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Wadley et al.

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[54] **APPLICATOR FOR UNIFORMLY APPLYING A SAMPLE TO BACTERIAL GROWTH MEDIA**

[75] Inventors: **Clark Searle Wadley**, Lake Forest; **Donald Sykes Kenney**, Northbrook, both of Ill.

[73] Assignee: **Abbott Laboratories**, North Chicago, Ill.

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Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 795,805, Feb. 3, 1969, abandoned.

[52] **U.S. Cl.** ..... **195/127**, 128/2 W, 195/103.5 R

[51] **Int. Cl.** **C12k 1/10**

[58] **Field of Search** ..... 195/103.5 R, 127, 139; 128/2 W

[56] **References Cited**

UNITED STATES PATENTS

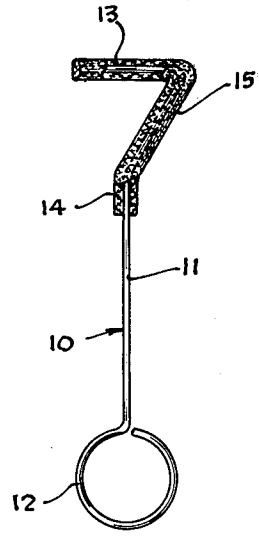
3,149,054 9/1964 Ryan ..... 195/103.5 R

Primary Examiner—Lionel M. Shapiro
Assistant Examiner—Robert J. Warden
Attorney, Agent, or Firm—Robert L. Niblack; Gildo E. Fato

[57] **ABSTRACT**

An applicator for uniformly applying a sample to bacterial growth media comprising a frame having absorbent material positioned over the tip of the frame and a sleeve of permeable material covering the absorbent material. The applicator is used in test procedures for determining the presence of microorganisms in a sample, the applicator distributing a uniform film of inoculum with even distribution of any organism which may be present in the sample. The applicator thereby permits quantitation of any organisms which may be present in a suspected sample since it applies a specific amount of the sample.

