APPENDIX-I

QUESTIONNAIRE OF UTI IN CHRONIC KIDNEY PATIENTS UNDERGOING HEMODIALYSIS

DATE: ……

SAMPLE NO.-……...

NAME- …… AGE- ……WEIGHT-……

ADDRESS- ……SEX-……:……

EDUCATION-……BLOOD PRESSURE-……

OCCUPATION-……BLOOD GROUP-……

MARITAL STATUS-……..MENOPAUSE-…….

GLOMERULAR FILTERATION RATE -……SERUM CREATININE -……..

PREVIOUS HISTORY-……

TRANSPLANTED ORGAN -……

TRANSPLANTED DURATION- ……

TRANSPLANTED KIDNEY – (left or right)-……..

DONOR (relationship and gender)-……

INFECTION - (CMV/HBV/HCV/HIV)-……

SYMPTOMS OF UTI (Dysuria, Frequency, Urgency, Fever, Flank Pain)……..

……

HISTORY OF BLOOD TRANSFUSION- (TAKEN/ GIVEN)-……

DIALYSIS-……

DIALYSIS NECESSITY - ……

DIABETES – (Yes/No)-…….

DURATION OF BEING DIABETIC -……...

HEALTH STATUS/HABIT -…….....

……...……...

QUESTIONNAIRE BY : BIBAS BASNET

मञ्जुंरीनामा/CONSENT/ASSENT

म / मेरो ……यस रिसर्च **"UTI in chronic kidney patients undergoing hemodialysis"** मा मेरो सो इच्छाले भाग लिएको हुँ । यो विद्यार्थीहरुको एक शोध हो । यसमा म आफू राजीखुशीले निम्न सर्तमा यो मञ्जुंरीनामामा सही छाप गरिदिएको छु । १ मेरो रगत/पिसाब यस अध्यनका लागि नमूनाका रुपमा दिएको छु ।२ मेरो व्यक्तिगत कुरा गोप्य रहने छन् ।३ म आफू राजीखुशीले कुनै पनि समयमा यो मञ्जुंरीनामा फिर्ता लिन पाउने छु ।४ मैले सबै जानकारी तथा जोखिम बुझेर कसैको दबाव बिना आफ्नो सो इच्छाले यो मञ्जुंरीनामामा मञ्जुंरी जनाएको हुँ । I/My……..... have agreed to participate in this research on "UTI in kidney patients" as a participant. I know this research is an academic process under the collaboration of National Kidney Centre, Banasthali and Central Department of Microbiology, Kirtipur. The procedures I agreed are the followings: 1. My urine or blood sample will be examined for this research. 2. My confidentiality will be maintained.3.My participation is voluntary. In any case I am free to withdraw my consent. 4. I have been explained and fully understand the risks involved and I have given this consent on my own will.

Name of participant (नाम)- Address(ठेगाना) -

Date (मिति)- Signature (सही)-

APPENDIX-II

Proforma

Clinical profile of the patient

Date:
…….

Urine Sample
no……...……

Lab code no.:
……..

Name of patient: …….…….....Age: ……... Sex:
……

Day 1:

Time of sample collection:
……..

Sample- Urine	
Chemical Examination	**Microscopic Examination**
pH-	Pus cells- /Hpf
Albumin-	RBC-- /Hpf
Sugar-	Epithelial cells-- /Hpf
	Casts- /Hpf

Culture of specimen on: a) MA……b) BA……..c) SDA…….

Incubation: a) Aerobic……b) Anaerobic…….

Day 2:

1. Reading of culture plates

Colony characteristics on MacConkey agar/Nutrient agar

Shape……..Size……Color……..Texture…….Lact
ose fermenting……..Lactose non
fermenting……

2. Gram staining test: …….Catalase test…….....Oxidase
test……..... Coagulase
test……...

Other biochemical

tests……

……

……..

The identified organism

was…….....

……

……..

