

URINARY VIABLE COUNTS – AN EVALUATION OF THREE METHODS

VJL HOW MBBS* AND N PARASAKTHI MBBS**

Summary

Viable bacterial counts were performed on 100 urine samples collected from inpatients with suspected Urinary Tract Infections at the University Hospital, Kuala Lumpur using the 'Microstix', 'Urostrip' and 'Standard Loop' methods. All 3 methods gave acceptable results when compared with the Miles and Misra surface viable count method.

When compared with the Miles and Misra method, identical results were obtained by the 'Microstix', 'Urostrip' and 'Standard Loop' in 69 or 71%, 77 or 79% and 74 or 76% – out of a total of 97 specimens (accurate counts could not be made on 3 out of the 100 specimens).

It is concluded that with the ease of use, the short time and minimum skill required, the 'Microstix' is ideal for use in large scale urine screening surveys but as it fails to detect yeasts and slow growing Streptococci the 'Urostrip' method is to be preferred for use in busy diagnostic laboratories. 'The Standard Loop' method gave acceptable results and was easier to read but was relatively more expensive and time consuming.

The diagnosis of urinary tract infections in a bacteriological laboratory depends on quantitative methods of bacterial culture. Qualitative methods are not adequate as the mere presence of bacteria in any urine sample cannot be accepted as proof that infection is present.

Quantitative methods have to be used to differentiate between a true infection in which bacteria are present and actively multiplying in the urine along some part of the Urinary tract and contamination of urine by bacteria colonising the vaginal-urethral area as it is voided.

Kass^{1,2} demonstrated that bacterial counts of $>10^5$ organisms per ml are indicative of a genuine urinary tract infection, counts of $<10^3$ organisms per ml are suggestive of contamination and counts between 10^4 and 10^5 are of doubtful significance and should ideally be re-assessed.

Viable bacterial counts using either the pour plate or surface viable count method of Miles and Misra³ are accurate but very few laboratories can afford either the time or the expense of either of these methods for routine use.

In the Diagnostic Bacteriology Laboratory, University Hospital, Kuala Lumpur, viable counts are done routinely on all urine specimens. These specimens comprised 34% of all

clinical specimens examined by this laboratory in 1975. For economic reasons, this laboratory has had to rely on the semiquantitative 'Urostrip' method (Edwards Instruments Co., Australia) for urinary viable counts.⁴

With the availability of the newer 'Microstix' method (Ames Division, Miles Laboratories, Elkhart, Indiana) this study was undertaken to compare the relative merits of the 'Microstix', 'Urostrip' and 'Standard Loop' methods for semiquantitative bacterial examination of urines. These were compared with the surface viable count method of Miles and Misra.

MATERIALS AND METHODS

100 mid-stream urine samples were collected over a 10 day period from patients with suspected urinary tract infections seen at the University Hospital, Kuala Lumpur. All urine samples were refrigerated immediately after collection and all were tested with the four methods selected within 3 hours of collection. For the 3 methods requiring culture media, both Human blood agar (10% Human Blood in Blood Agar Base Oxoid CM55) and MacConkey Agar (Oxoid CM7) were used. All inoculated materials were incubated at 37°C for 18-24 hours.

* & ** Lecturer, Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

* Address for reprint requests.

The Miles and Misra Surface Viable Count Method

10-fold dilutions of each urine sample were made using normal saline as the diluent. 0.02 ml volumes of the undiluted urine and of each dilution from 10^{-1} to 10^{-5} were dropped onto labelled sectors on the surface of well dried MacConkey Agar and Blood Agar plates (Figure 1) using a calibrated 50 dropper rinsed in boiling water between use. Counts were made on areas showing between 3 and 30 colonies, and the viable counts was calculated as follows:

A third pad detects the presence of nitrates in the sample, turning pink within 30 seconds if nitrates are present. The manufacturer's instructions were followed strictly:— the strip was dipped in undiluted urine for 5 seconds, excess urine was drained off, 30 seconds was allowed to elapse for the nitrite test to develop and be read, then the strip was inserted in a clear plastic self-sealing pouch provided by the manufacturer. The pouches were then incubated at 37°C for 18–24 hours. The viable count was estimated using the Manufacturer's guide (Figure 2).