3 Claims, 5 Drawing Figures



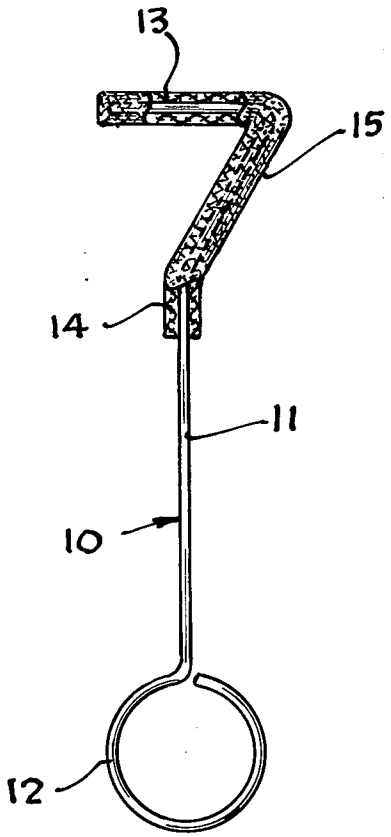


FIG. 1

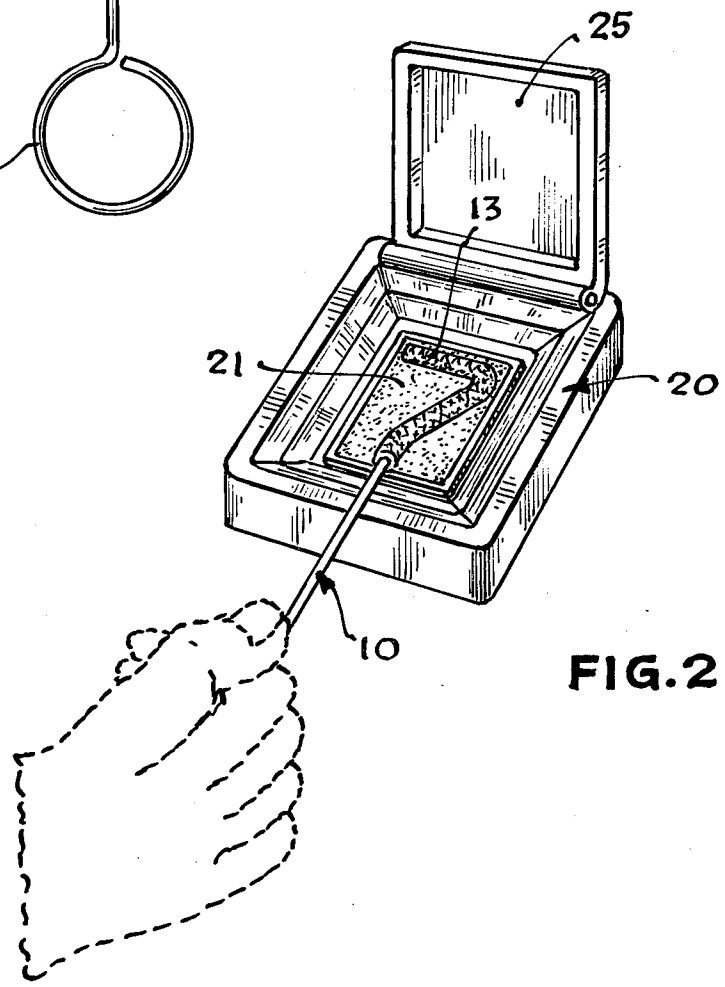


FIG. 2

FIG.3 EFFECT OF VARIATIONS IN THE DIPPING TIME

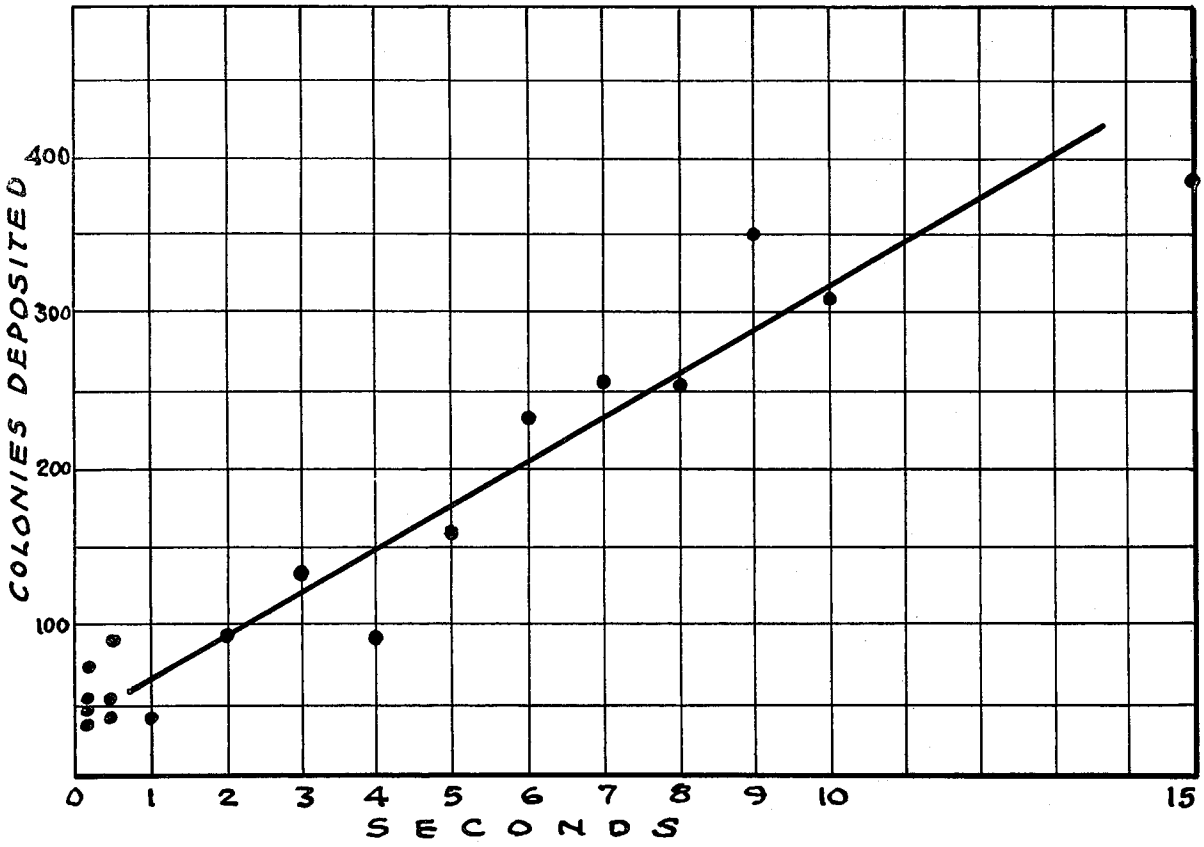


FIG.4 EFFECT IN VARIATIONS IN THE EQUILIBRATION TIME

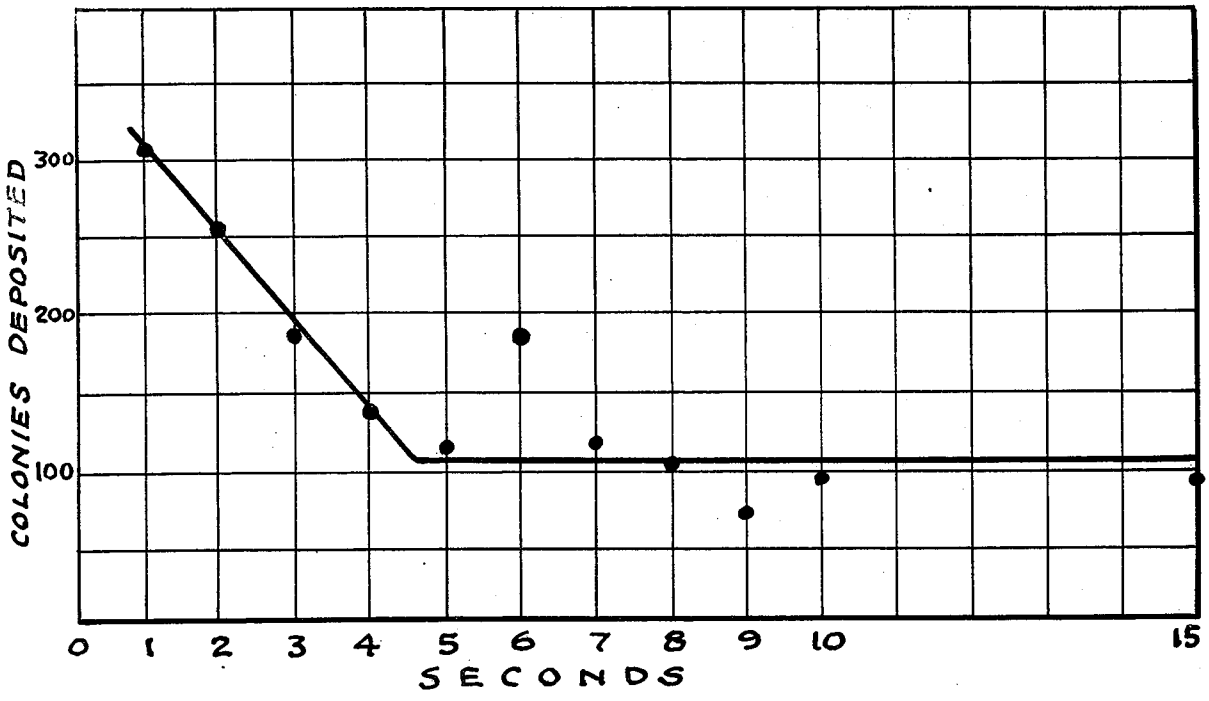
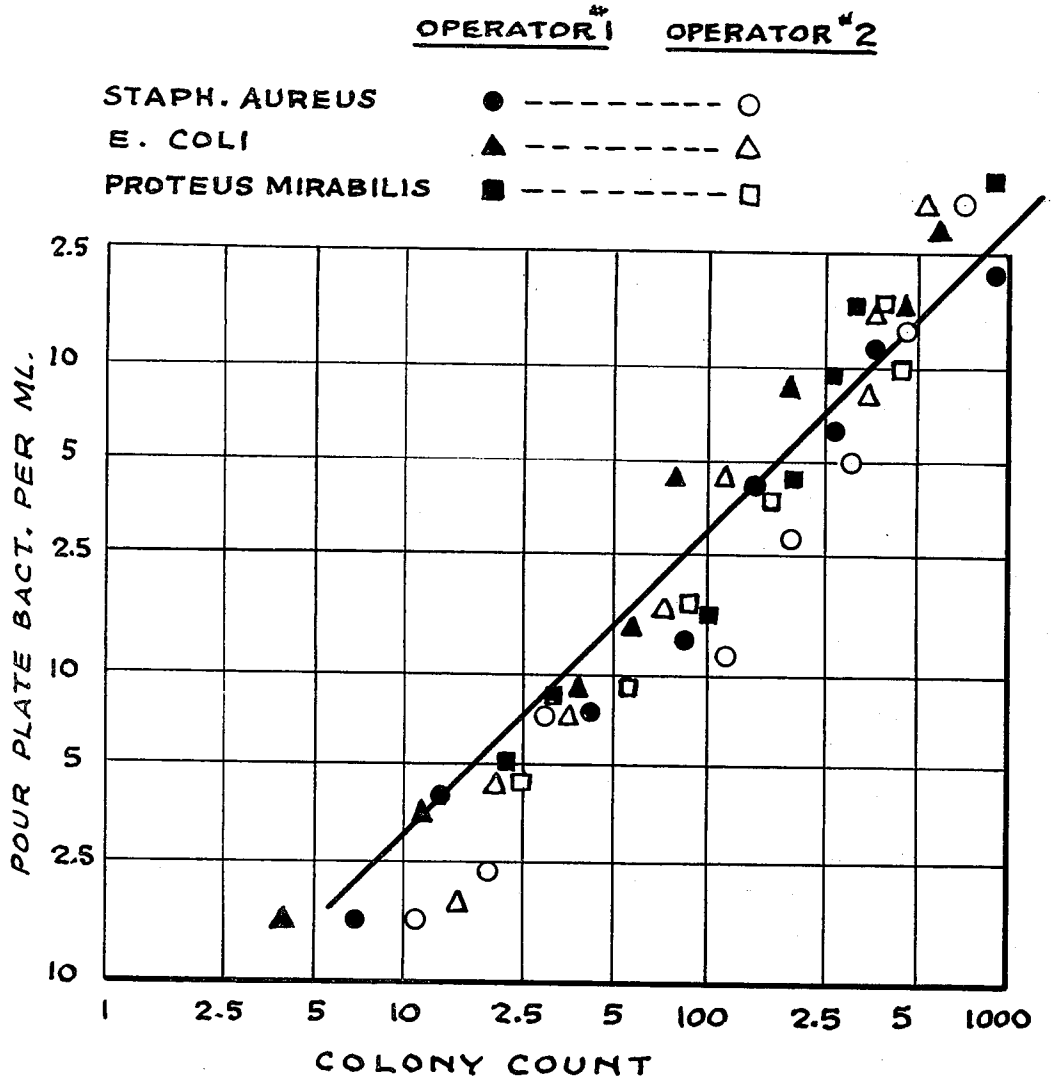


FIG. 5

RELATIONSHIP BETWEEN POUR PLATE AND COLONY COUNTS



APPLICATOR FOR UNIFORMLY APPLYING A SAMPLE TO BACTERIAL GROWTH MEDIA

This application is a Continuation-in-Part application of U.S. application Ser. No. 795,805, filed Feb. 3, 1969, now abandoned.

BACKGROUND OF THE INVENTION

To determine the presence of disease causing bacteria in a sample of urine for example, a sample is spread on bacterial growth medium and the resultant growth of bacteria is observed. Bacteria in urine is diagnostic of urinary tract infection when the level of bacteria per milliliter of urine is in the range of 100,000 or greater. While the conventional pour plate dilution method can be used to determine the number of bacteria per milliliter of sample, more rapid methods are desirable so that treatment of the infection can be rapidly and effectively undertaken. One method to determine the presence of a disease causing baceteria in a sample involves the inoculation onto a blood agar plate or similar media of the suspected urine sample for example, with a calibrated loop. A calibrated loop, however, requires considerable skill and may result in uneven or overlapping growth of colonies if not utilized properly.

