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ORIGINAL ARTICLES

A NEW CONVENIENT EGG-ENRICHED MEDIUM FOR THE CULTURAL DIAGNOSIS OF GONORRHOEA

By

CHACKO, C. W.* and NAIR. G. M.**

Neisseria gonorrhoea is considered as the most fastidious species among the Neisseria group of bacteria, to be isolated and maintained in artificial culture. Most strains of the gonococcus do not grow on simple nutrient broth agar, in primary cultivation outside from persons infected with it. The variety of new "enriched" and "selective" media and modification of the older ones, that have been prescribed and used from time to time, points to the difficulties involved in the cultural method of sure diagnosis of gonorrhoeae. Most media described are complex and the constituents used for enrichment, are not easily available, everywhere, so that the good results reported with them by the authors, could not often be reproduced by others. As a result, there has been constant research for a sensitive simple non-commercial medium which could be easily prepared and reproduced giving consistant isolation of the gonococcus in culture. Such a medium will raise the standard of cultural identification which only gives an accurate diagnosis of gonorrhoeae, particularly in the female.

In this report, a " trypsin-digest-beef-extract-agar " base medium, enriched with the contents of hen's egg, that may be very conveniently prepared in every laboratory and found to be effective in the primary cultural isolation and diagnosis of gonorrhoeae, is described.

TECHNIQUE OF PREPARATION OR THE MEDIUM

Gonococcus Medium base: A trypsin digest of beef-extract used as the " basal medium " for the gonococcus has been prepared as follows:

100 gms. of minced " beef " muscle, free from fat is weighed into a 1000 ml. Pyrex Conical flask and 500 ml. of distilled water is added to it. They are mixed well, if available, in a " Waring blendor ". 15 ml, of, " one normal " NaCH is added and the mixture is kept in a Water-bath at 75° to 80°C, for 5 minutes, The flask

* Dr. C. W. Chacko, M. B. & B. S., Ph. D , D. T. M., F. A. M. S., Professor of Serology Central V. D. Reference Laboratory, Institute of Venereology, Madras Medical College, Madras-3, India.

** G. M. Nayar, B. Sc., Assistant to Serologist, Central V. D. Reference Laboratory, Institute of Venereology, Madras Medical College, Madras-3, India.

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from the Water-bath is cooled to 40°C, and 0.20 gms, of " Trypsin powder " (1 : 250 DIFCO) is added and placed in a dry incubator, at 37°C for 6 hours for " digestion ". Now, 0.7 ml. of glacial acetic acid is added and the whole mixture is boiled for 10 minutes to stop the digestion. It is then kept cool in a refrigerator over-night and filtered through a gauze, the very next day.

500 ml. of 1% NaCL is prepared in a separate 1000 ml. flask and it is well mixed with the above filtrate. 1 gram of glucose, and 5 gms. of di-sodium mono-hydrogen phosphate ($Na_2 HPO_4$) is then added to it. The PH is adjusted to 7.2-7.4 by using a pH comparator using " Bromothymol blue " as indicator. It is then distributed in 200 ml. quantities, in conical flasks and to each 200 ml., 4 grams of Bacto Agar (DIFCO) is added. The agar is dissolved in a boiling waterbath and autoclaved at 10 lbs. pressure for 30 minutes. This stock of base medium can be preserved at room temperature, until used.

Enrichment of the Basal Medium: Hen's egg, fertilized or unfertilized, readily available everywhere from the market, is used for enrichment. The shell is cleaned with soap and water or with 70% alcohol and dried. Tincture of Iodine is applied on the surface containing the air sac and the iodine is removed with 70% alcohol. The shell is broken over the air-sac portion, with the flat surface of a sterile scalpel and it is removed with a pair of small sterile scissors, to an approximate diameter of 1 to $1\frac{1}{2}$ cms. The shell membrane is opened with a pair of small sterile forceps and introducing a long sterile " Nichrome wire loop " into the egg, through the cut opening, the " yellow and the white " of the egg are thoroughly mixed by rotating the loop, and holding the egg near a flame to avoid the possibility of any aerial contamination.