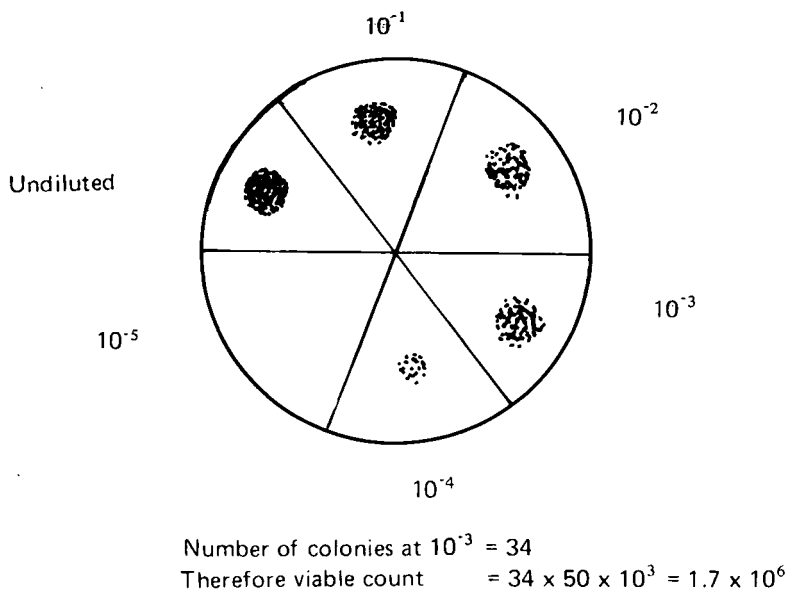


FIGURE 1 The Miles and Misra surface viable count method.

No. of colonies x 50 x dilution factor = No. of organisms/ml of undiluted urine

(1) The 'Microstix' Dip Strip Method

This method uses a clear polystyrene strip with 2 miniaturised and dehydrated culture media pads. The proximal pad contains culture media and a bile salt to inhibit the growth of Gram positive organisms. The distal pad contains medium that supports the growth of both Gram positive and Gram negative organisms.

The pads also contain colourless triphenyl tetrazolium which is reduced in the presence of bacteria to produce discrete red spots on the pad. The density of these spots is then used to indicate the number of bacteria present.

(2) The 'Urostrip' Filter Paper Method

A commercially available filter paper strip – 'Urostrip' with a special 'foot' area (Figure 3) was dipped in each undiluted urine sample. The strip was held upright touching the edge of the container to drain off any excess urine. The 'foot' area was then impressed onto the surface of a MacConkey agar and blood agar plate.

With this method it is possible to test between 6–8 urine samples on each agar plate. After incubation the number of colonies growing on each 'foot' area was counted and the viable count was estimated using the manufacturer's tables:—

