



For the Detection, Enumeration and Identification of UTI Pathogens

1. INTENDED USE

**MICROPRO™ BCS (Broth Culture System)** is a complete system that detects, enumerates and identifies most pathogens involved in Urinary tract infections, in less than five hours. It consists of (1) Broth Culture System that uses a Turbidimetry-based analyzer for the detection and enumeration of UTI pathogens (2) ID System for Biochemical identification of the UTI organisms.

2. INTRODUCTION

Urinary tract infection or UTI, is a major cause of morbidity in humans and one of the most frequently encountered infections that needs to be detected and diagnosed at the very earliest. These infections can occur at different points in the urinary tract, including bladder where it is called cystitis, in the urethra-urethritis and in severe cases when infection moves upwards and affects the kidneys, where it is known as pyelonephritis. Symptoms of a lower urinary tract infection can include painful urination and either frequent urination or urge to urinate (or both); while the symptoms of pyelonephritis include fever and flank pain in addition to the symptoms of a lower UTI. About 40% of women and 12% of men have a urinary tract infection at some time in their life. The main causative agent of most UTI's is *Escherichia coli*, though other bacteria, viruses or fungi may rarely be the cause. Most of the UTI are caused by the eight common urinary pathogens that account for approximately 97% of infections. The primary pathogen involved is *Escherichia coli*. The fairly common secondary pathogens are *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus spp.*, *Pseudomonas aeruginosa*. The secondary pathogen which is rarely involved is *Citrobacter spp.* The common urethral or genital flora include the *Lactobacillus*, *alpha haemolytic streptococci*, *Gardrerelle vaginalis*, *Corynebacteria spp.* etc.

Diagnostic testing of UTI includes urinalysis and urine culture. Urinalysis is a screening test, while the urine culture is considered the gold standard and the only way to make conclusive diagnosis of an UTI. Antibiotic sensitivity can also be performed with these cultures, making them useful in the selection of an appropriate antibiotic for treatment. Conventional diagnostic methods include quantitative culture on solid media, Gram staining of selected colonies and biochemical identification testing. This process can take more than twenty four to forty-eight hours before the microorganisms can be reported to the physicians.

**MICROPRO™ BCS** comprises of a novel way to detect and diagnose UTI by the Turbidimetry-based Bacterial broth culture detection. This system ensures that the entire process of detection and enumeration of UTI organisms is completed in four hours.

Apart from this a separate **MICROPRO™ ID** kit is available for the biochemical identification of UTI pathogens. The Biochemical analysis of UTI organisms can be completed in less than twenty- five minutes.

3. SPECIMEN COLLECTION AND PREPARATION

(a). First morning voided, midstream and clean catch urine is recommended for testing. Urine from first morning catheterization or suprapubic taps can also be used. Follow standard recommended procedures for collection of urine specimen. (b). Fresh urine should be used for testing. If a delay in testing is anticipated, store the specimen immediately at 2-8°C and ensure that the test is performed within 3 hours. (c). It is recommended that sterile containers are used to collect specimen. Contamination of specimen must be avoided.

Store at 2-8°C	Manufacturer	Batch Number	Production Site
Catalogue Number	Consult Instructions for use	Use by	Authorised Representative

**Microxpress®**

A Division of

**T TULIP DIAGNOSTICS (P) LTD.**

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Qarad b.v.b.a. Cipalstraat 3, B-2440 Geel, Belgium

