URIFAST 2

Rapid Antimicrobial Susceptibility Testing of urinary Enterobacteriaceae

20 tests (REF 22297)

CPB 0138_EN-2024-06 NCE
Single use test

1 - INTENDED USE

The URIFAST 2 kits have been designed to determine in 4.5 hours to 6.5 hours the susceptibility of *Enterobacteriaceae* to the most common antibiotics used in the treatment of bacterial urinary tract infections. One kit is sufficient for 20 tests.

2 - INTRODUCTION

Urinary tract infections are the second most important reason for consulting a family practice doctor after respiratory infections. In hospitals, urinary tract infections are the most frequent source of nosocomial infections and are difficult to treat and prevent. Most urinary tract infections are benign. However, normally asymptomatic infections, may become pathological in nature as a consequence of infection localized high up in the urinary tract, as a result of the recurrent nature of infection, or by the opportunistic infection of a weakened environment. This can result in complications such as cystitis, chronic prostatitis, or even pyelonephritis. Precise bacterial diagnosis as well as the rapid identification of the antibiotic most suitable for treatment are essential to prevent the development of complicated infections as well as to increase the chances of curing severe infections.
Diagnosis of urinary tract infections depends on a clinical and biological analysis. This includes the isolation of the germ responsible as well as the assessment of antibiotic susceptibility. The methods for determining minimum inhibitory concentrations (MIC) by diffusion in agar medium as well as by microdilution in liquid medium are lengthy and have proved unsuitable for routine diagnosis or emergency situations.

3 - PRINCIPLE

The URIFAST 2 test is used to evaluate the susceptibility of the *Enterobacteriaceae* to various antibiotics in a liquid medium. The test is carried out by taking one bacterial colony isolated on an agar plate, which is then resuspended, using a standardized technique using the PRESTO ABG inoculator (1) in modified Müeller-Hinton medium containing bromothymol blue indicator. The bacterial suspension thus obtained is distributed equally into the wells of a URIFAST 2 tray containing the antibiotics.
After incubating for 4.5 hours and 6.5 hours at 34 - 37°C, the absence of a colour change in the well indicates the absence of bacterial growth and hence susceptibility to the antibiotic being tested (2).
The antibiotics included in the URIFAST 2 tray are tested at their critical concentrations as determined by the EUCAST (The European Committee on Antimicrobial Susceptibility Testing) and the "Comité de l'Antibiogramme de la Société Française de Microbiologie" (CA-SFM) (3).

4 - REAGENTS

Packaging

Reagent	Quantity	Description
URIFAST 2	10	Tray of 2x10 wells containing the antibiotics at one concentration and individually packed in an aluminium sachet with a desiccant. Each divisible tray allows the testing of two specimens. Each half tray is for single use.
M4H+	20	Vial of 5 mL modified Müeller-Hinton medium containing bromothymol blue. Single use reagent
R4H+	1	Vial containing 5 mL of surfactant for use with *Proteus mirabilis* strains. Reusable reagent if stored at 2 - 25°C until its expiration date
PRESTO ABG	25	Stainless steel inoculator to standardize the inoculum
Closing System	20	Protective translucent plastic cover of the inoculated tray or half tray

Composition of the URIFAST 2 tray:
-well 1 (C+): Growth control
-well 2 (AMX): Amoxicillin 8 µg/mL
-well 3 (AMC): Amoxicillin + Clavulanic acid 8/2 µg/mL
-well 4 (CXM): Cefuroxime 8 µg/mL
-well 5 (CPD): Cefpodoxime 1 µg/mL
-well 6 (ETP): Ertapenem 0.5 µg/mL
-well 7 (CIP): Ciprofloxacin 0.25 µg/mL
-well 8 (FOS): Fosfomycin 8 µg/mL
-well 9 (NIT): Nitrofurantoin 64 µg/mL
-well 10 (SXT): Trimethoprim / Sulfamethoxazole 2/38 µg/mL

Composition of the M4H+ medium in g/L:
Mueller-Hinton broth supplemented with cations (Beef infusion, casein hydrolysate, starch) 22
Yeast extract..... 1.5
Glucose..... 10
Bromothymol blue..... 0.1
pH: 7.7 +/- 0.1

5 - PRECAUTIONS

The reagents are intended solely for *in vitro* use and must be handled by authorized personnel.
The urine samples and inoculated reagents are potentially infectious; they must be handled with caution, in observance of hygiene rules and the current regulations for this type of product in the country of use.
The reagents contain raw materials of animal origin and must be handled with caution.
Do not use reagents that have been damaged or that have been poorly conserved before use.
Do not use reagents after the expiry date. Use a fresh pipette tip for each specimen.