3. Antibiotic Susceptibility Test

Antibiotic discs	Zone of inhibition (mm)	Result		
		Resistant	Intermediate	Sensitive
Amikacin				
Cephalexin				
Cefotaxime				
Ceftriaxone				
Cotrimoxazole				
Cefoxitin				
Imipenem				
Nalidixic acid				
Nitrofurantoin				
Ofloxacin				
Norfloxacin				

APPENDIX-III

Materials used during the study

Equipments-

Autoclave:	Life +
Centrifuge:	Techno Fab
Refrigerator:	Hitachi, Japan
Hot air oven:	Ambassador
Incubator:	Ambassador
Water bath:	Boekel 148003, Japan
Weighing balance:	Choyo MP, Japan
Microscope:	Olympus, Japan
Test tubes/ Petri plates-	Borosil

Media used-

Nutrient agar,
MacConkey agar,
Blood agar,
Mueller Hinton agar,
Mueller Hinton broth,
Sabouraud Dextrose agar,
MR/VP medium,
Triple Sugar Iron agar,
Simmons Citrate agar and
Sulfide Indole Motility agar.
The entire media used were from Hi Media Laboratories Pvt. Limited.

Chemical used-

Hydrogen peroxide,
Kovac's reagent,
Barritt's reagent,
Methyl red,
Oxidase,
Crystal violet,
Alpha-napthalamine,
Hydrochloric acid,
Phenol red and
Grams staining reagents.

Antibiotics used-

Amikacin (15µg),

Cefotaxime (30µg),

Cephalexin (30µg),

Cotrimoxazole (25µg),

Norfloxacin (10µg),

Nalidixic acid (30µg),

Nitrofurantoin (300µg),

Cefoxitin (30µg),

Ofloxacin (5µg) and

Imipenem (10µg).

These entire antibiotic discs used were from Hi Media Laboratories Pvt. Limited.

Standard test organisms-

E. coli **(ATCC 25922),** ***S. aureus*** **(ATCC 25923),** ***K. pneumoniae*** **(ATCC),** ***P. mirabilis*** **(ATCC)**

APPENDIX- IV

A. Composition and preparation of different media

1. MacConkey agar

Composition	Gm/lit
Peptic digest of animal tissue	17.0
Protease peptone	3.0
Lactose	10.0
Bile salt	1.5
Sodium chloride	5.0
Neutral red	0.03
Agar	15.0
Final pH at 25 °C	7.1±0.2

51.3 gram of the medium was dissolved in 1000 ml of distilled water and then boiled to dissolve completely. The media was autoclaved at 121°C (15 lbs pressure for 15 minutes. The sterilized medium was then poured into sterile petridishes and was allowed to cool.

2. Nutrient agar

Composition	Gm /lit
Peptic digest of animal tissue	5.0
Beef extract	1.5
Yeast extract	1.5
Sodium chloride	5.0
Agar	15.0
Final pH at 25°C	7.4±0.2

28 gram of the medium was dissolved in 1000 ml of distilled water and then boiled to dissolve completely. The media was autoclaved at 121°C (15 lbs pressure for 15 minutes. The sterilized medium was then poured into sterile petridishes and was allowed to cool.

3. Blood agar base (Infusion agar)

Composition	Gm/lit
Beef heart infusion form	500.0
Tryptose	10.0
Sodium chloride	5.0
Agar	15.0
Final pH at 25°C	7.3±0.2

42.5 gram of the medium was dissolved in 1000 ml of distilled water and then boiled to dissolve completely. The media was autoclaved at 121°C (15 lbs pressure for 15 minutes. After cooling to about 50-55°C, 5% v/v defibrinated sheep blood was added septically, then mixed with gentle rotation and poured into the sterilized petridishes and was allowed to cool.

4. Nutrient broth (NB)

Composition	Gm/lit
Peptic digest of animal tissue	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Final pH at 25°C	7.4±0.2

13.0 gram of the medium was dissolved in 1000ml of distilled water and then boiled to dissolve completely. The media was then dispensed into the tubes about 3ml in each and autoclaved at 121°C (15 lbs pressure for 15 minutes. The sterilized medium was then allowed to cool to room temperature.

