

CULTURE DIAGNOSIS OF GONORRHOEA—A COMPARISON BETWEEN TWO STANDARD LABORATORY METHODS AND A COMMERCIAL GONOCOCCAL CULTURE KIT (KVADRICULT®)

Dan Danielsson,¹ Eric Sandström,² Jan Kjellander,¹
Harald Moi¹ and Gösta Wallmark²

¹*Departments of Clinical Bacteriology and Dermatovenereology, Central County Hospital, Örebro,*
and ²*Departments of Clinical Bacteriology and Dermatovenereology,*
Södersjukhuset, Stockholm, Sweden

Abstract. The efficiency of a commercial kit—Kvadricult®—intended for the culture diagnosis of gonorrhoea and specially designed to simplify “bedside” inoculation, was compared in two laboratories with their standard laboratory gonococcal (GC) culture methods. The yields of specimens inoculated at “bedside” were compared with those inoculated after transport for 4–18 hrs in a modified Stuart transport medium. The highest positive yield was obtained by “bedside” inoculation with approximately equal results for the conventional methods and the Kvadricult system. Compared with “bedside” inoculation, the loss of positive specimens after transport was less than 10% in one laboratory but more than 17% in the other, which was found to be due to inferiority of the GC medium used in this laboratory, the reasons for which are discussed. Prolonged transportation of specimens for 2 days increased the loss of positive specimens, rising to as much as 55% even at the laboratory with the more efficient GC medium. These losses can obviously be avoided by transport for less than 24 hours—or better still, by “bedside” inoculation. From this point of view we have found the Kvadricult system efficient and easy to handle.

Key words: Gonorrhoea; Laboratory diagnosis; Culture methods

Gonorrhoea is one of those few diseases that with some accuracy can be diagnosed by direct microscopy of properly stained smears, for example by Gram's technique or by means of fluorescent antigonococcal antibodies. However, the development of improved methods for culturing *Neisseria gonorrhoeae*, the increased incidence of gonorrhoeal cases, the documentation of a considerable number of asymptomatic gonorrhoea not only among women but also among men, and the

need for sensitivity testing, are all factors that have increased the need for the laboratory identification of gonococcal (GC) organisms by culture.

In Sweden, diagnosis of gonorrhoea by means of culture has been used routinely over the past 15 years. For this purpose specimens have usually been sent to the laboratory in a modified Stuart transport medium (STM) (6, 12). Since GC organisms are inherently sensitive to prolonged storage during transportation, inoculation of specimens on GC media in the clinic (“bedside” inoculation) gives higher positive yield (1, 3).

A new system—Kvadricult®, Orion Diagnostica Corp, Helsinki, Finland—has been adapted to simplify “bedside” inoculation. It combines the use of non-selective and selective media in one plate, and uses the candle extinction principle for generating CO₂.

In the present work the cultural yields with the Kvadricult system were compared with those of two conventional methods after inoculation “bedside” and after transport of specimens in a modified Stuart medium. The study was carried out at the bacteriological laboratories of two hospitals, since this also gave us an opportunity to compare two different GC culture media.

MATERIALS AND METHODS

Patients and participating laboratories

Specimens obtained from patients visiting the clinics for venereal diseases at the Central County Hospital in Örebro (118 specimens from 28 men and 29 women) and at

Table 1. Comparison of the yields obtained in Laboratory A (118 specimens from 28 men and 29 women) and Laboratory B (97 specimens from 64 men and 15 women) with Kvadricult inoculated "bedside", and with conventional agar plates inoculated "bedside" and after transport of the specimens (4–18 hours) in modified Stuart medium (see Materials and Methods)

Kvadricult® method	Conventional method			
	"Bedside"		After transport	
	Positive	Negative	Positive	Negative
Laboratory A:				
Positive	46	5	37	14
Negative	0	67	1	66
Laboratory B:				
Positive	78	1	74	5
Negative	5	13	4	14

Södersjukhuset, Stockholm, Sweden (139 specimens from 82 men and 28 women) were included in the study. The specimens were handled and examined as described below at the bacteriological laboratories of these hospitals, hereafter referred to as Laboratory A and Laboratory B, respectively.

