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Evaluation of Griess test and Calibrated Loop–Direct Streak Method Determination of Infected Urine

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The experiment was performed to test the efficiency of Griess test compared to the colony counting method, which when positive giving more than 10^5 cell/ml. of urine streaked from a calibrated loop. The first morning mid-stream voided urine obtained from 258 clinical patients and 223 pregnant woman in Nakorn-Chiang Mai Hospital.

The total urine specimens of 481 were culture in blood agar and Mc Conkey agar plates by the means of calibrated-loop direct streak. The number of bacteria were counted and other differential media were used to differentiate the organism. Griess test was also performed parallel to the test described above.

Out of 258 specimens form the clinical patients 61 specimens were positive for colony count having more than 10^5 cell/ml. Showed 90.2% positive for Griess test. The organism found mostly was E. coli 34%.

The colony counting in pregnancy urines showed 12 out of 223 specimens to be positive. These were only 9 specimens out of 12 specimens positive in Griess test (75%). The organism mostly found was also E. coli (50%).

In 1870, Griess, a German Chemist. developeh a reagent for detection of nitrites in solution. The reagent, an acid solution of sulfanilic acid and alphanaphthylamine, undergoes a diazotization reaction with nitrites to form a red azo dye. Cruichshank and Moyes using this reagent demonstrated a direct correlation between the presence of nitrite in urine and the presence of colifrom urinary tract infec-

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tion. During this time, it has become apparent that 3 factor are of prime importance in determining whether are positive Griess reaction will be found :-

1. The presence of a adequate numbers of nitrate reducing bacteria.
2. The presence of nitrate in urine.
3. That the bacteria be incontact with the urine for sufficient time to allow the reduction of nitrate to nitrite.

The investication of this paper is to evaluate the Griess test and calibrated loop - direct streak method determination of infected urine.

Materials and Methods

Urine specimens obtained from Nakorn Chiang Mai Hospital by means of mid - stream voided urine. Blood agar and Mc Conkey agar or Eosin Methylene Blue agar (EMB) plates were used for culture. The 4 mm. diameter calibrated loop contained 0.01 ml. of urine.

Preparation of the Griess reagent : One and One-half of sulfanilic acid (chemically pure) were dissolved in 450 ml. of 10% acetic acid. This solution was added to a solution of 0.6 gm. alphanaphthylamine (Chemically pure) in 60 ml. of boiling distilled water and filtered through Whatman no 1 filter paper. This combined reagent, now colorless, was stored in a tightly stoppered dark bottle to prevent oxidation. The reagent in this form remained stable for two to four weeks and decomposition could be noticed by the appearance of a pinkish color in the solution. The activity of the reagent could be tested by adding a few drops of few milliliter of 10% sodium nitrite solution, the development of a red color meaned the reagent was in a good condition.

One calibrated loop of infected or pregnancy Urine was streaked on blood agar plate and on McConkey or EMB.

One mililiter of the same urine was added to 1 ml. of Griess reagent, the development of a pink or red color in a solution of seconds was considered to be a positive test :

After overnight incubation the plates were examined by counting and identification of organisms.

Result

Of those 258 urine were collected from December 1971 to February 1972. The relationship of colony counts in urine specimens to positive griess reaction (Table 1) showed 79 no growth but 1 Griess test positive (1.3%). 47 urine were colony count less than 10^4/1 ml. positived 3 Griess test (6.4%). 71 urine were counted between 10^4-10^5/1 ml. positived 31 Griess test (43.6%). 61 urine were counted more than 10^5/1 ml. positived 55 Griess test (90.2%).

223 pregnancy urines showed 63 no growth, and Griess test negatived: 88

urines were counted less than 10^4/1 ml. positived 4 Griess test (4.5%). 60 urines were counted between 10^4-10^5/1 ml. negatived Griess test. 12 urines counted more than 10^5/ml. positivod 9 Griess test (75%).

The bacteria isolated from 61 infected clinical urine specimens show on table II, the mostly found organisms was E. coli.

The bacteria isolated from 12 infected pregnancy urine specimens mostly found E. coli, show on table III.

The correlation of specimen of bacteria to Griess reaction and specimen yielding greater than 10^5 cell/ml. urines, show on table IV.

Discussion

This experiment indicated that Griess test gave a good result and should be used in the laboratory becouse it is practical, rapid, less time consuming and less cost than the calibrated-loop direct stteak. The disadventages of the Griess test are the false negative and that the organisms cannot be isolated for further differentiation and sensitivity test as in the calibrated-loop direct streak method.

Table I. The relationship of colony counts in urine specimens to positive Griess reactions.

colony count/ml. urine	no growth	$< 10^4$	10^4-10^5	$> 10^5$
a) Clinical specimen				
Number of Specimen	79	47	71	61
Number of positive Griess test	1	3	31	55
percent of specimen with positive Griess test	1.3	6.4	43.6	90.2
b) Pregnancy Specimen				
Number of Specimen	63	88	60	12
Number of positive Griess test	–	4	–	9
percent of specimen with positive Griess test	–	4.5	–	75.0
c) Clinical specimen, Pregnancy specimen				
Number of specimen	142	135	131	73
Number of positive Griess test	1	7	31	64
percent of specimen with positive Griess test	0.70	5.2	24.5	87.7

Table II. Bacteria isolated from 61 infected clinical urine specimens.

Organism found	No. of organism found	% of organism found
E. coli	27	34.0
Proteus mirabilis	15	18.9
Pseudomonas aeruginosa	7	8.9
Klebsiella species	6	7.6
Paracolon bacilli	5	6.3
Staphylococci	4	5.1
Achromobacter	4	5.1
Aerobacter	3	3.8
Alkalageneous	2	2.6
Beta – streptococci group A	2	2.6
Salmonella typhi	2	2.6
Citrobacter	1	1.8
Enterococci	1	1.3

Table III. Bacteria isolated from 12 infected pregnancy urine specimens.

organism found	No. of organism found	% of organism found
E. coli	6	50
Paracolon bacilli	2	16.7
Klebseilla species	1	8.3
Staphylococci	1	8.3
Beta – Streptococci group. A	1	8.3
Enterococci	1	8.3

Table IV. Correlation of kind of bacteria to Griess Reaction.
Specimens yielding greater than 10^5 cell/ml. urine.

organism	No. of positive culture	Griess Test positive	% Griess Test positive
E. coli	33	32	96.96
Proteus mirabilis	15	14	93.33
Pseudomonas aeruginosa	7	7	100
Klebsiella species	7	7	100
Paracolon bacilli	7	7	100
Staphylococci	5	3	60
Achromobacter	4	4	100
Beta Strep. Group. A	3	0	0
Alcaligenes species	2	2	100
Salmonella typhi	2	2	100
Enterococci	2	0	0
Citrobacter	1	1	100

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