The " Gonococcus medium base " prepared is melted in a boiling waterbath and cooled to 55°-50°C, and the contents of an hen's egg is added directly into the base medium and homogenised thoroughly by rotating with hand. This gives an approximate concentration of 10% enrichment of the medium with whole egg contents. This may be reduced to 5 percent by dilution in the basal medium without affecting the potency of the medium and actually reducing the yellow colour and opacity of the medium with advantage.

The medium is poured into PETRI DISHES about 4 mm. high and allowed to solidify at room temperature. The egg medium is incubated at 37°C for 24 hours before use to test for sterility. Now the medium can be preserved at room temperature and longer at refrigeration temperature. When poured into plates, the medium is moderately opaque with a tinge of yellow colour It has been found to have good surface tension and therefore it is easily and satisfactorily inoculated with culture materials.

The materials for the comparative evaluation of the sensitivity of the egg medium to support the growth of the gonococci were obtained from the male and female patients clinically suspected of gonorrhoeal infection. The male cases had " acute urethritis ", and female cases had obvious " discharga per vagina " or were contacts of known cases of male gonorrhoeae. The material was collected with platinum loop

of 4 mm. diameter from the urethra of males, and from the cervix, urethra and vagina of the females and petri dishes of each of the 3 media under study were directly and immediately inoculated by the multiple streak method, with same amount of inoculum taken separately. After inoculating the plates, direct smears of the materials were made on slides for identification of the gonococcus microscopically by the gram staining technique, at the same time. They were then incubated at 36–37°C, in a MacIntosh and Fieldes Jar, in an atmosphere of 5–10% CO . This has been brought out by burning a candle inside the closed jar, till the flame extinguished itself. The moisture inside the jar was maintained by placing a moist cotton-ball inside. The plates were examined after 24 hours and if negative again after 48 hours of incubation.

The results of the incidence of primary isolation of the gonococcus in the Egg medium, have been compared first with the same base medium supplemented by ASCITIC FLUID which was obtained from " Cirrhosis " patients, under sterile conditions and free from antibiotics. Then the conventional " Bacto G. C. medium base " enriched with BACTO HAEMOGLOBIN and BACTO SUPPLEMENT B, of DIFCO origin was also included in the trials. These 3 media were inoculated with suitable materials from male and female patients, under parallel and standard environmental conditions.

The morphology of the colonies of the gonococcus that developed in the 3 media were more or less similar. They were raised transluscent discs measuring approximately 1 to 2 mm. in diameter with entire or slightly crenated margin, easily distinguishable with experience, " Oxidase reaction " was carried out by spraying on the plate an one percent solution of para-amino-dimethyl-aniline Oxalate. The colonies of the gonococcus appeared immediately ' Pink ', which turned " MAROON " soon, and finally ' black ' in colour in a few minutes. The purity of the culture and identification of the gonococci were ensured by their GRAM negative staining, and bio-chemical reaction fermenting glucose only with acid and no gas. The results obtained have been analysed as shown in the following tables.

Table I

PRIMARY CULTURE OF THE GONOCOCCUS

TRYPSIN-DIGEST-BEEF-EXTRACT-AGAR, base medium enriched with EGG compared with the same medium enriched with ASCITIC FLUID

Specimens	MALES		FEMALES			
	Egg	Ascitic Fluid	Egg		Ascitic Fluid	
Smear + Culture +	71	69	16	30	15	19
Smear − Culture +	4	—	14		4	
Smear + Culture −	−	2	—		1	
Smear − Culture −	6	10	88		98	
Total	81	81	118		118	

