

AN IMPROVED OUTFIT FOR THE ISOLATION OF BRUCELLA, SALMONELLA, ETC. BY BLOOD CULTURE*

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The method developed by the author for cultivation of blood in brucellosis (1, 2) requires a layer of agar medium placed on the side of a bottle while a certain amount of broth remains at the bottom of the same bottle. The usefulness of this double medium relies on the facility of detection of positive cultures without cumbersome transfers from liquid to solid media. From those who have adopted this method some use rectangular bottles, others use cylindrical bottles but in all cases there is some trouble with the layer of agar which may drop from the wall particularly when the outfit is exposed to careless handling. Because of this inconvenience, the bottle has not been widely used in laboratories or clinics without proper facilities to prepare the outfit.

In order to correct the indicated deficiency we endeavoured to obtain a bottle with characteristics detailed in sketch on page 565, in which there is a sort of cavity at one side resulting from a double depression in the mould which produces inside ridges along the walls of the bottle. The agar medium is allowed to harden in this "canal" and remains in a stable position capable of standing rough handling and shaking. The liquid medium has no deteriorating effect on the slant and the outfit is properly protected, first with a rubber stopper (the size of those that are supplied with penicillin vials), which is held in a tight position by a perforated metal cap which is further pro-

ected with another cap screwed over the top of the bottle. Media prepared in this outfit may be safely mailed with no loss in the shape of the slant. A further advantage of this outfit consists in the possibility of referring positive cultures to specialized laboratories by the simple procedure of removing aseptically the mixture of broth and blood, replacing the stoppers and sending the bottle through the mail.

Instructions for use of the new bottle

The agar proportion of the solid medium may be raised to 3.5% in order to insure better slants. About 15 ml. of the melted agar are placed in the bottle and the wider metal stopper screwed in place. The bottles are sterilized at 15 lb. for 20 minutes and the medium allowed to harden in the "canal". Then the broth, previously sterilized, is added in amounts of about 15 ml. and the bottle stoppered with the rubber stopper supported by the perforated metal cap. The wider metal cap is finally replaced. When the CO₂ is required this may be added after injecting the blood by means of a syringe previously filled with the gas which has been filtered through sterile cotton. Before use, the bottles are tested for sterility by incubating 10 days at 37° C. Transfer from broth to slant is performed by allowing the mixture of blood and broth to run over the agar at intervals of 48 hours. The negative bottles are discarded 20 to 30 days after being inoculated.

* Document WHO/Bruc/110, 1 June, 1955.

REFERENCES

- (1) A practical method for routine blood cultures in brucellosis, *Proc. Soc. Exp. Biol.*, 64:114, 1947.
- (2) Joint FAO/WHO Expert Committee on Brucellosis, 2nd Report, *Wld. Hlth. Org. Techn. Rep. Ser. No. 67*, May 1953.