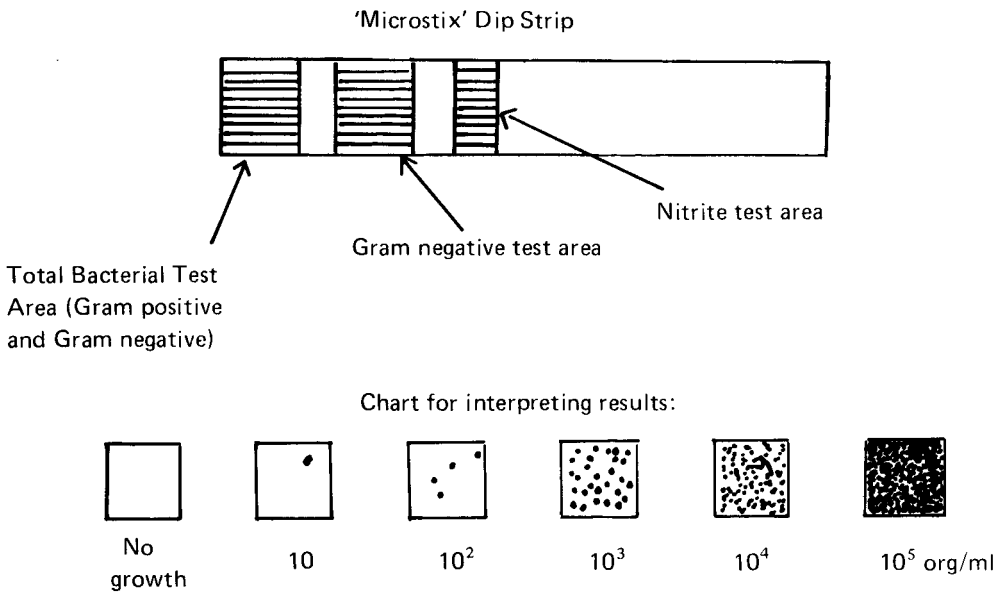


FIGURE II The 'Microstix' dip strip method

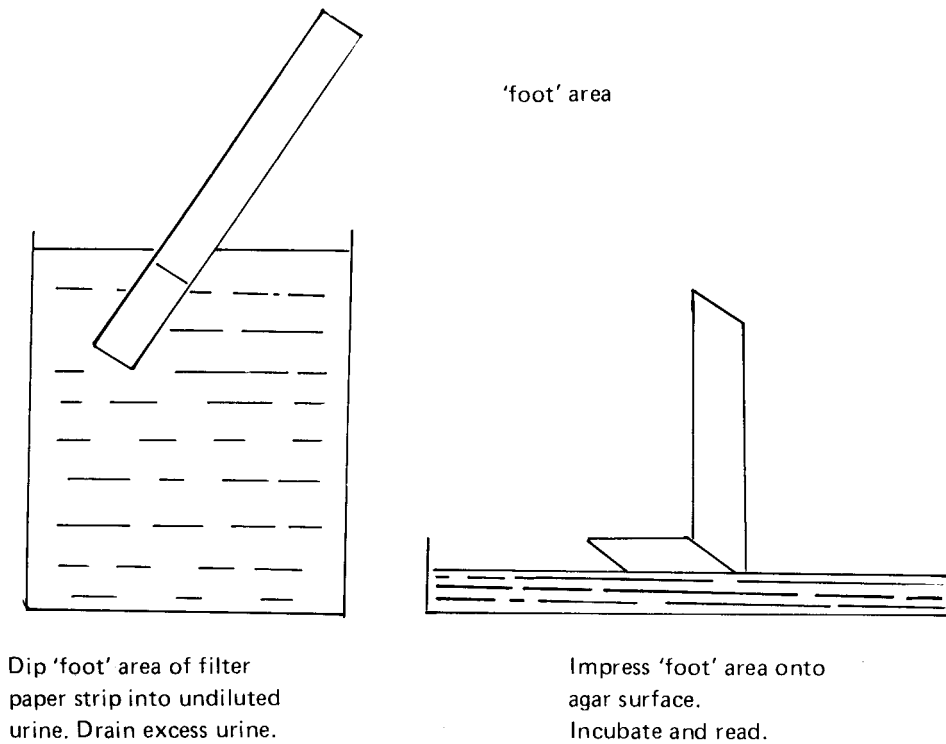
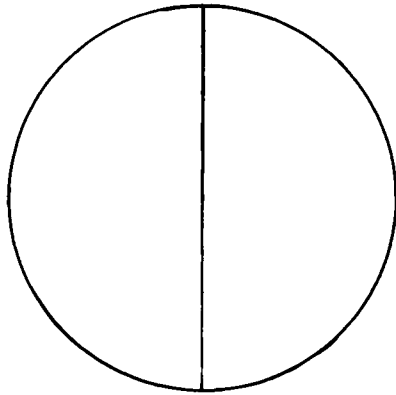


FIGURE III The 'Urostrip' filter paper method.

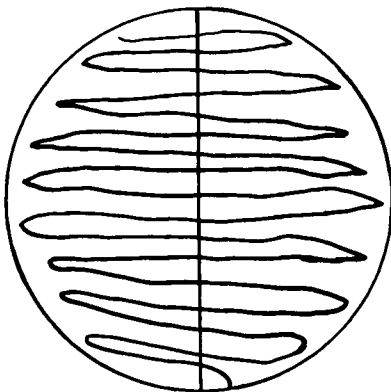
<u>Bacilli</u>	<u>Cocci</u>	<u>Viable count (organisms/ml)</u>
0 colonies	0 colonies	0 to 10^3
1 – 5 colonies	1 – 8 colonies	10^3 – 10^4
6 – 25 colonies	9 – 30 colonies	10^4 – 10^5
26 or > colonies	31 or > colonies	$> 10^5$

(3) *The Calibrated 'Standard Loop' Stroke Plate Method*

A calibrated wire loop (3 mm. internal diameter) was used to transfer a constant volume of undiluted urine onto the surface of each agar plate (MacConkey & blood) and streaked along the midline of each plate (Figure 4). Subsequent streaks were made at close intervals at right angles to the original streak.



Initial streak made along midline of plate



Subsequent streaks made at right angles to initial streak.

FIGURE IV The calibrated 'Standard Loop' stroke plate method.

