A Field Trial of the Clinicult System for Detection of Asymptomatic Gonorrhea in Women

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IDENTIFYING AND TREATING the asymptomatic woman who is infected with gonorrhea has become a high priority in gonorrhea control in the United States during the past 5 years. The advent in 1964 of a selective medium—Thayer-Martin *(1)*—made possible a practical, though sophisticated, system of routine screening for gonococcal disease. Nationwide experience, which now reflects the results of more than 1/2 million tests, shows a positivity rate of approximately 5 percent *(2)*.

An ideal field culture medium would be specific, sensitive, reproducible, and easy for relatively untrained personnel to use. The medium should be stable and have a prolonged shelf life. Positive results should lend themselves to easy confirmation.

Since Thayer and Martin developed their selective medium, several modifications have appeared in response to specific difficulties encountered in its use in communitywide screenings. The time and distance that separates the screener from the processing laboratory are critical in obtaining accurate results. If more than 8 to 12 hours elapse between the time the specimen is obtained and the time the seeded culture plate is placed in an incubator with proper CO_2 environment, the "die-off" of the fragile gonococcus organism is significant.

Transgrow *(3)* and Clinicult *(4)* are two modifications of the original Thayer-Martin medium that have recently been developed to bridge the time-distance gap. Transgrow, which provides a CO_2 environment within the bottle, permits growth during transmittal to the laboratory. This growth is enhanced if the culture is pre-incubated for 15 to 24 hours before shipment. Many screeners, however, do not have access to an incubator for this purpose.

The Clinicult system (developed by Smith Kline Diagnostics of Philadelphia) goes a step further by providing a CO_2 environment and immediate incubation within the screener's own office. It also provides for performance of a presumptive oxidase test in the physician's office after the culture has incubated for a minimum of 48 hours. The system is designed so that a positive oxidase test can be delivered or mailed to a competent laboratory for further confirmation.

The obvious advantages of the Clinicult method of screening are as follows:

1. The presumptive test results can be obtained by the screener without transportation affecting the results.
2. The screener can have a presumptive report within 48 hours after the culture has been taken.
3. An obvious advantage to the public health laboratory is that a large number of negative cultures need not be processed in the central laboratory. Thus, the volume of tests to be performed in the public health laboratory is reduced by at least 90 percent and, in turn, the unit cost of each case found is reduced.

A basic question, however, remains: Can the screener using the Clinicult system be motivated and taught to carry out successfully the following procedures?

1. Properly obtain the specimen and inoculate the medium.
2. Prepare the tube for incubation by initiating the CO_2 environment.
3. Properly control the 48-hour period of incubation.

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4. Accurately interpret the growth of colonies and the oxidase test.

5. Promptly dispatch suspicious cultures to the laboratory for confirmation before the gonococcal organisms die (within 72 hours).

Methods and Procedures

Selection of screening and participants. Since many types of clinical facilities may conduct gonorrhea screening, our study project was designed to include as wide a variety of these facilities as possible. All screeners were grouped into three general categories:

Group 1. The central venereal disease clinic of the Seattle-King County Health Department was to represent the control since the experience, training, and motivation of the clinic's staff should provide the most ideal situation for producing accurate screening results. The results it obtained could probably also be applied to smaller health departments with minimal laboratory services.

Group 2. Family planning clinics and hospital outpatient (obstetrics-gynecology) clinics were considered to have stable staffs and to focus on the special needs of women. Their staffs' experience with gynecologic conditions should be a strong plus factor in enabling them to closely follow the study protocol.

Group 3. The private general practitioners and obstetrician-gynecologists who were selected had all expressed a willingness to participate in the study. Such offices, however, are often busy with such a wide range of medical conditions that careful attention to detail may be overlooked. Because of the physician's busy schedule, the office nurse or receptionist was primarily responsible for conducting the tests after the physician had taken the specimen. This third group represented the testing locale where most errors are likely to occur.

