks when stored in plastic bags providing there is no change in the appearance of the medium to suggest contamination or deterioration.

PH of medium: This should be within the range 7.1–7.5 at room temperature.

2.9. Hydrochloric acid:

To make 100 ml:

Hydrochloric acid, concentrated 8.6 ml

Distilled water to 100 ml

1. Half fill a 100 ml volumetric flask with distilled water.
2. Add the 8.6 ml concentrated hydrochloric acid. Make up to the 100 ml mark with distilled water, and mix well. Transfer to a screw-cap container.

Caution: Concentrated hydrochloric acid is corrosive, therefore handle it with care.

Does not mouth pipette.

3. Label the bottle and store it at room temperature. The reagent is stable indefinitely.

2.10. Mannitol salt agar:

This medium is best prepared from ready to use dehydrated powder, available from most suppliers of culture media.

Contents: Peptone, *Lab-Lemco* powder, mannitol, sodium chloride, phenol red, agar

The medium is usually used at a concentration of 11.1 g in every 100 ml distilled water (concentration may vary depending on manufacturer).

1. Prepare the medium as instructed by the manufacturer. Sterilize by autoclaving at 121°C for 15 minutes.

2. When the medium has cooled to 50–55°C, mix well, and dispense it aseptically in sterile Petri dishes. Date the medium and give it a batch number.

3. Store the plates at 2–8°C preferably in plastic bags to prevent loss of moisture.

Shelf-life: Several weeks providing there is no change in the appearance of the medium to suggest contamination, deterioration, or alteration of PH.

PH of medium: This should be within the range 7.3–7.7 at room temperature.

2.11. Kligler iron agar (KIA):

This medium is best prepared from ready to use dehydrated powder, available from Oxoid Ltd, code CM0033, and other suppliers of culture media.

Contents: Peptone, *Lab-Lemco* powder, yeast extract, sodium chloride, lactose, glucose (dextrose), ferric citrate, sodium thiosulphate, phenol red, agar.

The medium is usually used at a concentration of 5.5 g in every 100 ml distilled water (concentration may vary depending on manufacturer).

1. Prepare as instructed by the manufacturer.

When cooled to 50–55°C, *mix well* and dispense in 6 ml amounts in large size tubes.

2. Sterilize by autoclaving (with caps loosened) at 121°C for 15 minutes.
3. Allow the medium to solidify in a sloped position to give a butt 25–30 mm deep and a slope 20–25 mm long (the butt should be longer than the slope). Date the medium and give it a batch number.
4. Store in a cool dark place or at 2–8°C.

Shelf-life: About 3 weeks or longer providing the tube caps is tightly screwed and there is no change in the appearance of the medium to suggest contamination, deterioration, or an alteration of PH.

PH of medium: This should be within the range 7.2–7.6 at room temperature.

2.12. Lugol's iodine solution:

To make 1 liter:

Potassium iodide 20 g
Iodine 10 g
Distilled water To 1 liter

- 1 Weigh the potassium iodide, and transfer to a brown bottle premarked to hold 1 liter.
2. Add about a quarter of the volume of water, and mix until the potassium iodide is completely dissolved.
3. Weigh the iodine, and add to the potassium iodide solution. Mix until the iodine is dissolved.

Caution: Iodine is injurious to health if inhaled or allowed to come in contact with the eyes, therefore handle it with care in a well ventilated room.

4. Make up to the 1 liter mark with distilled water, and mix well. Label the bottle, and mark it *Toxic*. Store it in a dark place at room temperature. Renew the solution if its color fades.

2.13. Mueller Hinton Agar:

G/l

Beef dehydrated infusion from 300.0 casein

Hydrolysate.....17.5

Starch.....1.5

Agar.....17.0

PH: with range 7.3 ± 0.1 .

Sterilization: in autoclave at 121°C for 15 minutes.

2.14. Nutrient Agar:

This medium is best prepared from ready to use dehydrated powder, available from most suppliers of culture media.

