Appendix (1)

Colonial morphology, gram stain,

Biochemical tests and susceptibility test of *M.catarrhalis*

1. Colonial morphology of *M.catarrhalis* on sheep blood agar

Colonies were gray to white, opaque, smooth, dry, non hemolytic and 1-3mm in diameter after 24 hours of incubation. With an inoculating loop, colonies can easily be slid across the agar surface, like hockey pucks, and can be stacked like disks as shown in figure 1.

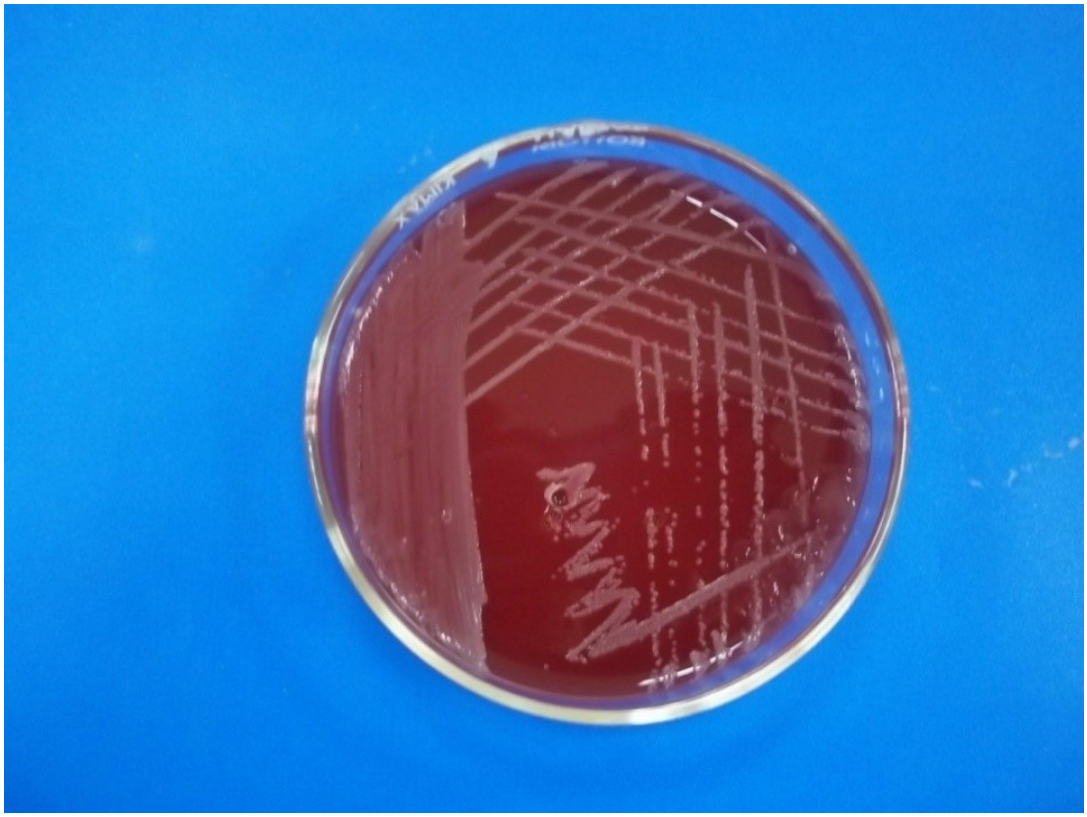


Figure (1) Colonial morphology of *M.catarrhalis* on sheep blood agar

2. Colonial morphology of *M.catarrhalis* on chocolate agar

M.catarrhalis colonies were pinkish - brown and can be pushed along the surface of the agar like a hockey puck. The colonies of clinical isolates were compared with reference strains as shown in figure 2 and 3.