Table 2

PRIMARY CULTURE OF THE GONOCOCCUS
EGG MEDIUM COMPARED WITH THE 2 OTHER CONVENTIONAL
ENRICHMENT MEDIA

Specimens	MALES			FEMALES		
	Egg	Ascitic fluid	Bacto Haemoglobin Yeast Concentrate	Egg	Ascitic fluid	Bacto Haemoglobin Yeast concentrate
Smear+ Culture+	56	56	56	7	7	7
				-19	-15	-19
Smear− Culture+	4	—	—	12	8	12
	-60					
Smear+ Culture−	—	—	—	—	—	—
Smear− Culture−	4	8	8	80	84	80
Total	64	64	64	99	99	99

Table 2 A

ALL 3 MEDIA without POLYMYXIN B+MYCOSTATIN

Specimens	MALES			FEMALES		
	Egg	Ascitic fluid	Bacto Haemoglobin Yeast Concentrate	Egg	Ascitic fluid	Bacto Haemoglobin Yeast concentrate
Smear+ Culture+	3	3	3	2	2	2
				-5		-5
Smear− Culture+	−	−	−	3	−	3
Smear+ Culture−	−	−	−	−	−	−
Smear− Culture−	3	3	3	42	45	42
Total	6	6	6	47	47	47

Table 2 B

ALL 3 MEDIA made SELECTIVE with POLYMYXIN B+MYCOSTATIN

Specimens	MALES			FEMALES		
	Egg	Ascitic fluid	Bacto Haemoglobin Yeast Concentrate	Egg	Ascitic fluids	Bacto Haemoglobin Yeast Concentrate
Smear+ Culture+	53	53	53	5	5	5
	57			14	13	14
Smear − Culture+	4	—	—	9	8	9
Smear+ Culture −	—	—	—	—	—	—
Smear − Culture −	1	5	5	38	39	38
Total	58	58	58	52	52	52

COMMENTS ON THE RESULTS

In the table 1, is shown certain advantage noticed by replacing " Ascitic fluid " an usual source for enriching a base medium, with whole content of fresh hen's "egg", in the primary isolation of the gonococcus from male and female patients.

Form 81 male cases of " acute urethritis ", gonococci were isolated in the egg medium, in all the 71 samples in which the original smears of their urethral discharges were found to have intracellular gram-negative diplococci, resembling gonococci. This is in contrast to 69 samples of them from which gonococci could be isolated in medium enriched with Ascitic fluid. In 4 cases where the smears were negative for apparent gonococci, the egg medium supported the growth of the gonococcus while the Ascitic fluid medium could not do so in Culture. This apparent advantage for the egg medium in only a few instances, may not be considered very significant. However, the high sensitivity of the egg medium to pick of the gonococcus from acute male gonococcal urethritis at least as well as the conventional Ascitic fluid medium may be obvious. It may be noted in this connection that in the 6 instances of 81 acute urethritis cases in the male, where gonococci could not be grown in the egg medium, they could not be demonstrated in the original smears too, suggesting possibly that they were " non-gonococcal urethritis " cases.

In 118 females cases evaluated with the two media, the egg medium supported the growth of the gonococcus in 30 samples. This included 16 in which the original smears were positive, and 14 in which they were negative for the gonococcus. This is against 19 samples where the Ascitic fluid medium supported the growth of the

gonococcus, including 15 which were originally smear-positive and 4 which were smear-negative. Here again, some evidence of an apparent advantage for the " egg medium " over the " Ascitic fluid medium " may be noted.