The bacterial count was estimated as follows:—

<u>No. of colonies</u>	<u>Viable count (organisms/ml)</u>
0 – 50 colonies	10^3 or <
51 – 200 colonies	10^4 – 10^5
> 201 colonies	$> 10^5$

RESULTS

Growth

Twenty of the urine samples did not show any growth at all. In 16 samples there was growth of a single bacterial specie. Sixty one samples showed a mixed growth of 2 or 3 species with 1 specie clearly predominating in 23 samples. Three samples showed growth that was too mixed (> 3 types) for accurate counts to be made. These were eliminated from the study.

The Miles and Misra method detected a significant bacteriuria in 35 out of the 97 samples. Eighteen samples gave growth of doubtful significance while the remaining 44 samples gave growth suggestive of contamination.

Microorganisms isolated from positive samples

Table I shows the micro-organisms isolated from the positive samples i.e. samples with bacterial counts $> 10^5$ organisms/ml. Of the 19 samples with a mixed growth only the predominant organism is included in the table. Gram negative rods made up 31 of the 35 isolates (88%) while 2 strains of *Staph. albus* and 1 strain each of *Staph. pyogenes* and *Candida albicans* made up the remaining 4 isolates. Tables II, III and IV show the 'Microstix', 'Urostrip' and 'Standard Loop' compared with the Miles and Misra Method.

Microstix Method

The 'Microstix' detected 37 positive samples, (Table II) 30 of which were also detected by the reference test. The remaining 7 'Microstix' positive samples were of doubtful significance in the reference test. This test completely missed 3 positive samples which later grew *Candida albicans*, *Staph. pyogenes* and an *Enterococcus*. Identical results were obtained with the Miles and Misra method in 69 out of 97 samples or 71.1%. The Nitrite test was positive in only 4/97 samples or 4.12%.

TABLE I

BACTERIA ISOLATED FROM SPECIMENS THAT GAVE SIGNIFICANT GROWTH

Bacterial Species	Number
<i>Esch. coli</i>	11
<i>Klebsiella</i> species	8
<i>Ps. aeruginosa</i>	4
<i>Proteus</i> species	3
<i>Enterococci</i>	3
<i>Acinetobacter anitratus</i>	2
<i>Staph. albus</i>	2
<i>Staph. pyogenes</i>	1
<i>Candida albicans</i>	1
Total	35

TABLE II

RESULTS OF VIABLE COUNTS 'MICROSTIX' COMPARED WITH THE MILES AND MISRA

'Microstix' method (organisms/ml)	Miles and Misra surface count method (organisms/ml)							Total
	<10	10	10 ²	10 ³	10 ⁴	>10 ⁵	UR**	
<10	18	1	3	2	0	3*		27
10	2	2	2	2	0	0		8
10 ²	1	0	6	1	0	0		8
10 ³	0	0	0	3	1	0		4
10 ⁴	0	0	0	1	10	2	1	13
>10 ⁵	0	0	0	0	7	30	2	39
UR	0	0	0	0	0			
Total	21	3	11	9	18	35	3	100

* includes 1 strain of *Candida albicans*, 1 *Staph. pyogenes* and 1 *Enterococcus*.

** UR = unreadable.

'Urostrip' Method

The 'Urostrip' detected 34/35 positive samples (Table III) and missed 1 positive sample which later grew a strain of *Klebsiella*. Identical results were obtained in 77/97 samples or 79.4%.

'Standard Loop' Method

All 35 samples with a significant bacteriuria by the Miles and Misra method were detected by the 'Standard Loop' test (Table IV). The accuracy of this test was 76.3% with identical results obtained in 74/77 samples.

Comparison of the Results

Table V summarises the results of the viable

bacterial counts. All 3 methods detected more positive samples than the reference method. 'Microstix', 'Urostrip' and 'Standard Loop' gave false positive rates of 2/35 (5.7%), 4/35 (11.4%) and 4/35 (11.4%) respectively.

All 3 tests detected identical numbers of false negative samples giving a false negative rate of 6.8%.

