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A Method for the Reclamation of Agar.

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The possibility that a shortage of agar may occur in the near future has prompted experiments to devise a simple method for the reclamation of used agar media. Methods of reclamation reported by several German bacteriologists during World War I have not proved entirely satisfactory in the Laboratory, Station Hospital, Fort Bragg, North Carolina.

It has been found that plain nutrient agar media, which are extensively used, may be easily and satisfactorily reclaimed by employing an alternate freezing and thawing technic. Probably this process is not feasible from a commercial standpoint. However, it has proved successful where one is concerned with relatively small quantities. With simple modifications the procedure can be adapted to the purification of Endo's agar, eosin-methylene-blue agar, or any other dye containing agar media. Although not yet attempted, agar media containing sugar can probably be similarly reclaimed. Blood agar medium may be treated in the same manner as plain nutrient agar.

The procedure for reclamation of plain nutrient agar without added dye is here described. Pool the agar medium to be processed and autoclave at 15 lb. pressure for 15 min. Adjust the reaction to pH 7.0. Place the melted agar in an ice cube tray and permit to solidify at room temperature. Make parallel cuts lengthwise and crosswise of the agar mass. Freeze completely in the freezing compartment of an electric refrigerator. Break the frozen mass into conveniently sized pieces, place in a gauze sack and let thaw at room temperature. The water carries with it much of the unused soluble ingredients and impurities leaving partially purified solid agar which is insoluble in cold water. Add sufficient distilled water to double the original volume of medium. Melt thoroughly in a boiling water bath or Arnold steamer and cool to 55°C. Add egg white, with thorough stirring, in the proportion of the white of one egg for each 2 liters of media. Boil until coagulation is complete, then filter through a hot wet absorbent cotton filter. (If available, a filter with steam or boiling water jacket will simplify filtration by permitting use of filter paper). Solidify by standing at room temperature, cut into cubes and freeze in the ice compartment as before. Break up the mass and let thaw in a coarse paper filter. Add sufficient distilled water to the residuum to make one-half the original volume. Then again melt, solidify, cut into cubes, freeze and thaw on coarse paper filter as before. Air dry the resultant agar masses at 55°C.

For the reclamation of agar media containing dye, it is advisable, after the first freezing and thawing, to place the solid agar in a washed salt or sugar sack and dialyze in running water for 12 to 24 hr. Most of the dye will be removed by this treatment. If the agar is to be used again in the preparation of the same dye medium, it is necessary only to complete the process as outlined above. Complete decolorization of the agar may be accomplished, if desired, by boiling with animal charcoal.

Dried agar reclaimed by this procedure was used in the preparation of nutrient agar and compared with standard nutrient agar (Difco). Preliminary experiments have shown no significant difference in these two media when used in parallel plate count determinations.