Culture procedures

1) *Kvadricult method*. This system consists of a rectangular culture dish divided into two equal compartments (5×5 cm), one containing non-selective and the other selective GC culture medium. At the time of the study (Nov. 1975 through April 1976) the medium was, according to the manufacturer, based on BBL GC medium base enriched with Isovitalex, horse blood and horse serum. The selective medium containing polymyxin B (20 IU/ml), trimethoprim (3.5 g/ml) and nystatin (12.5 IU/ml). CO₂-generation is based on the candle extinction principle. After the plate is inoculated it is placed in a small box, together with a candle that is lighted, before the lid of the box is closed, and placed in an incubator at 35–36°C in the clinic or in the laboratory. The plates were used within 8 weeks of preparation.

2) *Conventional culture*. GC agar plates with or without vancomycin (3.0 g/ml), colistin (7.5 g/ml) and nystatin (12.5 IU/ml) were made up at Laboratory A of BBL GC agar base enriched with Bacto hemoglobin and Isovitalex according to the manufacturer's instructions, and at Laboratory B of Oxoid Columbia Agar enriched with 100 ml heated horse blood and 85 ml horse serum per liter agar. The prepared plates were normally used within one week.

Collection and handling of specimens

Specimens were taken from the urethra of males and from urethra, cervix and rectum of females with swabs im-

pregnated with charcoal (6). The collected material was thoroughly suspended in 1 ml of trypticase soy broth (TSB) as described previously (2). The original swab was removed from the TSB and replaced by four new swabs. By this technique each swab will be loaded with approximately the same amount of material and will therefore be suited to comparative studies. The culture yield from each of these swabs was shown by Danielsson et al. (2) to be at least as high as that from the original swab.

The swabs were used for "bedside" inoculation and transport to the laboratory as follows.

1) *"Bedside" inoculation*. With two of the swabs, non-selective and selective conventional culture plates and a Kvadricult® plate were immediately inoculated at the clinic. The conventional agar plates were put in a candle jar and placed in the clinic's incubator at 35–36°C together with the Kvadricult plates in their candle extinction boxes. After incubation for 2 days the jars and the boxes were transferred to the laboratory for bacteriological examination.

2) *Transport of swabs in Stuart medium*. The other two swabs were inserted separately into a modified Stuart transport medium (STM) (6) and transported to the laboratory. The transportation time was 4–18 hours; in a smaller study it was extended to 2 days. One of the swabs was used to inoculate two conventional GC agar plates, one with non-selective, the other with selective culture medium, that were placed for 2 days in a CO₂-incubator (5% CO₂) at 36°C with high humidity. The other swab was used to inoculate a Kvadricult plate which was then put in the candle extinction box and placed in the incubator at 35–36°C.

Bacteriological examinations

After 2 days' incubation, the conventional agar plates and the Kvadricult plates were subjected to ocular inspection and oxidase testing. The presence of gonococci in oxidase-positive colonies was confirmed by immunofluorescence tests, Gram-staining and co-agglutination or fermentation as described elsewhere (4, 5).

RESULTS

The yields obtained at the two laboratories (A & B) with conventional and Kvadricult® (trial) methods are summarized in Tables I and II.

Table I shows that in Laboratory A "bedside" inoculation gave 5 (10.9%) more positives with the trial method than with the conventional one. Under corresponding conditions in Laboratory B the conventional method gave 4 (4.8%) more positives than the trial method. After transport of specimens to the laboratories for 4–18 hours in a modified Stuart medium the conventional method gave a loss of 17.4% in Laboratory A in contrast to 6% in Laboratory B. Compared with the trial method the loss of positive specimens after transport and culture

Table 11. Comparison of the yields obtained in Laboratories A & B with the conventional and Kvadricult® methods with regard to non-selective and selective culture media

A total of 166 specimens from 64 men and 34 women were examined by "bedside" inoculation and after transport in modified Stuart medium for 4–18 hours (see Materials and Methods)

Positive yield in	Inoculation "bedside"				Inoculation after transport			
	Conventional		Kvadricult		Conventional		Kvadricult	
	NS	S	NS	S	NS	S	NS	S
Laboratory A	43	38	48	48	37	31	48	46
Laboratory B	40	40	37	39	36	37	36	38
Total	83	78	85	87	73	68	84	84

on routine medium was 25.5% in Laboratory A but only 1.3% in Laboratory B.

