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EVALUATION OF VARIOUS METHODS USED FOR THE DETECTION OF SIGNIFICANT BACTERIURIA IN HUMANS

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A VARIETY OF procedures can be employed for the detection of significant bacteriuria in humans. Urine which have been collected aseptically can be tested by the more direct conventional bacteriological methods, such as Gram-stained smears and/or cultural procedures (qualitative and quantitative), which have been known for many decades. More recently other indirect tests, such as the Greiss[1], Triphenyl Tetrazolium[2], and Electronic procedures[3], have been more widely used.

The primary considerations, which must be well understood in the diagnosis of urinary tract infections, concern (a) the method of collection and (b) the interpretation of the laboratory data. It is well known that catheterization is one of the age-old procedures for obtaining urine specimens aseptically from females and occasionally from males. During the past decade, many investigators[4-8] have shown that clean voided urine specimens from patients with significant bacteriuria usually contains 100,000 organisms per milliliter and that these results compare favorably with urine specimens collected by urethral catheter, percutaneous puncture of the bladder, or direct puncture of the exposed bladder. As a result, the "Clean Catch Mid-Stream—CCMS" procedure has replaced the catheterization technique in a large majority of cases. However, it should be emphasized that some of the CCMS specimens may possibly be contaminated, due to improper cleansing of the genitalia.[9]

The interpretation of the data should be based on the recommendations reported by Kass, in 1956,[10] and Sanford *et al,*[6] in the same year—namely, that a quantitative count be performed on the urine specimens collected aseptically by the CCMS procedure. The bacterial count in specimens of cases having a significant bacteriuria has been established as 100,000 bacteria or greater per milliliter of urine.[6,10] If mid-stream specimens are processed without delay from non-infected cases, the cultures rarely demonstrate numbers which approach this critical level.[10,11] This conclusion has been confirmed by other investigators.[9] It should be stressed that this method has frequently been compared with the more conventional procedures, such as Gram-stained smears[9] or wet mounts of centrifuged or uncentrifuged urine,[9] as well as with the battery of cultural procedures employing a variety of solid and fluid media.

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However, one disadvantage of the quantitative plating procedure is that it is laborious and time-consuming for many bacteriological laboratories. Another disadvantage may be the emphasis that is placed on the "magic number" of 100,000 cells per milliliter without seriously considering the clinical aspects of the case. Therefore, it should not be considered as an absolute figure but rather as a guide to diagnosis. The significance of the bacteriological count should be assessed in terms of its relation to clinically apparent urinary infections.[9]

The need for early detection of bacteriuria, especially in asymptomatic cases, has led other investigators to search for methods which, perhaps, might be more rapid, simpler, and possibly more accurate than the more conventional qualitative and/or quantitative bacteriological techniques. Among these are three tests which have been proposed. The first is the Greiss procedure[1] which has been known for many years. It depends on the ability of the organisms to reduce nitrates to nitrites. The method is simple but fails to detect 20-30 per cent of the infections.[1]

A second procedure, which is a relatively simple test for significant bacteriuria, is the reduction of a colorless soluble chemical known as 2, 3, 5 triphenyl tetrazolium chloride (TTC) to its red insoluble triphenyl formazan. Simmons and Williams,[2] in 1962, reported that the TTC test detected 94 per cent of 480 urines which contained at least 100,000 organismus per milliliter. Studies on file at Knickerbocker Biologics* state that 85 per cent of the urine specimens tested with TTC in field studies gave a positive result which correlated with a quantitative count of 100,000 cells per milliliter or greater. The results were more favorable with gram-negative rather than with gram-positive organisms.

A third procedure, which employs an electronic device called the Coulter Counter,** was described, in 1962, by Truant *et al.*[3] This electronic apparatus contained a 30 u fused aperature tube on the stand and either a Coulter model A or B was used in conjunction with a plotter. The results showed agreement with the quantitative plate method in 90-96 per cent of 10,000 urine specimens. The urine was pre-cultured for 2-3 hours and processed on the Coulter apparatus in 100 seconds with interpretation being immediate on the basis of the oscilloscope pattern and the plotted graph.[3] The disadvantages are (1) the cost of the instrument, (2) the time required in preparing the clean, sterile broth and (3) the fact that not all positive specimens were detected. Because of the two latter objections, the author investigated the possibility of using raw urine.