5. Muller Hinton agar (MHA)

Composition	Gm/lit
Beef infusion	300.0
Casein acid hydrolysate	17.5
Starch	1.5
Agar	17.0
Final pH at 25°C	7.3±0.2

38gram of the medium was dissolved in 1000ml of distilled water and then boiled to dissolve completely. The media was autoclaved at 121° C (15 lbs pressure for 15 minutes. The sterilized medium was then poured into sterile petridishes and was allowed to cool.

B. Composition and preparation of different biochemical test media

1. Simon Citrate Agar

Composition	Gm/lit
Magnesium sulphate	0.2
Mono ammonium dihydrogen phosphate	1.0
Dipotassium phosphate	1.0

Sodium citrate	2.0
Sodium chloride	5.0
Bromothymol blue	0.08
Agar	15.0
Final pH at 25°C	6.8±0.5

24.2 gram of the medium was dissolved in 1000 ml of distilled water and then boiled to dissolve completely. 3 ml of media was then dispensed in each tube and autoclaved at 121°C (15 lbs pressure for 15 minutes. The sterilized medium was then allowed to settle at slant forming position.

2. Urea agar base (Christensen urea agar)

Composition	Gm/lit
Peptic digest of animal tissues	1.0
Dextrose	1.0
Mono potassium phosphate	0.8
Dipotassium phosphate	1.2
Sodium chloride	5.0
Agar	15.0
Phenol red	0.012
Final pH at 25°C	6.8±0.2

24.0 gram of the medium was dissolved in 950 ml of distilled water and then boiled to dissolve completely and autoclaved at 121°C for 15 minutes. After cooling to 50°C, 50 ml of sterile 40% urea solution was poured into the medium and mixed with gentle rotation. Then 5 ml of the medium was dispensed in each tube and slant was prepared.

3. Sulphide indole motility (SIM) agar

Composition	Gm/litre
Peptic digest of animal	30.0
Beef extract	3.0
Peptonised iron	0.2
Sodium thiosulphate	0.025
Agar	3.0
Final pH at 25°C	7.3±0.2

36.23 gram of the medium was dissolved in 1000 ml of distilled water and boiled to dissolve completely. Then it was dispensed in the test tube about 4 ml and autoclaved at 121°C (15 lbs pressure for 15 minutes. The sterilized medium was allowed to cool down.

4. MR-VP medium

Composition	Gm/lit
Buffered peptone	7.0

Dextrose	5.0
Dipotassium phosphate	5.0
Final pH at 25°C	6.9± 0.2

17.0 gram of the medium was dissolved in 1000 ml of distilled water and boiled to dissolve completely. 3ml was dispensed in each test tube and autoclaved at 121°C (15 lbs pressure for 15 minutes.

5. Triple sugar iron (TSI) agar

Composition	Gm/lit
Peptic digest of animal tissue	10.0
Casein enzymatic hydrolysate	10.0
Yeast extract	3.0
Beef extract	3.0
Lactose	10.0
Sucrose	10.0
Dextrose	1.0
Sodium chloride	5.0
Ferrous sulphate	0.2
Sodium thiosulphate	0.3
Agar	12.0
Phenol red	0.024
Final pH at 25°C	7.4± 0.2

65.0 gram of the medium was dissolved in 1000 ml of distilled water and boiled to dissolve completely. Then it was dispensed into the test tubes and autoclaved at 121°C (15 lbs pressure) for 15 minutes. The sterilized medium was then allowed to set in slant with a butt of 1 inch thickness.