#### 4. SYSTEM CONTENTS

S.No.	Component	Purpose	Qty.	Cat. No.
Installation Pack			1 pack	MSIP01
1	<b>MICROPRO™ BCS Analyzer</b> + one cable cord and two fuses	Turbidimetry- based analyzer system for detection and enumeration of UTI pathogens	1 unit	MA0001
2	Blanking Cuvettes (3 ml)	Cuvettes for zero blanking the <b>MICROPRO™ BCS Analyzer</b>	2 Nos.	MABC01
3	Printer Paper Roll	Paper Roll for Printing the test results	1 No.	MAPR01
4	<b>MICROPRO™ ID Well stand</b>	Stand for ID Test wells	1 No.	MIDS01
5	Fixed Volume Micropipette 100 µL	Micropipette for dispensing urine specimen into Barcoded Cuvette (A) & Testing vial (B)	1 No.	MAFP01
6	Fixed Volume Micropipette 300 µL	Micropipette for dispensing the Broth culture growth into ID Test wells	1 No.	MIFP03
7	User Manual		1 No.	
Reagent Packs				
1	<b>MICROPRO™</b> - Broth Culture kit a) Barcoded Cuvettes (A) - black cap (3 ml) b) Test Vials (B) - green cap (1.5 ml) c) 100 µL Microtips	Ready to use, Barcoded, Broth culture cuvettes for use on <b>MICROPRO™ BCS Analyser</b> & also for ID Test  Ready to use, Broth culture test vials for ID test only  Gamma - Sterile microtips with filter barrier for use with 100 µL Fixed volume pipette	2 x 25 Nos.  2 x 25 Nos.	MBCS01
2	<b>MICROPRO™</b> - ID kit a) 8 different ID reagents (5 ml) b) <b>MICROPRO™</b> ID wells	Reagents for biochemical identification of the UTI pathogens  Breakable test wells for use in the Biochemical ID Test	8 x 1 Nos. 12 wells x 25 Nos.	MIDR01
3	Quality Control kit consisting of a) <b>MICROPRO™</b> UTI Control - red cap (0.5 ml) b) Buffer Media c) 1 mL Micropipette tips	Eight different lyophilized UTI Controls  Media for reconstitution of the lyophilized UTI Controls  Sterile filter barrier microtips for 1 mL Micropipette for the Quality Control test	8 x 1 Nos. 1 No. 10 Nos.	MSQC01

CAT. NO.	ANTIMICROBIAL AGENT	SYMBOL	DISC CONTENT	PACK SIZE
TRT5				5 Carts
TRV5	Trimethoprim	TR	5 mcg	5 Vials
TR01				Single vial
COTT25				5 Carts
COTV25	Trimethoprim / Sulphamethoxazole	COT	1.25 /23.75 mcg	5 Vials
COT001				Single vial
VAT30				5 Carts
VAV30	Vancomycin	VA	30 mcg	5 Vials
VA001				Single vial

#### Biogram™ Pack size:

5 Cartridges (Each cartridge contains 50 discs) & Vials (5 vials = 5 x 100 discs in each vial and single vial = 1 x 100 discs in vial).

Discs in routine use should be stored at 2-8°C. Longer term storage should be at -20°C.

