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Impedimetric Screening for Bacteriuria

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A rapid, automated instrumental procedure for distinguishing urine cultures containing greater than 10^5 organisms per ml is described. The method is based upon the measurement of changes in impedance that take place as microorganisms alter the chemical composition of the medium. The time required to detect impedance change is inversely related to the initial concentration of microorganisms in the sample. By defining an impedance-positive culture as one that gives detectable impedance change within 2.6 h, 95.8% of 1,133 urine cultures tested were correctly classified as containing more than or fewer than 10^5 organisms per ml. Selection of a longer detection time decreases false negative results at the cost of increased false positive results. Impedance screening is compared with screening data reported in the literature using adenosine-5'-triphosphate detection, microcalorimetry, electrochemical measurements, and optical microscopy.

A screening test for bacteriuria, if rapid, accurate, and inexpensive, could have many advantages. It would speed a preliminary report to the clinician who could prescribe treatment on the basis of positive laboratory evidence of infection. It would eliminate the unrewarding plating and reading of negative cultures that in most laboratories comprise the large majority of urine cultures. Finally, it would encourage screening of asymptomatic populations that are not presently screened for economic reasons.

A number of rapid methods have been described. These include measurement of bacterial adenosine-5'-triphosphate by luciferase (1, 4, 11), production of radioactive carbon dioxide by metabolism of labeled substrates (J. P. Kilbourn and J. L. Bramhall, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, M278, p. 112), measurement of the potential generated by growing microorganisms using a platinum and a calomel electrode (7), measurement of heat generated by metabolizing microorganisms using a microcalorimeter (2; K. A. Bettelheim, S. M. O'Farrell, S. Al-Salihi, E. J. Shaw, and A. E. Beezer, Abstr. 2nd Int. Symp. Rapid Methods Auto. Microbiol. 1976, p. 8), measurement of the impedance of a suspension of organisms filtered from urine and eluted into water (12), and measurement of particle size and distribution by electronic-pulse height analysis (L. S. Gall and W. A. Curby, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, C88, p. 40). In addition, a number of investigators have used microscopic examination of urine as a screening procedure (8–10).

This paper reports on the use in 1,133 clinical urine cultures of an impedance-measuring instrument to detect samples containing more than 10^5 microorganisms per ml, a concentration of microorganisms commonly associated with the presence of urinary tract infection. It should be pointed out that Kass (6) showed that over 10^5 of one single type of microorganism per ml was indicative of a urinary tract infection, whereas the method described here screens for over 10^5 total organisms per ml. It is occasionally the case that a urine culture will contain over 10^5 organisms but less than 10^5 of any single type per ml.

The principle upon which impedance monitoring is based (3) can be briefly summarized here. As microorganisms grow and metabolize, the chemical composition of the supporting medium is altered as nutrients are consumed and metabolic end products are produced. Associated with this change in chemical composition is a corresponding change in the resistance to the flow of an alternating current (i.e., the impedance) when a pair of electrodes are placed in the medium. When a small concentration of microorganisms is present, the change in impedance is not detectable. However, if the organisms are allowed to replicate, they will, in time, reach numbers sufficient to cause a detectable impedance change. This concentration of microorganisms is designated the threshold concentration. The threshold concentration depends, in part, on how detectable impedance change is defined. When detection is defined as the first noticeable change in impedance ratio, the corresponding threshold concentration is about 10^5 organisms per ml. When detection is defined as 0.8% change in impedance ratio, a larger and more clearly

obvious change, the corresponding threshold concentrations for most organisms are in the range of 10^6 to 10^7 organisms per ml.

The time required to reach the threshold concentration is called the detection time. It is clear that the smaller the initial concentration, the longer the time required to reach threshold. The detection time is, thus, a function of the initial concentration; i.e., as the initial concentration is reduced, the detection time increases. Detection time is also a function of the generation time of the population of microorganisms being measured and is prolonged when organisms are growing with slow generation times and shortened with more rapid growth. If a population of organisms has similar generation times, the detection time can be used to estimate initial concentrations. The application of this principle to estimating the initial concentrations of microorganisms in clinical urine samples, especially in establishing whether a sample contains more than or less than 10^5 organisms per ml, is the subject of this paper.

A preliminary report based on part of this data has already been presented (B. Nunke, S. W. Dufour, and S. J. Kraeger, Abstr. Annu. Meet. Amer. Soc. Microbiol. 1975, C25, p. 31).