C. Composition and preparation of different staining reagent

1. Gram stain

a. Crystal Violet Solution

Crystal violet	20.0 gm
Ammonium oxalate	9.0 gm
Ethanol or Methanol	95.0 ml
Distilled water	1000 ml

Preparation: 20 grams of crystal violet was weighed in a clean piece of paper and transferred to a clean brown bottle. Then 95.0 ml of ethanol was added and mixed until the dye is completely dissolved. To the mixture, 9.0 grams of ammonium oxalate dissolved in 200 ml of distilled water was added. Finally the volume was made 1000 ml by addition of distilled water.

b. Lugol's Iodine

Potassium iodide	20 gm
Iodine	10gm
Distilled water	1000ml

Preparation: To 250 ml of distilled water, 20 gm of potassium iodide was dissolved and 10 gm of iodine was mixed to it until it was dissolved completely. Finally the volume was made 1000 ml by addition of distilled water.

c. Acetone Alcohol Decolouriser

Acetone	500 ml
Ethanol (absolute)	475 ml
Distilled water	25.0ml

Preparation: 475 ml of ethanol was added to 25ml of distilled water and mixed and kept in a clean bottle. Then immediately 500ml of acetone was added to the bottle and mixed well.

d. Safranin (Counter stain)

Safranin (2.5% in 95% ethanol)	10.0 ml
Distilled water	100.0 ml

Preparation: 2.5% of safranin solution was prepared in 95% ethanol and 10ml of prepared suspension was mixed in 100ml of distilled water.

e. Normal saline

Sodium chloride	0.85 gm
Distilled water	100.0 ml

Preparation : 0.85 gram of sodium chloride was weighed and added to a bottle containing 100ml of distilled water and mixed well to dissolve the salt completely and autoclaved. Then it was stored.

Procedure for Gram staining procedure

Thin film of material to be examined was prepared and dried. The smear was heat fixed and allowed to cool before staining. The slide was flooded with crystal violet and allowed to remain without drying for 10 to 30 seconds. Then the slide was rinsed with tap water, shaking off excess. Then the slide was rinsed with iodine solution and allowed to remain on the solution without drying for 1 minute. The slide was then washed with tap water shaking off excess and flooded with acetone alcohol decolouriser for 10 seconds and rinsed immediately with tap water until no further color flows from the slide with the decolouriser. The slide was flooded with counter stain Safranine for 30 seconds and washed off with tap water. The slide was blotted between two sheets of bibulous paper and examined microscopically under oil immersion at 100X (Forbes *et al.* 2002).

Procedure for Germ tube test

A small inoculums made from isolated colony of yeast cell is suspended in 0.5ml of serum. Then the tube was incubated at 35^{o}C for 3hours. A drop suspension was observed under microscope. Then the sporulating yeast cells observed indicated the germ tube test positive by *Candida albicans.*

D. Biochemical test reagents and preparation procedures

a. Catalase Test

Catalase reagent (3% H2O2)

Hydrogen peroxide	1.0 ml
Distilled water	9.0 ml

Preparation: To the 9.0 ml of distilled water, 1ml of hydrogen peroxide was added and mixed well so as to make 3% solution of hydrogen peroxide.

b. Oxidase test

Oxidase strip soaked in oxidase reagent

Tetra mthyl para–phenylene diamine dihydrochloride (TPD)	1gm
Distilled water	100.0 ml

Preparation: 1 gram of TPD was dissolved in 100ml of distilled water and strips of Whatmann no.1 paper was soaked and drained for about 30 seconds. Then the strip was freeze and stored in dark bottle tightly.

c. Indole test

Kovac's indole reagent

Para dimethyl amino benzaldehyde	2.0 gm
Isoamyl alcohol	30.0 ml
Concentrated hydrochloric acid	10.0 ml

Preparation: In 30 ml of isoamyl alcohol, 2 gm of para amino benzaldehyde was dissolved and transferred to clean brown bottle. Then to this solution, 10 ml of concentrated hydrochloric acid was added and mixed well.

d. Methyl red test

Methyl red solution

Methyl red	0.05 gm
Ethyl alcohol	28.0 ml
Distilled water	22.0 ml

Preparation: 0.05 gm of methyl red was dissolved in 28ml of ethanol and transferred to a clean brown bottle. To this 22ml of distilled water was added and mixed well.

e. Voges Proskauer test

Barritt's reagent

Solution A

Alpha-Napthol	5.0 gm
Ethyl alcohol	100.0 ml

Preparation: 5 gm of α-Napthol was dissolved in 25ml ethanol and transferred into clean bottle. Then final volume was made 100ml by adding ethanol.