Table II summarizes the yields obtained in the two laboratories with the conventional and Kvadricult methods with regard to non-selective and selective culture media inoculated "bedside" and after transport of specimens.

It will be seen that at Laboratory A the "bedside" inoculation of non-selective medium of the trial method gave 5 (11.6%) more positives than non-selective of the conventional one, and the selective as much as 10 (26%) more positives. At Laboratory B the non-selective medium of conventional plates gave 3 (7.5%) more positives than non-selective medium of the trial method, and the selective 1 (2.5%) more positive.

After transport of specimens the results were almost identical for the trial and conventional methods at Laboratory B, with a loss of 3–10% as compared to "bedside" inoculation. The Table also shows that there was no appreciable loss at Laboratory A for the trial method used "bedside" or after transport of specimens. On the other hand the conventional method gave after transport of specimens a loss of 6 (14%) and 7 (18.4%) for non-selective and selective media respectively. Compared with the trial method used "bedside", these figures will be 22.8% and 35.4% respectively.

Influence of the yield after transport of specimens for 2 days

In a series of 42 specimens from the urethra of 28 men and from cervix and/or urethra of 13 women with a preliminary diagnosis of gonorrhoea by direct microscopy, the yield after transport of speci-

mens for 2 days was compared with the yield of "bedside" inoculation. This study was carried out at Laboratory B on their conventional GC medium which was shown to be more efficient than that of Laboratory A. Thirty-six out of the 42 specimens were positive by "bedside" inoculation but only 16 after transport of specimens for 2 days, i.e. there was a loss of 55%.

DISCUSSION

The primary purpose of this investigation was to test the efficiency of the Kvadricult® system for culture diagnosis of gonorrhoea but we also wanted to see if there were any obvious differences between two conventional and commercially available GC culture media, one of which is widely used. The findings in the present investigation are, from these points of view, of interest and merit some comments.

Since the introduction of the so-called Stuart transport medium (STM) for GC (13, 14) the rational approach in Sweden and other Scandinavian countries has been transport of GC specimens to the diagnostic laboratories in a modified STM (6, 12) for culture on selective media (9, 15)—or on both selective and non-selective media which will give a higher positive yield (3, 11). Compared with "bedside" inoculation there was a loss in one of the laboratories (Lab. B) of only 6–10% of positive specimens after transport for less than one day. These findings are in agreement with those previously reported (1, 2, 3). In this laboratory the Kvadricult method and the conventional technique gave approximately the same yields "bedside" or after transport of specimens. In the other laboratory

(Lab. A), however, there was a substantial loss of positive specimens with the conventional method after transport of specimens. This loss was still more pronounced when compared with the results obtained at Laboratory A with the Kvadricult® method.

The inferiority of the GC medium used at Laboratory A was a surprise, since it has proved efficient in earlier investigations (2, 3) and since each batch of medium, according to the information provided by the manufacturer, is controlled and certified at the Center for Disease Control in Atlanta, USA. We first thought it to be a non-recurrent phenomenon at laboratory A, but corresponding observations have since been reported from other laboratories in Sweden. These findings point to the need for a continuous quality control of culture media for such sensitive organisms as *Neisseria gonorrhoeae*, not only under laboratory but also under clinical conditions.

There can be several reasons for the differences between the two media used, for example poor quality of the agar base, or different epidemiological situations with regard to gonococcal auxotypes. One obvious difference, however, was the addition of horse blood and horse serum to the agar base used at Laboratory B and corresponding additives were used by the manufacturer of Kvadricult®. Besides growth-promoting factors, these additives might also give an osmotic stabilization of the medium which would probably favour the fragile and osmotically sensitive gonococcal organisms.