Protocol design. A time limit of 4 to 5 months was scheduled for completion of the study of approximately 5,500 women. Since group 3 (patients of private physicians) was expected to have the lowest positivity rate, the major portion of the screened patients had to be obtained from that group. Therefore, the goal for group 3 was to provide 50 percent of all patients screened. The family planning clinics and the venereal disease clinic were to supply the remainder.

Since the Thayer-Martin medium is presently considered the most reliable method of isolating gonococci, it was chosen as the reference medium for the control clinic. The venereal disease clinic had previously worked, on a trial basis, with the Clinicult system.

The Transgrow medium had been used without preincubation by private physicians in the area for about 18 months. During this time, the physicians were obtaining approximately 2.5 percent positive cultures on specimens from their patients. Since the physicians in the area were familiar with Transgrow, we decided to use this system as the reference medium for groups 2 and 3.

A daily courier service was established to deliver all media and supplies. The courier returned seeded Transgrow cultures for processing in the central laboratory at the same time. The seeded Clinicult tubes were incubated for 48 hours in incubators that had been especially provided for use in the participating physicians' own offices. After the incubation, the physician performed the oxidase test on one side of the medium stick and recorded the results. The Clinicult tube was saved for the courier to pick up the next time he visited the office.

The Transgrow cultures were delivered to the Seattle-King County Health Department Laboratory for processing and reading. The Clinicult tubes were delivered to the Washington State Department of Social and Health Services Laboratory (2 blocks away) for independent processing and confirmation of results. To minimize the effects of variation in the shelf-life of various media, quality control measures were instituted to assure that only fresh media were used in all three systems. Collation of the results was carried out independently of either the laboratory staff or the screening physicians.

The selection of patients for screening was guided by criteria already in effect in most of the study facilities. If a woman needed a pelvic examination, a cervical culture was to be taken on her initial visit. If the patient was currently receiving antibiotic treatment or having a scheduled recheck, she was not to be entered in the study.

A sterile cotton applicator swab was used to obtain specimens for seeding each of the two culture systems. The screener was to alternate patients so as to seed one culture system first one time and the other system first the next time. This same procedure was followed in the venereal disease clinic, where Clinicult and Thayer-Martin plates were the two media used. The Thayer-Martin plates were immediately delivered to the laboratory for incubation (within 30 minutes), since the clinic and laboratory are adjacent to each other.

The streaked Transgrow culture used in the physicians' offices was held at room temperature until it was picked up by the courier. The seeded Clinicult tube, with the activated CO_2 tablet, was placed in the special incubator designed for that system. A minimum of 48 hours was allowed for incubation before the initial oxidase test was performed in the physician's office. All Clinicult tubes, regardless of the result of the oxidase test, were picked up by the courier after a presumptive reading by the clinician and delivered to the State laboratory for further testing. The screener was advised to wait for results of the Transgrow culture or Thayer-Martin plate before making a final diagnosis of gonorrhea and initiating treatment.

When the Clinicult tubes were returned to the State laboratory, they were examined by a microbiologist for evidence of colony growth. The undisturbed side of the medium stick was subjected to the oxidase test, and suspicious colonies were immediately removed for gram

staining and confirmation by fluorescent antibody staining. The results were recorded as positive, negative, or unsatisfactory.

All data collected from screeners and the two laboratories were transferred to a computer program maintained by the Washington State Department of Social and Health Services laboratory. The following items of information were collated: identification of screenee, type of medium used, study group of screener (venereal disease clinic patient, family planning clinic patient, or patient of private physician), result of oxidase test on Clinicult medium in the screener's office, and results of laboratory verification tests from both laboratories.

Laboratory procedure. After 48 hours of incubation in a CO_2 environment, the Thayer-Martin media plates used for group 1 were examined for colony growth. If typical colonies were present, the oxidase test was performed. The oxidase-positive growth was then quickly transferred to a slide for gram staining to demonstrate the presence of gram-negative diplococci. The fluorescent antibody technique was then used to confirm the gram-stained slide result. If the oxidase test was negative after 48 hours of incubation, the culture was determined to be negative.