This study was undertaken primarily to compare the more conventional procedures commonly used for determining significant bacteriuria with the newer methods. Comparative studies, using both the direct and indirect methods, have been completed on more than 10,000 urine specimens obtained from "In Patient" and "Out-Patient" Services.

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*Knickerbocker Biologics, Pfizer Laboratories Division, Charles Pfizer and Co., Inc., New York, N. Y., supplied the product "Uroscreen".

**The Coulter Counter is manufactured by Coulter Electronics, Hialeah, Florida.

MATERIALS AND METHODS

I. *Qualitative Examination.*

(a) *Culture Media.*

An aliquot of 0.1 ml of undiluted urine was inoculated onto either one or all of the following solid media: sheep's blood agar (5-7 per cent sheep's blood), phenylethyl alcohol agar and desoxycholate. All three media were used for specimens requiring a more rapid and complete identification of isolates. For example, if the electronic studies showed that a urine specimen had a significant number of organisms then some time was saved by using the phenylethyl alcohol and desoxycholate in conjunction with a sheep's blood agar (SBA) plate. However, if the electronic examination was negative then only a blood plate was inoculated. This was done in order to assure detection of those specimens which contained more than 100,000 organisms per milliliter but which might be missed by the electronic procedure. Another reason for the blood plate was to give the bacterial flora and semi-quantitative data of the urine specimen for those clinicians who so desired.

(b) *Triphenyltetrazolium Chloride (TTC).*

This compound was used according to the methods adapted from Simmons and Williams[2] and described by Knickerbocker Biologics, who supplied the product — "Uroscreen" — to us for testing purposes. Two milliliters of freshly collected urine (or urine which had been stored at 4° C. for 24 hours) was inoculated into the "Uroscreen tube" and shaken. The mixture was incubated at 37° C. for four hours. The mixture must not be shaken or disturbed during this period. If the precipitate is disturbed before the reading is obtained, the resuspended precipitate must be centrifuged. A pink to red deposit denotes a positive test. This colored precipitate (triphenyl formazan) may appear in 1-2 hours if the bacterial count of the urine is high (millions of cells per milliliter).

In addition to the TTC test, all specimens were examined by standard bacteriological plating methods and quantitative bacterial counts.

(c) *The Greiss Test.*

Interest has been renewed periodically in the Greiss nitrate reduction test as a means of detecting significant bacteriuria in humans (Smith *et al*).[1] The Greiss test can be done by adding 1 ml of Greiss reagent[1] to 1 ml of urine. The development of a pink to red color in a matter of seconds is considered a positive test. A modified procedure discussed by Smith *et al*,[1] which we used more frequently in this study, consisted of incubating the urine for one hour at room temperature with 0.5 ml of a 10 per cent solution of potassium nitrate before testing with the Greiss reagent.

All urine specimens treated in each series of experiments were, also, tested by standard bacteriological methods (i.e. SBA and quantitative plates), as well as by the electronic method.

II. *Quantitative Procedures.*

Several semi-quantitative and quantitative procedures were performed in an attempt to evaluate the degree of correlation which could be expected by the various methods. The techniques which were employed in these studies consisted of the following:

(a) The use of 1.0 and 5.0 ml pipettes,
(b) calibrated 6.0 mm nichrome loops,
(c) 10^{-3} dilution pour-plate procedure,
(d) electronic method using (i) Trypticase Soy or Brain-Heart Infusion broth, or (ii) raw, "Filterfuged"* urine.

(a) *Pipette Method.*

A sterile 1.0 ml pipette (or 5.0 ml at times) was used to aseptically transfer 0.1 ml of undiluted urine to the upper third of agar plates (sheep's bloor agar, phenylethyl alcohol and/or desoxycholate). The plates were streaked with the point of the pipette so as to obtain isolated colonies in one-half or more of the plate area even with specimens containing more than 3 x 10^6 organisms per ml. This was achieved by rotating the plate during the inoculation procedure, as in the case with "isolation streaking" with a sterile loop.

*Filterfuge tube (see Technical Bulletin FF-1) is manufactured by International Equipment Company, Needham Heights, Mass.

The classification used for reporting purposes was based on the number of organisms in the initial or primary zone of inoculation (see Table I).

