

# Validation of FLEXICULT™ SSI-Urinary Kit for Use in the Primary Health Care Setting

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The efficacy of the FLEXICULT™ SSI-Urinary Kit for point-of-care diagnosis and susceptibility testing of urinary tract pathogens was evaluated. The kit, which was exclusively developed for urine culture in the primary health care setting, is designed as an ordinary Petri dish divided into 6 compartments: 1 large one for quantitative analysis and 5 smaller ones for susceptibility testing. The agar in each small compartment contains 1 of 5 antimicrobials (trimethoprim, sulfamethoxazole, ampicillin, nitrofurantoin and mecillinam) at a concentration adjusted to the breakpoint, and growth in these compartments indicates resistance. The kit was tested in-house with 116 urinary tract pathogens and by 19 general practitioners in a field trial with 121 diagnostic urine specimens. The kit was flooded with the urine specimens for a couple of seconds, incubated overnight and read the following day. Quantitative readings were evaluated by comparing with standardized inoculi and the susceptibility tests were compared with the MIC value of the strain for each of the 5 antimicrobials. In the field trial, the quantitation had an overall error rate of 4% and correctly determined susceptibility in 93% of the tested bacteria. Although identification of the isolates is not a feature of this kit, it is suitable for point-of-care diagnosis and for susceptibility testing of uncomplicated urinary tract infections in the primary health care setting.

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## INTRODUCTION

Unjustified prescribing accounts for a significant fraction of the total use of antimicrobials worldwide (1–4). The World Health Organization recommends research into rapid point-of-care diagnostic tools as 1 way of limiting such unjustified use (5). Empiric treatment of urinary tract infections (UTIs) is generally based only on clinical signs without proper microbiological diagnosis.

The majority of patients suffering from UTIs consult their primary health care facility for advice. In Denmark this is the general practitioner (GP). For diagnosis, Danish GPs often use some kind of point-of-care diagnostic procedure, i.e. a dipstick for nitrite and leukocyte esterase, microscopy or incubation of the urine specimen in a suitable commercially available kit. If appropriate, they subsequently perform susceptibility testing. However, these procedures have disadvantages. Microscopy, dipsticks and many of the kits are unable to measure urine bacterial counts in the range  $10^3$ – $10^5$ , and most GPs do not perform a simultaneous susceptibility test. Additionally, if a UTI is diagnosed and susceptibility testing is performed, the final results will often only be available 2 d after the sample was taken. Consequently, and given the fact that the overall costs of UTIs to society are related to the appropriateness of the empiric treatment (6, 7) and the increasing resistance of urinary tract pathogens in Denmark, the Statens Serum Institute (SSI) developed a kit for overnight, point-of-care, diagnosis and susceptibility testing of urinary tract pathogens: the FLEXICULT™ SSI-Urinary Kit. The aim of this study was to validate this kit.

## MATERIALS AND METHODS

### *FLEXICULT™ SSI-Urinary Kit*

The kit is designed as an ordinary Petri dish but with higher sides. The Petri dish contains Mueller–Hinton BBL-II agar (Becton Dickinson, Basle, Switzerland) and is divided into 6 compartments: 1 large one for quantitative analysis and 5 smaller ones for susceptibility testing (Fig. 1). The agar in each of the smaller compartments contains 1 of 5 antimicrobials at the following concentrations ( $\mu\text{g/ml}$ ): trimethoprim, 16; sulfamethoxazole, 700; ampicillin, 32; nitrofurantoin, 256; and mecillinam, 128.

