

A MEDIUM FOR
THE CULTIVATION OF
T. CRUZI AND THE
LEISHMANIAS (OFFUTT)



Difco Blood Agar Base is a convenient and satisfactory foundation medium for the cultivation of *T. cruzi* and the Leishmanias from stock cultures and animal sources.

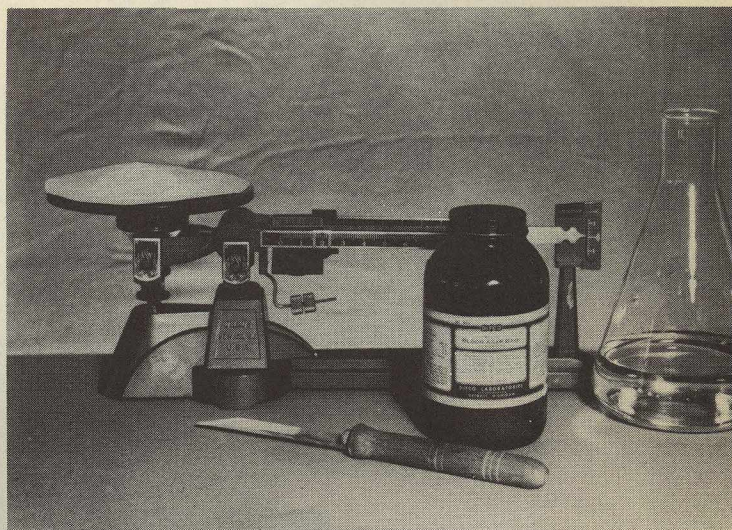
The base is prepared as directed on the bottle and sterilized at 121.6° C. for 15 minutes. It may be stored in the refrigerator for several months until needed. The base is then melted, 5 percent fresh rabbit blood added, the medium tubed in 4 to 5 cc. amounts, and slanted. There will be very little water of condensation formed, so about 1 cc. of sterile normal saline is added to each slant, and the tubes incubated at 37° C. for 24 hours to test for sterility.

This medium may be inoculated with blood, bone marrow, and tissue from spleen and liver. It is essential that aseptic precautions be used in the inoculation and handling of cultures, for none of the blood flagellates will grow in the presence of bacteria or fungi. The cultures are incubated at 22° to 25° C. for at least 2 weeks. Addition of a rubber stopper prevents evaporation of the fluid at the base.

Stock cultures of these organisms grow very well on this medium. Transfers every 14 days give most satisfactory results, but the organisms will remain viable much longer if the proper temperature is maintained.

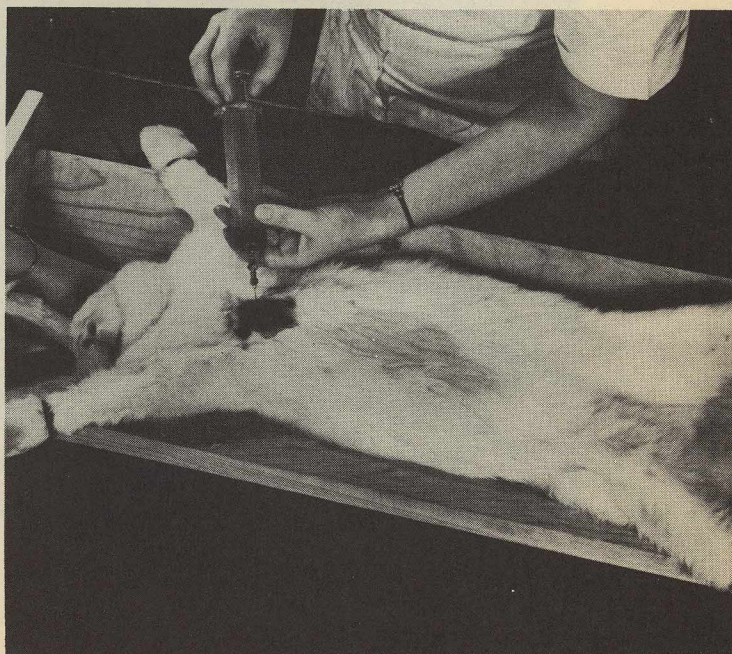
Sterile rabbit blood is obtained by direct heart puncture.