6 - SAMPLE COLLECTION AND HANDLING

Susceptibility testing should be carried out on colonies isolated within maximum 18 to 24 hours of incubation on agar (TSA, chromogenic agar, sheep blood agar, CLED, Mac Conkey).

7 - STORAGE OF REAGENTS

All the reagents are ready to use.
The M4H+ medium and the trays are stable at 2 - 8°C as well as R4H+ medium at 2 - 25°C in their original packaging until the expiry date given on the kit.
Should only one half of a URIFAST 2 tray be used, store the other half in its original, tightly closed packaging. This half tray is stable for 6 days at 2 - 8°C.
Do not freeze the reagents in the kit.

8 - MATERIAL REQUIRED BUT NOT PROVIDED

Densitometer and 0.9% NaCl medium
Paraffin oil
Incubator at 34 - 37°C.
Waste container for contaminated waste.
Adjustable volume pipette (10 - 100 µl) and sterile tips

9 - METHOD

9.1 a) Preparation of the inoculum using the PRESTO ABG inoculator

The PRESTO ABG inoculator provides a one-step standardized inoculum, which is adapted for carrying out the test.
Choose one clearly identified medium sized colony (minimum diameter 1 mm)
Using the PRESTO ABG inoculator, stab vertically into the center of the colony through the agar until lightly touching the base of the dish, making sure the inoculator remains perpendicular to the agar at all times.
Gently withdraw the inoculator with a single movement, maintaining it vertically at all times.
Release the inoculum immediately into a vial of M4H+ medium mixing thoroughly by swirling the inoculator for 5 to 10 seconds so that a smooth suspension is obtained.
Close the vial and shake vigorously for 3 to 5 seconds. Dispose of the inoculator in the waste container.

Notes
Do not flame sterilise the inoculators before use.
The PRESTO ABG inoculators are disposable and for single use only. Never re-use an inoculator for a second inoculum.
The inoculator should not be wetted nor used in a 2-step process.

9.1 b) Preparation of the inoculum using a densitometer

From 2 or 3 isolated colonies, carry out an inoculum equivalent to 2 +/- 0.1 Mac Farland in a vial of 0.9% NaCl.
Inoculate the vial of M4H+ medium with 35 µL of the suspension medium adjusted to 2 +/- 0.1 Mac Farland.

9.2 Inoculation of the wells

Carefully open the bag containing the tray, remove the tray, separate the 2 tests if necessary and return the unused test to the bag, making sure to close it as tightly as possible.
Then proceed with the following steps:
- Identify the half gallery
- Inoculate each of the 10 wells of the tray with 100 µL of inoculated M4H+ medium
- Add two drops of paraffin oil to the NIT well
- For *Proteus mirabilis*, add 25 µL of R4H+ reagent to the AMX, AMC, CXM, CPD, ETP wells.
- Clip a cover onto the inoculated tray to avoid spilling the contents of the wells afterwards
- Incubate at 34 - 37°C for 4.5 hours to 6.5 hours.

Note: Once inoculated, the inoculum in M4H+ can be stored for up to one hour at room temperature on the bench.

10 - READING AND INTERPRETATION

At the end of the URIFAST 2 test, 3 main colours may be seen, with possible intermediate colours appearing in the wells:
- blue : the original colour, indicates absence of bacterial growth.
- green, green-yellow or yellow indicates bacterial growth.

10.1 - Validation (control wells)

Refer to the color chart (CPD 0138) provided in the kit

Check that the medium corresponding to the growth control has turned yellow, green or dark green. This proves that the reagents are functional, and that the procedure has been carried out correctly. If the medium has remained blue or dark blue, continue incubation for a further 2 hours without exceeding 6.5 hours of incubation. If there is no colour change to yellow, green or dark green, repeat the test.