MATERIALS AND METHODS

Instrumentation. The impedance-measuring instrument used in all experiments was a BACTOMETER 32 microbial monitoring system (Bactomatic, Inc., Palo Alto, Calif.), which has been described elsewhere (5).

Electrodes. All sample chambers used in these experiments were in clusters of 16, comprised of 8 sample chambers and 8 reference chambers, known as printed-circuit modules (Fig. 1) and have been described elsewhere (5). In this study, both gold-plated and stainless-steel electrodes were used. After use, modules were decontaminated with Amphyl (Sterling Drug), rinsed thoroughly, and soaked overnight in 7X detergent (Linbro). Before sterilization, the module was extensively rinsed (1 h minimum) with tap and distilled water. Our experience showed that this procedure was required not only to reduce any residual phenolic compounds to nontoxic levels, but also to assure more uniform electrode response.

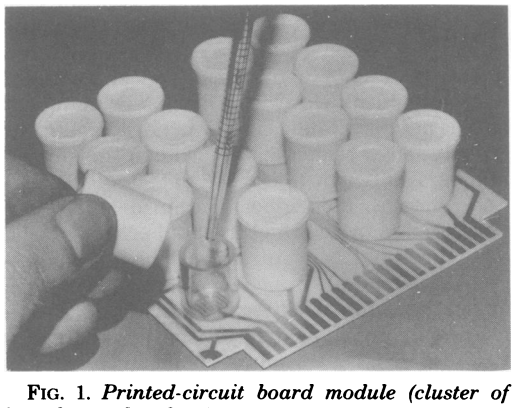

FIG. 1. *Printed-circuit board module (cluster of impedance chambers).*

Impedance measurements. Ethylene oxide-sterilized modules were aseptically filled with medium, 1.0 ml in all reference chambers and 0.5 ml in all sample chambers. All chambers were capped, and the electrodes were allowed to equilibrate at room temperature for at least 1 h before obtaining urine samples. Modules were generally prepared the previous day.

After equilibration, duplicate samples of fresh urine (0.5 ml) were pipetted into sample wells. When all sample wells were filled, they were recapped and the module was then inserted into the incubator portion of the BACTOMETER 32 microbial monitoring system, which had been preheated to 35°C. A 40-mV sinusoidal signal of 2,000 Hz was then passed across both reference and sample wells in series, and the impedance change was then automatically and continuously recorded by the instrument's strip chart recorder.

Impedimetric detection time. To distinguish the onset of impedance change caused by microbial growth from nonmicrobial changes, such as drift, noise, and temperature fluctuation, a detection standard was used. An accelerating change of 0.8% in impedance had to take place before an impedance change was ascribed to microbial activity. The time at which the accelerating-impedance change reached 0.8% was designated the detection time. Sometimes, especially with large concentrations of microorganisms, there was a strong, immediate, nonaccelerating change in impedance. If this change exceeded 10% in 1 h, it was designated as detection.

Cultures. All clinical urine specimens were randomly selected and obtained from Kaiser Foundation Hospital (Santa Clara, Calif.) and transported to our laboratory. Three plates from each sample were prepared: two dilutions were plated onto blood agar and one dilution was plated on Levine eosin-methylene blue agar. All plates were prepared by using a calibrated-loop technique. Organisms from positive cultures were identified by conventional microbiological procedures.

Media. All media were made from commercially prepared dehydrated bases obtained from Baltimore Biological Laboratory (Cockeysville, Md.) according to manufacturer's directions. These included brain heart infusion (BHI), BHI with 0.05% agar, Trypticase soy broth, Columbia broth, and thioglycolate broth.

Other media, blood culture medium with and without 0.05% agar contained BHI base (BBL), 0.5% yeast extract, 0.025% sodium polyanetholsulfonate, 0.0001% vitamin K (dissolved in 95% ethanol), and 0.005% hemin.

RESULTS AND DISCUSSION

The medium and the sample inoculum size were determined by preliminary studies performed with urine samples known to have high concentrations of organisms. Six media, BHI,

blood culture medium without agar, blood culture medium with agar, Trypticase soy broth, Columbia broth, and thioglycolate broth, were compared with respect to their promotion of rapid growth, impedance drift, and response size. After 62 urine samples were analyzed, BHI was chosen, not because it was demonstrated to be clearly superior, but because the other media were no better with respect to the above parameters.