Table I

Schema For Comparing Semi-Quantitative Data

Count On SBA Plate Using Pipette	Report	Quantitative Plate Colony Count
1 — 50*	Rare	0 — 1,000
50 — 100	Few	1,000 — 10,000
100 — 300	Few-Moderate	10^4 to 10^5
300 — 500	Moderate	10^5 to 3 x 10^6
More than 500	Numerous	Greater than 10^6

*Number of colonies.

(b) *Calibrated 6.0 mm Nichrome Wire Loop Technique.*

Several standardized 6.0 mm nichrome wire loops, which were calibrated to deliver 0.02 ml of urine, were used to inoculate the agar plates. After depositing the urine in the initial zone of inoculation (covering an area of one-quarter of the plate surface), the loop was flamed, cooled, and a second streak area produced on the next one-quarter of the plate. This technique was repeated until four quadrants had been inoculated, taking special precautions to avoid "carry-over" from the initial area by touching the primary inoculation area on the last streaking procedure.

The schema for comparing and interpreting the counts obtained with the loop were similar to those of Table I, with the slight exception that the "loop-plate colony count" was usually slightly less but certainly not by a factor of 20, as is theoretically possible on a volume basis.

(c) *Dilution Pour-Plate Technique.*

A 1:1000 dilution of the urine specimen was prepared by adding 1.0 ml to 9.0 ml of sterile saline and shaking manually or by means of a turbo-shaker for several seconds. A 1.0 ml aliquot of this sample was transferred after "adequate mixing" to another 9.0 ml sample of sterile saline, mixed and, finally, 1.0 ml was transferred into another 9.0 ml volume of sterile saline and shaken. One milliliter aliquots from the last tube dilutions (10^3) were transferred to each of two sterile plates and 15 ml Trypticase Soy Agar at 45-48° C. were poured into each plate. The contents were mixed immediately by gently rotating the plates. They were incubated at 37° C. and counts were made at 24 and 48 hours.

(d) *Electronic Studies Using (i) Broth.*

One milliliter aliquots of urine were transferred to clean, sterile sputum bottles (50.0 ml volume) containing 10.0 ml of sterile Trypticase or Brain-Heart Infusion Broth (15 per cent Supplement C may be added). The broths were always prepared with Abbott's normal saline* and subsequently filtered through 0.3 u or 0.45 u millipore filters.

Special care must be taken to prepare these bottles and the broth so as to avoid a high particle count which would produce a false positive graph and/or oscilloscope picture. This aspect of the procedure is so important that several controls (uninoculated broths) are routinely checked at the time of preparation and other controls are incubated and tested daily with the routine specimens. After inoculating the 1.0 ml aliquots into the 10 ml of sterile, filtered broth, the mixture was shaken for two to three hours at 37° C. If the specimens appeared turbid at two hours, they were processed electronically at this time, otherwise they were checked after three hours of incubation. The electronic test procedure was performed as follows: The 10 ml specimen was diluted with sterile Abbott's saline to a 40 ml volume (final urine dilution of 1-40 ml) and was processed through a 30 u fused aperature tube.[3] The electrical characteristics were observed on the oscilloscope. The particles were automatically counted on the model B Coulter whose aperature current setting was 1 and amplification setting was at 0.25. The data was automatically plotted on graph paper by the attached Coulter plotter. The data was interpreted according to the reports by Truant *et al.*[3]

*Abbott's normal saline has a low particle count. It is obtained from Abbott Laboratories, North Chicago, Ill.

(d) *Electronic Studies Using (ii) Raw Urine.*

Since the author's first report, in 1962, a new method for processing urines has been developed. The procedure involves the filtration of raw urine specimens by centrifugal force. The urine passes through a paper prefilter (15 to 50 u porosity) which permits bacteria to pass into a stainless steel Filterfuge tube.* The procedure consists of the addition of 3.0 ml of raw urine to the upper section of the assembled filterfuge tube (containing only one mid-section with screen and a millipore paper clarifying filter).** The tube is sealed with its teflon liner and cap.** The tubes are centrifuged at 2700 RPM for three minutes. With the aid of rubber gloves the operator removes the mid-section and upper section containing the screen and filter and places these parts in 5 per cent phenol. One milliliter of urine filtrate is removed from the lower section and diluted with 24 ml of Abbott's saline in clean sputum jars. Electronic tests are performed as described in the previous section (*di*). The comparative studies, also, included the testing of another 1.0 ml aliquot of the urine filtrate. This sample was diluted to 10^3 and two pour-plate counts were obtained. Aliquots of 0.1 ml of the urine filtrate were used for plating on culture media (sheep's blood agar, phenylethyl alcohol and desoxycholate).