10.2 - Reading and interpretation (wells with antibiotics)

10.2. a) - Reading

Refer to the color chart (CPD 0138) provided in the kit

Bacterial growth is indicated by a colour change of the medium: The medium has turned green, green-yellow or yellow. The medium remains blue when there is an inhibition of growth.
The strains are characterized as being sensitive, intermediate or resistant to the antibiotics according to the following criteria:
- the strain is said to be *Sensitive* when its growth is inhibited at the critical concentration of the antibiotic,
- the strain is said to be *Resistant* when its growth is not inhibited at the critical concentration of the antibiotic.

Specific Cases:
For uncomplicated urinary tract infections, sensitivity to FOS is only interpreted for the species ***Escherichia coli***.
For two antibiotics, CIP and SXT, the strain is said to be *Sensitive* (S) when its growth is inhibited at the critical concentration of the antibiotic. The strain is said to be *Intermediate* (I) or *Resistant* (R) when its growth is not inhibited at the critical concentration of the antibiotic.

10.2. b) – Interpreting the results

The enterobacteria exhibiting **natural resistance (R)** to the antibiotics are listed in the table below. Whatever the result obtained with the URIFAST 2 tray, these resistance profiles must be taken into account when interpreting the results.

Example: *Proteus mirabilis* must be interpreted as being Resistant to nitrofurantoin irrespective of the result obtained with the URIFAST tray.

Espèce	AMX	AMC	CXM	NIT
Klebsiella spp.	R			
C. diversus	R			
E. aerogenes	R	R		
S. marcescens	R	R	R	R
P. mirabilis				R
P. vulgaris	R		R	R
M. morganii	R	R		R
P. stuartii	R	R	R	R
Y. enterocolitica	R	R		

11 - QUALITY CONTROL

In order to verify the standardization of the method, it is recommended that periodic quality control assessments be carried out using the reference strain, *Escherichia coli* ATCC 25922:

C+	AMX	AMC	CXM	CPD	ETP	CIP	FOS	NIT	SXT
+	S	S	S/R	S/R	S	S	S	S	S

12 - SOURCES OF ERROR

Improper use of the PRESTO ABG inoculator can cause false results, the prick should not be conducted on a colony smaller than 1mm in diameter and it should not be carried out on non-isolated colonies.
The following causes of errors can be observed:
- reading the results when the growth control has not shown a complete colour change
- incubation temperature (34 - 37°C) not maintained throughout the incubation period
- risk of wells drying out and their contents spilling if the Closing System is not used and correctly clipped
- performing the test from strains isolated after more than 24 hours of incubation may generate false results
- respect the incubation time. Reading after 3.5 hours is not sufficient and readings above 7 hours are too high

13 - LIMITS OF THE METHOD

As for all antibiogram methods, the conditions used during sample isolation determine the quality of the results.
The method has only been tested on colonies isolated on specific agar for urinary samples (§6).
Wells should not be covered by the label during incubation.
For certain formats of isolation media, such as Urinax CL / MC, if the isolated colonies are too small to be pricked with a Presto ABG, it is recommended that the inoculum be calibrated using a densitometer (§9.1.b).
As the *S. marcescens* species may exhibit an inhibition defect with the ETP antibiotic, it is necessary to confirm an ETP resistant result obtained with the URIFAST 2 test with a third method.
Some strains with MICs close to the stated concentration of the well may generate intermediate colors close to the limit of categorization between *Sensitive* / *Resistant.*

14 - PERFORMANCE CHARACTERISTICS

Study on clinical strains

URIFAST 2 performance evaluations were carried out in two laboratories:
- The CNR laboratory associated with Antibiotic Resistance and on a panel of 102 strains,
- ELITech MICROBIO's R&D laboratory on a panel of 92 strains, and 10 *E. coli* strains (only for the FOS antibiotic)

The clinical studies were carried out against the liquid micro dilution reference method for determining the MICs, except for the FOS antibiotic, the results of which were compared to the agar diffusion MIC technique, as recommended by EUCAST and the CA-SFM.
Following new recommendations from EUCAST (January 2021), the FOS antibiotic is only analyzed and compared for the *E. coli* species.
The percentages of agreement with the **exact MIC** of strains or with the **+/- 1 deviation dilution of the MIC** are presented below:

Summary table of the URIFAST 2 test performance by antibiotic at the exact MIC*

	ATB	AMX	AMC	CXM	CPD	ETP	CIP	FOS	NIT	SXT	Global
	Number of tested strains	**200**	**200**	**200**	**200**	**200**	**200**	**90**	**200**	**200**	NA
	Number of resistant strains	144	78	59	64	8	41	2	48	65	NA
	Number of sensitive strains	56	122	141	136	192	157	88	152	135	NA
	Number of intermediate strains	NA	NA	NA	NA	NA	2	NA	NA	NA	NA
Reading after 4:30	**Agreement %**	98	90	90,0	86,5	92	98	100	78	95	91,4
	ME %	1	7,5	9,5	13	7,5	1	0	18,5	4	7,3
	me %	NA	NA	NA	NA	NA	1	NA	NA	0	0,1
	VME %	1	2,5	0,5	0,5	0,5	0	0	3,5	1	1,1
Reading after 6:30	**Agreement %**	96	88,5	92,5	92,5	93	97	98,9	70	92	90,7
	ME %	3	7,5	6	7,5	7	2	1,1	30	7	8,3
	me %	NA	NA	NA	NA	NA	1	NA	NA	0	0,1
	VME %	1	4	1,5	0	0	0	0	0	1	0,9

* The black frames represent the recommended reading times corresponding to the best performances at 4.5 hours or 6.5 hours
Legend: ME: Major Error, me: minor error, VME: Very Major Error

Summary table of the URIFAST 2 test performance by antibiotic at MIC +/- 1 deviation dilution*

	ATB	AMX	AMC	CXM	CPD	ETP	CIP	FOS	NIT	SXT	Global
	Number of tested strains	**200**	**200**	**200**	**200**	**200**	**200**	**90**	**200**	**200**	NA
	Number of resistant strains	144	78	59	64	8	41	2	48	65	NA
	Number of sensitive strains	56	122	141	136	192	157	88	152	135	NA
	Number of intermediate strains	NA	NA	NA	NA	NA	2	NA	NA	NA	NA
Reading after 4:30	**Agreement %**	99	92	92,5	88,5	93,5	100	100	94	95	94,6
	ME %	0,5	6,5	7,5	11	6	0	0	5	4	4,8
	me %	NA	NA	NA	NA	NA	0	NA	NA	0	0
	VME %	0,5	1,5	0	0,5	0,5	0	0	1	1	0,6
Reading after 6:30	**Agreement %**	97,0	92,5	96,5	94,0	94,5	99,0	98,9	88	92	94,4
	ME %	2,5	5,5	3,5	6	5,5	1	1,1	12	7	5,1
	me %	NA	NA	NA	NA	NA	0	NA	NA	0	0
	VME %	0,5	2	0	0	0	0	0	0	1	0,4

* The black frames represent the recommended reading times corresponding to the best performances at 4.5 hours or 6.5 hours

The tables below describe the **distribution of observed discrepancies, by antibiotic after 4.5 hours or 6.5 hours of incubation:**

Breakdown of discrepancies observed at 4.5 hours of reading, by antibiotic and by MIC value*

	AMX		AMC		CXM		CPD		ETP		CIP			FOS		NIT		SXT		
CMI	ME	VME	ME	VME	ME	VME	ME	VME	ME	VME	ME	me	VME	ME	VME	ME	VME	ME	me	VME
0,06							1		9									1		
0,125					1		5		1									2		
0,25							6		2		2							1		
0,5			2				10		3			2						3		
1			3		2		4											1		
2			1		2															
4	1		7		10			1		1										
8	1		2		4															
16	1			2		1										1				
32				1												9				2
64				2												27				
128	1																5			

* The grey cells correspond to the announced antibiotic concentration

Breakdown of discrepancies observed at 6.5 hours of reading, by antibiotic and by MIC value*

	AMX		AMC		CXM		CPD		ETP		CIP			FOS		NIT		SXT		
CMI	ME	VME	ME	VME	ME	VME	ME	VME	ME	VME	ME	me	VME	ME	VME	ME	VME	ME	me	VME
0,06									7		2							2		
0,125					1		1		1									2		
0,25							5		3		2							4		
0,5	3		1				6		3			2						5		
0,75														1						
1			2		1		3											1		
2			1																	
4	2		7		5															
8	1		4		5											1				
16		1		4		3										7				
32				2												16				2
64				2												36				
128		1																		

* The grey cells correspond to the announced antibiotic concentration

15 – WASTE ELIMINATION

Waste should be disposed of in accordance with the hygiene rules and current regulations for this kind of product in the country of use.

16 - BIBLIOGRAPHY

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The changes from the previous version are highlighted in grey.

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