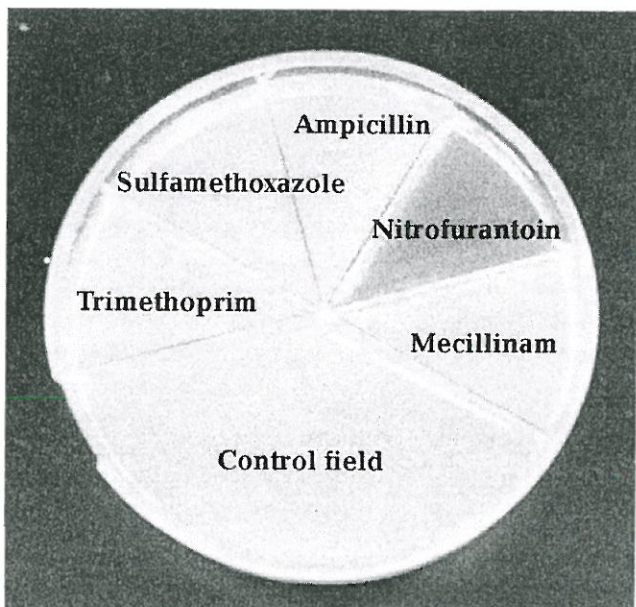


Fig. 1. The FLEXICULT™ SSI-Urinary Kit.

The agar plate is flooded with the urine specimen for a couple of seconds and then incubated at 35°C overnight. The following day the plate is read. When reading the compartment for quantitative analysis the lower limit is  $\approx 10^3$  colony-forming units (cfu) per milliliter. As the concentrations of the antimicrobials in the 5 smaller compartments are adjusted in accordance with break-points, growth on these compartments indicates resistance of the pathogen in question and hence a potential risk of treatment failure.

The overall validation of the FLEXICULT™ SSI-Urinary Kit was performed by means of 2 separate studies. Firstly an in-house validation of the field for quantitative assessment and those for susceptibility testing and subsequently a field trial in which the kit was tested in collaboration with GPs.

#### *In-house validation*

In 1997, Kern et al. (8) studied a total of 228 consecutive urine samples from patients with clinically suspected UTIs attending GP clinics in Roskilde County, Denmark. From 187 dipslides (Uricult®, Orion Diagnostica, Espoo, Finland) with significant growth in that study, 202 urinary tract pathogens were identified according to routine procedures at the SSI. All strains had their MICs for trimethoprim, sulfamethoxazole, ampicillin, nitrofurantoin and mecillinam determined according to NCCLS guidelines (9). A sample of 113 different strains from this collection and 3 additional ATCC strains were used for the in-house validation of the FLEXICULT™ SSI-Urinary Kit.

#### *Quantitation*

The ability of the FLEXICULT™ SSI-Urinary Kit to quantify the number of bacteria in urine samples was validated using 5 bacterial strains (*Escherichia coli*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Klebsiella pneumoniae* and *Citrobacter freundii*) adjusted to  $\approx 2-6 \times 10^8$  cfu/ml as measured by optical density (540 nm) using a colorimeter. Suspensions of bacteria were diluted in 50 ml of sterile urine (fresh, filtered and pooled urine from healthy humans who had taken no antimicrobials during the previous month) to various concentrations. Each bacterial suspension was poured into a FLEXICULT™ SSI-Urinary Kit for 1-2 s in accordance with the instructions supplied with the kit. Excess urine was poured off and the FLEXICULT™ SSI-Urinary Kit was incubated, bottom up, overnight at 35°C. The densities of the colonies on the kit were compared to the cfu/ml values determined by serial dilution on 5% blood agar plates (SSI) after overnight incubation at 35°C.