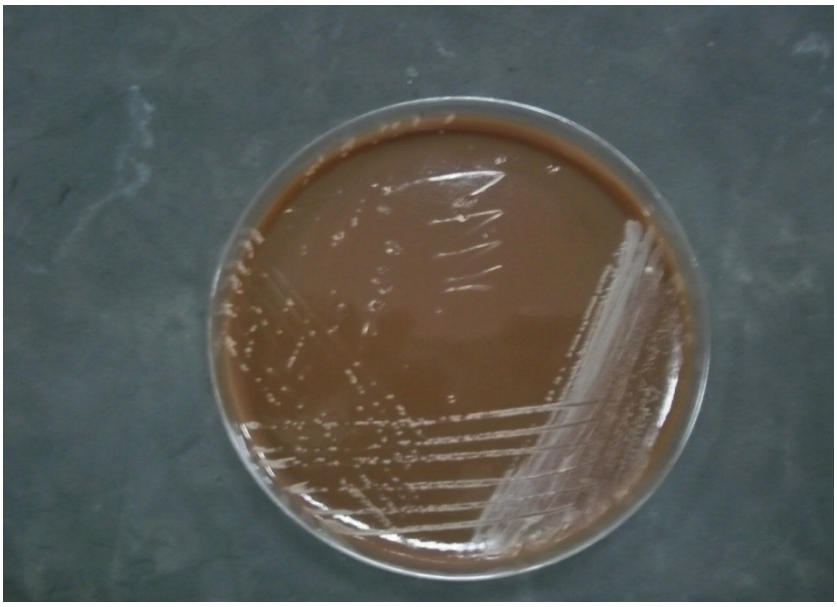


Figure (2) Colonial morphology of isolated *M.catarrhalis* on chocolate agar

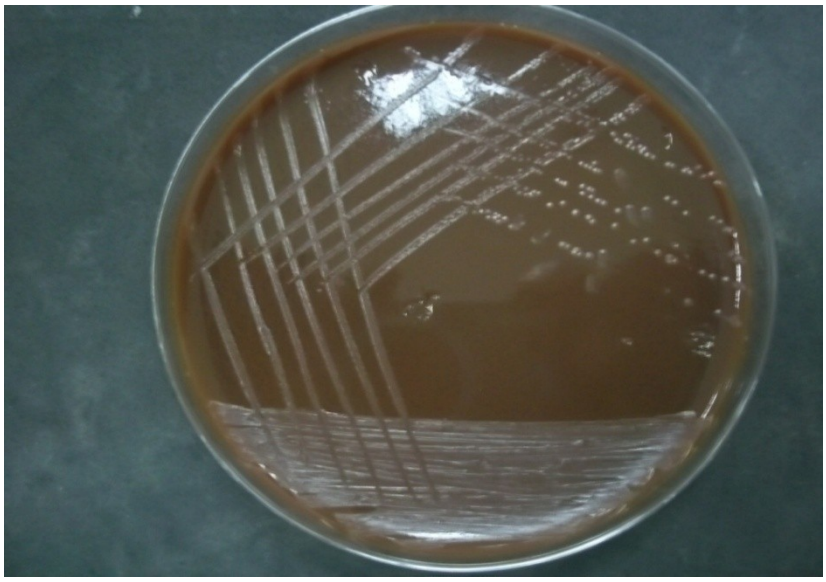


Figure (3) Colonial morphology of ATCC23246 on chocolate agar.

3. Growth of *M.catarrhalis* on nutrient agar

M.catarrhalis isolates were grown on nutrient agar at 35°C and their colonial morphology compared with the reference strains as shown in figure 4 and 5.