Solution B

Potassium hydroxide (KOH)	40.0 gm
Distilled water	100.0 ml

Preparation: 40 gm of KOH was dissolved in 25ml of distilled water and transferred into the clean bottle and final volume was made 100ml by adding distilled water.

f. Turbidity standard equivalent to McFarland 0.5

1% V/V solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99 ml of distilled water. 1% W/V solution of Barium Chloride was prepared by dissolving 0.5 gram of dehydrate barium chloride in 50 ml of distilled water. Then to the 99.5 ml of 1% sulphuric acid solution, 0.5 ml of barium chloride solution was mixed and stirred continuously. Then the solution was transferred into the clean screw capped tube and stored at dark place until use. The tubes was stored gingerly and used for six months during the course of study.

E. ZONE SIZE INTERPRETATIVE CHART (CLSI interpretation)

Antibiotics	Disc content (µg)	Resistant (mm)	Intermediate (mm)	Sensitive (mm)
Amikacin	**30**	**≤14**	**15-16**	**≥17**
Cefotaxime	**30**	**≤14**	**15-22**	**≥23**
Cephalexin	**30**	**≤14**	**15-17**	**≥18**
Cotrimoxazole	**25**	**≤10**	**11-15**	**≥16**
Ofloxacin	**5**	**≤12**	**13-15**	**≥16**
Norfloxacin	**10**	**≤12**	**13-16**	**≥17**
Nalidixic acid	**30**	**≤13**	**14-18**	**≥19**

Nitrofurantoin	**300**	**≤14**	**15-16**	**≥17**
Cefoxitin	**30**	**≤14**	**15-17**	**≥18**
Imipenem	**10**	**≤13**	**14-15**	**≥16**

(Based on Results obtained using Mueller Hinton Agar).

F. Morphology and Cultural characteristics of bacteria isolated from urine sample

Bacteria	Morphological characteristic	Cultural characteristics
Escherichia coli	**Gram negative rod, 1-3µm x 0.4- 0.7µm, Aerobic and anaerobic, Nonsporing, Motile, noncapsulated**	**MA- Bright pink colonies due to lactose fermentation, smooth, glossy, translucent** **BA- Large 1-2 mm, grayish white, moist, smooth, convex and opaque. The colonies may appear mucoid and some strains are haemolytic.**
***Klebsiella* spp.**	**Gram negative, short, thick rods of 1-2 µm x 0.8 µm in size, nonsporing, nonmotile and capsulated.**	**MA and BA- Large dome shaped moist and usually viscous or mucoid colonies**
***Proteus* spp.**	**Gram negative rod, 1-3 µm x 0.4-0.6 µm, noncapsulated**	**MA- Non lactose fermenting colonies.** **BA- When cultured aerobically most strains are swarming type and have a fishy odour.**

Staphylococcus aureus	**Gram positive, Spherical cocci, 0.8-1.0 µm, Non sporing, facultative anaerobe, Non motile except for rare strains, non capsulated. They are arranged in characteristics grape like clusters or in small groups , pairs , singles and short chain (less than five cocci in line)**	**MA- Small (pin head size), 0.1-0.5 mm, Pink or pink orange due to lactose fermentation. Some strains are non lactose fermenting. BA- Large 2-4 mm, Circular, smooth with glistening surface, entire edge, soft butyrous consistence and opaque and pigment appearance. The pigmentation is golden yellow to cream coloured. Some strains are Beta haemolytic when grow aerobically.**