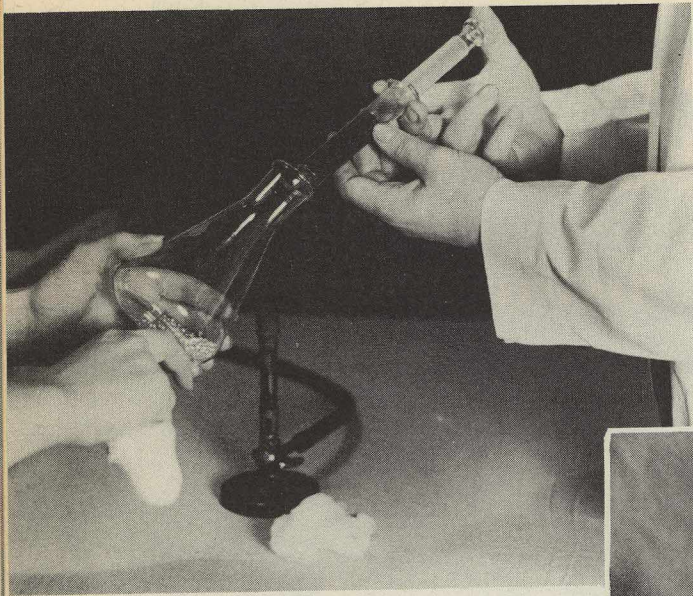
The hair is shaved off an area over the heart and the skin cleaned with alcohol and painted with tincture of iodine. A sterile 10 cc., 20 cc., or 50 cc. syringe may be used, with an 18 or 20 gauge needle, depending on the amount of blood desired.



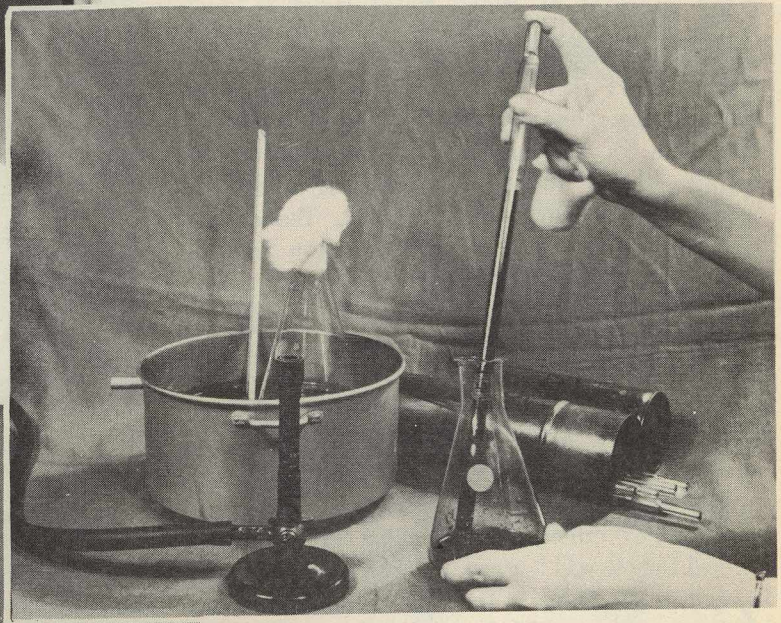
Difco Blood Agar Base is a convenient foundation for the cultivation of *T. cruzi* and the Leishmanias.

Eight grams is suspended in 200 cc. of cold distilled water and dissolved by boiling. It is sterilized at 121.6° C. for 15 minutes and stored in the refrigerator until ready for use.

