# Southern Branch

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## Preparation of Culture Media for Routine Cultures of Feces for Pathogenic Amebas.

CARLO J. TRIPOLI. (Introduced by F. M. Johns.)

*From the Laboratory of Clinical Medicine, Tulane University of Louisiana.*

As an aid to the microscopic examination of feces for the diagnosis and differentiation of pathogenic amebas, routine cultures should, theoretically, occupy a prominent place in the clinical laboratory. As described in the literature, Dobell's modifications[1] of Boeck and Drbohlav's original media[2] for the cultivation of pathogenic amebas is apparently best suited to routine use, as it furnishes a definite source of assimilable carbohydrate as food for the amebas, and further provides a mild antiseptic (acriflavine) which inhibits the growth of such frequently found bacterial flora as would preclude the obtaining of a positive culture. In obtaining and maintaining a large number of cultures of *Endameba histolytica* for the past year, many details of producing a uniformly successful medium have been studied. The following method in detail, of preparing these media has seemed to give uniform results:

I. *Ingredients of media:*

(a) Serum-Ringer solution: Beef or human blood (preferably the latter) is obtained and after clotting is placed for 24 hours in a refrigerator. The serum is pipetted off and mixed with Ringer's solution in the proportion of one part of serum to 8 parts of Ringer's solution.[3] Sterilization is accomplished by passing the fluid

[1] Dobell, C., and Laidlaw, P. P., *Parasitology*, 1926, xviii, 206.

[2] Boeck, W. C., and Drbohlav, J., *Am. J. Hyg.*, 1925, v, 371.

[3] Ringer's solution consists of NaCl, 9.0 gm.; KCl 0.2 gm.; $CaCl_2$ 0.2 gm. to 1000 cc. distilled water.

[4] Seitz-Wertz filters made by Empire Laboratory Supply Co., Inc., N. Y., model No. 1787/3.

through the *large type* Seitz-Wertz filter.[4] This filtered, sterile, diluted serum is pipetted off with a 50 cc. sterile pipette and placed in sterile flasks, 50 cc. to 100 cc. to each flask. These flasks are kept as cold as possible until ready for use.

(b) Starch: The best kind of starch is rice starch.[5] It possesses small grains, and is not easily hydrolyzed. This is weighed out in 0.2 gm. quantities, placed in small filter paper tubes which are made by rolling a 3½x5 cm. piece of thin filter paper about a pencil and in which the starch is kept in place by lightly crimping the ends of the paper tube. The starch in the tubes of filter paper is placed in test tubes which are then plugged with cotton and covered with several layers of wrapping paper which is well tied down over the end of the tube. The tubes are autoclaved at 15 pounds pressure for 15 minutes. After autoclaving, the paper covering is removed and the tubes are placed in a hot air oven or incubator to evaporate the little moisture that has condensed upon the walls of the tube.

(c) Acriflavine solution: A 1% solution of acriflavine[6] is prepared and sterilized in the autoclave. Acriflavine solution keeps fairly well when protected from light, preferably at a low temperature.

(d) Egg base: 4 eggs are emulsified with 50 cc. of Ringer's solution and poured into 1½x15 cm. tubes to a depth of approximately 2 cm. The tubes are autoclaved at 15 pounds pressure for 15 minutes in the upright position, which coagulates and sterilizes them, giving a flat surface upon which the amebas grow best. The pressure in the autoclave should be raised and lowered slowly in order to prevent bubbling of the media. The tubes are kept in the refrigerator until ready for use.

II. *Assembly of media:*

(a) The starch is added (0.2 gm.) to the serum-Ringer solution (50 cc.), placing the small paper tubes containing the starch into the flask. By slight agitation the paper opens up and liberates the starch. (b) Acriflavine is added by transferring 0.1 cc. of the 1% solution, which gives an ultimate dilution of 1-50,000. Some samples of acriflavine require a greater concentration (1-25,000) to retard the growth of unfavorable flora. (c) 4 to 5 cc. of sterile serum-Ringer's solution plus the starch and acriflavine are then poured into the tubes containing the coagulated egg. A preliminary

[5] Rice starch prepared by Eli Lilly & Co. (Lilly's authentic starches).

[6] Neutral acriflavine, "National," the National Aniline and Chemical Co., N. Y.

inactivation of the serum has not been found necessary. The tubed culture media should be stored in the refrigerator until used. At the time of inoculation the media should be warmed to 37° C. An equal number of tubes should be prepared omitting the acriflavine. The pH of the prepared media varies from 7.2 to 7.8 and "needs no adjustment."

III. *Method of Inoculating, Examining and Transplanting Cultures:*

A particle of fecal material about the size of a small green pea is pickel up on a wooden applicator and is transferred to a tube of plain and a tube of acriflavine charged media which have been warmed to about body temperature. With soft and liquid stools, which are more apt to contain vegetative amebas, the material used for inoculation should be obtained fresh from the patient. Proctoscopic removals, rectal washings, etc., should similarly be inoculated immediately. With formed or hard stools, which are more apt to contain cysts, the cold stool will suffice even though it be several hours old. We have obtained the same results with cysts as obtained fresh from the patients and with those several hours old.

The tubes are incubated in an upright position at 37° C.

The growth of amebas reaches its maximum in from 48 to 72 hours.

Examination of the cultures is made by skimming the debris of starch and bacteria from the surface of the coagulated egg with a capillary pipette having a large lumen (1 mm.) which has been nicked and broken with a square tip, and equipped with a Wright's rubber bulb. A drop of material is removed, placed on a slide, cover glass added, and examination made with 16 mm. objective.

To transplant the culture a drop of the material is transferred to fresh media.

Initial growth is usually obtained in both plain and acriflavine charged media. Occasionally one or the other variety only will give a positive culture.

In a large series of cultures all proven cases have given cultures of the pathogenic amebas. Non-pathogenic strains occasionally grow, but do not withstand successive transplanting in the above 2 media described. Fresh cultures should be "carefully nursed" and transplanted every 36-48 hours. Older cultures may be transplanted every 3 to 6 days.