The most practical use of the cultural method is in the diagnosis of gonorrhoeae in the females who again provide the best clinical material for assessing the sensitivity of any culture medium for the gonococcus. 30 instances of isolation of the gonococcus out of 118 females cases in this study in the egg medium, may be considered to be too low to be impressive. However, the failure in 88 instances may be viewed against the clinical background of these female patients with only a sign of " vaginal discharge " from which gonococci could not be demonstrated in the smears from cervix, vagina or urethra, and without any conclusive clinical evidence to establish infection with gonococcus in them unequivocally. In fact in the 30 successful cultures In the egg medium, direct original smears were negative in 14 cases including 6 cases with micro-purulent discharge, and 8 cases which were asymptamatic contacts of known cases of male gonorrhoea. Therefore, the egg medium may be considered to give useful results in the primary cultural isolation and diagnosis of gonorrhoeae in the female too. Even if significant difference in the sensitivity of the two media in the primary isolation of the gonococcus has not been shown in this limited preliminary study, this medium may be given the benefit certain advantages and attention. The egg can be obtained anywhere more easily and conveniently than the Ascitic fluid which is indeed difficult to get in quality always, everywhere.

In the table 2 is shown the comparative results, when another conventional medium for the culture of the gonococcus, particularly the popular BACTO-G. C.-medium base, enriched with BACTO-HAEMOGIOBIN and BACTO SUPPLEMENT B, from DIFCO, has also been included in the comparitive assay of the " egg medium ".

It may be particularly seen the number of instances of primary isolation of gonococcus from male and female cases, in the egg media, compared very well with that of the popular G. C. medium from DIFCO. The general trend noted in table I evaluating only 2 media, is seen also in the table 2, with the high percentage of isolation in male urethritis cases, and low in female cases, in all the 3 media. Whenever gonococci were demonstrated microscopically in the direct discharge smears, they were successfully grown in all the 3 media and, where the direct smears were negative, the egg and DIFCO medium have appeared to let the gonococci grow in them apparently more frequently than in the Ascitic fluid medium. Howevever, it may said in favour of the Ascitic fluid medium that it is comparatively less opaque than the other two and the colonies of the gonococcus could be more easily picked up and counted in it.

The Bacto G. C. medium from DIFCO, has been reported upon by Carpenter et al (1949) as the most useful among 12 conventional media evaluated by them in the primary isolation of the gonococcus. Therefore the preliminary findings in this study, that the egg medium may do, as well as, the DIFCO-BACTO medium, appeared worthy

of attention and follow up. This medium enriched with " haemoglobin " and " yeast concentrate " and available commercially in dehydrated form from DIFCO, is certainly convenient to use, as it can be re-constituted by the less trained technical personel, with dependable and reproducible results. However, this medium has to be imported from abroad prohibiting its routine use in all countries. Enrichment by the same nutrient materials in the form of "blood" of human or animal origin, as in " Chocalate agar ", and " Yeast extract " may be locally managed non-commercially but it is suggested that, supplementing with fresh egg content would be a more easier proposition. In fact, it has been found in a few trials in this study, that BACTO G. C. medium base, enriched with egg contents only, can also support the growth of the gonococcus, well. Even ordinary nutrient agar enriched with egg can support the growth of the gonococcus to a limited degree but the basal medium used in this study is to be preferred. The egg medium described in this study can be easily prepared in any bacteriological laboratory, by the less-trained technicians and can be preserved in stable form under normal temperature variations for routine use everywhere.

Isolation of the gonococcus from infected secretions is often difficult because of over-growth by other contaminating micro-organisms, in all enrichment media devised for the gonococcus. THAYER anu MARTIN (1964) have advocated the use of a combination of " polymyxin B " and " RISTOCETIN ", to conventional enrichment media to make them " selective " for the gonococcus by suppressing the gram negative and gram positive contaminants, respectively. Ristocetin has not been available in this study. Polymyxin B and in addition " Mycostain " effective against fungi, incorporated in the 3 media, have been tried. The results obtained is shown in the tables 2A and 2B. Out of the 64 male cases shown in table 2, specimens from 6 (table 2A) have been inoculated in the media without, and from 58 (table 2 B) inoculated in the media with the above antibiotics. Out of 99 cases of females, specimens from 47 were inoculated into the media without and specimens from 52 were inoculated into the media with the above antibiotics. There is an increase noticed in percentage isolations of a gonococci in the media when made selective with the above antibiotics as seen in the table 2B over these seen in the table 2A. Further, it is mentioned that it has been possible to isolate gonococci from " rectum " of a passitive case of " sodomy ", included in this group for the first time in this Institute, in all the 3 media, obviously by making them " selective " with antibiotics. Thus the egg enriched media has appeared in this preliminary study to let itself be made " selective " too, with the incorporation of the antibiotics polymyxin B and Mycostatin, with improvement in its practical utility. With the addition of Ristocetin too, if available, it may be expected to do better still in the primary cultural diagnosis of gonorrhoeae and its control, in difficult situations like female and rectal gonorrhoeae.