The Transgrow cultures used for groups 2 and 3 were returned daily to our laboratory without preincubation. All bottles were checked for the presence of CO_2 before being placed in a regular incubator for 48 hours. A small strip of filter paper, wetted with phenolthalein solution, was suspended in the bottle above the medium surface. Decolorization of the indicator signified CO_2 was present. The identification of *Neisseria gonorrhoeae* colonies was carried out in the same manner as for those on the Thayer-Martin plates.

Results

During the 4½-month study period, 5,141 female patients were screened. The venereal disease clinic cultured specimens from 720 patients; the hospital and family planning clinics screened specimens from 1,983. Although 42 private physicians participated in screening 2,438 women, 90 percent of the specimens from these women were processed by 8 physicians.

Since Thayer-Martin and Transgrow were the reference media, the results from these tests were selected as correct unless the State laboratory could prove the presence on Clinicult tubes of *N. gonorrhoeae* that had been missed by either of the other two systems.

Table 1 shows the number of positive cultures and the positivity rates for each group. An unsatisfactory result on a specimen was called nonpositive (negative) since the culture system had failed to detect a positive result. For example, overgrowth on the culture plate was the predominant cause of unsatisfactory results on Thayer-Martin medium. Unsatisfactory results with Clinicult were caused by errors in technique. Oxidase was applied to both sides of the "paddle," thus destroying colonies. The CO_2 tablet was not added to the tube. In some instances, the second side of the culture paddle was not seeded. The total number of positives represents the combined number of isolates obtained by both laboratories. If we assume that this total represents the "theoretical 100 percent" of infected women within the group tested, then we can determine a sensitivity rate for each screening system. Although the overall yield of positives was more than 5 percent of the women screened, the positivity rate among private patients (group 3) was only 1.76 percent.

Table 1. Sensitivity of various media in isolations of *Neisseria gonorrhoeae*, Clinicult field trial, Seattle, Wash., February–July 1973

Study groups	Positive cultures and positivity rates			
		Clinicult system		
	Total[1]	Screeners	State laboratory	Reference system[2]
Group 1 (720 venereal disease clinic patients):				
Number	198	179	184	191
Percent	100.0	90.4	92.9	96.5
Group 2 (1,983 family planning clinic patients):				
Number	54	47	51	45
Percent	100.0	87.0	94.4	83.3
Group 3 (2,438 patients of private physicians):				
Number	43	26	33	39
Percent	100.0	60.4	76.7	90.7

[1]Positives were confirmed by isolations obtained in either or both laboratories used in study.
[2]Thayer-Martin was reference medium used for group 1 and Transgrow reference medium used for groups 2 and 3.

A specificity rate for each screening system was calculated by the same method. A confirmed nonpositive result means that neither of the confirming systems was able to detect the gonococcal organism. The total number of negative cultures therefore represents the "theoretical 100 percent" of women assumed to be free of infection. Using this basis of comparison, table 2 illustrates the high level of specificity that was attained in all three systems used by the screeners.

It can readily be seen that the group 3 screeners (private physicians) had the greatest percentage of errors in using Clinicult, as compared with Transgrow. The discordant results are shown in table 3. The probability that the two reference media would not always be correct proved true, as is shown by the number of false-negative and unsatisfactory tests obtained by the two laboratories.

Thirteen Clinicult tests that the State laboratory called oxidase positive were not finally identified as containing *N. gonorrhoeae*. A definite identification of these oxidase-producing organisms was not made.