Contents: Peptone, *Lab-Lemco* powder, yeast extract, sodium chloride, agar.

Nutrient agar is usually used at a concentration of 2.8 g in every 100 ml distilled water (concentration may vary depending on manufacturer).

1. Prepare as instructed by the manufacturer. Sterilize by autoclaving at 121°C for 15 minutes.

2. Dispense aseptically in the required amounts (i.e. 3 ml to make nutrient agar slopes, 5 ml to make nutrient agar deeps, or the amounts required to make blood agar or other media). Date the medium and give it a batch number.

3. Store in a cool dark place.

Shelf-life: Up to 2 years providing there is no change in the appearance of the medium to suggest contamination or deterioration.

PH of medium: This should be within the range pH 7.2–7.6 at room temperature.

For use: Transfer a small amount of the reagent to a brown dispensing bottle.

2.15 Oxidase reagent:

Prepare fresh before use. To make 10 ml:

Tetramethyl-*p*-phenylenediaminedihydrochlorid..... 0.1 g

Distilled water.....10 ml

dissolve the chemical in the water. The reagent is not stable. It is therefore best prepared immediately before use.

2.16 Physiological saline:

To make 1 liter:

Sodium chloride 8.5 g

Distilled waterTo 1 liter

1. Weigh the sodium chloride, and transfer it to a leak-proof bottle premarked to hold 1 liter.
2. Add distilled water to the 1 liter mark, and mix until the salt is fully dissolved.
3. Label the bottle, and store it at room temperature.

The reagent is stable for several months. Discard if it becomes contaminated.

2.16. Peptone water:

To make about 65 bottles:

Peptone..... 2 g

Sodium chloride1 g

Distilled water200 ml

1. Dissolve the peptone and salt in the water. Dispense in 3 ml amounts in screw-cap bottles (Bijou type are suitable).
2. Sterilize by autoclaving (with caps loosened) at 121°C for 15 minutes. When cool, tighten the bottle caps. Date the medium and give it a batch number.

3. Store in a cool dark place.

Shelf-life: Up to 2 years providing the medium shows no change in volume or appearance to suggest contamination.

PH of medium: This should be within the range pH 7.0–7.

2.17. Safranin(Counter stain):

Safranin	200 mL
95% ethanol.....	200 mL
Distilled water.....	800 mL

2.18. Turbidity standard (McFarland's):

Preparation of turbidity standard:

1. Prepare a 1% v/v solution of sulphuric acid by adding 1 ml of concentrated sulphuric acid *to* 99 ml of water. Mix well. *Caution:* Concentrated sulphuric acid is hygroscopic and highly corrosive, therefore do not mouth pipette, and never add the water to the acid.
2. Prepare a 1% w/v solution of barium chloride by dissolving 0.5 g of dehydrate barium chloride ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) in 50 ml of distilled water.
3. Add 0.6 ml of the barium chloride solution to 99.4 ml of the sulphuric acid solution, and mix.
4. Transfer a small volume of the turbid solution to a capped tube or screw-cap bottle of the same type as used for preparing the test and control inocula.

When stored in a well-sealed container in the dark at room temperature (20–28°C), the standard can be kept for up to 6 months.

2.19 MR-VP Broth:

Enzymatic Digest of Casein	3.5 g
Enzymatic Digest of Animal Tissue.....	3.5 g
Dextrose	5 g

Potassium Phosphate 5 g

Final pH: 6.9 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Appendix (3)

Instruments:

3.1. Autoclave:

Dixon's surgical instruments LTD	UK
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3.2. Hot Air Oven:

Leader engineering widens Cheshire	UK
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3.3. Incubator:

Gallenkamp:	UK
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3.4. Microscope:

Olympus Optical LTD	Japan
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3.5. Refrigerator:

Coldair	Europe
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3.6. Sensitive balance:

KERN model A15120-4	Germany
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