



### UTI Controls

#### 1. INTENDED USE

The **MICROPRO™** UTI control kit consists of eight different UTI pathogens, each available in a lyophilized format for the validation of **MICROPRO™ Broth Culture System**.

#### 2. INTRODUCTION

**MICROPRO™ Broth Culture System (BCS)**, a novel method for diagnosis of UTI infection, detects and enumerates the UTI organisms in less than 4 hours. This method uses a proprietary broth culture medium, the Barcoded Cuvettes (A) and the Test Vials (B), for the growth of the UTI organisms. For samples that are positive, significant bacteriuria in the broth culture medium, the causative UTI pathogen can be identified biochemically within twenty-five minutes using the **MICROPRO™** ID kit.

The **MICROPRO™** UTI control kit consists of eight different UTI pathogens/ controls for performing quality control of

- (1) **MICROPRO™ BCS** Broth culture media - the Barcoded Cuvettes (A) and Test Vials (B)
- (2) **MICROPRO™** ID reagents
- (3) Assay procedure of both broth culture system and identification system.
- (4) **MICROPRO™ BCS** Analyzer

#### 3. TEST PRINCIPLE

The properties of the UTI lyophilized control reagents, when reconstituted with an appropriate buffer media, are similar to the UTI positive specimen. As these UTI controls have assigned values, when substituted in place of specimen they serve as reagent and procedure quality control for both, **MICROPRO™** Broth culture and **MICROPRO™** ID assays.

#### 4. KIT CONTENTS

The **MICROPRO™** UTI control kit (Cat No. MSQC01) consists of the following :

S.No.	Component	Purpose	Qty.
1.	<b>MICROPRO™</b> UTI Controls - red cap (0.5 ml)	Eight different UTI Controls (lyophilized) for Quality Control	8 Nos.
2.	Buffer Media (5 ml)	Media for reconstitution of lyophilized UTI Controls	2 Nos.
3.	1 ml Microtips	Sterile microtips for dispensing Buffer Media in UTI Controls	10 Nos.

#### 5. STORAGE AND STABILITY

Store the UTI Controls and the Buffer Media at 2-8 °C.

**DO NOT FREEZE.** Avoid exposure to light.

The shelf life of the UTI Control kit and the Buffer Media is as per the expiry date mentioned on respective carton/ bottle packaging.

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

1 ml pipette, 100 µl micropipette, 300 µl micropipette, Soybean Casein Digest Agar or Mueller Hinton Agar Plates, Bacteriological Incubator set at 35-37°C, Marker Pen, Tissue Paper, 70% IPA, Bactericidal Handrub, Gloves and Mask.

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

## Micropress®

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## 7. PRECAUTIONS

- (a) For laboratory use only.
- (b) Bring all the reagents 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 insert before use.
- (e) Take Universal Precautions. Treat all reagents and controls 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 potent Handrub before and after test procedure.
- (i) Visually examine the UTI Control kit and the Buffer Media 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 UTI Control vial and the Buffer Media vial with tight caps should be opened carefully to avoid injury due to breakage of glass.
- (k) Do not open the UTI Control vial and the Buffer Media vial until ready to use.
- (l) Minimize exposure to light.

## 8. CLEANING AND DECONTAMINATION

- (a) Spills of potentially infectious material should be cleaned up immediately with absorbent tissue paper and the contaminated area should be decontaminated with disinfectants such as 0.5% freshly prepared sodium hypochlorite (10 times dilution of 5% sodium hypochlorite (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.

## 9. TEST PROCEDURE

### A). Quality Control of **MICROPRO™ Broth Culture System**

#### a) Reconstitution of UTI Controls

Note: The UTI Controls kit consists of eight different UTI controls/ pathogens available in lyophilized form. For reconstitution of UTI controls, maintain a sterile environment, while working in Laminar Air flow or in between two Bunsen burners.

1. Open the red cap and rubber stopper of one UTI control vial and place them on a sterile area.
2. Using 1 ml pipette and a sterile microtip provided with the kit, reconstitute the UTI control with 1 ml Buffer Media and then pipette in and out several times to ensure proper dispensing and mixing of lyophilized cultures in the Buffer Media.
3. After reconstitution, using the same tip, perform streaking on Soybean Casein Digest Agar or Mueller Hinton Agar Plates to check for contamination.
4. Discard the tip in 0.5% freshly prepared sodium hypochlorite solution and put the rubber stopper and red cap back on the UTI control vial.
5. Gently swirl the vial to ensure proper mixing of lyophilized cultures in the broth.
6. Using a fresh tip repeat steps 1 to 5 for rest of the UTI control vials.
7. Place the reconstituted UTI Controls kit along with the stand in bacteriological Incubator set at 35-37°C for 16 hours.