Table 2. Specificity of various media in isolations of *Neisseria gonorrhoeae*, Clinicult field trial, Seattle, Wash., February–July 1973

Study groups	Confirmed negative cultures and negativity rates[1]			
		Clinicult system		
	Total	Screeners	State laboratory	Reference system[2]
Group 1 (720 venereal disease clinic patients):				
Number	522	517	522	522
Percent	100.0	99.0	100.0	100.0
Group 2 (1,983 family planning clinic patients):				
Number	1,929	1,917	1,929	1,928
Percent	100.0	99.4	100.0	100.0
Group 3 (2,438 patients of private physicians):				
Number	2,395	2,384	2,395	2,395
Percent	100.0	99.5	100.0	100.0

[1]Negatives were confirmed by failure of either laboratory to obtain isolations.
[2]Thayer-Martin was reference medium used for group 1 and Transgrow reference medium used for groups 2 and 3.

As the field trial progressed, it became evident that some private physicians were obtaining close correlations between their two screening systems and that others were not. Once screeners were enlisted in the study, they were not removed unless they specifically asked to be relieved or had screened for a reasonable length of time without finding any positive tests. Of the 11 physicians who began the study, 3 dropped out before completion of the trial. Thirty-one new physicians were recruited to help obtain the desired number of screening tests.

The variations in the correlations obtained by private physicians can best be seen in table 4. Screener A correctly identified all six positive specimens presented to him. In one case he called a specimen positive that could not be confirmed as positive. Screener B, however, correctly identified only one of six positive specimens presented to him. Clearly, screener A's location would be acceptable for use of the Clinicult media and screener B's location would not. In spite of careful observations, we have no explanation for screener B's result other than poor motivation on the part of the nurse or receptionist in following through with the required procedures. Analysis of the data from the entire group revealed a statistically significant difference between the private physicians' results and the laboratory results obtained with Transgrow media.

Discussion

The apparent biases in our study were as follows:

1. The Transgrow medium was not commercially produced, but was prepared by the Seattle-King County laboratory.

2. The Transgrow media was not pre-incubated. Bonin *(5)* has demonstrated that pre-incubation increases the Transgrow medium's sensitivity.

3. The advertised shelf life of Clinicult is 6 months. No media used in this study were more than 6 weeks old and, in fact, the media usually were used within 2 to 3 weeks after production.

4. All media from the private physicians' offices were picked up by a courier, and therefore our study does not provide an evaluation of these systems when the media are mailed.

5. There is a possible bias in that eight physicians accounted for at least 90 percent of all the testing in group 3.

6. A bias exists in drawing conclusions about the general use of Clinicult by physicians because the monitor in our study made every effort to detect faulty technique or equipment. The special incubators of the physicians were checked for proper temperature control (36° C). The physicians' staffs were observed performing the initial oxidase test, and they were shown good

Table 3. Discordant results obtained with Clinicult, Thayer-Martin, and Transgrow culture media, Clinicult field trial, Seattle, Wash., February–July 1973

Study group and culture medium	False positives	False negatives	Unsatisfactory specimens[1]
Group 1			
Clinicult	5	17	7
Thayer-Martin	0	2	17
Group 2			
Clinicult	12	6	20
Transgrow	0	9	1
Group 3			
Clinicult	11	16	32
Transgrow	0	4	0

[1]Unsatisfactory specimens were those which were overgrown or that could not be read as positive or negative because of technical errors.

Table 4. Extreme variations in two private physicians' readings of Clinicult test results, Clinicult field trial, Seattle, Wash., February–July 1973

Clinicult tests	Tests performed by—	
	Physician screener A	Physician screener B
Total tests performed	259	397
Read by physician as positive	7	2
Confirmed by State laboratory	6	6
Confirmed by Transgrow	6	6
Found to be false positive by State laboratory	1	1
Read by physician as negative but found to be false negative by one or the other laboratory	0	5
Sensitivity achieved (percentage)	100.0	16.7

NOTE: The State laboratory had only the second side of the Clinicult "paddle" for confirmation tests. The Seattle-King County Health Department laboratory had the Transgrow culture on the same patients.

oxidase-positive colonies before they attempted to read their own results. Any errors detected were corrected as they were observed.