Filterfuge Tube Cleaning Technique.

The stainless steel filterfuge tube separates into several sections (see Technical Bulletin FF-1 supplied by the International Equipment Co., Needham Heights, Mass.). After processing the urine specimen, the tubes are disassembled with sterile surgical rubber gloves and the parts are placed in five per cent phenol for cold disinfection. The urine filtrate from the bottom section is harvested. The bottom section of the tube is also placed in the five per cent phenol for cold disinfection (15 to 30 minutes). The filterfuge tubes are cleaned as follows:

1. Rinse the parts in tap water, shake.
2. Transfer to alkaline detergent for five minutes, shake, use brush on the screens and parts.
3. Rinse apparatus in tap water.
4. Transfer to 0.1 N HC1 for five minutes, rinse in tap water and, lastly, in Abbott's saline.
5. Allow to dry, taking care not to contaminate with dust or lint, etc.
6. Reassemble with prefilter in mid-section.

An alternate cleaning procedure is being evaluated which consists of:

1. Autoclaving the parts at 15 pounds for 15 minutes.
2. Brushing screens, sections and washer.
3. Rinsing in tap water and Abbott's saline.
4. Carefully drying and avoiding dust contamination.
5. Re-assembling with pre-filter in mid-section.

Results

I. *Qualitative Procedures.*

(a) *Culture Media.* The attempt to find a media which would enhance the growth of the majority of organisms which might be present in urine specimens was successful. Trypticase Soy Broth and Brain-Heart Infusion produced good growth of the majority of organisms from positive urine specimens within two to three hours. Strains of *Pseudomonas,* however, were more difficult to grow in these fluid media (see Table II). As a result, the electronic procedure did not always detect the cases of bacteriuria due to *Pseudomonas.* A search for a better media which would grow this and other urine

*The Filterfuge tubes were obtained from the International Equipment Company, Needham Heights, Mass.

**Please read instruction in Technical Bulletin FF-1 obtained from International Equipment Co., Needham Heights, Mass.

isolates was undertaken. The addition of either Bacto - Supplement C*** or ferrous ammonium sulfate enhanced the growth of more *Pseudomonas* strains more than did

Table II

Growth of Pseudomonas In Brain-Heart Infusion
And Trypticase Soy Agar After 3 hours at 37° C

No. of *Pseudo.* strains (pure culture)	Growth in BHI*	Growth in T-Soy**
10	3 strains	3 strains
No. of *Pseudo.* present in urine		
3	2 strains	2 strains

*BHI — Brain Heart Infusion.
**T-Soy — Trypticase Soy Broth.

ascitic fluid and horse serum (see Table III. The data shown in both Table II and IV indicates that more strains of *Pseudomonas* will grow from urine specimens than from pure cultures in both unsupplemented and supplemented broth.

Table III

Possible Enhancement of 10 Strains of Pseudomonas
By the Addition of Supplements in BHI

	Horse Serum		Ascitic Fluid		Supplement C		Ferrous Ammonium Sulfate	
Concentration	3%	15%	3%	15%	3%	15%	1 ug/ml	30 ug/ml
No. of strains showing growth in 3 hrs. at 37°	None	2	2	2	3	7	7	7

Table IV

Effect of Horse Serum in T-Soy Broth
On the Growth of Pseudomonas

		Growth in 3 hrs. at 37° C.	No Growth in 3 hrs. at 37° C.
No. of *Pseudo.* strains (Pure culture)	10	1	9
No. of *Pseudo.* tested in urine	6	5	1

***Bacto-Supplement C was obtained from Difco Laboratories, Detroit, Michigan.

(b) *Triphenyl Tetrazolium Chloride.* This procedure was evaluated in two separate studies (see Tables V and VI). Initially, 85 urine specimens were tested by means of TTC, Greiss, Electronic and Quantitative Plate techniques. It can be seen from the results in Table V that the TTC and Greiss tests identified slightly more (3 per cent) negative urines than the Electronic procedure. The Electronic procedure showed more equivocal results in the quantitative categories of from 10^1 to 10^5 cells per ml, but this procedure predicted more positive urines (greater than 100,000 cells per ml) than did the Greiss or the TTC.