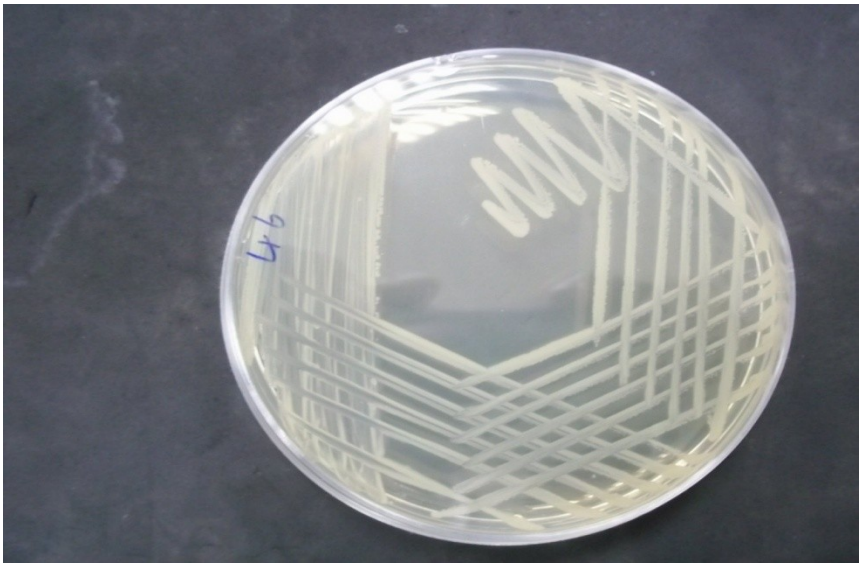


Figure (4) Colonial morphology of isolated *M.catarrhalis* on nutrient agar

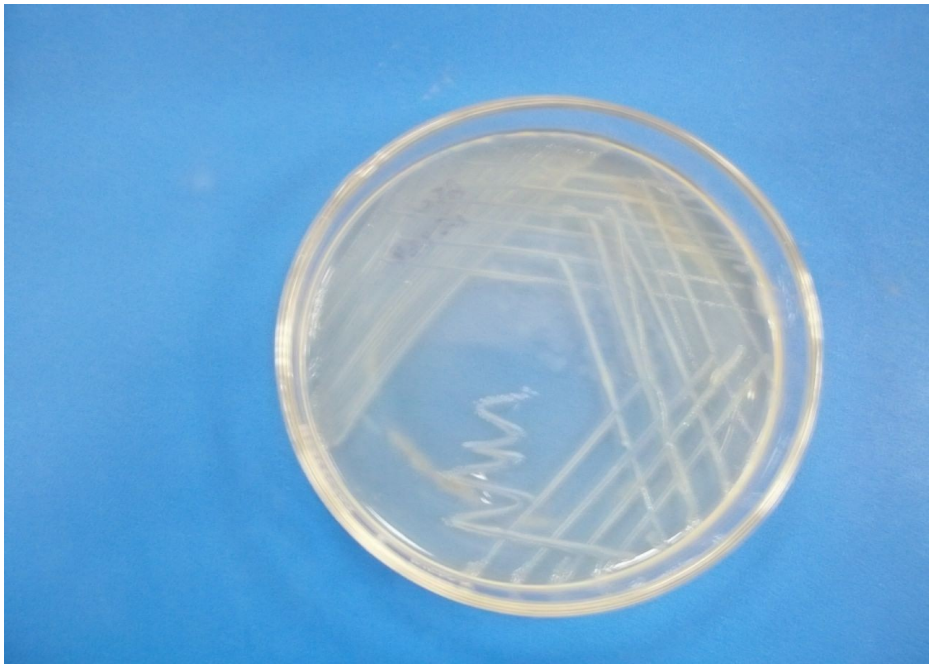


Figure (5) Colonial morphology of ATCC 25238 on nutrient agar

4. Gram stain of *M.catarrhalis*

M.catarrhalis was appearing on Gram stain as large kidney – shaped gram negative cocci as show in figure 6.

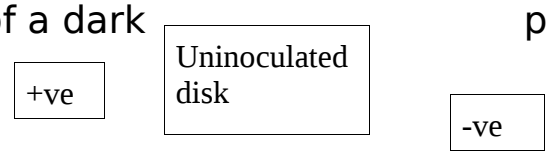
Figure (6) Gram stain of *M.catarrhalis* (large kidney –shaped gram negative cocci) (arrow).

5. Oxidase test

The light pink oxidase test disk serves as an artificial substrate, donating electrons to cytochrome oxidase and in the process becoming oxidized to a purple and then dark purple compound in

the presence of free O_2 and the oxidase. All clinical isolates were positive for oxidase test as shown in figure 7.

Figure (7) Oxidase test, positive test (*M.catarrhalis*) is indicated by the development of a dark purple color.



6. Catalase test

Both *M.catarrhalis* clinical isolates and reference strains were positive for catalase test which indicated by release of immediate oxygen bubbles as shown in figure 8 & 9.

Figure (8) Catalase test of isolated *M.catarrhalis*, the rapid elaboration of oxygen bubbles indicate positive result.

