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Dr. Mueller's Medium for the Gonococcus
For 1,000 c.c. Starch Agar

Reference: Mueller, J. H., & Hinton, J. *Ann. Proc. Soc. Exper. Biol. & Med., Utica*, 48: 330-333, Oct., 1941.

Directions. Add 17 g. dry shredded agar to 500 c.c. of tap water in a 2 L. flask. Autoclave at 15 lbs. for 25 min. to dissolve, (or longer if solution is not effected during that time.) While still hot, add the following solution which may be prepared while the agar is being autoclaved:

Beef heart or meat infusion.*	300 c.c.
Casein hydrolysate.**	Varies with lot No.
Starch paste.***	100 c.c.
Para-amino benzoic acid, 1%.****	5 c.c.
Water	75 c.c.

Adjust pH to 7.6 *****

If the beef-heart infusion has been prepared ahead of time, reheat in steam-bath. Add 75 c.c. hot water. The whole mixture must remain hot while tubing, or else it will solidify and become too difficult to handle. It may be distributed at once either into previously autoclaved and stoppered test-tubes (about 20 c.c. each for pours, 5 c.c. for slants) or into flasks of 120 to 200 c.c.. Original directions call for autoclaving not more than 10 min. at 10 lbs. In our laboratory the time has been extended to 30 min. with no bad effects to the strains employed.

Over autoclaving spoils the medium for the more delicate, recently isolated strains. The flasks can be used to pour plates at once. The tubes may be melted in boiling water and used as needed. Cultures should be placed in a tin can or jar with a tightly fitting cover. A lighted candle is lowered into the container, the lid put on, and the covered container incubated at 35°-36°C for 18 to 24 hours.

* Meat infusion:- 450 gm. meat (chopped lean beef or beef-heart from which all fat has been removed) - 500 c.c. water. Suspend meat in water, bring to active boiling, strain through cheese-cloth and filter through paper, Autoclave in 200 c.c. quantities, 10 min., 10 lbs., and

store in ice-box, in stoppered bottles with a few c.c. of chloroform.

** Casein hydrolysate:- Difco product supplied under trade name "Casamino Acids". This consists in a complete hydrochloric acid hydrolysate of casein from which the greater part of the acid has been removed by vacuum distillation, and the remainder neutralized with sodium hydroxide. The resulting solution has been decolorized with charcoal and dried. The material contains considerable salt. The quantity to be used must be determined, at present, for each lot. Lot S-64123 calls for 17.5 g. for 1,000 c.c. of medium, Lot Rx No.42228, Ref. No.340245 for 10 gm.

*** Suspend 1.5 g. ordinary starch (corn starch or laundry starch, not "soluble starch") in 10 c.c. cold water. Pour slowly into 90 c.c. boiling water, while stirring and bring to active boil.

**** Para-amino benzoic acid:- Suspend 1 g. in 75 c.c. water. Add strong sodium hydroxide, drop by drop, with shaking until dissolved, (about 0.3 gm. NaOH required.) Dilute to 100 c.c.. The solution keeps well. This is used to counteract the effect of the sulfonamide drugs used in the treatment of cases from which strains have been isolated. This solution can therefore be omitted if laboratory strains of gonococcus are being exclusively used.

***** Titration and adjustment of reaction may be carried out as usual, or if desired, 2 c.c. of 0.2% solution of phenol red in sodium hydroxide may be added per liter, and enough acid or alkali then added to bring the batch to a definite red color. (18 c.c. of 1 N NaOH added to 1 L. of material as prepared in this laboratory, while Dr. Mueller's technician adds 28 c.c. of 1 N NaOH.