A Comparison of the Methods

The approximate cost of materials per test, labour charges based on the average technician's salary (\$525 per month) amount of glassware and time required to perform each test and the degree of skill required are summarised in Table

TABLE III
RESULTS OF VIABLE COUNTS 'UROSTRIP' COMPARED WITH THE
MILES AND MISRA

'Urostrip' method (organisms/ml)	Miles and Misra surface count method (organisms/ml)							Total
	<10	10	10 ²	10 ³	10 ⁴	>10 ⁵	UR**	
<10	20	1	0	0	0	1*		22
10	1	2	0	1	0	0		4
10 ²	0	0	3	1	0	0		4
10 ³	0	0	8	7	2	0		17
10 ⁴	0	0	0	0	11	0	1	12
>10 ⁵	0	0	0	0	5	34	2	41
UR	0	0	0	0	0	0		
Total	21	3	11	9	18	35	3	100

* *Klebsiella* species

** UR = unreadable

TABLE IV
RESULTS OF VIABLE COUNTS 'STANDARD LOOP' COMPARED WITH
MILES AND MISRA

'Std. Loop' method (organisms/ml)	Miles and Misra surface count method (organisms/ml)							Total
	<10	10	10 ²	10 ³	10 ⁴	>10 ⁵	UR**	
<10	18	2	2	0	0	0		22
10	1	0	1	0	0	0		2
10 ²	2	1	6	2	0	0		11
10 ³	0	0	1	6	5	0	1	13
10 ⁴	0	0	1	1	9	0	0	11
>10 ⁵	0	0	0	0	4	35	2	41
UR	0	0	0	0	0	0		
Total	21	3	11	9	18	35	3	100

** UR = unreadable

VI. The ease of interpretation of each test and the minimum time interval between receiving the sample and the despatch of results of a theoretical positive sample growing a Gram negative rod is also included in the table.

DISCUSSION

This is a very small study undertaken to compare the relative merits of 3 locally avail-

able screening tests for bacteriuria – 'Microstix' a dip strip chemical test and 2 semiquantitative culture tests one making use of a filter paper 'foot' pad and the other a calibrated loop.

The 'Urostrip' was found to be the cheapest, most rapid to perform and most accurate of the 3 methods studied giving identical results in 79.4% of the specimens, a sensitivity* of 97.7% and a specificity** of 97.1%. This test required

*Sensitivity = $\frac{\text{No. of specimens } > 10^5 \text{ org./ml by test method}}{\text{No. of specimens } > 10^5 \text{ org./ml by Miles \& Misra}} \times 100$

**Specificity = $\frac{\text{No. of specimens } < 10^3 \text{ org./ml by test method}}{\text{No. of specimens } < 10^3 \text{ org./ml by Miles \& Misra}} \times 100$

TABLE V
RESULTS OF VIABLE BACTERIAL COUNTS BY THE 4 SELECTED METHODS

organisms/ml	Miles & Misra* surface viable count method	'Microstix' dip strip method	'Urostrip' filter paper method	'Standard Loop' method
>10 ⁵	35	37	39	39
10 ⁴	18	13	11	11
<10 ³	44	47	47	47
Total no. of specimens	97	97	97	97
False positive number and rate		2/35(5.7%)	4/35(11.4%)	4/35(11.4%)
False negative number and rate		3/44(6.8%)	3/44(6.8%)	3/44(6.8%)
Identical results		69/97(71.1%)	77/97(79.4%)	74/97(76.3%)

*The Miles and Misra method was used as a reference for evaluating the results obtained by the other 3 methods.

only minimal technical skill, only 20 seconds and 1/6–1/8 of a plate of blood agar and MacConkey agar (to facilitate the identification of Gram negative rods).

As the foot area is small (14 x 6 cms.) the test can be difficult to read and a subculture may be necessary if the growth is mixed.

The 'Standard Loop' was found to have a Sensitivity of 100%, specificity of 95.5% and identical results in 76.3% of the samples. The test took 70 seconds to perform and requires some degree of basic training in streaking techniques. It is easier to perform if whole agar plates are used but with experience half plates can be as satisfactory. This makes the test more costly than the 'Urostrip'.

The accuracy of this test varies with the technique and skill of the tester and also with the loop itself. Norden⁵ has shown that with repeated heating and cooling the dimensions of the loop can be altered by as much as 50%. This can be avoided either by frequent calibrations or by changing to new loops frequently.