#### 17. REFERENCES

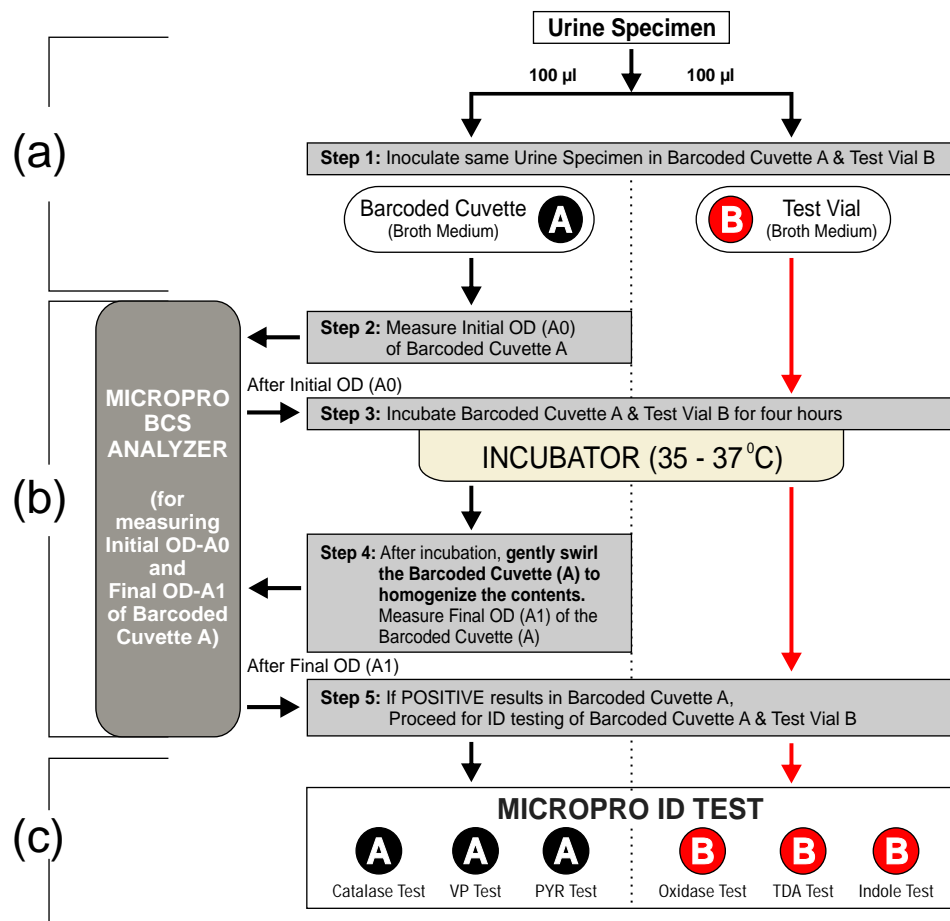
- (1). Practical Medical Microbiology, Mackie and MacCartney, Vol 2, 13 th ed, Churchill Livingston 1989, Edited by J,G Collee Duguid, A.G. Fraser, B.P. Marmion. (2). Detection, Prevention and Management of Urinary Tract infections, C.M Kunin, 4th Edition, 1987, (3). McPherson RA, Ben-Ezra J. Basic examination of urine. In: McPherson RA, Pincus MR, eds. (Henry's Clinical Diagnosis and Management by Laboratory Methods.) 22nd ed. Philadelphia, PA: Elsevier Saunders; 2011:chap 28. (4). Hooton TM, Bradley SF, Cardenas DD, et al. Diagnosis, prevention, and treatment of catheter-associated urinary tract infection in adults: 2009 International Clinical Practice Guidelines from the Infectious Diseases Society of America. Clin Infect Dis. 2010; 50(5):625-663. (5). Ban KM, Easter JS. Selected urologic problems. In: Marx JA, Hockberger RS, Walls RM, et al, eds. Rosen's Emergency Medicine: Concepts and Clinical Practice. 7th ed. Philadelphia, Pa: Mosby Elsevier; 2009: chap 97. (6). Dean AJ, Lee DC. Bedside laboratory and microbiologic procedures. In: Roberts JR, Hedges JR, eds. Clinical Procedures in Emergency Medicine. 5th ed. Philadelphia, Pa: Saunders Elsevier; 2009:chap 68. (7). MacFaddin, Jean F. "Biochemical Tests for Identification of Medical Bacteria." Williams & Wilkins, 1980, pp 173 – 183. (8). Bachoon, Dave S., and Wendy A. Dustman. Microbiology Laboratory Manual. Ed. Michael Stranz. Mason, OH: Cengage Learning, 2008. Exercise 15, "Normal Flora of the Intestinal Tract" Print. (9). Bergey's Manual of Systematic Bacteriology, Vol. 1. Baltimore, Williams and Wilkins, 1984. (10). Nicola F1, Centorbi H, Bantar C, Smayevsky J, Bianchini H., Utility of pyrrolidonyl-arylamidase detection for typing Enterobacteriaceae and non-fermenting Gram-negative bacteria, Rev Argent Microbiol. 1995 Oct-Dec;27(4):204-9. (11). Gordon J, McLeod JW. Practical application of the direct oxidase reaction in bacteriology. J Pathol Bacteriol 1928; 31:185-90. (12). Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC Jr. Color atlas & textbook of diagnostic microbiology. 5th ed. Philadelphia: JB Lippincott, 1997. (13). L Essers and K Radebold, Rapid and reliable identification of Staphylococcus aureus by a latex agglutination test. J Clin Microbiol. Nov 1980; 12(5): 641–643. (14). Kloos, W.E. and P.B. Smith. Staphylococci. 1980. Manual of Clinical Microbiology, 3rd ed. E.H. Lennette, A. Balows, W.J. Hausler, Jr. and J.P. Truant, ed. ASM, Washington, D.C. (15). Finegold, S.M. and E.E. Sweeney. 1961. New Selective and Differential Medium for Coagulase-Positive Staphylococci Allowing Rapid Growth and Stain Differentiation. J. Bacteriol.; 81:636-641. (16). Data on file: Microxpress (P) Ltd.