#### b) Inoculation of the UTI Controls into the Barcoded Cuvette (A) and the Test Vial (B)

1. After 16 hours of incubation, retrieve and place the eight reconstituted UTI Controls to be tested on flat clean table top.

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2. Retrieve eight sets of Barcoded Cuvettes (A)- black cap and the Test Vials (B)- green cap, as one set of Barcoded Cuvette (A)- black cap and Test Vial (B)- green cap is to be used for one reconstituted UTI control.
3. Label each of the Barcoded Cuvette (A)- black cap and the Test Vial (B)- green cap set with UTI control name.
4. Retrieve 8 pouches of 100 µl micropipette tips. The individual microtip pouches must be opened just before the inoculation process so as to minimize contamination risk.
5. Open the black cap of one of the Barcoded Cuvette (A) and the green cap of its corresponding Test Vial (B) and keep aside. Do not remove the yellow coloured rubber stopper.
6. Remove the cap and rubber stopper of the respective UTI Control vial, pipette out 100 µl of the well mixed reconstituted UTI Control and transfer it into the opened Barcoded Cuvette (A) by piercing the microtip through the rubber stopper.
7. While removing out the microtip from the Cuvette, hold the rubber stopper tight so that the rubber stopper does not come out along with the microtip. Discard the microtip. Ensure that the UTI Control mixes well with the broth by gently swirling the vial.
8. Using another fresh microtip, pipette out the remaining 100 µl of reconstituted UTI control from the same UTI Control vial and transfer it to the corresponding opened Test Vial (B) by piercing the micropipette tip through the stopper of the Test Vial. Again ensure that the UTI Control mixes well with broth in the Test Vial (B) by gently swirling the vial.
9. Repeat step (5-8) for the rest of the reconstituted UTI controls. Use fresh microtips each time.
10. The Barcoded Cuvettes (A) and the Test Vials (B) can then be recapped with their original black and green cap respectively and placed in the Cuvette stand (card board fitment) supplied with the kit.

Note: For the rest of the procedure, namely

- c) Blanking of the MICROPRO™ BCS Analyzer
- d) Measuring Initial Absorbance (A0)
- e) Incubation of the inoculated Barcoded Cuvettes (A) and the Test Vials (B)
- f) Final Absorbance (A1) of the Barcoded Cuvettes (A).

Follow the procedure from the MICROPRO™, Broth Culture System pack insert, refer Segment- 10. TEST PROCEDURE, Section (B), (C), (D)& (E)

- g). Result interpretation of Barcoded Cuvettes (A)-black cap inoculated with UTI controls  
The, difference in the final (A1) and initial (A0) OD readings of the UTI controls, i.e, Delta-OD Values obtained in the Results of the MICROPRO™ BCS Analyzer for each of the Barcoded Cuvettes (A) with different UTI Controls should match with the Expected Delta-OD Values given in the following table (Table -1) for the eight different UTI Controls.

Table - 1

UTI Controls	Expected Values CFU/ml	UTI Controls	Expected Values CFU/ml
<i>E. coli</i>	10 <sup>3</sup> < 10 <sup>8</sup> CFU/ml	<i>Proteus spp.</i>	10 <sup>3</sup> < 10 <sup>8</sup> CFU/ml
<i>Enterococcus faecalis</i>	10 <sup>3</sup> < 10 <sup>8</sup> CFU/ml	<i>Pseudomonas aeruginosa</i>	10 <sup>3</sup> < 10 <sup>8</sup> CFU/ml
<i>Streptococcus pyogenes</i>	10 <sup>3</sup> < 10 <sup>8</sup> CFU/ml	<i>Citrobacter spp.</i>	10 <sup>3</sup> < 10 <sup>8</sup> CFU/ml
<i>Proteus mirabilis</i>	10 <sup>3</sup> < 10 <sup>8</sup> CFU/ml	<i>Klebsiella pneumoniae</i>	10 <sup>3</sup> < 10 <sup>8</sup> CFU/ml

## B) Quality Control of MICROPRO™ ID System

Note: A MICROPRO™ ID well strip consists of twelve breakable wells. Six wells are required to perform the six tests for identifying a single UTI Control. (refer Table 2)

W1	W2	W3	W4	W5	W6
Catalase test	Voges-Proskauer (VP) test	Pyrrolidinyl Arylamidase (PYR) test	Oxidase test	Tryptophan deaminase (TDA) Test	Indole test

For identifying eight UTI Controls, use four MICROPRO™ ID well strips and break each strip equally into two to get eight of 6-well strips.