Table V

Summary Of Results Obtained Using The Greiss, TTC, Electronic And Quantitative Plate Procedures On 85 Specimens.

Quant. Count Category	Corresponding Number and Per Cent for Each Group			
	Negative	1 — 10^4	10^4 — 10^5	More than 10^5
No. in each Quant. Group	30 (35%)	23 (27%)	7 (8%)	25 (30%)
Greiss	29 (34%)	0 (0%)	3 (4%)	16 (19%)
TTC	29 (34%)	0 (0%)	2 (2%)	17 (20%)
Electronic	26 (31%)	7±* (8%)	5±* (6%)	20 (24%)

* ± refers to doubtful or equivocal results.

(c) *Quantitative Studies.* The second study consisted of a comparison of the standardized loop (6.0 mm diameter), TTC, the Electronic technique and the quantitative count. In Table VI, one can see that, again, there is slightly better (4 per cent) prediction of the negative urine specimens by the standardized loop and the TTC procedure, as compared to the Electronic method. However, the Electronic procedure predicted a higher percentage (6-8 per cent) of the positive urines than did the other two methods.

Table VI

Summary of Results Obtained Using the Standardized Loop, TTC, Electronic and Quantitative Plate Procedures on 49 specimens

Quant. Category	Corresponding Number and Per Cent of Specimens			
	Negative	1 — 10^4	10^4 — 10^5	More than 10^5
No. in each Quant. Group	21 (43%)	0 (0%)	1 (2%)	27 (56%)
Standardized Loop	20 (40%)	0 (0%)	1 (2%)	26 (54%)
TTC	19 (39%)	0 (0%)	1 (2%)	24 (50%)
Electronic	17 (35%)	0 (0%)	1 (2%)	27 (56%)

The results obtained by using the standardized loop did not completely agree with the quantitative count data as frequently as that obtained by the pipette inoculation technique. This is due in part to the evalution system. The data collected by the loop and pipette procedures was based on the classification seen in Table I. However, the aliquot inoculated by the loop is 1/10 to 1/20 the volume of that transferred by the pipette. Therefore, a classification somewhat similar, but based on a statistical evaluation under "in use conditions," would provide better agreement between the three methods. This study is presently in progress.

Another study which was undertaken consisted of a comparison of 175 urine specimens using the Electronic and the pipette procedures (routine bacteriological procedure on urine service). The data demonstrated the close correlation of the two methods (see Table VII). It will be noted that there is very good agreement, especially in the completely negative (Group A) and the strongly positive urines (see Group G).

Table VII

SUMMARY OF DATA OBTAINED ON 175 URINE SPECIMENS USING PIPETTE AND "ELECTRONIC BROTH" METHODS

	#	Group (%)	Total (%)
A. Total no. of neg. urines* with neg. SBA	35		20.00
1. Neg by SBA plate and − by Coulter.	34	97.14	19.43
2. Neg. by SBA plate and ± by Coulter.	0		
3. Neg. by SBA plate and + by Coulter.	1	2.86	0.67
B. Total no. of neg. urines with "rare"* no. of colonies on SBA plate.	69		39.43
1. "rare" on SBA plate and − Coulter.	62	89.86	35.43
2. "rare" on SBA plate and ± Coulter.	5	7.25	2.86
3. "rare" on SBA plate and + Coulter.	2	2.89	1.14
C. Total no. of urines with "few"* no. of colonies on SBA plate	18		10.29
1. "few" on SBA plate and − Coulter.	15	83.33	8.57
2. "few" on SBA plate and ± Coulter.	1	5.56	0.57
3. "few" on SBA plate and + Coulter.	2	11.11	1.14
D. Total no. of urines with "few-mod"* no. of colonies on SBA plate	6		3.43
1. "few-mod" on SBA plate and − Coulter.	5	83.33	2.86
2. "few-mod" on SBA plate and ± Coulter.	0		
3. "few-mod" on SBA plate and + Coulter.	1	16.67	0.57
E. Total no. of urines with "mod"* no of colonies on SBA plate.	9		5.15
1. "mod" SBA plate and − Coulter.	5	55.55	2.86
2. "mod" SBA plate and ± Coulter.	2	22.22	1.14
3. "mod" SBA plate and + Coulter.	2	22.22	1.14
F. Total no. of urines with "mod-num"* no. of colonies on SBA plate.	4		2.28
1. "mod-num" SBA plate and − Coulter.	4	100.00	2.28
2. "mod-num" SBA plate and ± Coulter.	0		
3. "mod-num" SBA plate and + Coulter.	0		
G. Total no. of urines with "num"* no. of colonies on SBA plate.	34		19.43
1. "num" on SBA plate and − Coulter.	1	2.94	0.57
2. "num" on SBA plate and ± Coulter.	1	2.94	0.57
3. "num" on SBA plate and + Coulter.	32	94.12	18.29

*See classification listed in Table I for approximate quantitation of each category.