#### *Susceptibility testing*

In order to include a variety of urinary pathogens, 116 strains from the same strain collection were used for validation of the susceptibility testing feature of the FLEXICULT™ SSI-Urinary Kit: *E. coli* ( $n = 59$ , including ATCC 35218 and ATCC 25922); *Klebsiella* spp. ( $n = 9$ ); *Proteus* spp. ( $n = 8$ ); *Enterobacter* spp. ( $n = 5$ ); *Citrobacter* spp. ( $n = 6$ ); *Pseudomonas* spp. ( $n = 5$ , including ATCC 27853); *Enterococcus* spp. ( $n = 16$ ); *Staphylococcus* spp. ( $n = 4$ ); and *Acinetobacter* spp. ( $n = 4$ ). Bacteria were grown overnight on 5% blood agar (SSI), suspended in saline and adjusted to  $\approx 2-6 \times 10^8$  cfu/ml as determined by optical density measurement (540 nm) using a colorimeter. The bacterial suspensions were then diluted in 50 ml of sterile urine (as above) to  $\approx 10^5$  cfu/ml. Each bacterial suspension was tested with the FLEXICULT™ SSI-Urinary Kit as described. Growth on each of the compartments containing antimicrobials was compared to the corresponding MIC values of the strains. Note that the FLEXICULT™ SSI-Urinary Kit used for this part of the study had a nitrofurantoin concentration of 128 µg/ml.

#### *Field trial*

Nineteen GPs from Roskilde County in Denmark volunteered to participate in the validation of the FLEXICULT™ SSI-Urinary Kit. The GPs were asked to use the kit in addition to their routine diagnostic procedures [i.e. direct phase-contrast microscopy, dipstick (nitrite and leukocyte esterase) or a dipslide method] for patients with symptoms of UTI. The kit was subsequently kept in a small table incubator overnight and read in accordance with recommendations. The GPs recorded the number of bacteria in the control field as well as growth or lack of growth in the 5 compartments containing an antimicrobial agent. The kit was then sent to our laboratory by regular mail. Simultaneous with the diagnostic sample, a urine sample ( $\approx 10$  ml) was collected in a sterile tube containing 85 mg of boric acid (Vacutainer® Urine C&S Transport Kit No. 364948; Becton Dickinson), in order to preserve the bacteria until the bacterial count could be determined at the SSI. This bacterial count was compared to the number of bacteria in the control field recorded by the GPs.

In order to properly validate the quantitative analysis of the FLEXICULT™ SSI-Urinary Kit in the field trial, GPs would have to determine the number of cfu/ml of urine by means of serial dilution on 5% blood agar for every sample included in the study. However, this would have been impossible because of the unacceptably high workload involved and the lack of laboratory facilities at the GPs offices. Therefore, we felt it necessary to validate the performance of the Vacutainer® Urine C&S Transport Kit prior to the field trial in terms of preservation of frequent pathogens of the urinary tract. According to the manufacturer's information, the Vacutainer® Urine C&S Transport Kit keeps the bacterial count in the urine specimen stable for a period of 48 h at ambient temperature and comparable to those of urine specimens from UTIs kept refrigerated for the same length of time. However, this could only be confirmed for *Klebsiella* spp. and *S. saprophyticus*. With respect to *E. coli*, *Enterococcus* spp., *S. aureus* and *Pseudomonas* spp., the ability of the Vacutainer® to preserve the bacteria beyond 48 h was reduced when kept at room temperature as compared to when refrigerated (data not shown).

In spite of these limitations, we felt compelled to accept the Vacutainer® Urine C&S Transport Kit for our field trial. To minimize the possible growth or death of bacteria during transport, the Vacutainer® was refrigerated immediately after the specimen was taken until being sent, by regular mail, to our laboratory the following day. On arrival the urine specimen was either immediately refrigerated or examined. This procedure ensured that the period for which the urine specimen was at ambient temperature was kept to < 24 h.

Bacteria growing on the FLEXICULT™ SSI-Urinary Kit had their MIC values for trimethoprim, sulfamethoxazole, ampicillin, nitrofurantoin and mecillinam determined according to NCCLS guidelines using standard procedures (9), and the MICs were compared with the ability of the strains to grow in the various compartments containing antimicrobials in the kit.