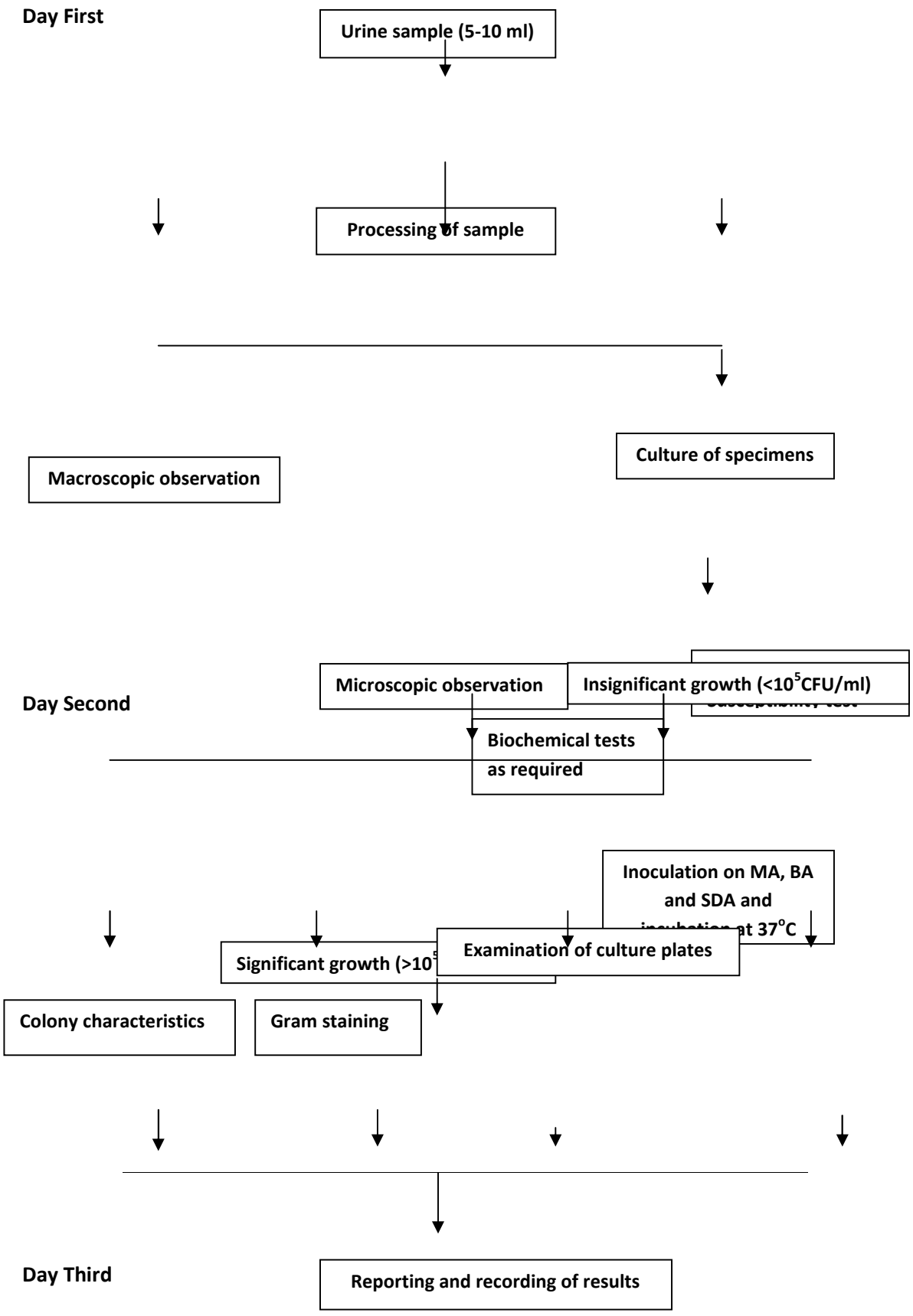
Day First
Urine sample (5-10 ml)
Processing of sample
Macroscopic observation
Culture of specimens
Day Second
Microscopic observation
Insignificant growth (<10^5CFU/ml)
Biochemical tests as required
Inoculation on MA, BA and SDA and incubation at 37°C
Examination of culture plates
Significant growth (>10^5
Colony characteristics
Gram staining
Day Third
Reporting and recording of results

Figure 1: Flow Diagram of Protocol of Urine sample

(Source- Cheesbrough M, 2000)