(d) *i. Electronic Studies Using Broth Procedure.* A more complete summary of results, which have been obtained on 6,616 urine specimens by the quantitative and the Electronic techniques, is presented in Table VIII. The Electronic procedure employed during the past three years has repeatedly predicted between 90 and 96 per cent of all negative and positive specimens. Note that slightly more difficulty is encountered in detecting positive urine specimens containing 100,000 to 1,000,000 organisms per ml. All specimens showing a ± (doubtful) electronic result are tested by the quantitative plate procedure. Those specimens which are positive are plated on SBA, phenylethyl alcohol and desoxycholate agar for definitive identification.

(d) *ii. Electronic Studies Using the Filterfuge Tubes.* The results obtained with 150 "raw urine specimens," which had been filtered with the aid of stainless steel Filterfuge tubes and Millipore paper clarifying filters have been very encouraging, to say

Table VIII

SUMMARY OF 6,616 URINE SPECIMENS SHOWING THE RESULTS OF THE QUANTITATIVE AND "ELECTRONIC BROTH" PROCEDURES

		% of Group	% of Total
Negative Urines:	2,259		34.14 %
No. neg. by quant. and − by Coulter:	2,080	92.08 %	31.45 %
No. neg. by quant. and ± by Coulter:	132	5.85 %	1.99 %
No. neg. by quant. and + by Coulter:	47	2.18 %	0.70 %
No. of urines + by quant. from 500 — 9,999 cells/ml.:			
1. No. + by quant.:	1,474		22.279%
2. No. + by quant. and + by Coulter:	267	18.11 %	4.035%
3. No. + by quant. and ± by Coulter:	122	8.27 %	1.84 %
4. No. + by quant. and − by Coulter:	1,195	81.07 %	18.21 %
Equivocal Urines:			
No. of urines + by quant. from 10,000 — 99,999 cells/ml.:			
1. No. + by quant.:	671		10.126%
2. No. + by quant. and + by Coulter:	98	14.605%	1.48 %
3. No. + by quant. and ± by Coulter:	84	12.519%	1.269%
4. No. + by quant. and − by Coulter:	489	72.876%	7.36 %
No. of urines + by quant. from 100,000 — 999,999 cells/ml.:			
1. No. + by quant.:	461		6.96 %
2. No. + by quant. and + by Coulter:	199	43.16 %	3.01 %
3. No. + by quant. and ± by Coulter:	104	22.55 %	1.57 %
4. No. + by quant. and − by Coulter:	158	44.29 %	2.38 %
Positive Urines:			
No. of urines + by quant. 1,000,000 and greater cells/ml.:			
1. No. + by quant.:	1,639		24.77 %
2. No. + by quant. and + by Coulter:	1,556	92.11 %	22.78 %
3. No. + by quant. and ± by Coulter:	70	4.03 %	1.01 %
4. No. + by quant. and − by Coulter:	68	3.96 %	0.98 %

the least. Filtration of urine specimens through the Millipore paper prefilters removed much of the debris and permitted the majority of the bacterial cells to pass as part of the filtrate (Table IX). Some specimens showed as much as a ten-fold drop in total count due to filtration. The graphs obtained on the "filtered negative urines" were more readily interpreted than those of the non-filtered urines, especially when the sample was initially cloudy and possibly contained a precipitate.

For purposes of checking the reliability of the technique on positive urine specimens (counts of 100,000 celles or greater), a total of 140 known positives were examined before and after filtration by both bacterial counting and electronic procedures. A cross section of results is shown in Table IX. If the counts were one million or greater, there was no difficulty in detecting a positive urine specimen. However, if the counts were less than one million (100,000 to 1,000,000), the electronic result was frequently ± (doubtful), which meant that the technologist was alerted to prepare a quantitative plate count on these. Some of the counts in the range of 10,000 to 100,000, also, gave a doubtful (±) graph. These were, also, processed by quantitative plating procedures. It was encouraging to note that only three of 140 positive urine specimens (1.5 per cent) were missed by using the direct electronic technique on prefiltered samples.