Various urine-BHI ratios were investigated, ranging from 1% urine and 99% BHI to 100% urine. The predominantly urine mixtures have the advantage of maintaining a high concentration of microorganisms and, hence, of shortening detection times. They have the disadvantage of introducing a large variable, the urine itself, which can differ considerably from patient to patient with respect to pH and ionic content. Conversely, a small inoculum such as 10 μl of urine minimizes the variability of the urine and dilutes inhibitory substances, but it markedly cuts down the initial concentration of organisms in the impedance chamber, thus prolonging detection times. (Detection times with 1% urine and 99% BHI were typically 3 h later than those with 100% urine.) A workable compromise was found with 0.5 ml of urine and 0.5 ml of medium.

Detection times for 1,133 urine specimens plotted against initial concentration are shown in Fig. 2. Each urine specimen is represented by a single point on the graph, the coordinates of which define the detection time in hours and the initial concentration in colony-forming units. Detection times greater than 12 h are shown at the extreme right hand of the graph along a common vertical line. The numbers next to larger black circles indicate the number of samples having the same detection time and initial concentration displayed together as one large data point.

The horizontal line at 10^5 organisms per ml indicates the level chosen to divide positive and negative urine cultures. Thus, all points above the line represent cultures defined as positive by plate count, and all points below the line represent cultures defined as negative. By this criterion, 188 samples were positive and 945 samples were negative.

We may choose a time called the cutoff time that will divide the cultures into those detected before the cutoff (and hence are impedance positive) and those detected after the cutoff time (and are thus impedance negative). One such cutoff time is represented by the vertical line at 2.6 h. All points now fall into four quadrants. The points in the upper-left quadrant represent cultures positive both by plate count and by

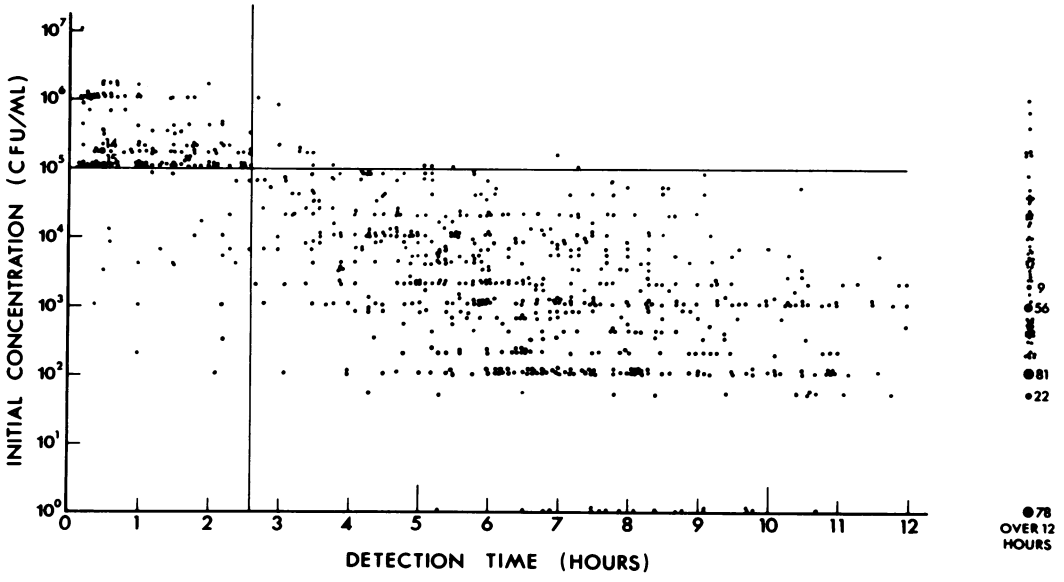

FIG. 2. *Detection times of 1,133 randomly selected urine samples plotted against the initial concentration in colony-forming units per milliliter (by plate count). The horizontal line divides all cultures into those with greater or fewer than 10^5 organisms per ml. The vertical line divides all cultures into impedance positive (detection time, ≤2.6 h) or impedance negative (detection time, >2.6 h). Some points are larger than the others and have a number next to them. The number represents the number of data points occupying the same position on the graph. All samples with detection times greater than 12 h are shown at the extreme right-hand side of the graph.*

impedance. Conversely, the points in the lower-right quadrant represent cultures negative by both criteria. Cultures designated as positive by impedance but negative by plate count are represented by points in the lower-left quadrant and are false positive results. Cultures positive by plate count but negative by impedance are false negative results and are represented by points in the upper-right quadrant.