CAT. NO.	ANTIMICROBIAL AGENT	SYMBOL	DISC CONTENT	PACK SIZE
FFT200 FFV200 FF0001	Fosfomycin	FF	200 mcg	5 Carts 5 Vials Single vial
GENT10 GENV10 GEN001	Gentamicin	GEN	10 mcg	5 Carts 5 Vials Single vial
IPMT10 IPMV10 IPM001	Imipenem	IPM	10 mcg	5 Carts 5 Vials Single Vial
LET5 LEV5 LE01	Levofloxacin	LE	5 mcg	5 Carts 5 Vials Single Vial
LZT30 LZV30 LZ001	Linezolid	LZ	30 mcg	5 Carts 5 Vials Single Vial
MRPT10 MRPV10 MRP001	Meropenem	MRP	10mcg	5 Carts 5 Vials Single vial
MZT75 MZV75 MZ001	Mezlocillin	MZ	75mcg	5 Carts 5 Vials Single vial
MXFT5 MXFV5 MXF01	Moxifloxacin	MXF	5 mcg	5 Carts 5 Vials Single vial
NITT300 NITV300 NIT0001	Nitrofurantoin	NIT	300 mcg	5 Carts 5 Vials Single vial
NXT10 NXV10 NX001	Norfloxacin	NX	10 mcg	5 Carts 5 Vials Single vial
OXT1 OXV1 OX01	Oxacillin	OX	1mcg	5 Carts 5 Vials Single vial
PIT100 PIV100 PI0001	Piperacillin	PI	100 mcg	5 Carts 5 Vials Single vial
PTZT100 PTZV100 PTZ0001	Piperacillin/Tazobactam	PTZ	100/10 mcg	5 Carts 5 Vials Single vial
TEIT30 TEIV30 TEI001	Teicoplanin	TEI	30 mcg	5 Carts 5 Vials Single vial
TET30 TEV30 TE001	Tetracycline	TE	30 mcg	5 Carts 5 Vials Single vial
TGCT15 TGCV15 TGC001	Tigecycline	TGC	15 mcg	5 Carts 5 Vials Single vial
TOBT10 TOBV10 TOB001	Tobramycin	TOB	10 mcg	5 Carts 5 Vials Single vial

## 5. TEST PRINCIPLE

The entire system is based on three fundamentals:

- Proprietary broth culture medium to support growth of typical UTI pathogens.
- Detection and enumeration of UTI based on Turbidity.
- Identification of the UTI pathogen based on well-characterized biochemical tests.



## 6. STORAGE AND STABILITY

(a). Store the Barcoded Cuvettes (A)-black cap, Test Vials (B) -green cap and the ID reagents at 2-8 °C. (b). DO NOT FREEZE. Avoid exposure to light. (c). The shelf life of the Barcoded Cuvettes (A)-black cap, Test Vials (B) -green cap and the ID reagents is as per expiry date mentioned on respective carton/ bottle packaging.

## 7. MATERIAL REQUIRED BUT NOT PROVIDED WITH THE KIT

Bacteriological Incubator (at 35 - 37 °C), Marker Pens, Timer, Tissue Paper, 70% IPA, Bactericidal Handrub, Gloves, Masks and Removable Stickers.

## 8. PRECAUTIONS

(a). For laboratory use only. (b). Bring all reagents and specimen to room temperature (20 - 30°C) before use. (c). Do not use the kits beyond expiry date. (d). Carefully read the User Manual and package inserts before use. (e). Take Universal Precautions. All human body fluids should be treated as potentially infectious. (f). Always be prepared for any accidental spillage. In case of accidental spillage clean the area thoroughly and wipe with 70% IPA at least three times. (g). It is recommended that basic Personal Protective Equipment like gloves and masks are used at all times. (h). Use a Bactericidal Handrub before and after test procedure. (i). Visually examine the Barcoded Cuvettes (A) with black caps and the Test Vials (B) with green caps to ensure there is no physical damage, microbial contamination, discoloration, precipitation, evaporation or other signs of deterioration. If any of these is observed, do not use these reagents and contact Service provider immediately. (j) The Barcoded Cuvettes (A) and the Test Vials (B) are capped tightly, open carefully to avoid injury due to breakage of glass. (k). Minimize exposure to light. (l). Ensure that the broth culture media (Barcoded Cuvettes (A), the Test Vials (B)) attain room temperature before inoculation and for Biochemical Identification test.

## 9. CLEANING AND DECONTAMINATION

(a). Spills of potentially infectious material should be cleaned up immediately with absorbent paper tissue and the contaminated area should be decontaminated with disinfectants such as 0.5% freshly prepared sodium hypochlorite (10 times dilution of 5% sodium hypochlorite i.e. household bleach) before continuing work. (b). Sodium hypochlorite should not be used on an acid-containing spill unless the spill-area is wiped dry first. Materials used to clean spills, including gloves, should be disposed off as potentially biohazardous waste e.g. in a biohazard waste container.