SUMMARY OF THE INVENTION

The present invention relates to an applicator for uniformly applying a suspected sample to bacterial growth media and comprises a frame having an absorbent material on the tip thereof and preferably a sleeve of permeable material positioned over the absorbent material. When dipped into a sample, the external sleeve permits easy access of the sample to the inner absorbent material. The effect is that a small amount of the sample is quickly taken up by the applicator. After an appropriate dipping time, the time applicator is held in the sample, and the equilibration time, the time after removal of the applicator from the sample and before it is applied to the media, is determined, the applicator can be used to quickly and conveniently apply sample to media. Further, the applicator permits the application of a specific amount of sample to the media so that quantitation of any organisms in the sample can be achieved. Additionally, the applicator can be used to uniformly and successively innoculate a number of media.

DRAWINGS

The invention will be better understood upon consideration of the following description with reference to the drawings, in which:

FIG. **1** is a side elevational view, partly in cross-section of an applicator for applying suspected sample to media.

FIG. **2** is a perspective view of a container with media therein and illustrating inoculation by means of the applicator.

FIG. **3** is a graph illustrating the effect of variations in the time the applicator is held in the sample.

FIG. **4** is a graph illustrating the effect in variations of the equilibration time after the applicator is removed from the sample.

FIG. **5** is a graph illustrating the relationship between the pour plate dilution method and use of the applicator.

DETAILED DESCRIPTION

Referring to the drawings, FIG. **1** illustrates a suitable applicator **10** comprising an elongated wire frame **11** having a loop **12** at one end for convenience in handling, and a transversely disposed tip **13** at the other end. Fitted over the tip **13** and a portion of the frame **11** is an absorbent or wick-like material **14** such as for example, cotton cording. Preferably, a sleeve **15**, of a permeable material which may be a woven synthetic fabric such as rayon, is positioned over the absorbent material **14**. When dipped into the sample, the absorbent material **14** absorbs a small quantity. The absorbent material **14** permits rapid absorbtion of the sample to thereby prevent dripping which would result in improper application of the sample to the media surface with consequent unsatisfactory results. With the applicator **10**, using a light downward stroke, a technician can deposit a highly uniform film of inoculum, one that distributes any organisms evenly over the entire top surface of the bacterial growth medium. This greatly facilitates the quantitation of the bacteria since it minimizes the uneven or overlapping growth of colonies that may result from a lack of experience when using a calibrated loop of the prior methods. The width of the tip **13** should approximate the width of the media. For example, with a medium width of one inch, the tip **13** should likewise be one inch. While the use of a sleeve **15** covering the absorbent material **14** is preferred, a single absorbent material is satisfactory. Likewise, while cotton cording is preferred for the absorbent material **14**, other materials can be used. With each material or combination of materials, the appropriate conversion factor must be determined. The diameter of the material **14** placed on the tip **13** of the applicator can be selected, together with the width thereof, to provide a suitable dipping and equilibration time.

FIGS. **3**, **4** and **5** illustrate the determination of a factor for converting colony count on the medium using the applicator **10** to bacterial count per milliliter of sample. FIG. **3** illustrates the effect of the dipping time, the time the applicator **10** is held in the urine sample, for example. As illustrated, a straight line function results between the length of time of dip of the applicator **10** in the sample and the number of bacteria deposited upon the surface of the medium. From these results, a one second dipping time was selected. FIG. **3** illustrates the results obtained when the equilibration time was varied. If insufficient time is allowed for the urine to be absorbed by the applicator **10**, a high count will be obtained. Once the urine sample is absorbed, the amount deposited on the medium is consistent. This absorption occurs within 5 seconds. A 5 second equilibration time was therefore selected since this results in a faster test procedure by reducing the time the applicator **10** must be held between dipping and streaking of the sample on the medium.