### a) Reagent Addition

- Place the eight (6 wells) strips in the MICROPRO™ ID Well stand in a row.
- Paste a Removable Sticker at the end of the each well strip row in the well stand to write the UTI Control name.
- Retrieve the eight MICROPRO™ ID reagents and place it on the table. Allow it to attain room temperature.
- As shown in Table 3, to the First well strip, Dispense  
2 drops of R1 reagent into first well.  
2 drops of Reagent R-2.1, R-2.2 & R-2.3 each to well No.2  
2 drops of Reagent R-3 to well No.3  
2 drops of Reagent R-4 to well No.4  
2 drops of Reagent R-5 to well No.5  
2 drops of Reagent R-6 to well no.6.

The corresponding well number is also mentioned on each ID reagent bottle labels.

Table 3

Well Nos	W1	W2	W3	W4	W5	W6
MICROPRO ID Reagents	R1	R2 2.1	R3	R4	R5	R6
		R2 2.2				
		R2 2.3				
Reagent Volume	2 drops	2 drops 2 drops 2 drops	2 drops	2 drops	2 drops	2 drops

- Continue Step No 4 for the next seven well strip rows.

### b) Sample Addition

- From the Cuvette stand, retrieve one set of Barcoded Cuvette (A) incubated with UTI control and its corresponding Test Vial (B). Check the UTI control name on the Barcoded Cuvette (A) and note it down on the removable sticker of the first row of the well strip.
- Remove the black cap and the rubber stopper of the Barcoded Cuvette (A) and place it on a clean surface.
- Using a 300 µl Fixed Volume Pipette dispense 300 µl of the growth from Barcoded Cuvette (A) into the first 3 wells of the first ID well strip.
- Recap the Barcoded Cuvette (A) with black cap along with the stopper tightly, place it back in the cuvette stand.
- Retrieve the Test vial (B) green cap of the same UTI Control, open the green cap and rubber stopper placing them on a clean surface.

- Using the 300 µl Fixed Volume Pipette dispense 300 µl of the growth from the Test Vial (B), in to the next 3 wells of the same ID well strip.
- Recap the Test Vial (B) with its green cap along with the stopper tightly, place it back in the cuvette stand.
- Repeat the steps (8-13) for rest of the UTI Control Barcoded Cuvette (A) and the Test vial (B) sets.
- Start the timer. Read the results after 25 minutes.

### c) Result Interpretation:

Check the results of the UTI Controls as shown below

UTI Controls	Result Interpretation of wells						Positive results in wells
	W1 Catalase Test	W2 VP Test	W3 PYR Test	W4 Oxidase Test	W5 TDA Test	W6 Indole Test	
<i>E. coli</i>	Bubbles	-	Salmon color	-	-	Pink ring	1 & 6
<i>Enterococcus faecalis</i>	-	Light Dark Pink	Dark cherry red	-	-	-	2 & 3
<i>Streptococcus pyogenes</i>	-	-	Dark cherry red	-	-	-	3
<i>Proteus mirabilis</i>	Bubbles	-	Salmon color	-	Light Dark Brown	-	1 & 5
<i>Proteus spp.</i>	Bubbles	-	Salmon color	-	Light Dark Brown	Pink ring	1, 5 & 6
<i>Pseudomonas aeruginosa</i>	Bubbles	-	Salmon color	Blue to Purple	-	-	1 & 4
<i>Citrobacter spp.</i>	Bubbles	-	Dark cherry red	-	-	-	1 & 3
<i>Klebsiella pneumoniae</i>	Bubbles	Light Dark Pink	Salmon color	-	-	-	1 & 2

Note: - represents no change  
Salmon color represents negative result.

### 13. REFERENCES

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