## 10. TEST PROCEDURE

The user is requested to familiarize with the working of the **MICROPRO™ BCS** Analyzer before embarking on the Test Procedure. (Kindly refer the **MICROPRO™ BCS** Analyzer user manual).

### 1. Detection and Enumeration of the UTI pathogens

The detection and enumeration procedure involves the following steps:

#### A). Inoculation of the Barcoded Cuvettes (A) -black cap and the Test Vials (B)- green cap with the patient's urine specimen

Note: (a). After inoculation only the Barcoded Cuvettes (A)-black cap, are to be used for testing on the **MICROPRO™ BCS** Analyzer. (b) The Test Vials (B)-green cap along with the Barcoded Cuvettes (A)-black cap are for Biochemical identification of the UTI organisms using **MICROPRO™** ID kit.

1. Retrieve the required number of Barcoded Cuvettes (A)- black cap and the Test Vials (B)- green cap corresponding to the number of samples to be tested and place it on a stand or a flat clean table top. Kindly note that a single set of Barcoded Cuvette (A)- black cap and the Test Vial (B)- green cap is to be used for one patient.
2. Write patient IDs/names specifically within the space indicated on each set of Barcoded Cuvette (A)- black cap and the Test Vial (B)- green cap.
3. Retrieve the required number of 100 µl micropipette tip pouches from the pack. A pair of microtip is to be used for testing one patient sample. The Individual microtip pouches must be opened just before the inoculation process so as to minimize contamination risk.
4. Open the black cap of the Barcoded Cuvette (A) and the green cap of the Test Vial (B) of one patient and keep aside. Do not remove the yellow coloured rubber stopper.
5. Pipette out 100 µl of well-mixed urine from the sample container and transfer it to the opened Barcoded Cuvette (A) by piercing the pipette microtip through the stopper of the Cuvette and pressing the plunger of micropipette up to the bottom. Do not release the plunger. Holding the rubber stopper tight in its place, slowly remove the microtip, ensuring that the rubber stopper does not come out along with the microtip. Ensure that urine is mixed well with the broth in the Barcoded Cuvette (A) by gently swirling it.

## 16. **BIOGRAM™** Antibiotics Disc as per CLSI standards

CAT. NO.	ANTIMICROBIAL AGENT	SYMBOL	DISC CONTENT	PACK SIZE
AKT30 AKV30 AK001	Amikacin	AK	30 mcg	5 Carts 5 Vials Single vial
AMPT10 AMPV10 AMP001	Ampicillin	AMP	10 mcg	5 Carts 5 Vials Single vial
AMCT30 AMCV30 AMC001	Amoxicillin/Clavulanate	AMC	20/10 mcg	5 Carts 5 Vials Single vial
ATT30 ATV30 AT001	Aztreonam	AT	30 mcg	5 Carts 5 Vials Single vial
CZT30 CZV30 CZ001	Cefazolin	CZ	30 mcg	5 Carts 5 Vials Single vial
CPMT30 CPMV30 CPM001	Cefepime	CPM	30 mcg	5 Carts 5 Vials Single vial
CTXT30 CTXV30 CTX001	Cefotaxime	CTX	30 mcg	5 Carts 5 Vials Single vial
CECT30 CECV30 CEC001	Cefotaxime/Clavulanic acid (Not as per CLSI)	CEC	30/10 mcg	5 Carts 5 Vials Single Vial
CXT30 CXV30 CX001	Cefoxitin	CX	30 mcg	5 Carts 5 Vials Single vial
CAZT30 CAZV30 CAZ001	Ceftazidime	CAZ	30 mcg	5 Carts 5 Vials Single vial
CACT30 CACV30 CAC001	Ceftazidime/Clavulanic acid (Not as per CLSI)	CAC	30/10 mcg	5 Carts 5 Vials Single Vial
CTRT30 CTRV30 CTR001	Ceftriaxone	CTR	30 mcg	5 Carts 5 Vials Single vial
CXMT30 CXMV30 CXM001	Cefuroxime	CXM	30 mcg	5 Carts 5 Vials Single vial
CIPT5 CIPV5 CIP01	Ciprofloxacin	CIP	5 mcg	5 Carts 5 Vials Single vial
CDT2 CDV2 CD01	Clindamycin	CD	2 mcg	5 Carts 5 Vials Single vial
CLT10 CLV10 CL001	Colistin	CL	10 mcg	5 Carts 5 Vials Single vial
ETPT10 ETPV10 ETP001	Ertapenem	ETP	10 mcg	5 Carts 5 Vials Single Vial

#### 14. PERFORMANCE DATA

##### Internal Evaluation

- A) In an in-house evaluation, 110 urine specimens were tested simultaneously both on **MICROPRO™ BCS** and on standard solid Agar medium.