Using a 1 second dipping time and 5 second equilibration time, the following method was employed to obtain the necessary factor to convert the number of colonies on the medium to the number of bacteria per milliliters of urine sample. Suspensions of 18 hour broth cultures were prepared in 1% peptone water at 40°C. Two fold dilutions were made to cover the range from 10^3 to 10^6 bacteria per milliliter. The organisms used were *Staphylococcus aureus* 209P, *Escherichia coli* Upham Maine, and *Proteus mirabilis* Finland No. 9.

Two technicians, working independently, used the same suspensions as urine samples inoculating each level on bacterial growth media using the applicator **10.** Each technician made pour plate counts of each sample in the conventional manner using duplicate plates of brain heart infusion agar. The results are presented in Table 1.

TABLE 1

RESULTS OF SAMPLE COUNTS

Organism	Sample	Pour Plate Count (Organisms/Ml)	Colony Count (colonies/cup) 1	2	3	Avg
Technician No. 1						
Staph aureus	A	1.2×10^6	*	*	*	*
	B	6.2×10^5	*	*	*	*
	C	2.0×10^5	1100	725	1000	950
	D	1.2×10^5	575	300	230	368
	E	6.1×10^4	238	224	291	276
	F	4.1×10^4	179	118	138	145
	G	1.3×10^4	90	97	64	84
	H	7.6×10^3	40	37	46	41
	I	3.8×10^3	13	21	6	13
	J	1.6×10^3	8	10	4	7
E. coli	A	1.5×10^6	*	*	*	*
	B	6.4×10^5	*	*	*	*
	C	2.8×10^5	445	490	825	587
	D	1.6×10^5*	380	505	440	442
	E	8.5×10^4*	159	210	225	198
	F	4.4×10^4*	79	108	56	81
	G	1.4×10^4	47	53	74	58
	H	9.0×10^3	12	49	50	37
	I	3.6×10^3	8	11	17	12
	J	1.6×10^3	5	4	2	4
Proteus mirabilis	A	3.8×10^6	*	*	*	*
	B	1.7×10^6	*	*	*	*
	C	7.7×10^5	625	925	1000	850
	D	4.2×10^5	775	1100	800	892
	E	1.6×10^5	320	390	280	330
	F	9.6×10^4	275	282	235	263
	G	4.4×10^4	245	125	200	190
	H	1.6×10^4	108	117	74	100
	I	8.6×10^3	33	30	33	32
	J	5.2×10^3	18	25	23	22
Technician No. 2						
Staph aureus	A	1.3×10^6	*	*	*	*
	B	7.2×10^5	*	*	*	*
	C	3.4×10^5	750	800	675	725
	D	1.4×10^5	600	300	530	477
	E	5.1×10^4	320	300	330	317
	F	2.8×10^4	210	167	215	197
	G	1.2×10^4	85	188	76	116
	H	7.5×10^3	36	30	26	31
	I	2.3×10^3	6	29	21	19
	J	1.6×10^3	7	13	14	11
E. coli	A	1.3×10^6	*	*	*	*
	B	7.5×10^5	*	*	*	*
	C	3.4×10^5	450	725	375	517
	D	1.6×10^5	186	700	256	381
	E	8.5×10^4	227	600	238	355
	F	4.4×10^4	153	98	118	123
	G	1.6×10^4	52	93	83	76
	H	7.6×10^3	41	44	23	36
	I	4.3×10^3	6	19	39	21
	J	1.8×10^3	11	10	25	15
Proteus mirabilis	A	5.0×10^6	1225	1300	1050	1192
	B	1.8×10^6	1275	725	850	950
	C	1.0×10^6	500	1100	775	792
	D	4.9×10^5	325	425	305	352
	E	1.6×10^5	390	380	385	385
	F	1.0×10^5	400	475	425	433
	G	3.8×10^4	135	165	157	152
	H	1.7×10^4	57	77	131	88
	I	9.0×10^3	36	81	41	53
	J	4.4×10^3	21	19	29	23

* Too numerous to count

FIG. **5** illustrates the average of the results obtained by the two technicians. Over the range of interest, generally 10^3 to 10^6 bacteria per milliliter of sample, the points describe a straight line function. The slope of the straight line function may be expressed as a simple factor using the general formula,

$$M = \frac{Y_2 - Y_1}{X_2 - X_1},$$

where X_1 is the pour plate count arbitrarily chosen and Y_1 is the corresponding colony count obtained on a growth media using the applicator **10.** X_2 and Y_2 are similar points arbitrarily chosen at a higher location on the graph.