## RESULTS

#### *In-house validation*

Fig. 2 shows the results of the quantitation study. The number of colonies on the control field of the kit reflects the concentration of bacteria in the standard urine sample. The detection limit of the kit was determined to be  $\approx 5 \times 10^2$  cfu/ml. Theoretically, there should be  $\approx 15-20$  colonies on the control field of the kit if the urine sample contains  $10^3$  cfu/ml,  $10^5$  cfu/ml should yield a semi-confluent growth on the control field and at  $10^7$  cfu/ml the

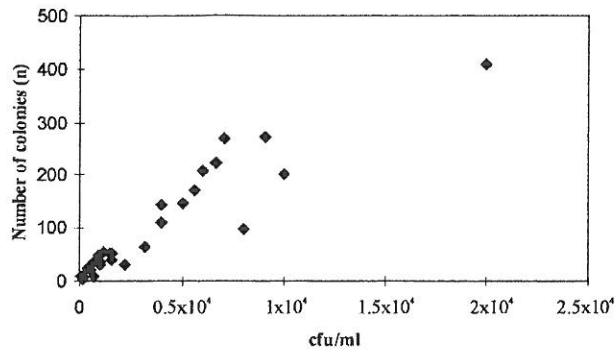


Fig. 2. Number of colonies on the control field of the FLEXICULT™ SSI-Urinary Kit for different values of cfu/ml.

growth should be confluent. However, this will only be true if the length of contact between the urine sample and the control fields of the kit is limited to a few seconds, as described in the instructions supplied with the kit. With a longer contact time, more urine will be absorbed by the agar and, as a consequence, the density of colonies will

Table I. In-house susceptibility testing of 116 bacterial strains using the FLEXICULT™ SSI-Urinary Kit. Growth and lack of growth is compared to the respective MIC values

FLEXICULT™	Reference method	
	MIC > concentration in the kit	MIC ≤ concentration in the kit
Growth on kit	125	16
No growth on kit	13	426

increase and there is a risk of false-positive cultures (data not shown).

Fig. 3 shows the distribution of MIC values for the 116 bacteria used for evaluation of susceptibility and growth on the FLEXICULT™ SSI-Urinary Kit. The kit correctly identified resistance and susceptibility in 90% and 96% of the tested strains, respectively. The overall accordance with MIC values for susceptibility testing was 95% (Table I). In