Total Samples = 110	<b>MICROPRO™ BCS</b>	PLATE CULTURE
Positive for UTI	14	14
Negative for UTI	76	70
Contamination	20	26

From the above evaluation it is observed that

- For Positive UTI samples: **MICROPRO™ BCS** showed 100% correlation with PLATE CULTURE
  - For Negative samples: 76 samples were reported negative by **MICROPRO™ BCS** and 70 samples by PLATE CULTURE.
  - Contamination was observed in 20 samples tested by **MICROPRO™ BCS** and 26 samples by PLATE CULTURE method.
- B) Precision: Stability studies were performed on Broth culture media (Barcoded Cuvettes (A) and Test vials (B) for color change, pH, conductivity and OD at 546 nm for three different temperatures 2-8°C, Room temperature and 37°C for a period of 15 days and no variation was observed in color and marginal variation was observed in the other parameters
- C) Performance: Repeatability and Reproducibility were performed with same sample inoculated in five Barcodes Cuvettes (A) and Initial and Final OD measured. Despite Variation in Initial and Final OD for the same sample inoculated in five cuvettes, the result obtained is same, owing to the delta OD, which diminishes the variation from Cuvette to Cuvette resulting in 100% reproducibility of results.

#### 15. REMARKS

- (A) False negative results can occur if the contents of the Barcoded Cuvette (A) are not mixed properly prior to measuring the Final absorbance A1 as the culture growth tends to settle down in the Barcoded Cuvette (A)/Test Vial (B) during incubation.

To dislodge the cultures settled during incubation, the correct way is to: Hold the cap of the Barcoded Cuvette (A)/ Test Vial (B) and gently swirl it to form a homogeneous suspension. Do not shake vigorously or turn the vial upside down during shaking. ( See Fig 1)



Fig 1

- (B) Use only the gamma sterile tips provided for the sample pipette (100 µl). Avoid using ETO sterilized tips as, there will be ETO residue left on the tip surface which might get added into the broth during inoculation, leading to growth inhibition.
- (C) Procedure for dispensing sample in Broth culture cuvettes (A&B):
- (1) Place your thumb over the top of the pipette plunger and press down to the first stop. Hold the plunger in this position. (2) Place the pipette tip into the sample at the proper immersion depth and release the pressure on the plunger slowly. Be sure that the pipette is either vertical or within 20° off vertical. This will allow the piston to rise, aspirating sample fluid into the pipette tip. Do not remove your thumb from the plunger abruptly or let the plunger rise up quickly. This will result in aspirating incorrect sample volumes. (3) Once the plunger reaches the starting position, pause for a second or two to ensure that the aspiration of the sample fluid is complete. Be sure there are no bubbles in the tip. (4) Remove the pipette tip from the sample volume. (5) To dispense, pierce the cuvette stopper with the pipette tip and then press the plunger down slow and steadily passing the first stop all the way to the bottom of the piston stroke. Wait 1-2 seconds to allow complete dispensing of the sample fluid. Do not release the plunger now. (6) Remove the pipette tip from the Broth culture cuvette and then release the plunger. (7) Press the ejector button to remove the pipette tip or carefully pull the tip off with your fingers and disposed of the tip in the proper biohazard container according to the safety requirements of your facility.

Note: For detailed pipetting procedure, refer Section 15 C.