Reading from Table 1, two examples of the calculations are presented in the following table for each or-

ganism. Each point represents the average of the values obtained by each of the technicians.

TABLE 2

Organism	SAMPLE	Y_1	Y_2	X_1	X_2	M
Staph aureus	H & D	7,600	140,000	36	422	342
	I & H	3,100	7,600	16	36	225
E. coli	I & E	4,000	85,000	16	276	312
	I & D	4,000	160,000	16	412	394
Proteus mirabilis	J & F	4,800	98,000	22	348	286
	I & G	8,800	41,000	42	171	250

Average M = 302

The average of the slope between the chosen points on the graph of FIG. **5** was found to be about 300 and represents the conversion factor.

The applicator **10** and suitable medium can be used to determine the presence of bacteria in a urine sample, for example, as follows. A suspected urine sample is first shaken to obtain a uniformily mixed sample. The cover **25** of the tray **20** is lifted to expose the medium **21** as illustrated in FIG. **2.** The applicator **10** is then dipped into the specimen, just covering the transverse tip **13** and held in place for 1 second before removal. After an equilibration pause of 5 seconds, the medium **21** is streaked as illustrated in FIG. **2,** completely covering the length of the medium **21** surface. The external sleeve **15** offers to the urine sample quick absorption and easy excess to the inner absorbent material **14.** The effect is that a small amount of the urine sample is quickly taken up by the sleeve **15** and absorbent or wick-like material **14** so that the applicator **10** deposits a uniform inoculation on the media with even distribution of any organisms which may be present. After inoculation, the tray **20** containing the inoculated media **21** is incubated. After incubation, quick quantitation of the bacteria is achieved by counting the colonies of the medium **21** and multiplying by the factor of 300 obtained as previously described to result in the number of bacteria per milliliter of urine sample. After quantifying the bacteria, if the bacteria are present at a level of about 100,000 per milliliter of urine or greater, indicating a urinary tract infection, the particular organism responsible can be identified by conventional methods.

As described, the applicator **10** can be employed in a simple three step method: (1) dipping the applicator **10** in the suspected sample for a preselected time; (2) removing the applicator **10** from the sample and allowing a preselected equilibration time before applying the sample to the medium; and (3) inoculating the medium by streaking the sample on the medium with the applicator **10.** In the event a plurality of media are required, such as duplicate samples or special growth media for identification purposes, the applicator **10** can be used to successively streak each of the media after only a single dipping of the applicator **10** into the sample. Uniform inoculation of each of the media will result. In this manner, at least four media can be successively inoculated.

What is claimed is:

1. An applicator for applying a sample to be examined to bacterial growth media, said applicator comprising:

an elongated frame having a tip disposed at one end transverse to said frame;

absorbent material fitted over the tip of said applicator to absorb the sample and including a permeable sleeve positioned over the absorbent material, said sleeve providing to the sample easy access to the absorbent material, whereby said applicator, when placed in the sample, will absorb a precise quantity of sample and distribute the sample uniformly on the surface of the medium.

2. The applicator of claim **1** wherein the absorbent material comprises cotton cording and the permeable sleeve comprises a woven synthetic fabric.

3. A method of applying a sample to be examined for the presence of bacteria to bacterial growth media, said method comprising:

providing an applicator comprising an elongated frame having a tip disposed at one end transverse to the frame and including absorbent material fitted over the tip of the applicator to absorb the sample;

dipping the tip of the applicator in the suspected sample for a preselected time;

removing the applicator from the sample;

allowing a preselected equilibration time to permit the absorbent material to absorb the sample; and

inoculating the medium by streaking the sample on the medium with the applicator.

* * * * *