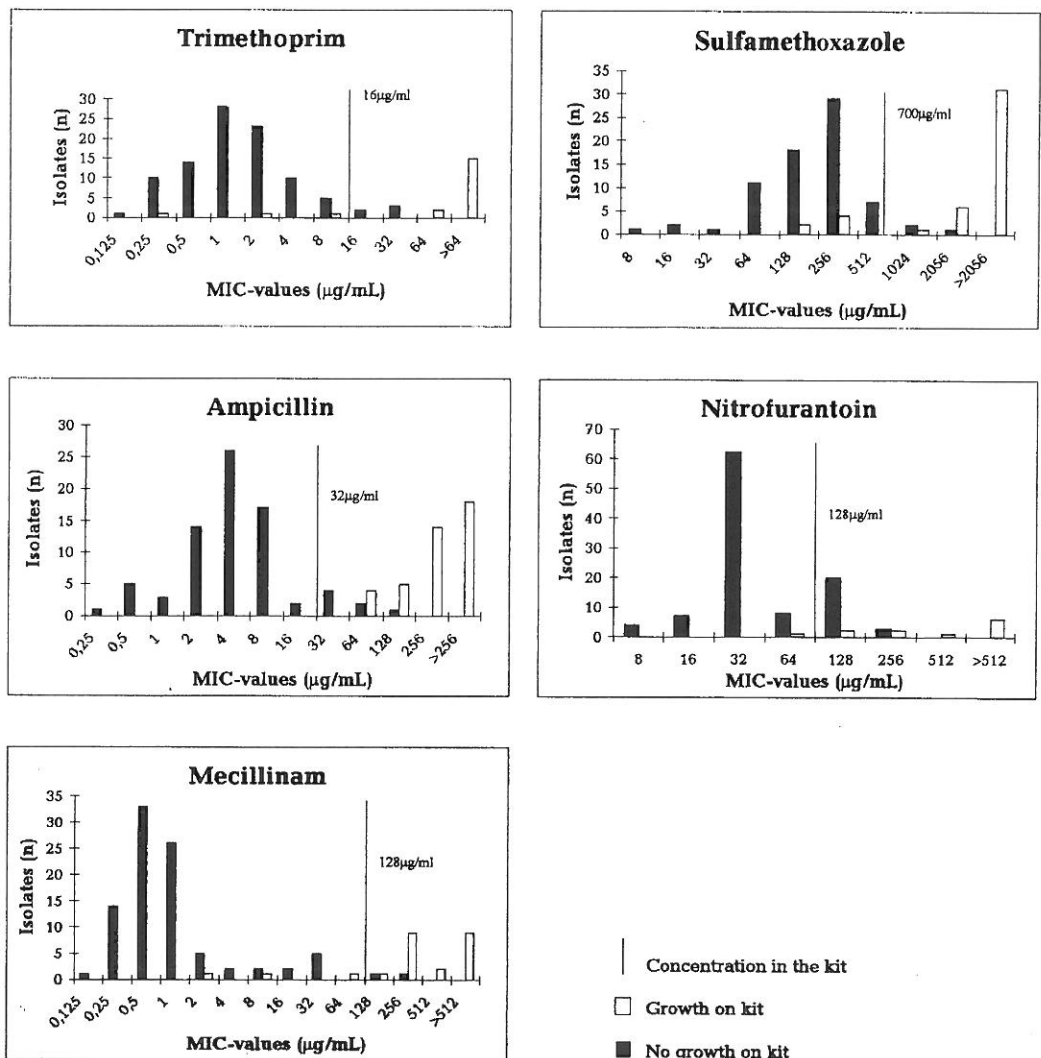


Fig. 3. Distribution of MIC values for 116 bacteria and their growth or lack of growth in the antimicrobial-containing compartments of the FLEXICULT™ SSI-Urinary Kit.

29 instances susceptibility testing using the FLEXICULT™ SSI-Urinary Kit was incorrect. In 14 of these failures the discrepancy between positive and negative growth on the kit and the corresponding MIC value was within 1 MIC dilution, which is acceptable when using standard methods. The remaining 15 discrepancies occurred for 12 different bacteria: 3 for trimethoprim (2 *Enterococcus* spp. and 1 *E. coli*); 7 for sulfamethoxazole (4 *Klebsiella* spp., 2 *Enterococcus* spp. and 1 *Pseudomonas* spp.); 1 for ampicillin (*Enterobacter* spp.); 3 for mecillinam (1 *Enterococcus* spp. and 2 *E. coli*); and 1 for nitrofurantoin (*Klebsiella* spp.).

#### Field trial

A total of 121 urine samples from patients who consulted participating GPs with suspected UTI were tested for bacteriuria using the FLEXICULT™ SSI-Urinary Kit and compared with urine samples obtained simultaneously for control purposes. Of the 121 urine samples, 42 (4 Enterobacteriaceae) had  $< 10^3$  cfu/ml, 6 (1 Enterobacteriaceae) had  $10^3$ – $10^4$  cfu/ml, 12 (7 Enterobacteriaceae) had  $10^4$ – $10^5$  cfu/ml and 61 (54 Enterobacteriaceae) had  $> 10^5$  cfu/ml (Table II). Discrepancies in the quantitative analysis between the FLEXICULT™ SSI-Urinary Kit and the control urine samples occurred in 16% of the specimens. In 12/20 urine samples with discrepancies, contaminants hampered the determination of the number of cfu in samples transported using the Vacutainer® Urine C&S Transport Kit. Two urine samples containing *Proteus* spp. were insufficiently preserved in boric acid and 1 other control urine sample had obviously not been preserved despite the boric acid, as the GP noted his microscopy result as “ $< 10^3$  CFU/ml” and the count in the quantitation compartment was similar. This leaves 5 urine samples for which there were unexplained discrepancies between growth on the FLEXICULT™ SSI-Urinary Kit and the control urine, and an overall error rate of 4%.