A much larger series of comparative studies is presently in progress in order to better evaluate reliability of this method for all types of urine specimens. If the subsequent data confirms our initial findings, then this technique will be adopted routinely on all urine specimens submitted to our Bacteriology Laboratory. It is likely that this procedure will, also, be used as a screening device on all specimens submitted daily for urinalysis study.

The technique requires only 1-3 ml of urine, 3-5 minutes of centrifugation and 100 seconds for processing by the automatic Coulter counter and plotter. If disposable Filterfuge tubes were available, the technique would be simplified considerably.

Table IX

CROSS SECTION OF RESULTS OBTAINED WITH URINE SPECIMENS PROCESSED BY THE FILTERFUGE PROCEDURE

Urine Specimen No. for 1964	Appearance of Urine	Quantitative Count*		Electronic		SBA Plate
		Before FF*	After FF	Before	After	
3-25-19	Clear	3000	2000	−	−	rare *S. albus* rare *Corynebacterium sp.*
3-25-25	Precipitate	−	−	−	−	negative
3-28-9	Cloudy with Precipitate	$2x10^6$	10^6	+	+	numerous *E. coli*
3-28-25	Clear	TNTC**	TNTC	+	+	numerous *A. aerogenes*
3-29-1	Clear	$133x10^4$	$33x10^4$			moderate *Proteus* rare *Enterococci*
3-30-18	Clear	TNTC	$86x10^4$	+	±	moderate to numerous *Proteus sp.*

* FF Bacterial count (colonies per ml.) of urine sample in Filterfuge tube before and after filtration by centrifugation at 2700 RPM.

** TNTC refers to too numerous to count, or more than $3x10^6$ colonies.

Discussion

The Electronic procedure was capable of detecting positive urine specimens (greater than 100,000 organisms) which had been inoculated into Brain Heart Infusion or Trypticase-Soy Broth and incubated two to three hours.[3] A search for a supplemented medium which would assist in detecting a higher percentage of positive urine specimens containing significant numbers of *Pseudomonas* was successful. It has been demonstrated that either Difco Supplement C or ferrous ammonium sulfate will accomplish this end. A larger series of experiments will be conducted to determine which procedure is best. There is some indication that *Pseudomonas* isolates grow more readily from urine specimens than from the pure culture state (see Tables II and IV). Attempts to use one per cent carbohydrate solutions (glucose, lactose, and sucrose) as supplements to stimulate the growth of *Pseudomonas* were unsuccessful.

The Greiss and TTC methods showed fewer (2-3 per cent) false positives than did the Electronic method, but the latter showed few false negatives. The Electronic method also identifies more doubtful positives (±) which must be followed-up by qualitative and quantitative studies. But this assists the technologist in detecting a larger percentage of positives. Since the Electronic technique can be done in 2-3 hours, the refrigerated urine specimens showing doubtful positives or definite positives can be plated the same day onto the desired media and the quantitative plates can be initiated. The susceptibility tests may be performed from the "electronic broth culture" or urine specimen, providing the bacteriologist and clinician evaluate and interpret the results on the basis of this methodology.

Although the standardized 6.0 mm nichrome loop had many operational advantages, the relatively small volume plated (0.02 ml.) precludes its use in estimating viable counts in urine samples, especially those of low bacterial density. A statistical study using four spots or more will be attempted on a larger number of specimens.

Electronic Studies Using the Filterfuge Tubes. The prefilter of urine avoids the excessive plugging which may occur with cloudy and/or precipitated specimens. The interpretation of the plotted data was easier to interpret at times because there was less debris (particles) being counted and plotted. The peaks of the curves occur more frequently in the first few windows (threshold settings) rather than in the latter half, as occurs with the 2-3 hour incubated samples. This indicates that the bacterial cells in prefiltered urine frequently have a smaller cell volume than do the cells actively multiplying in the broth.

It is hoped that this method will permit us to use the 18 or 25 u orifice tube in the hopes of increasing the sensitivity (prediction of positives) of the method without increasing the problems related to the plugging of the aperature tube.