The well known and inherent sensitivity of the GC organisms to prolonged transportation (2 days) was confirmed in this study, since more than half of the positive specimens were lost. These great losses can obviously be avoided by shortening the transport time (less than 24 hours), or better still by "bedside" inoculation. The Transgrow system (10), aimed to combine transport and culture, looks attractive in this respect but raises technical problems (2, 8), and it will also miss the Vancomycin-sensitive strains (2, 11). These disadvantages are overcome by the Kvadricult system which is specially designed for "bedside" inoculation. It combines the use of non-selective and selective media in one plate and easy, direct access to the surface of the culture medium both for inoculation, inspection and testing of suspected colonies. It also uses the candle extinction principle for generating CO₂ which has been shown to be most efficient (7). We

have found the Kvadricult system easy to handle in an out-patient clinic for venereal diseases and also in other clinics. The loss of positive specimens by transportation will be minimized in this way and after incubation of the Kvadricult boxes for 1 or 2 days in the clinic they can be transferred to a laboratory for subsequent confirmation of gonococcal-like colonies, and, if needed, sensitivity testing.

REFERENCES

1. Danielsson, D.: The demonstration of *N. gonorrhoeae* with the aid of fluorescent antibodies. 6. A comparison of conventional methods and fluorescent antibody (FA) techniques with counterstaining for the demonstration of *N. gonorrhoeae*. *Acta Dermatovener (Stockholm)* 45: 74, 1965.
2. Danielsson, D., Laurell, G. & Öman, S.: Culture diagnosis of gonorrhoea—A comparison of the results obtained by culture in the clinic, after transport in Stuart transport medium and with the use of Transgrow. *Acta Path Microbiol Scand Sect B* 80: 343, 1972.
3. Danielsson, D. & Johannisson, G.: Culture diagnosis of gonorrhoea. A comparison of the yield with selective and non-selective gonococcal culture media inoculated in the clinic and after transport of specimens. *Acta Dermatovener (Stockholm)* 53: 75, 1973.
4. Danielsson, D. & Kronvall, G.: Slide agglutination method for the serological identification of *Neisseria gonorrhoeae* with antigonococcal antibodies adsorbed to protein A-containing staphylococci. *Appl Microbiol* 27: 368, 1974.
5. Danielsson, D. & Forsum, U.: Diagnosis of *Neisseria* infections by defined immunofluorescence. Methodologic aspects and applications. *Ann NY Acad Sci* 254: 334, 1975.
6. Gästrin, B., Kallings, L. O. & Marcetic, A.: The survival time for different bacteria in various transport media. *Acta Path Microbiol Scand* 74: 371, 1968.
7. James-Holmquest, A. N., Wende, R. D., Mudel, R. L. & Williams, R. F.: Comparison of atmospheric conditions for culture of clinical specimens of *Neisseria gonorrhoeae*. *Appl Microbiol* 26: 466, 1973.
8. Jephcott, A. E., Morton, R. S. & Turner, E. B.: Use of transport- and culture medium combined with immunofluorescence for the diagnosis of gonorrhoea. *Lancet* 11: 1311, 1974.
9. Martin, J. E., Peacock, W. L. & Thayer, J. D.: Further studies with a selective medium for cultivating *Neisseria gonorrhoeae*. *Br J Vener Dis* 41: 199, 1965.
10. Martin, J. E. & Lester, A.: Transgrow, a medium for transport and growth of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. *HSMHA Health Rep* 86: 30, 1971.
11. Reyn, A. & Bentzon, M. W.: Comparison of a selec-

- tive and a non-selective medium in the diagnosis of gonorrhoea to ascertain the sensitivity of *Neisseria gonorrhoea* to vancomycin. Br J Vener Dis 48: 363, 1973.
12. Ringertz, O.: A modified Stuart medium for the transport of gonococcal specimens. Acta Path Microbiol Scand 48: 105, 1960.
13. Stuart, R. D.: The diagnosis and control of gonorrhoea by bacteriological cultures. Glasgow Med J 27: 131, 1946.
14. Stuart, R. D., Toshach, R. S. & Patsula, M. T.: The problem of transport of specimens for culture of gonococci. Canad J Publ Hlth 45: 73, 1954.
15. Thayer, J. D. & Martin, J. E.: A selective medium for cultivating *Neisseria gonorrhoeae* and *Neisseria meningitidis*. Publ Hlth Rep 79: 49, 1964.

Received November 15, 1976

D. Danielsson, M.D.
Department of Clinical Bacteriology
Central County Hospital
S-701 85 Örebro
Sweden