Figure (9) Catalase test of ATCC25240 and ATCC 25238, the rapid elaboration of oxygen bubbles indicate positive result.

7. DNase test for *M.catarrhalis*

Hydrolysis of DNA by DNase enzyme was detected by clear zone around inoculum for both clinical isolates and reference strains as shown in figure 10 &11.

Figure (10) DNase activity of isolated *M.catarrhalis* on DNA agar medium which shows clear zone (positive test)

Figure (11) DNase activity of *M.catarrhalis* ATCC25240, ATCC25238 and ATCC23246 on DNA agar medium which shows clear zone (positive test)

8. Reduction of nitrate

M.catarrhalis has ability to reduce nitrate to nitrite which is indicated by the development of red color as shown in figure 12. Both clinical isolates and reference strains were positive for nitrate test.

Figure (12) Nitrate test of the 3 isolated *M.catarrhalis*: development of red color indicates the reduction of nitrate to nitrite (positive test).

Zinc dust was used to determine whether nitrate had been reduced to nongaseous end products. Zinc dust will reduce any nitrate remaining in the medium to nitrite, and a red color will develop. After the addition of zinc there was no red color developed and the nitrate in the medium was already reduced, indicating a positive nitrate reduction reaction. The development of a red color after the addition of zinc dust confirmed a true negative reaction as shown in figure 13.

Figure (13) Nitrate test of reference strains: After the addition of zinc powder there was no red color develops and the nitrate in the medium was already reduced by *M.catarrhalis*, indicating a positive nitrate reduction reaction.

9. Tributyrin-Strips

Diagnostic test for the differentiation between *M.catarrhalis* and *Neisseria*. The test principle is an enzyme hydrolysis of tributyrin.

This reaction causes color change of acidobasic indicator. Results can be read after several hours when the red color changes to yellow indicates positive result. Both the clinical isolates and reference strains show positive test as shown in figure 14.

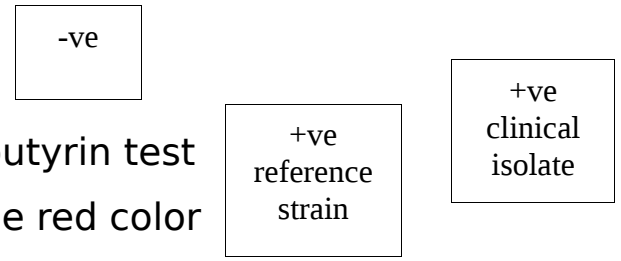


Figure (14) Tributyrin test for *M.catarrhalis* which shows the red color changes to yellow in case of positive result.

10. Susceptibility test

Figure (15) Sensitivity of isolated *M.catarrhalis* to amoxyclav, azithromycin, ceftriaxon, chloramphenicol, ciprofloxacin, co-trimoxazole and cephalexin.

Figure (16) Sensitivity of ATCC 23246 to amoxyclav, azithromycin, ceftriaxon, chloramphenicol, ciprofloxacin, co- trimoxazole and cephalexin.

11. β-lactamase detection test

β-Lactamase was detected by the chromogenic nitrocefin disks. All clinical isolates of *M.catarrhalis* were positive for β-lactamase production but the reference strains were not β-lactamase producer as shown in figure 17.

Figure (17) β-lactamase which is rapid strip test contains nitrocefin which is a chromogenic cephalosporin that changes color from yellow to red when its β-lactam ring is hydrolyzed by β-lactamase enzyme (positive).

+ve +ve +ve -ve +ve

Appendix (2) Preparation of Reagents and Culture Media

1. Amies Transport Medium with Charcoal

Amies Transport Medium with charcoal is used for transportation and preservation of microbiological specimen.

Composition

Ingredients	Gms / Litre
Sodium chloride..……..	3.000
Potassium chloride ……	0.200
Calcium chloride……	0.100
Magnesium chloride……..	0.100
Monopotassium phosphate……..0.200	
Disodium phosphate…….1.150	
Sodium thioglycollate……1.000	
Charcoal…….10.000	
Agar ……4.000	

Final pH (at 25°C)……..7.2±0.2

Directions

Suspend 19.75 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in screw cap bottles or tubes in 6 ml or desired quantity. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Cool in an upright position. Turn the tubes several times while agar is solidifying, to maintain uniform suspension of charcoal particles.