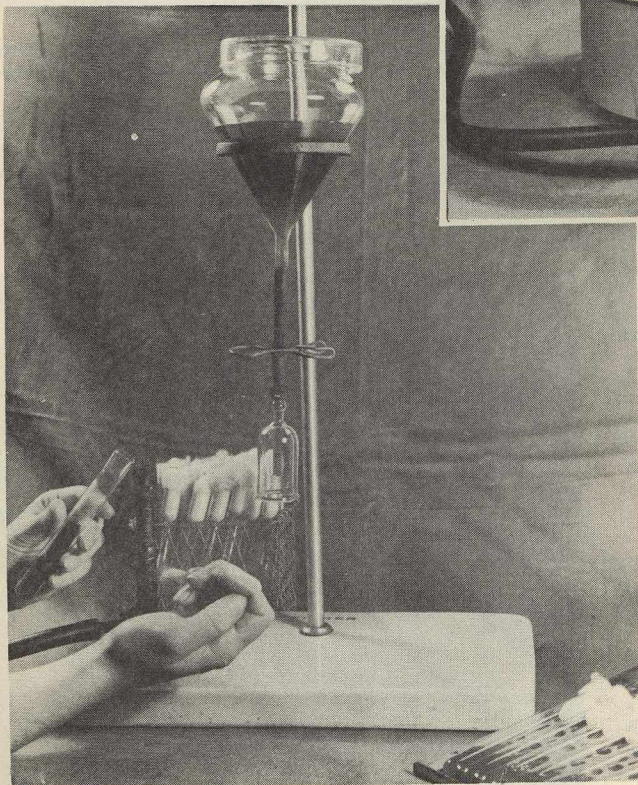




◀ The blood is defibrinated by shaking with glass beads in a sterile flask.

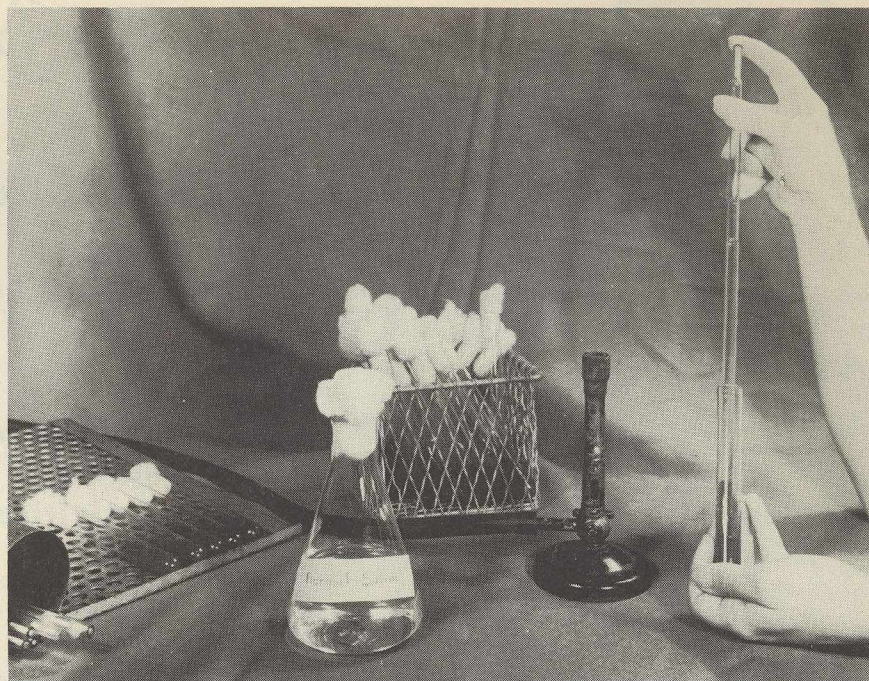


▲ The agar base is melted and cooled to 45° - 50° C. and 10 cc. (5 percent) of the defibrinated blood is added to each 200 cc.



◀ The medium is tubed in 4 - 5 cc. amounts with a dispenser and allowed to solidify in a slanted position.

About 1 cc. of sterile normal saline is added to each slant. ➡



To test the completed medium for sterility, the tubes are incubated for ➡ 24 hours at 37° C. and examined for bacterial contamination.