Egg media have been used satisfactorily for the isolation and cultivation of the " tubercle bacillus ", of pathogenic " clostridia " and of " spore-bearing serobes ". " Donovania granulomatis " have also been grown secondarily in egg-yolk-medium after they have been primarily isolated in growing chick embryo yolk sac. The

" gonococcus " does not appear to have been reported to grow in artificial culture in media enriched with egg constituents, earlier than in this study. But Walsh et al (1963) has shown that developing Chick embryo served as a " live selective medium " for the growth and maintenance of virulent gonococcus and to re-constitute its virulence. Obviously there are un-named nutrient and growth factor or factors available in the egg for the gonococcus. In fact, the efforts, to confirm WAISH S observations and the success in growing the gonococcus in the allantoic fluid of embryonated Chick eggs in this study, led to the idea of experimenting with egg contents as a source of special nutriment to the gonococcus in non-living artificial media. It was found that gonococci could be successfuly grown and maintained in the basal medium supplemented not only with the yolk and allantoic fluid from embryonated Chick eggs, but also with yolk or whole contents of fresh hen's eggs, fertilized or non-fertilized, and even using " duck egg ". Several local, fresh and stock strains of the gonococcus, have been maintained successfully in repeated passages in the egg medium, prepared from several local strains of Chick eggs over a significant period in this study.

It is known that the gonococcus is slow growing and more exacting than most other bacteria in its growth requirements of moisture, salt concentration, pH of the medium, incubation temperature, and carbondioxide and oxygen. It has been found to be exceedingly susceptibie to the toxic effect of a variety of substances commonly present in ordinary media. It can grow well on an agar media consisting of meat infusion, pedtones, glucose, buffered with phosphate, and enriched with whole blood, haemoglobin, serum, Ascitic fluid, hydrocele fluid and placental extract. The effect of natural proteins like blood, serum and Ascitic fluid has been ascribed to their detoxifying effect on the " inhibitors " present in peptones and agar.

According to McLEOD, " meat-extract " alone supported the gonococcus poorly, and " PEPTONE " though helpful, was not well tolerated when concentrated. There may be substances in the peptone which were actually lethal to the gonococcus and the blood added to the medium might protect it from such substances. " Heated blood ", seemed to give better result. Substances inhibiting the growth of the gonococcus have been found in "agar", a "fatty acid" whose action may be counteracted by the addition "starch" or "charcoal" to the medium.

Lankford and Snell (1943) found that Glutamine or Glutamic Acid was necessary for the growth of certain strains of the gonococcus for the construction of its cell proteins with the amino acid. Lankford and Skagg (1946) reported that Glutamine is a necessary supplement to " Bacto Proteosn Peptone No. 3 Haemoglobin Agar ", in the cultural investigation of the patients, in about 25 percent of the strains of the gonococci in certain areas. Certain other strains grew upon that medium only in the presence of relatively thermostable factor present in Yeast and other natural substances. Although this factor may be replaced by a relatively high concentration of Thiamine, the high activity of Yeast extract could not be explained on the basis of its " thamine content " alone. This thermostable factor in

Yeast extract has been found to be Co-carboxylase. It has been reckoned that certain strains of gonococci effected a two-stage phosphorylation of Thiamine and the compound Di-Phosphothiamine had been found have " co-carboxylase " activity.

