Yonsei Medical Journal
Vol. 19, No. 1, 1978

Modified Dextrose Starch Agar for the Preservation of *Neisseria gonorrhoeae*

Yunsop Chong, Sung Ok Kim and Samuel Y. Lee

Department of Clinical Pathology, Yonsei University College of Medicine, Seoul, Korea

To seek a practical and inexpensive method to preserve gonococcal cultures, a few methods were compared. The following methods kept the cultures alive for only a short period of time: skim milk at -20°C; tryptic soy broth with 15% glycerol at -20°C; cystine tryptic agar at 35°C. Most of the test cultures survived for more than 4 weeks in the following media: one half strength dextrose starch agar; one half strength dextrose starch agar with ferric nitrate; one half strength dextrose starch agar with antimicrobic CNV. Dextrose starch agar could be substituted by GC medium base with a slight modification. It is concluded that preservation of gonococci in one half strength dextrose starch agar with CNV, which produces less frequent contamination, is a practical method to maintain cultures for teaching and quality control.

Increased incidences of gonorrea has become a renewed problem all over the world since the nineteen sixties (Godden, 1973). For bacteriological studies of gonococci and for epidemiological investigation of the infection it is essential to keep clinical isolates. Preservation of stock culture is also necessary for teaching and for quality control of clinical bacteriology procedures.

Gonococci are one of the most difficult bacteria to store (Stokes, 1970). Lyophilization (Bartlett, 1974) requires special facilities and a complex process which preclude application for the storage of clinical isolates on the spot. Freezing in liquid nitrogen (Ward and Watt, 1971) is not feasible for common clinical laboratories.

It has been stated that cystine trypticase agar (CTA; Vera, 1948) and one half strength dextrose starch agar (DSA-½; Difco, 1971) at 35°C could keep stock cultures of gonococci. Since these methods do not require any special equipment nor complex process, we were interested in their usefulneess. As to the preserving temperature, La Scolea *et al.* (1975) kept their cultures at -75°C in trypticase soy broth (TSB) with 15% glycerol (TSB-G). We experienced that at -20°C delicate anaerobes kept quite well in 10% skim milk. Therefore we made a preliminary study to compare the survival period of gonococci at -20°C in TSB-G and in skim milk, and at 35°C in CTA and in DSA-½.

Since the preliminary test showed the best result with DSA-½, this medium was chosen for further studies. It has been reported that deprivation of dextrose from culture media causes autolysis and hence death of gonococci (Morse and Bartenstein, 1974). Ferric nitrate in media

* Received March 14, 1978

is known to maintain virulent colony types (Kellogg, Peacock, Deacon, *et al.*, 1963). Occasional contamination by bacteria or fungi was a practical problem encountered with DSA-½ culture and certain control measures such as addition of antimicrobic CNV (Difco) seemed to be necessary. Therefore, studies were made on effects of i) higher concentration of dextrose, ii) presence of ferric nitrate and iii) addition of CNV in DSA-½ upon the survival of gonococci.

A commercial product of DSA is not commonly available in clinical laboratories. Preparing the medium from separate ingredients was quite tedious work and an attempt was made to substitute for this medium by a modification of GC medium base (Difco). The survival time on this medium was tested.

MATERIALS AND METHODS

Ten per cent skim milk and TSB-G which was made by addition of 15% glycerol (Tedia) to TSB (Difco) were dispensed 0.5 ml each in glass vials. CTA (Difco) with 0.5% dextrose (CTA-D), DSA-½, and modified GC medium base (MGC) were tubed in 4 ml amounts in 75x100 mm screw cap test tubes. DSA-½ was prepared by incorporating proteose peptone No. 3 (Difco) 0.75%, dextrose 0.1%, soluble starch (Showa) 0.5%, sodium chloride 0.25%, disodium phosphate (Merck) 0.15%, gelatin (Difco) 1% and agar (Difco) 0.5%. Modified DSA-½s were prepared as follows: DSA-½ with 0.5% dextrose (DSA-½D) by adding 0.4% dextrose to DSA-½; DSA-½D with ferric nitrate (DSA-½DF) by adding 0.005% ferric nitrate (Kanto) to DSA-½D. CNV-added DSA-½DF (DSA-½DFC) was prepared by adding 1% CNV (Difco) to DSA-½DF after sterilization and cooling of the media to 45°C.