By changing the cutoff time, the numbers of false positive and false negative results can be altered. Short cutoff times minimize false positive but increase false negative results. Long cutoff times minimize false negative but increase false positive results.

Maximum agreement between the two methods is achieved with a cutoff time of 2.6 h. With this criterion, 95.8% of all samples are correctly classified, with 22 false positive samples and 26 false negative samples. Thus, 1.9% of all samples are false positive and 2.3% of all samples are false negative. These numbers are summarized in Table 1, where the upper-left box contains the number of samples found positive by both systems; upper right, those positive by plate count only; lower left, those positive by impedance only; and lower right, those negative by both systems. Also summarized in Table 1 are the percentages of false negative and false positive samples (as percentages of the total number of cultures) and positive samples missed (as a percentage of the number of cultures found positive by plate count).

Maximum agreement may not be the most desirable criterion for selecting a cutoff time. Current practice in many clinics is to treat all patients on the basis of history, symptoms, and a urinalysis, using a culture only to verify the diagnosis. A rapid screen would enable the clinician to withhold treatment until he had specific laboratory confirmation of bacteriuria. Increasing the cutoff time, thereby minimizing false negative at the expense of increasing false positive results, will assure that fewer cases of bacteriuria are missed. The additional false positive samples so engendered would mean that more patients would receive unnecessary treatment. A greater number of positive samples would also mean more work for the microbiologist, assuming that all cultures positive by the screen would need further workup.

To reduce false negative results, and thereby reduce the percentage of positive cultures missed, one can increase the cutoff time; this has the effect of increasing false positive samples as well. Using 3.5 h as the cutoff time, to reduce the false negative results and the percentage of positive cultures missed, gives agreement of 93.3% with 14 false negative and 55 false positive samples. Thus, the percentage of all samples that are false negative falls from 2.3 and 1.2%. The false positive cultures rise from 1.9 to 4.9%, and the percentage of positive samples missed drops from 13.8 to 7.5% (Table 2).

Another way to view these percentages is in terms of the percentage of samples impedimetrically classified positive or negative for which the plate count yielded the same classification. The 2.6-h cutoff yields 184 impedance-positive cultures, with 162, or 88%, also positive by plate count, and 949 impedance-negative cultures, with 923, or 97.3%, also negative by plate count. Increasing the cutoff time to 3.5 h increases the latter percentage to 98.5% at the expense of decreasing the former to 76%.

There are several likely reasons for the false negative results that occurred with the impedance method. One source of false negative data is urine samples that contain antimicrobial agents. In only a few cases was information available that urine samples came from patients actively being treated with antibiotics. In some of these specimens, we noted that the impedimetric assessment was negative, whereas the plate count was positive. This may result from the fact that a greater dilution of antimicrobial agents takes place on the surface of a culture plate than is possible in an impedance chamber. The small volume of a calibrated loop is spread over the entire surface of the plate, and presum-

TABLE 1. *Number of positive and negative samples obtained by culture and by impedance using a 2.6-h cutoff*

Culture (organisms/ml)	Impedance[a] (cutoff time)	
	+ (≤2.6 h)	− (>2.6 h)
+ (≥10^5)	162	26
− (<10^5)	22	923

[a] Number of false positive samples, 22/1,133 (1.9%); false negative samples, 26/1,133 (2.3%); positive samples missed, 26/188 (13.8%); agreement, 1,085/1,133 (95.8%).

TABLE 2. *Number of positive and negative samples obtained by culture and by impedance using a 3.5-h cutoff*

Culture (organisms/ml)	Impedance[a] (cutoff time)	
	+ (≤3.5 h)	− (>3.5 h)
+ (≥10^5)	174	14
− (<10^5)	55	890

[a] Number of false positive samples, 55/1,133 (4.9%); false negative samples, 14/1,133 (1.2%); positive samples missed, 14/188 (7.4%); agreement, 1,064/1,133 (93.9%).

ably the volume into which the antibiotic can diffuse is, by contrast, very large. Alternatively, antibiotics may be bound to agar in the plate count procedure, whereas this is not possible in the liquid medium of the impedance chamber.

Some false negative cultures may also be caused by slower-growing organisms that would not reach threshold as rapidly as, for example, *Escherichia coli*. It should be noted, however, that many organisms with generation times longer than *E. coli*, such as staphylococci, streptococci, lactobacilli, yeast, etc., were detected as impedance positives in this study—presumably due to very high initial concentrations. A list of the organisms encountered in the cultures designated positive by plate count appears in Table 3.