The authors were impressed by the simplicity of the 'Microstix' method. All the materials required for the test i.e. dip strips, labels, incubation pouches and even a miniature incubator were provided by the manufacturer. Though the incubator proved too small for routine use in this laboratory it later proved very useful in the clinic of a general practitioner. The reading of the test was especially easy and at a glance

the viable count could be estimated reasonably accurately even by untrained persons. Kunin and Degroot⁶ have described its use for self inoculation by patients and Gillenwater et al⁷ reported on its satisfactory use for home inoculation, home incubation at room temperatures (kitchen) and self interpretation of the results by parents of 289 children. With experience there was no need to compare every specimen with the manufacturer's guide, only the doubtful ones thereby cutting down on the reading time. With increasing familiarity with the test and good planning e.g. prelabelling, the time taken could be cut down to 40 seconds. The major drawback of the 'Microstix' is the fact that this is a chemically based test. Therefore the pad areas have to be impressed onto agar surfaces and streaked to allow recovery of the organisms for identification and sensitivity tests. This delays the issue of final reports by about 24 hours.

Other workers have reported difficulties with interference from high concentrations of antimicrobial drugs in the urine and with *Pseudomonas aeruginosa* masking colour changes in the pads.^{8,9}

The nitrite test was a disappointment. Only 4 out of 97 samples were positive in our hands while Skelton¹⁰ reported the test to be positive in 59% of children with urinary tract infections. Similarly poor results were obtained in a urine survey now in progress involving 4015 school-

girls in which only 8 tests were positive (personal observation). All 12 nitrite positive samples in our experience eventually grew Gram negative rods. The 4 nitrite positive samples in this study gave counts of between 10^8 and 10^9 organisms/ml.

The poor results in these cases many be due to the fact that the urine samples were neither waking samples nor samples of urine collected more than 4 hours after the last voiding as recommended by the manufacturers. Strict adherence to these instructions may perhaps improve on the results.

An ideal method for detecting significant bacteriuria is one that is simple, inexpensive, accurate and convenient to use under the conditions requiring its use. It should also not require too much skill.

Of the 3 methods tested, the 'Urostrip' and 'Standard Loop' require the use of culture media plates not readily available to private clinics in this country. The 'Microstix' is the only satisfactory method of the 3 for private practitioner's clinics.

All 3 methods can be useful in a busy diagnostic laboratory. However, the 'Microstix' is affected by antimicrobial agents and fails to detect yeasts and slow growing streptococci, thereby restricting its usefulness. This problem can be avoided if a $\frac{1}{4}$ or $\frac{1}{2}$ plate of Blood agar is streaked with loopful of undiluted urine in addition to using the 'Microstix'.

Though the 'Urostrip' was slightly more difficult to interpret when compared with the 'Standard Loop' it was found to be simple, fast, inexpensive and gave acceptable sensitivities and specificities. Other workers have reported similar findings.^{1,2,13} As such it is recommended that the method of choice in this laboratory and in other similarly placed laboratories should still be the 'Urostrip'.

For large scale urine surveys the method chosen should be reasonably accurate and very fast and simple to use. The materials required should be minimal, stable and easily trans-

ported. Therefore the most suitable method for this purpose will be the 'Microstix'.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance and criticisms from their colleagues in the preparation of this paper. This study is supported in part by Vote F grant No. 152/75 from the University of Malaya.

REFERENCES

1. Kass, EH: Asymptomatic infections of the Urinary tract *Trans Ass Amer Phycns*, 69: 56-63, 1956.
2. Kass, EH: Bacteriuria in the diagnosis of infections of the urinary tract *Arch Intern Med*, 100: 709-714, 1957.
3. Miles, AA, Misra, SS, and Irwin, JD: The estimation of the bactericidal power of the blood *J Hyg (Lond)*, 38: 732-749, 1938.
4. Ryan, WL Hoody, S, and Luby, R: A simple quantitative test for bacteriuria. *J Urol*, 88: 838-841, 1962.
5. Norden, CW, and Kass, EH: Bacteriuria of pregnancy — a critical review. *Ann Rev Med*, 19: 431-470, 1968.
6. Kunin, CM, and Degroot, JE: Self-screening for significant bacteriuria evaluation of dip strip combination nitrite/culture test. *JAMA*, 231: 1349, 1974.
7. Gillenwater, JY *et al*: Home urine cultures by the dip-strip method: Results in 289 cultures *Pediatrics*, 58: 508-512, 1976.
8. Craig, WA, Kunin, CM, and Degroot, JE: Evaluation of the new urinary tract infection screening devices *Appl Microbiol*, 26: 196, 1973.
9. Morrow, JW, *et al*: Quantitative urine culture by reagent strip incubation. *Urology*, 5: 56, 1975.
10. Skelton, IJ, *et al*: Urinary tract infections in childhood *Med J Aust*, 882-885, 1977.