The accuracy of susceptibility testing using the FLEXICULT™ SSI-Urinary Kit compared to MIC values was tested with 67 different strains isolated from different kits (6 isolates from kits with  $< 10^3$  cfu/ml, 2 isolates from kits with  $10^3$ – $10^4$  cfu/ml, 5 isolates from kits with  $10^4$ – $10^5$  cfu/ml and 54 strains from kits with  $> 10^5$  cfu/ml). The kit correctly identified resistance and susceptibility in 90% and 94% of the tested strains, respectively. The overall accor-

Table II. Accuracy of bacterial quantitation using the FLEXICULT™ SSI-Urinary Kit in the field trial

Cfu/ml	Quantitation by GPs; n (%)	Accordance with control urine (%)
$< 10^3$	42 (35)	83
$10^3$ – $10^4$	6 (5)	100
$10^4$ – $10^5$	12 (10)	17
$> 10^5$	61 (50)	95
Total	121 (100)	84

Table III. Field trial susceptibility testing of 67 strains using the FLEXICULT™ SSI-Urinary Kit. Growth and lack of growth is compared to the respective MIC values

FLEXICULT™	Reference method <sup>a</sup>	
	MIC > concentration in the kit	MIC ≤ concentration in the kit
Growth on kit	54	17
No growth on kit	6	257

<sup>a</sup> One MIC value for trimethoprim was missing.

dance with MIC values for susceptibility testing was 93% (Table III).

In 23 instances there were discrepancies between positive and negative growth on the FLEXICULT™ SSI-Urinary Kit and the corresponding MIC values. In 1 instance this discrepancy was within 1 MIC dilution, which is acceptable when using standard methods. Fifteen bacteria were retested in the FLEXICULT™ SSI-Urinary Kit. Consequently, 17/23 mismatches between positive and negative growth on the kit and the respective MIC values were corrected. Five discrepancies remained unexplained.

#### DISCUSSION

UTIs are among the most frequent bacterial infections worldwide and the management of patients with symptoms of UTI constitutes a considerable proportion of the workload of primary health care systems. As many as 50% of women report having had at least 1 UTI in their lifetime (10, 11). In fact, patients with acute UTIs account for  $\approx$  3% of all visits to GPs (11). In addition to clinical signs and symptoms, the diagnosis of UTI relies on the detection of pathogenic microorganisms in the urine. Traditionally, growth of  $> 10^5$  cfu/ml of urine indicates infection. However, studies have shown that urine specimens from symptomatic women with pyuria often contain  $< 10^5$  cfu/ml of urine (12–14) and therefore the diagnostic criteria for symptomatic patients have been debated. At present the recommendation is that a count of a common urinary pathogen of  $\geq 10^3$  cfu/ml may indicate a UTI (15). However, it should be emphasized—as in our field trial—that the majority of symptomatic patients have urine counts  $> 10^5$  cfu/ml.

In Denmark, most patients with UTIs are treated by their GPs. For diagnosis, they use direct phase-contrast microscopy, dipsticks (for nitrite and leukocyte esterase) or the dipslide methodology. All these methods have relatively low specificity and/or sensitivity for diagnosing bacterial UTI, in particular if the bacterial count is  $< 10^5$  cfu/ml (16–22). Some GPs perform susceptibility testing by applying paper disks containing antimicrobials to the dipslides or antimicrobial-containing tablets on separate agar plates. These methods are time-consuming and some lack control for the inoculum.