One should examine the data from the point of view of work saved. If one relies upon the screen and does no further work with cultures that are screened as negative, considerable labor is saved. This savings is most marked when the percentage of positive samples is generally small, as might be expected in an outpatient clinic. If all impedance negative samples were discarded and only impedance positive cultures were quantitatively cultured, a cutoff time of 3.5 h would yield 79.8% reduction in work. The work saved is even greater with the shorter cutoff time, i.e., 83.8%.

TABLE 3. *Distribution of organisms in 188 urine samples positive by culture (>10^5/ml)*

Organism	No. of cultures	%
Escherichia coli	107	56.9
Proteus mirabilis	13	6.9
Staphylococcus aureus	12	6.4
Klebsiella pneumoniae	9	4.8
Enterococcus	7	3.7
Pseudomonas aeruginosa	5	2.7
Enterobacter sp.	3	1.6
Streptococcus sp.	2	1.1
Lactobacilli and diphtheroids	2	1.1
Serratia marcescens	1	0.5
Candida sp.	1	0.5
Unidentified gram-negative rods	18	9.6
Unidentified gram-positive cocci	4	2.1
Mixed organisms	4	2.1

In Table 4 the impedance screen is compared with other techniques. The data are all cast into the same form as Tables 1 and 2, and are taken from the cited references. The results of each technique are compared with plate count results by using the same criterion for a positive culture of 10^5 organisms or greater per ml. All techniques are rapid, ranging in time from a few minutes for microscopy to 4 h for the electrochemical. Also, in general, the agreement obtained was high for all methods (Table 5). Microcalorimetry had the lowest agreement due to the large number of false positive results; however, the very low percentage of positive samples missed is a very strong feature of this method. Impedance has the best overall agreement (see either Table 1 or 2) but does not have as low a percentage of positive cultures missed as were seen with microcalorimetry or microscopy. The luciferase method missed over ¼ of the samples positive by plate count, which is a severe limitation to this procedure. More confidence can be placed in results from those studies having the largest sample sizes. Thus, it is possible that further studies may lead to slightly different conclusions with those methods where the sample size is very small.

The use of microscopy, although reasonably accurate and by far the most rapid, is not widespread. This may be due to the burden it places on the staff performing the work, which is exacting and tedious. Adenosine-5'-triphosphate assays require chemical manipulation of the sample, which is a barrier for many laboratories

TABLE 4. *Comparison of the number of positive and negative samples obtained by culture and various screening procedures*

Culture	Screening procedure							
	Electrochemical[a]		Microcalorimetry[b]		Adenosine-5'-triphosphate[c]		Microscopy[d]	
	+	−	+	−	+	−	+	−
+	37	3	146	4	46	17	712	31
−	2	26	44	105	20	265	188	1633

[a] Data taken from Fig. 1 of reference 7.
[b] See reference 2.
[c] See reference 1.
[d] See reference 8. Only the results from defining a positive result by culture as samples having >10^5 organisms per ml and a positive result by microscopy as multiple cells are shown.

TABLE 5. *Agreement of various screening procedures*

Screening procedure	No. of samples	False positive samples (%)	False negative samples (%)	Positive samples missed (%)	Agreement (%)
Electrochemical[a]	68	2/68 (2.9)	3/68 (4.4)	3/40 (7.5)	63/68 (92.6)
Microcalorimetry[b]	299	44/299 (14.7)	4/299 (1.3)	4/150 (2.7)	251/299 (84.0)
Adenosine-5'-triphosphate luciferase[c]	348	20/348 (5.7)	17/348 (4.9)	17/63 (27.0)	311/348 (89.4)
Microscopy[d]	2,564	188/2,564 (7.3)	31/2,564 (1.2)	31/743 (4.2)	2,345/2,564 (91.5)

[a] Data taken from Fig. 1 of reference 7.
[b] See reference 2.
[c] See reference 1.
[d] See reference 8.

in terms of labor cost, and, in addition, the reagents are expensive. The major difficulty with microcalorimetry is that most microcalorimeters are single-channel instruments. Multiple-channel instruments have been made, but they are costly and not readily available. The electrochemical method is easy to use and shows great promise, but this method also appears to be difficult to extend (at low cost) to monitoring of large sample numbers. Thus, of the methods compared above, the impedance approach appears to offer advantages in ease of use and ability to handle large numbers of samples while retaining a high level of agreement with the plate count technique.

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