2. Bile Esculin Agar

Bile Esculin Agar is a differential medium recommended for isolation and presumptive identification of group D Streptococci from food and pharmaceutical products.

Composition

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Beef extract	3.000
Oxgall	40.000
Esculin	1.000
Ferric citrate	0.500
Agar	15.000
Final pH (at 25°C)	6.6±0.2

Directions

Suspend 64.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Mix and dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tube to solidify in slanted position.

3. Blood agar and chocolate agar

Blood agar base

Blood Agar Base is recommended as a base to which blood may be added for use in the isolation and cultivation of fastidious pathogenic microorganisms like *Neisseria* , Streptococci etc.

Composition

Ingredients	Gms / Litre
Beef heart, infusion from (Beef extract)……..	500.000
Tryptose……	10.000
Sodium chloride……..5.000	
Agar……	15.000
Final pH (at 25°C)……	7.3±0.2

Directions

Suspend 40 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add 5% v/v sterile defibrinated blood. Mix well and pour into sterile Petri plates.

Chocolate (heated blood) agar

When blood agar is heated, the red cells are lyzed and the medium becomes brown in colour. It is referred to as chocolate agar

1. Prepare as described for blood agar except after adding the blood, heat the medium in a 70 °C water bath until it becomes brown in colour. This takes about 10–15 minutes during which time the medium should be mixed gently several times.
2. Allow the medium to cool to about 45 °C, remix and dispense in sterile petri dishes as described for blood agar.

4. Columbia Blood Agar Base

Columbia Blood Agar Base is an efficient and enriched base for preparation of blood agar, chocolate agar and for various selective and identification media.

Composition

Ingredients	Gms / Litre
Peptone, special	23.000
Corn starch	1.000
Sodium chloride	5.000
Hemin	0.010
Agar	15.000
Final pH (at 25°C)	7.3±0.2

Directions

Suspend 44.01 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C before adding heat sensitive compounds.

For Blood Agar: Add 5% v/v sterile defibrinated sheep blood to sterile cool base.

For Chocolate Agar: Add 10% v/v sterile defibrinated sheep blood to sterile cool base. Heat to 80°C for 10 minutes with constant agitation.

5. Crystal violet Gram stain

To make 1 litre:

Crystal violet . . . . . 20 g

Ammonium oxalate . . . . . 9 g

Ethanol or methanol, absolute . . . .95 ml

Distilled water . . . .to 1 litre

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

2. Add the absolute ethanol or methanol and mix until the dye is completely dissolved.

3. Weigh the ammonium oxalate and dissolve in about 200 ml of distilled water. Add to the stain. Make up to the 1 litre mark with distilled water, and mix well.

4. Label the bottle, and store it at room temperature. The stain is stable for several months.

6. DNase agar

Contents

Ingredients	Gms / Litre
Tryptose	20.0
deoxyribonucleic acid	2.0
sodium chloride	5.0
agar	12.0

Disperse 39 g in 1L of distilled water. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50–55 °C, mix well and dispense in sterile petri dishes.

7. GC Agar Base

GC Agar Base, with added blood or hemoglobin and other supplements is recommended for selective isolation and cultivation of Gonococci.

Composition

Ingredients	Gms / Litre
Peptone, special	15.000
Corn starch	1.000
Dipotassium phosphate	4.000
Monopotassium phosphate	1.000
Sodium chloride	5.000
Agar	10.000

Final pH (at 25°C)……..7.2±0.2

Directions

Suspend 7.2 grams in 100 ml distilled water, to make a double strength base. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. For Chocolate Blood Agar, prepare single-strength medium using 3.6 grams in 100 ml of distilled water. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes and add 5% v/v defibrinated blood. Mix well and heat at 80°C for 10 minutes.

8. Hydrochloric acid, 1 mol/l (IN)

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

9. Hydrogen peroxide

3ml of 3% H_2O_2 (10 volume solution) dispense in sterile test tubes.

