RECLAMATION OF USED AGAR

By JAMES BRODIE, M.D., D.P.H. and DAVID STIVEN, D.Sc., Ph.D.

From the Department of Bacteriology, University of St Andrews, Medical School, Dundee

Owing to the entry of Japan into the war it is essential that agar should be used as economically as possible. Since October 1941, we have, in this laboratory, reclaimed used agar by the following simple method, which is a modification of the procedure described by Huang, Shen & Tang (1941).

The media from which the agar can be reclaimed by this procedure include Brilliant Green Eosin Agar, MacConkey's Neutral Red Agar, modified Leifson's Agar containing rosolic acid, ordinary Nutrient Agar, in short, all media except those containing tellurite. Attempts were made to reclaim these last without success.

The procedure is as follows.

Used plates, tubes, etc., are melted in the autoclave, and the agar is bulked, being poured into a small open dish to a depth of 1–2 in. When set this is chopped into fingers of about $\frac{1}{4}$ in. thick, and is collected in a wide-mouthed glass jar of about 3 l. capacity containing enough 10–20% alcohol to cover the agar. The agar is so collected until $1\frac{1}{2}$–2 l. are obtained. The mouth of the jar is now covered with 'linen scrim' and a glass tube passed through this to the bottom of the jar. Using rubber tubing, the glass tube is fitted to the water supply, the jar being placed in a sink, and cold water is allowed to run slowly through the material for at least 2 days. The preliminary treatment with alcohol has the advantage of removing a great deal of dye; thereafter the other constituents are removed by diffusion.

The excess of water is now drained off and the washed agar is melted in the autoclave, cooled to about 55° C., the *p*H adjusted to 7·6, and ox serum, one part to twenty of melted agar, added to clear. Steam for 40 min. and filter in a Buchner funnel through paper pulp. Distribute in shallow trays, allow to set, chop up and place the trays in a 55° C. incubator or a drying oven at between 50 and 80° C. After 24 hr. turn the drying agar and replace in the oven till dry.

When dry, scrape the agar off trays and store it in a screw-capped jar.

To convert reclaimed agar into nutrient agar, 25 g. of agar are required for every litre of medium. The agar is mixed with such a quantity of water that on the addition of nutrients, the final volume will be 1000 c.c. The procedure is to mix the agar with the water and dissolve in the autoclave. Remove the agar from the autoclave and allow it to cool to 55° C., adjust the *p*H to 7·6 and add ox serum in the proportion of 1 : 20. Also add the requisite volume of nutrient at 55° C. and *p*H 7·6 (Gladstone & Fildes (1940), very satisfactory). Steam the mixture for 40 min. The product is now filtered while hot through a Buchner funnel with paper pulp. At this stage it is necessary to standardize the strength of the gel according to the method described by Jenkins (1921), so that if the agar is too stiff it may be suitably diluted before being distributed into bottles which are steamed for 30 min. on three consecutive days to sterilize, after which they are ready for storage till required for use.

Stock non-nutrient agar may be made in the same fashion taking 25 g. of reclaimed agar to every 500 c.c. of water, the nutrient being added later as desired.

It is essential that a Jenkins test be performed since the setting qualities of different agars vary. The weight of agar herein stated has been found by experience to yield a product requiring the minimum of dilution.

Media made from reclaimed agar need not contain a percentage of new agar, for meningococci, gonococci and pneumococci grow well on media made as above.

In conclusion, it should be noted that no agar has been allowed to go to waste in this laboratory since October 1941, except that containing tellurite, and that the reclaiming procedure was applied to reclaimed agar over and over again without detriment.

CONCLUSIONS

1. Used agar can be reclaimed by the simple method described.
2. Reclaimed agar can itself be reclaimed many times without apparent detriment.
3. Media made from reclaimed agar are as good as media made from fresh agar.

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