MGC was prepared by adding 0.45% soluble starch, 1% gelatin, and 0.1% dextrose to GC medium base—½ (Difco). Three kinds of MGCs were prepared with the addition of no extra phosphate (MGC-A, pH 7.0), of 0.2% dipotassium phosphate (MGC-B, pH 7.1) and of 0.6% dipotassium phosphate with 0.05% mono potassium phosphate (MGC-C, pH 7.2).

Clinical isolates of gonococci were maintained by subcultures on chocolate agar. The cultures were propagated to chocolate agar plates for 24 hours. TSB-G and skim milk vials were inoculated with 3-mm loopfuls of the growth. The vials were capped tightly and immediately placed at -20°C in a freezing compartment of a refrigerator. CTA-D, DSA-½s and MGCs were inoculated with a 3-mm loop by making several stabs in the top of each media. The tubes were tightly capped and stored in a incubator of 35°C.

Survivals of gonococci were tested by making weekly subcultures. TSB-G and skim milk vials were taken out from the freezer, thawed quickly, and inoculated immediately. The vials were used once. CTA-D, DSA-½s and MGCs tubes were repeatedly used as inocula, and returned immediately into the incubator after each use. Inoculations were made onto chocolate agar with a 3-mm loop and incubated in a candle jar at 35°C for 48 hours. If any number of colonies developed, it was interpreted as the survival of the culture.

RESULTS

The preliminary test showed skim milk at -20°C kept alive 3 out of 6 cultures for only one week, while TSB-G kept 3 out of 6 for 2 weeks. CTA-D at 35°C showed better result, namely one out of 5 cultures survived for 4 weeks. But the best result was rendered by

Table 1. Survival Time of N. Gonorrhoeae Cultures on Various Storage Media

Survival (Weeks)	Numbers of culture			
	-20°C		35°C	
	TSB w/15% glycerol	Skim milk 10%	CTA w/0.5% dextrose	DSA-½
less than 1	0	3	0	0
1	3	3	2	0
2	3	0	2	0
3	0	0	0	0
4	0	0	1	4
Total	0	6	5	4

TSB : tryptic soy broth.
CTA : cystine tryptic agar.
DSA-½ : ½ strength dextrose starch agar.

DSA-½ on which all of the 4 cultures survived for 4 weeks (Table 1). From this result DSA-½ was chosen for further study.

DSA-½ and 3 kinds of modified DSA-½s were tested with 8 cultures of gonococci (Table 2). Depending on the test organism, survival time ranged from 1-16 weeks (mean 9.6) on DSA-½,7-17 weeks (mean 11.4) on DSA-½D, 4-16 weeks (mean 10.4) on DSA-½DF and 7-17 weeks (mean 11.5) on DSA-½DFC. The differences in mean survival times were statstically not significant ($P > 0.05$).

On the MGC-A (Table 3), gonococci survived for 5-12 weeks (mean 10.3), on MGC-B for 4-12 weeks (mean 9.9) and on MGC-C for 11-12 weeks (mean 11.9). The difference of mean survival times between MGC-A and MGC-C was statistically significant ($P < 0.01$).

Table 2. Survival Time of *N. gonorrhoeae* Cultures in DSA-½ and Modified DSA-½

Survival (Week)	Number of culture			
	DSA-½	DSA-½D	DSA-½DF	DSA-½DFC
1	1	0	0	0
4	0	0	1	0
7	2	1	1	1
8	0	0	0	1
9	2	2	3	2
10	0	1	0	0
11	0	1	0	1
13	1	1	0	0
14	0	0	1	0
15	1	1	1	1
16	1	0	1	1
17	0	1	0	1
Total	8	8	8	8
Mean survival time	9.6*	11.4*	10.4*	11.5*

DSA-½ : ½ strength dextrose starch agar.
DSA-½D : DSA-½ plus dextrose 0.4%.
DSA-½DF : DSA-½D plus 0.005% ferric nitrate.
DSA-½DFC : DSA-½DF plus 1% antimicrobic CNV.
* The differences of mean survival time in DSA-½ and in others were not significant ($P > 0.05$).