10. Kligler iron agar (KIA)

Kligler Iron Agar is recommended for the differential identification of gram-negative enteric bacilli on the basis of the fermentation of dextrose, lactose and H_2S production.

Composition

Ingredients	Gms / Litre
Peptic digest of animal tissue……..	15.000
Beef extract……	3.000
Yeast extract…….	3.000
Proteose peptone……..	5.000
Lactose……..	10.000
Dextrose……	1.000
Ferrous sulphate……	0.200
Sodium chloride……	5.000
Sodium thiosulphate…….	0.300
Phenol red…….	0.024
Agar……..	15.000
Final pH (at 25°C)…….	7.4±0.2

Directions

Suspend 57.52 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Mix well and distribute into tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubes to cool in slanted position to form slopes with about 1inch butts.

11. Lugol’s iodine solution

To make 1 litre:

Potassium iodide . . . . . 20 g

Iodine . . . . . 10 g

Distilled water . . . . . to 1 litre

1. Weigh the potassium iodide, and transfer to a brown bottle pre marked to hold 1 litre.

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.

4. Make up to the 1 litre 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 colour fades.

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

12. MacConkey agar

MacConkey Agar is a differential medium recommended for the selective isolation and differentiation of lactose fermenting and lactose non-fermenting enteric bacilli.

Composition

Ingredients	Gms / Litre
Peptic digest of animal tissue	17.000
Proteose peptone	3.000
Lactose	10.000
Bile salts	1.500
Sodium chloride	5.000
Neutral red	0.030
Agar	15.000
Final pH (at 25°C)	7.1±0.2

Directions

Suspend 51.53 gms of medium in 1000 ml distilled water. Heat to boiling with gentle swirling to dissolve the agar completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Avoid overheating. Cool to 45 - 50°C and pour into sterile Petri plates. The surface of the medium should be dry when inoculated.

13. Mannitol salt agar

Mannitol Salt Agar is used as a selective media for the isolation of pathogenic Staphylococci.

Composition

Ingredients	Gms / Litre
Proteose peptone	10.000
Beef extract	1.000
Sodium chloride	75.000
D-Mannitol	10.000
Phenol red	0.025
Agar	15.000
Final pH (at 25°C)	7.4±0.2

Directions: Suspend 111.02 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

14. Mueller Hinton Agar

Mueller Hinton Agar is used for testing susceptibility of common and rapidly growing bacteria using antimicrobial discs by the Bauer - Kirby method. Manufactured to contain low levels of thymine, thymidine, calcium and magnesium.

Composition

Ingredients	Gms / Litre
Casein acid hydrolysate	17.500
Beef heart infusion	2.000
Starch, soluble	1.500
Agar	17.000

Final pH (at 25°C)……7.3±0.2

Directions

Suspend 38 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and dispense as desired.

15. Nitrate broth

Composition

Ingredient Gms/ Litre

Nutrient broth ……..13.0

Potassium nitrate…… 2.0

Dissolve the ingredients in 1L distilled water; adjust solution to pH 7.0. Dispense 5 ml aliquots of the broth into tubes. Autoclave for 15 min at 121C.

Reagents: Sulfanilic acid solution (Nitrate reagent A): 0.8% in 5N acetic acid

Chemical Name: 4-aminobenzene sulfonic acid

Alpha-Naphthylamine solution (Nitrate reagent B): 0.6% in 5N acetic acid

Chemical Name: N,N-dimethyl-1 naphthylamine

Zinc powder, Reagent grade

16. Nitocefin disks

6mm diameter filter paper discs impregnated with nitrocefin.

17. Nutrient agar and nutrient broth

Nutrient agar

Nutrient Agar is used for the cultivation of less fastidious microorganisms, can be enriched with blood or other biological fluids.

Composition

Ingredients Gms / Litre

Peptic digest of animal tissue.....5.000

Sodium chloride.....5.000

Beef extract.....1.500

Yeast extract.....1.500

Agar.....15.000

Final pH (at 25°C).....7.4±0.2

Directions

Suspend 28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before pouring.

Nutrient broth

Nutrient Broth is used for the general cultivation of less fastidious microorganisms, can be enriched with blood or other biological fluids.