6. Using a fresh microtip, pipette out 100 µl of well mixed urine from same sample container and transfer it to the opened Test Vial (B) by piercing the micropipette tip through the stopper of the Test Vial and pressing the plunger of micropipette up to the bottom. Do not release the plunger. Holding the rubber stopper tight in its place, slowly remove the microtip, ensuring that the rubber stopper does not come out along with the microtip. Ensure that urine is mixed well with the broth in the Test Vial (B) by gently swirling the urine-broth mixture inside the vial.
  7. Repeat step 4-6 for all urine samples to be tested. Use fresh microtips each time.
  8. The Barcoded Cuvettes (A) and the Test Vials (B) must be recapped with their original black and green cap respectively and placed in the cuvette stand (card board fitment) supplied with the kit.
- B) Blanking the **MICROPRO™ BCS** Analyzer
- Blanking is to be done once a day or when the machine gets switched OFF during the procedure.
9. Switch ON the **MICROPRO™ BCS** Analyzer. The red power display LED will blink, Press power button followed by the "NEXT" key. The Analyzer will display "Insert Blank and press NEXT or EXIT".
  10. Retrieve one Blanking Cuvette provided with Analyzer. Clean the Blanking Cuvette with the dry tissue. Note: Always make sure to clean the Cuvette with a clean dry tissue paper before blanking or taking OD readings.
  11. Insert it in the reading chamber carefully ensuring that the arrow mark on the Blanking Cuvette aligns with the arrow mark next to the reading chamber of the Analyzer and press "NEXT".
  12. After Blanking, enter the lot number details of the Barcoded Cuvette (A) - black cap, in the Analyzer followed by the expiry details which is also mentioned on the Barcoded Cuvette (A) - black cap, in the Analyzer and press "NEXT".
  13. The main menu will be displayed, using the scroll down Arrow key, bring the cursor to Lot number to "Initial Absorbance" and press "NEXT"
- C) Measuring Initial Absorbance (Ao) of inoculated Barcoded Cuvette (A)-black cap on the **MICROPRO™ BCS** Analyzer
14. Retrieve one Barcoded Cuvette (A) inoculated with patient's urine sample.
  15. Insert it carefully in reading chamber as in Step No.11.
  16. The initial absorbance (Ao) for the particular Barcoded Cuvette (A) will be displayed on the Analyzer screen. Press "NEXT" key as this will ensure recording of the Initial absorbance (Ao) value in the memory of the Analyzer. Remove the Barcoded Cuvette (A) from the reading chamber and place it in the cuvette stand.
  17. Repeat steps (14-16) for rest of the inoculated Barcoded Cuvettes (A). Do not insert Test Vial (B) in the reading chamber.
  18. After measuring (Ao) values of all the Barcoded Cuvettes (A), do not switch OFF the Analyzer.
  19. Proceed for Incubation of the Barcoded Cuvettes (A) and the Test Vials (B).
- D) Incubation of the inoculated Barcoded Cuvette (A)-black cap and the Test Vials (B)-green cap
20. Incubate all the inoculated Barcoded Cuvettes (A) and the Test Vials (B) for 4 hours in a Bacteriological Incubator (at 35 - 37°C).
  21. After 4 Hrs. of incubation, proceed to measure the Final absorbance (A1) values of the Barcoded Cuvettes (A) on the **MICROPRO™ BCS** Analyzer.
- E) Measuring Final Absorbance (A1) of the incubated Barcoded Cuvettes (A)-black cap
22. In the display menu of the Analyzer using the scroll down Arrow key, bring the display cursor to "Final Absorbance" in the Main Menu and press "NEXT".
  23. Retrieve one incubated Barcoded Cuvette (A), before inserting it in Analyzer for measuring Final absorbance, kindly note **it is imperative to gently swirl the Barcoded Cuvette (A) to disperse**

**culture in the broth.** Do not shake the cuvette vigorously as this may result in false negative results. Check Section 15 Remarks. After mixing the contents, follow the procedure as in step (11).

24. On valid identification of the Barcoded Cuvette (A), the Analyzer will display the patient name, sample ID, test ID and Final absorbance. Press "NEXT" key as this will ensure recording of the Final absorbance (A1) value in the memory of the Analyzer. Remove the Barcoded Cuvette (A) from the reading chamber and place it in the Cuvette stand.
25. Repeat steps (23-24) for rest of the incubated Barcoded Cuvettes (A).
26. After completing the Final absorbance (A1) for all the incubated Barcoded Cuvettes (A) scroll down in main menu to bring the display cursor to "Results" and press "NEXT". The results will be displayed Sample Id Wise and the print out can be taken. Details of the "Result" menu management is mentioned in the **MICROPRO™ BCS** Analyzer user manual.

F) Result interpretation of Barcoded Cuvettes (A)-black cap

There are five types of results expected for the urine samples in **MICROPRO™ BCS** Analyzer, and based on these results further action is recommended.