The increasing problem of antimicrobial resistance is in itself an argument for performing susceptibility testing of pathogens from urine samples in suspected cases of UTI in order to optimize the treatment in accordance with the results. Although the problem of increasing resistance could be solved by recommending the use of the most recently marketed drug, to which most pathogens are susceptible, a far more rational approach would be to secure the initial prescription of the right drug for cases of well-documented UTI. The first-line drug for UTI in Denmark is sulfamethoxazole; however, resistance to sulfamethoxazole among *E. coli* isolates from cases of community-acquired UTI has increased over the past decade and is now reaching a prevalence of 32–43% in Denmark (23). For ampicillin, another commonly used agent for treatment of UTI, resistance is reaching 38–47% (23). In order to ensure optimal treatment and diminish further increase in resistance, sulfamethoxazole and ampicillin should only be prescribed following susceptibility testing.

The FLEXICULT™ SSI-Urinary Kit was developed exclusively for the diagnosis of uncomplicated UTI in the primary health care setting. The goal was to develop a simple diagnostic kit to enable GPs to perform point-of-care diagnosis of uncomplicated UTIs, with a higher sensitivity ( $10^3$  cfu/ml) compared to that of currently available commercial kits. In order to reduce the time between specimen collection and prescription of a relevant antimicrobial agent, we included a susceptibility test for 5 frequently used antimicrobials. The concentrations of antimicrobials in the kit were based on MIC distributions of 202 urinary tract pathogens obtained from patients with clinically suspected UTIs attending GPs in Roskilde County, Denmark (8), as well as breakpoints established by the Swedish RAF group (24), the British Society for Antimicrobial Chemotherapy (25) and the NCCLS (26). Because the kit was designed to cover all pathogens commonly associated with UTI it was not possible to use species-specific breakpoints. As *E. coli* causes  $\approx 80\%$  of acute infections in patients (27), breakpoints for this organism had a major influence on the decisions.

When asked, prior to the final design of the FLEXICULT™ SSI-Urinary Kit, the GPs stated that they would have preferred the kit to have 5 fields for susceptibility testing rather than 4 fields for susceptibility testing and 1 for identification of *E. coli* (unpublished survey). The panel of antimicrobials in the kit can be adjusted to suit local resistance frequencies and treatment recommendations or other selective media can be used. Finally, the kit is easy to use and read.

For the primary health care provider, the 2 important questions that need to be answered are ‘is this a case of UTI?’ and ‘which antimicrobial should be used?’. The FLEXICULT™ SSI-Urinary Kit provides simultaneous answers to these 2 questions. This is also true for the rare cases of UTIs caused by  $> 1$  pathogen.

The outcome of any diagnostic test will largely depend on the quality of the specimen taken and the FLEXICULT™ SSI-Urinary Kit is no exception. The kit supports the growth of all bacteria in the urine sample, making no discrimination between pathogens and contaminants, and, as for all diagnostic procedures for diagnosing UTIs, the specimen has to be collected as clean-catch, midstream urine (MSU). A sample of 25–50 ml of MSU, collected in a sterile container, is optimal for investigation purposes. The field trial of the FLEXICULT™ SSI-Urinary Kit underlined the importance of properly collected urine samples. Most of the samples investigated by the GPs with low numbers of cfu (i.e.  $< 10^3$  cfu/ml) consisted of contaminants and not urinary tract pathogens. Eight of the 18 samples with counts of  $> 10^3$  cfu/ml and  $< 10^5$  cfu/ml were Enterobacteriaceae, i.e. potential urinary pathogens. Contamination should be considered if there are a relatively low number of small, whitish colonies, with susceptibility to all the antimicrobial agents in the kit. The problem of distinguishing contaminants from causative agents will be minimized as GPs gain practical experience with the kit.

Despite these difficulties, the FLEXICULT™ SSI-Urinary Kit correctly identified resistant and susceptible bacteria in 90% and  $> 94\%$  of cases, respectively. The overall accordance with MIC values for susceptibility testing was  $> 93\%$ . In addition, the participating GPs considered the kit to be easy to handle and read. Therefore, the FLEXICULT™ SSI-Urinary Kit meets the need for a point-of-care diagnostic tool capable of dealing with the altered demands for the diagnosis and susceptibility testing of UTI in the primary health care setting. Further development of this kit using diagnostic agar will be pursued.

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