Composition

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Sodium chloride	5.000
Beef extract	1.500
Yeast extract	1.500
Final pH (at 25°C)	7.4±0.2

Directions : Suspend 13 grams in 1000 ml distilled water. Heat, if necessary, to dissolve the medium completely. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

18. Oxidase reagent

Prepare fresh before use.

To make 10 ml:

Tetramethyl-*p*-phenylenediamine . . . . . 0.1 g
dihydrochloride

Distilled water. . . . . 10 ml

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

19. Peptone water

Peptone Water is used as a growth medium and as a base for carbohydrate fermentation media.

Composition

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Sodium chloride	5.000
Final pH (at 25°C)	7.2±0.2

Directions

Suspend 15.0 grams in 1000 ml distilled water. Heat, if necessary, to dissolve the medium completely. Dispense in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

20. Physiological saline, 8.5 g/l (0.85% w/v)

To make 1 litre:

Sodium chloride . . . . . 8.5 g

Distilled water . . . . . to 1 litre

1. Weigh the sodium chloride, and transfer it to a leak-proof bottle pre marked to hold 1 litre.

2. Add distilled water to the 1 litre 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.

21. Simmons' Citrate Agar

Simmons Citrate Agar is recommended for differentiation the members of *Enterobacteriaceae* on the basis of citrate utilization.

Composition

Ingredients	Gms / Litre
Magnesium sulphate	0.200
Ammonium dihydrogen phosphate	1.000
Dipotassium phosphate	1.000
Sodium citrate	2.000
Sodium chloride	5.000
Bromothymol blue	0.080
Agar	15.000
Final pH (at 25°C)	6.8±0.2

Directions

Suspend 24.28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Mix well and distribute in tubes or flasks. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

22. Tributyrin-Strips

Composition:

(1 package contains 300 test strips)
Contains strips saturated with tributyrin and acidobasic indicator

23. Urea Agar Base (Christensen)

Urea Agar Base with the addition of urea is recommended for the detection of urease production, particularly by members of the genus *Proteus*.

Composition

Ingredients	Gms / Litre
Peptic digest of animal tissue	1.000
Dextrose	1.000
Sodium chloride	5.000
Disodium phosphate	1.200
Monopotassium phosphate	0.800
Phenol red	0.012
Agar	15.000
Final pH (at 25°C)	6.8±0.2

Directions

Suspend 24.01 grams in 950 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 10 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add 50 ml of sterile 40% Urea Solution and mix well. Dispense into sterile tubes and allow to set in the slanting position. Do not overheat or reheat the medium as urea decomposes very easily.

Appendix (3)

Instruments

Autoclave	Dixon’s surgical instrument LTD UK
Dryer	Leader engineering widnes Cheshire UK
Hot air oven	Leader engineering widnes Cheshire UK

Incubator	GALLENKAMD UK
Microscope	Olympus optical.LTD Japan
Refrigerator	Starlet Europe
Safety cabinet	Daihan lab tech co.LTD UK
Sensitive balance	KERN modle AIS 120-4 Germany

Appendix (4)

Questionnaire model

Hospital name……..

Specimen number……..

Age…….

Date……..

Gender: Male ☐
Female ☐

History of disease……
……..
……
……..
……
……..

Signs and symptoms…….
……
……
……

Underlying disease……
……

Is the patient under treatment? Yes ☐ NO ☐

The type of treatment……
……
……
…….

Appendix (5)

Table (14) Result of isolated organisms according to sex and age

Sample No	Sex	Age	Isolated organisms
1	male	4 Y	*S.aureus*
2	male	3 Y	*S.aureus*
3	male	1 Y	*S.aureus*
4	male	4 Y	No growth