A number of natural substances would supply Co-carboxylase including Liver extract Beef muscle extract, human, animal blood and Cow's milk, Ascitic fluid has been found to contain Co-carboxylase and glutamine.

Reyn et al (1964) found that Haemoglobin can replace " heated horse blood ", used in some media for the gonococcus. Accotding to Moller and Reyn (1964) a mixed Yeast and Liver autolysate, could replace Ascitic fluid as seown in the " Haemoglobin-Yeast-Liver (H. Y. L.) " medium described recently for culture of the gonococcus. Reising and Kellog (1964) has reported that Ferric Nitrate could replace Haemoglobin.

Cystine in 0.1 percent concentration, added to the conventional Chocolate Agar medium has been found to stimulate the growth of the gonococcus according to Higginbotham (1942). Boor (1942) also found that Cystine was useful but other Sulphur Compounds or even Sulphur itself were able to take its place. But the high optimal Cystine concentration reported by Boor it at variance with observation of Mcleod et al, (1927) that inhibition of the gonococcus occurred at that range.

Muelier and Hinton (1941) have shown that an essentially protein free medium of Casein Hydrolysate-Meat-Infusion-Starch Agar gave excellent growth of stock and freshly isolated strains of the gonococcus. Casein hydrolysate factor appeared distinct from that present in meat-infusion. The activity of Casein hydrolysate was shared between the amino-acids Histidine, Arginine and Lysine. The meat infusion factor was heat stable and dialysable and could not be replaced by any vitamins but yeast infusion partially replaced it.

Park (1964) considered that Adenine and Guanidine are useful nutritional requirements of the gonococcus.

In explaining the successful growth and maintenance of the gonococcus in the egg enriched medium in this study, the information available in the standard books on Nutrition and Bio-chemistry that, Thiamine, Glutathione, Arginine, Histidine, Lysine, Cystine, Sulphur, Iron. Magnesium, Phosphorous, Sodium, Chlorine and most Vitamins are present and available as " nutrient factors " in the hen's egg, particularly in its " yolk ", would be of significance.

It may also be mentioned in this connection that the basal medium used in this study, consisting of trypsin digest of beef-muscle extract, glucose, agar with pH 7.2 to 7.4. did not alone support the growth of the gonococcus. While the " whole egg contents " or " Yolk " alone, supplementing the above basal medium in a strength of 5 to 10 percent, supported the growth of the gonococcus, the "white" of the age egg alone did not do so. The water soluble, dialysable, portion of the whole egg contents, was again found to support, while its water insoluble, portion did not support the growth of the gonococcus when added as " supplement " to the basal medium. The water soluble dialysable portion of the egg content was thought to be heat-stable.

This is in view of the observation in this study that when heated at 60°C for 1 hour and added as supplement, it was found to be still effective, while autoclaved at 120°C for 15 minutes that portion did not support the growth of the gonococcus in the medium.

It has not been possible yet, to identify the actual nutrient or growth factor or factors present in this egg medium and found to support the growth of 105 local strains of the gonococcus in this study. But it is reckoned that, this particular basal medium with the added fresh egg contents, obviously provides most of the essential nutrient factors that gonococci has been found to require and provided in the various other enrichment media described by previous workers in this field. Egg medium is admittedly complex with reference to its constituents, and yet it may be considered useful and comparitively more convenient and simpler medium, that may be prepared easily by the technicians in less well oquipped laboratories, so that routine culture of the gonococcus particularly in the female cases, may be made possible everywhere,

The requirements of a culture medium for the gonococcus vary according to whether it is indicated for " direct primary diagnostic isolation ", for " transport " of suspected materials for cultural diagnosis " secondary culture " for " bio–chemical " and " drug sensitivity " tests, for " maintenance " and " preservation " for " metobolic " studies, and " antigen production. ".