S.No.	Result reported in <b>MICROPRO™ BCS</b> Analyzer	Interpretation of the results	Further Action
1	Negative	Negative for UTI	No further action required. Report with printout of result.
2	$10^3 < 10^5$ CFU/ml	Threshold or Evolving UTI infection	Proceed for Biochemical identification testing as recommended in Biochemical ID Kit packinsert.
3	$10^5 < 10^7$ CFU/ml	Positive (Frank UTI)	Proceed for Biochemical identification testing as recommended in Biochemical ID Kit packinsert.
4	$10^7 < 10^8$ CFU/ml	Positive (Frank UTI with High Count)	
5	$> 10^8$ CFU/ml	Very High Count. Mixed infection possible.	Proceed for Biochemical identification testing as recommended in Biochemical ID Kit packinsert.

Negative Test results:

- The Barcoded Cuvettes (A) with Negative results along with its Test Vials (B) are to be discarded as per local disposal guidelines, and sample to be reported negative with printout of the result.

Positive Test results:

- The Barcoded Cuvette (A) with Positive results along with the corresponding Test Vial (B) are to be retained in the Cuvette stand and then proceed for Biochemical identification testing using **MICROPRO™ ID** Kit.

Note (a) ODs displayed on the instrument screen are for indicative purpose only.

11. BIOCHEMICAL IDENTIFICATION OF UTI PATHOGENS

The **MICROPRO™ ID** test helps in identifying the eight possible UTI pathogens namely *E. coli*, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Proteus mirabilis*, *Proteus spp.*, *Pseudomonas aeruginosa*, *Citrobacter spp.*, *Klebsiella pneumoniae*. For the Test procedure refer the **MICROPRO™ ID**-kit Pack insert.

12. ANTIBIOTIC SUSCEPTIBILITY TEST

After the completion of Biochemical Identification tests, proceed for Antibiotic Susceptibility Test in four steps as follows:

- Step 1) After completing Biochemical Identification tests about 1 ml of broth will remain in **MICROPRO™** Barcoded Cuvette (A). Centrifuge the cuvettes at RCF of 5000 g for 10 minutes at room temp. A visible pellet will be observed. Discard the supernatant carefully by inverting the cuvette and retain the pellet. With a sterile loop, touch the pellet and transfer it to the tube of saline/peptone water. Dissolve the inoculum thoroughly to avoid clumping of the cells. Adjust turbidity of inoculum to match the standard, i.e., McFarland 0.5 equals approximately  $10^8$  CFU/mL.
- Step 2) Proceed for inoculation on Mueller-Hinton Agar plate. Visually examine the Mueller Hinton Agar plates prior to use, ensure that the plates are: free from visible contamination, poured to a uniform depth of approximately 4mm, not excessively wet and not cracked or dry. Sterile Ready Prepared Mueller- Hinton Agar Plates (Cat no. SP0012011) is available with **MICROXPRESS®**. Within 15 minutes of preparing the adjusted inoculum, dip a sterile cotton swab (Steristik, Cat no. AC0003) into the inoculum. Rotate the swab several times and press firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab. Streak the swab over the entire surface of the Mueller Hinton agar plate. Rotate the plate approximately 60° then repeat streaking motion. Rotate 60° again and repeat streaking for the third time. Complete inoculation by running the swab around the rim of the agar.
- Step 3) Dispense the required number of antibiotic disks to the agar surface with a sterile forcep or disc dispenser (forceps can be sterilized by flaming with alcohol. Avoid using over-heated forceps). Do not relocate any antibiotic disk after contact with the agar. After application, ensure that the antibiotic disk has made complete contact with the agar surface by touching the top of the disk with forceps. Antibiotic discs (**Biogram™**) recommended for UTI pathogens (as per CLSI) are available with **MICROXPRESS®**. (Refer antibiotics list mentioned in Section 16).
- Step 4) Keep the plates in the incubator set at 35-37° C for overnight and then look for zone of inhibition as per CLSI guidelines. Refer **Biogram™** Packinsert for detailed AST analysis.

13. QUALITY CONTROL PROCEDURES

Quality control of **MICROPRO™ BCS** system can be performed with **MICROPRO™** UTI Control kit which is available separately (Cat No MSQC01).

Quality control is to be performed for each batch to ensure that (1) Barcoded Cuvettes (A), Test Vials (B), the **MICROPRO™ ID** Kit reagents and the **MICROPRO™ BCS** Analyzer are working as per specifications. For the Test procedure refer the **MICROPRO™** UTI Control Kit pack insert.