Proposal to Bacteriologists and Clinicians. The methods mentioned herein for the detection of significant bacteriuria all have their advantages and disadvantages. It is well known that, if the facilities for bacteriological examination (isolation, quantitation, identification, and susceptibility testing are available, a more comprehensive examination is possible. If these are not available and/or the funds are a limiting factor, then

one of the abbreviated procedures (use of a single SBA plate, TTC, Greiss, or Electronic procedure) may serve a particular need—namely, to determine if a significant bacteriuria is present. However, one should stress the fact that these techniques may have a 5 to 30 per cent false negative and false positive "report rate." Therefore, it is extremely necessary to emphasize that these screening procedures are not definitive.

For the technologists working in hospitals with "In" and "Out" patients, the following procedures are suggested on the basis of experience gained in the Henry Ford Hospital Bacteriology Laboratory:

I. Electronic Procedure (Coulter Counter).

Method A: Broth Procedure

A. Materials:

1. 10 ml of filtered (0.3 u or 0.45 u Millipore Filter) sterile Trypticase Soy Broth dispensed into sterile 50 ml jars with screw caps.

B. Procedure:

1. 1 ml of urine is inoculated into the Trypticase Soy Broth.
2. This specimen is then placed in a shaker in the 37° C. incubator and shaken for three hours.
   a) The specimen is checked for growth macroscopically at the end of two hours. In any turbidity is noticed, it is removed from the incubator, diluted with 0.9 per cent sterile Abbott saline and processed by means of the Coulter.
3. At the end of three hours, the other specimens are removed from the incubator and diluted to approximately 50 ml with 0.9 per cent sterile Abbott saline and processed with the Coulter.

Method B: Filterfuge Tube Procedure

See details described in this paper.

II. Urine Culture Procedure.

A. Media:

1. Sheep's Blood Aga Plate: 5-7 per cent Sheep's Blood in Trypticase Soy Agar.
2. Desoxycholate Agar Plate.
3. Phenyl - ethyl alcohol Sheep's Blood Agar Plate: Can be used, especially for specimens positive or ± by Electronic method.

B. Procedure:

1. 0.1 ml of well-mixed urine is inoculated onto each of the above media. Isolation-streakings are made with the 1 ml pipette.
2. The inoculated plates are incubated at 37° C. and readings made on the following day.

III. Urine Quantitative Count.

A. Media:

1. Trypticase Soy Agar deeps—9 ml per tube kept melted in a 45°—48° C water bath.

B. Procedure:

1. A 1:1000 dilution of the urine is made as follows:
   a) 1 ml of urine is serially diluted through three tubes of sterile 0.9 per cent saline, each tube containing 9 ml. Use of separate pipettes, adequate shaking and aseptic handling should be stressed.
2. 1 ml of the 1:1000 dilution is placed in each of two sterile petri dishes.
3. 15 ml of the melted Trypticase Soy Agar is then added to the petri dish and the contents are well mixed by swirling.
4. The plates are allowed to harden and are incubated in the 37° C. incubator overnight.
5. The colonies on the plate are then counted as follows:
   a) All of the colonies on both plates are counted and the average number of colonies on the two plates is recorded.
   b) The total number of colonies per ml is calculated as follows:

$$\frac{\text{1) No. of colonies on 2 plates}}{2} \times 1{,}000 = \text{colonies/ml of urine.}$$

Summary and Conclusions

Strains of *Pseudomonas* were detected more frequently when cultured directly from urine specimens than from pure cultures. The addition of either ferrous ammonium sulfate or Supplement C to Brain Heart Infusion Broth increased the number of *Pseudomonas* which could be detected, using either pure culture inoculum or raw urine containing this organism. The results of the Greiss and TTC techniques show slightly better prediction of negative urines than the Electronic procedure, but the latter predicted more positive specimens. Thus, the Electronic method is less apt to miss the detection of bacteriuria than the TTC or Greiss procedure.

A comparison of the data obtained by "Electronic-Broth" procedures with the quantitative method shows agreement of 90 to 96 per cent on both negative and positive urines. The Electronic procedure for raw urines using the new "Filterfuge Technique" may well be a better method. It is recommended that a semi-quantitative (Electronic Procedure) test be used for the detection of significant bacteriuria and that this can be adequately accomplished by one of the two Electronic methods described herein. The qualitative and quantitative methods used in our laboratory are described and summarized in this paper.

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