The poor yield of positives in group 3 can be attributed to two factors. Since most physicians participating in the study had already been engaged in screening with Transgrow for a year or more, their reservoir of asymptomatic gonorrhea patients had steadily decreased. Moreover, some of the physicians who agreed to participate had a low yield of positives in the beginning. We did not have the flexibility to be highly selective in recruiting the screeners.

Personnel using Clinicult must be carefully trained in all aspects of the procedures. The colony growth of *N. gonorrhoeae* on Clinicult is smaller than on either the Transgrow or the Thayer-Martin media.

We mainly attribute the false-positive reports from screeners to overzealous readings, and not to the presence of nongonococcal oxidase-producers. Occasionally, flecks of solid material were noted in the Clinicult media, which produced purple-black reactions resembling oxidase-positive colonies when the oxidase reagent was applied. Possibly some of the positive readings made by the screeners were indeed positive, even though they could not be confirmed in either the State laboratory or the Seattle-King County laboratory.

Users of the Clinicult system need to be made aware that the initial oxidase-positive reading is only a presumptive positive, which must be confirmed by further testing before a final diagnosis of gonorrhea can be made. This point is illustrated by the State laboratory's observation of oxidase-positive colonies on Clinicult media from 18 patients that did not prove to be *N. gonorrhoeae*.

Until a reliable blood test becomes available for mass screening, we will have to rely upon culture systems despite their inherent inconvenience, sophistication, and expense. In this situation, the private practitioner who must use these systems can help public health agencies control gonorrhea only if he receives all the assistance that laboratories can give him.

Results of our study indiate that Clinicult was less accurate than Transgrow when used as a screening method by private physicians. We cannot therefore recommend its use in large-scale public health programs. This assessment does not preclude its successful used by physicians or other health workers if the test is performed with careful attention to detail.

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SYNOPSIS

Pedersen, A. H. B. (Seattle-King County Health Department), Kremers, Marshall Y., Dailing, Janet, Bonin, Paul, Brown, Charles D., and Jourden, Jack: *A field trial of the Clinicult system for detection of asymptomatic gonorrhea in women. Public Health Reports, Vol.* 90, September–October, 1975, pp. 430–434.

Clinicult, a selective medium for culturing *Neisseria gonorrhoeae*, was field-tested in a gonorrhea screening program in Seattle, Wash., in 1973. The results with this medium and with the Transgrow and Thayer-Martin culture systems were compared as to sensitivity and specificity. A total of 5,141 women from three patient groups were included in the study.

Group 1 consisted of 720 female patients of the venereal disease clinic of the Seattle-King County Health Department, who served as the control group. When this group was screened with the Clinicult and Thayer-Martin culture media, the Thayer-Martin medium proved superior in identifying positive carriers. Group 2 was composed of approximately 2,000 patients from five different facilities, including family planning clinics and hospital outpatient services. No statistical difference in accuracy was found between the two culture systems used for this group—Clinicult and Transgrow. Group 3 was comprised of approximately 2,500 female patients who were screened with the Clinicult and Transgrow cultures by their own private physician or his staff.

The Clinicult system proved significantly less effective than the Transgrow culture in identifying infected females in group 3. The physicians varied greatly in their ability to use the Clinicult system successfully. Possible reasons for their errors may have been *(a)* lack of motivation and of care by their office personnel in conducting the necessary additional procedures required with Clinicult, *(b)* the inhibitory nature of the medium, and *(c)* the failure of the medium to produce colonies of adequate size.

The staffs of communitywide screening programs for gonorrhea need to be highly selective in choosing the medical facilities in which to use the Clinicult culture system. When laboratory facilities are available for the full utilization of the Thayer-Martin medium, this system is preferable. When, however, standard culture procedures are not readily available, Clinicult, properly used, can reduce the central laboratory load by eliminating the need for processing negative cultures.