Table 3. Survival Time of *N. gonorrhoeae* Cultures in Modified GC Medium Base

		Modified GC medium base		
		A(pH 7.0)	B(pH 7.1)	C(pH 7.2)
No. of cultures tested		9	15	17
Survival time (week)	Mean	10.3	9.9	11.9
	Range	5–12	4–12	11–12

The difference of mean survival time in medium A and in B was not significant ($P > 0.05$), but in A and in C it was significant ($P < 0.01$).

DISCUSSION

Our attempts to preserve gonococci at -20°C either in TSB-G or in skim milk were not rewarding. CTA is known to keep gonococci alive for 10 days or more (BBL, 1974). This study showed that addition of dextrose to CTA did not extend the survival time. DSA-½ was found to be a practical medium. It kept most of the cultures alive for over 4 weeks.

Morse and Bartenstein (1974) reported that dextrose-limited gonococci culture was susceptible to autolysis. Hence it was our assumption that cultures may be deprived of dextrose during the long storage of gonococci in DSA-½, resulting in lysis of the organism. If so, a higher concentration of dextrose may render survival longer. But contrary to our expectation, 0.5% dextorse did not improve the survival time. Moreover, the DSA-½ used to store gonococci for 8 weeks still reacted with a dextrose oxidase stick, indicating 0.1% dextrose concentration was adequate.

Gonococci on successive subculture gradually change to nonvirulent type 3 and 4 colonies. Growth of virulent colony types 1 and 2 are stimulated by ferric nitrate (Kellogg, Peacock, Deacon, *et al.*, 1963). However the effect of this chemical on survival has not beeen studied. Although the colony types formed after storage on DSA-½ is not known, a test was done to find the effect of the chemical on survival. In this study no harmful effect of the chemical on the survival time was noted.

During the experimental as well as routine use of DSA-½ it was shown that occasional bacterial and fungal contamination can result in the loss of stock culture. Thayer-Martin medium contains an antibiotic combination, colistimethate, nystatin and vancomycin, to inhibit growth of various bacteria and fungi except gonococci (Thayer and Martin, 1966). The effect of these antibiotics on survival was tested and it was found that they do not give adverse effect on survival.

MGCs also preserved gonococci. The survival time on MGC-C (pH 7.2) was slightly longer than on MGC-A (pH 7.0) and the difference was statstically significant ($P < 0.01$). Morse and Bartenstein (1974) reported that the onset of autolysis was not related to decrease of pH, but it seems possible that in long term storage slightly decreased pH may be somewhat harmful to gonococci survival.

Although it is not certain whether the preserved organisms' characteristics such as antibiotic susceptibility remain unchanged, it is concluded from this study that DSA-½ or a modification of it or MGC can be used to store gonococci for teaching or quality control purposes by subculturing once every 4 weeks. It was also shown that DSA-½ could keep Professor Juni's strain #488 alive during the shipment from US to Korea at ambient temperatures which took 10 days.

REFERENCES

Bartlett RC: *Medical microbiology. Quality cost and clinical relevance. New York John Wiley and Sons 1974 p 227*

Difco: *Difco manual of dehydrated culture media and reagents for microbial and clinical laboratory procedures. 9th ed. Detroit Difco laboratories 1971 p 124*

Godden JO: *International symposium on gonorrhoea. Canad Med J 109:1043-1053, 1973*

Kellogg Jr DS, Peacock Jr WL, Deacon WE, et al: *Neisseria gonorrhoeae. I. Virulence genetically linked to clonal variation. J Bact 85:1274-1279, 1963*

La Scolea Jr LJ, Dul MJ, Young FE: *Stability*

of pathogenic colony types of Neisseria gonorrhoeae in liquid culture by using the parameter of colonal morphology and deoxyribonucleic acid transformation. J Clin Microbiol 1:165-170, 1975

Morse SA, Bartenstein L: *Factors affecting autolysis of Neisseria gonorrhoeae. Proc Soc Exp Biol and Med 145:1418-1421, 1974*

Stokes EJ: *Clinical bacteriology. 3rd ed. London Edward Arnold 1970 p 313*

Thayer JD, Martin Jr JE: *Improved medium selective for cultivation of N. gonorrhoeae and N. meningitidis. Pub Hlth Rep 81:559-562, 1966*

Vera HD: *A simple medium for identification and maintanance of the gonococcus and other bacteria. J. Bact 55:531-535, 1948*

Ward ME, Watt PJ: *The preservation of gonococci in liquid nitrogen. J Clin Path 24: 122-123, 1971*