The egg–enriched medium, that is stable under normal temperature changes and that may be made selective with incorporation of appropriate antibiotics, has appeared in this preliminary study, to yield a high proportion of positive culture results leading to quick identification. Thus it satisfies the difficult requirements for a primary diagnostic culture medium, for the gonococcus. It has been found to be useful in preliminary trials without the addition of antibiotics, for secondary cultivation the maintenance of the gonococcus for metabolic studies. The " sensisivity " estimation of the gonococci to penicillin, has also been tried by the " plate dilution " technique and it can be carried out in this egg medium. But the media to be used for drug sensitivity had better be a " shnthetic " and made of chemically known constituents and thus capable of standardization, so that rvsults in different laboratories may be more comparable. Gould et al (1944) have reported that the growth of several strains of gonococci has been obtained in a medium of known chemical constituents. But an entirely synthetic medium suitable to support the growth of all strains of the gonococcus, under all conditions, is not yet available. No media, accepted internationally by all concerned, exists at present for the cultivation of this Neisseria. There is the need and scope for using festidious strains of the gonococcus and specimens taken from sources with much contamination such as from female genitalia and " rectum ", for well controlled comparative studies of various media now in use. The egg medium observed to have potential worth in this preliminary study of it, is offered for independent experiments with it by those interested on a more extended scale to discover the qualitative and quantitative aspects of the culture of the gonococcus in this medium.

SUMMARY

The gonococcus has been found to grow well in artificial culture in a basal medium of Trypsin-digest-Beef-Muscle extract Agar enriched with the contents of hen's egg. This Egg enrichment medium has been compared with the same basal medium enriched with Ascitic Fluid and the Bacto G. C. Medium Base supplemented with Bacto Haemoglobin and Yeast Concentrate, by essaying them in parallel series for the primary diagnostic cultural isolation of the gonococcus, from male and female patients.

In preliminary trials, tne egg enriched medium has been found to support the growth and maintenance of several local fresh strains of the gonococcus, as well as the other two conventional enrichment media, in current use.

The Egg, apparently contains yet unnamed special nutrient factor or factors required by the gonococcus to grow in artificial culture. Egg can be obtained more conveniently anywhere than the the other conventional source or growth factors from Blood, Serum, Ascitic fluid and Yeast extract, in quality and quantity. The technique preparation of the egg medium described, lends itself with case, for application in practice, in bacteriological laboratories, anywhere. Therefore, the egg medium suggests itself as a good alternative cultural medium that may used with advantage in the routine diagnosis of gonorrhoea, everywhere. The various aspects of the egg medium is discussed and it is offered for more extensive study of potential worth discovered in this study.

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REFERENCES

1. Boor, A. K. and Miller, C. P.—Proc. Soc. Exp. Biol. and Med. 50, 22, 1942.
2. Carpenter, C. M. et al—Am. J. Syph. Gon. and V. D. 33, 164, 1949.
3. Gould, R. G. et al—J. Bact. 47, 289, 1944.
4. Higginbotham, M.—Am. J. Syph, Gon. and V. D. 26, 607, 1942.
5. Lankford, C. E. and Snell, E. E.—J. Bact. 45, 410, 1943.
6. Lankford, C. E. and Skagg, P. K.—Arch. Biochem. 9, 265, 1946.
7. McLeod, J. W., et al.—Brit. J. Exp. Path. 8, 25, 1927.
8. Moller, V. and Reyn, A.—WHO, VDT., Neisseria, 3.64.
9. Mulier, J. H. and Hinton, J.—Proc. Soc. Exp. Biol. and Med. 48, 330, 1941.
10. Thayer, j. D. and Martin, J. E.—Pub. Hlth. Rep. (U. S. A.), 79, 149, 1964.
11. Reising, Jr. G. and Keliog, D. S.—WHO, VDT., Neisseria, 3.64.
12. Reyn, A. et al.—WHO. VDT. Neisseria, 2.64.
13. Walsh, M. J. et al.—J. Bact. 86, 3, 178, 1963.