5	male	5 Y	*S.aureus*
6	female	1 Y& 6M	*S.aureus*
7	female	9 M	*K.pneumoniae*
8	female	6 Y	*S.aureus*
9	female	1 Y	*S.aureus*
10	male	6Y	*S.epidermidis*
11	male	3 Y	No growth
12	female	4 Y	*P.vulgaris*
13	male	10 M	No growth
14	female	1Y& 4M	*P.mirabilis*
15	male	7 M	No growth
16	male	10 M	No growth
17	male	1 Y& 6M	No growth
18	female	10 M	*K.pneumoniae*
19	male	5 Y	*P.aeruginosa*
20	female	2 Y	No growth
21	female	5 M	*S.aureus*
22	male	2 M	*S.aureus*
23	female	8 Y	*S.aureus*
24	male	7 Y	*S.aureus*
25	female	6 Y	No growth
26	female	9 Y	*S.aureus*
27	male	6 Y	*P.aeruginosa*
28	male	5 Y	No growth
29	female	5 Y	*P.mirabilis*
30	male	3 Y	*P.mirabilis*
31	male	2 Y	*S.marcescens*
32	female	4 Y	*S.aureus*
33	male	2 Y & 6 M	*H.influenzae*
34	male	2 Y & 9 M	*S.pyogenes*
35	male	7 Y	No growth
36	female	1 Y & 5 M	No growth
37	female	1 Y & 4 M	*E.coli*
38	male	5 M	No growth
39	female	1 Y	*S.aureus*
40	male	6 Y	*S.aureus*
41	male	3 Y	*P.mirabilis*
42	male	10 Y	No growth

43	male	3 Y	*H.influenzae*
44	male	4 Y	*E.faecalis*
45	female	5 Y	*M.catarrhalis*
46	female	3 M	*S.marcescens*
47	female	5 M	*S.aureus*
48	female	14 Y	*P.mirabilis*
49	female	6 M	*H.influenzae*
50	male	4 M	*S.aureus*
51	male	13 Y	*S.aureus*
52	male	7 M	*S.epidermidis*
53	female	12 Y	*S.aureus*
54	female	6 M	*S.aureus*
55	female	15 Y	Un identified
56	female	6 Y	*S.aureus*
57	male	2 Y	*S.epidermidis*
58	male	7 M	*S.aureus*
59	male	1 Y	*S.aureus*
60	male	13 Y	*P.aeruginosa*
61	male	3 Y	*P.mirabilis*
62	female	5 Y	*P.mirabilis*
63	female	7 M	*S.aureus*
64	female	1 Y & 7 M	*S.aureus*
65	male	1 Y & 8 M	*S.aureus*
66	female	1 Y	*S.aureus*
67	male	5 Y	No growth
68	male	3 Y & 6 M	Un identified
69	female	7 Y	*S.epidermidis*
70	male	1Y & 6 M	No growth
71	male	4 Y	*K.pneumoniae*
72	female	3 Y	*S.aureus*
73	female	2 Y	No growth
74	female	2 Y	*P.mirabilis*
75	male	5 Y	*P.mirabilis*
76	female	10 Y	*C.freundii*
77	female	1 Y & 8 M	*E.faecalis*
78	male	7 Y	Un identified
79	male	13 Y	*S.aureus*
80	female	1 Y	*S.epidermidis*

81	male	7 Y	No growth
82	male	7 M	*M.catarrhalis*
83	female	2 Y	*E.faecalis*
84	female	1 Y & 6 M	*S.aureus*
85	male	1 Y & 6 M	*S.aureus*
86	male	12 Y	*P.vulgaris*
87	male	6 Y	*P.aeruginosa*
88	male	2 Y	No growth
89	female	6 Y	No growth
90	female	2 Y	*S.aureus*
91	female	8 Y	*S.aureus*
92	female	7 Y	No growth
93	male	5 Y	*S.epidermidis*
94	female	10 Y	*P.aeruginosa*
95	male	12 Y	*P.aeruginosa*
96	male	3 Y	*P.mirabilis*
97	male	1 Y	*S.pneumoniae*
98	male	1 Y	*S.epidermidis*
99	male	5 Y	Un identified
100	male	8 Y	*K.pneumoniae*
101	male	9 Y	*P.aeruginosa*
102	male	8 M	*M.catarrhalis*
103	female	3 Y	*P.mirabilis*
104	male	1 Y & 3 M	*S.aureus*
105	male	1 Y & 4 M	*S.pyogenes*
106	female	9 Y	*S.pneumoniae*
107	male	5 Y	Un identified
108	female	3 Y	*H.influenzae*
109	male	5 Y	*S.marcescens*
110	female	8 Y	*S.marcescens*

Y: Year M: Month