Procedure for the Transfer of Small Discs of Agar-Containing Media Under Sterile Conditions and Some Applications of This Technique (Agar Disc Auxanography)

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Small agar discs are formed and transferred under sterile conditions. This technique permits the efficient and versatile use of the petri plate when many growth conditions and (or) cultures are being examined.

A number of applications of the petri plate have been designed to accommodate more efficiently studies involving the responses of organisms on solid media to a large number of nutritional conditions or the growth characteristics of large culture collections or both. These applications include the replica plating technique (4) as developed for this application (5), the auxanographic technique attributed to Beijerinck which continues to be of use for this purpose (3) and serves as the basis for the use of antibiotic sensitivity discs (1, 2), and the introduction of compartmentalized versions of the standard plate. The former methods suffer from certain disadvantages (2, 5), including problems related to the diffusion of the different substances used, cross-feeding, and the effects of different growth rates and motilities of the organisms employed. The last approach involves tedious media preparation or, if the plates are predispensed, may be too expensive for routine use, lack versatility, and expire. The present technique is free from these disadvantages and lends itself well to a number of unique applications.

The simple tool employed with the technique is assembled from 15-cm lengths of well-rounded glass rods 5 mm in diameter which have been individually selected to fit loosely into standard plastic household drinking straws 0.25 inch (0.63 cm) in diameter (Fig. 1A). All plastic straws we have tested can be autoclaved. The brand illustrated, manufactured by Union Carbide Corp., New York, N.Y., is generally available and possesses the necessary characteristics. The straws are cut to one-third length and autoclaved in wide-mouthed jars. The sterilized rod is inserted all the way into a sterile straw (Fig. 1B), tipped slightly from the vertical position, and carefully worked out of the jar. The fitted rod is inverted, and the straw is permitted to slide back until the tip of the rod is showing and is held in place with the finger (Fig. 1C). A piece of narrow tape is applied to the glass next to the straw in this position to prevent further retraction of the straw (Fig. 1D). The operation of the tool is illustrated with the straw retracted (Fig. 1E) and extended (Fig. 1F) by the action of the index finger. The tool will withstand a quick flaming from time to time as a precaution against air contaminants, but if the tip ever comes into contact with a nonsterile surface the straw should be discarded, the rod flamed with alcohol, and refitted.

Agar discs are cut by inserting the tool (with straw extended) directly down into the agar and to the glass surface beneath (Fig. 1G, left). The tool is then tipped from the perpendicular (Fig. 1G, center) and lifted straight up (Fig. 1G, right), the compartment formed by the straw and rod usually containing the small agar disc. The loaded tool is brought very close to the position on a dry empty petri plate, on which the disc is to be deposited, maintaining the nearly horizontal position (Fig. 1H) and then brought to a vertical position keeping the tip very close to the glass surface (Fig. 1I, left). If the agar disc does not fall onto the plate, the glass rod is extended, whereupon the disc usually falls in place (Fig. 1I, right). If the consistency of the medium is such that it sticks to the rod, it may be deposited by gently touching it to the glass surface and withdrawing the tool. The discs can be precisely aligned and, if tipped on the side, stood on end by the gentle action of the tool. Agar discs can best be prepared from a standard plate 9 cm in
FIG. 1. Agar disc auxanographic technique. (A-D) Construction of the tool, (E-F) operation of the tool, (G-I) formation and transfer of agar discs, and (J) completed auxanographic plate. The top row of agar discs in (J) are in the process of taking up nutrients from paper discs. A square cut from graph paper has been taped to the bottom of the plates in H and J and serves as a convenient means of identifying discs and recording results.
diameter poured with 30 ml of 1.5% agar, giving discs 5 mm in depth and 6 mm in diameter (0.14-ml volume) when first prepared.

Agar discs are usually inoculated from a master disc at the side of the plate to provide for a dilution of the original culture by touching the center of the disc with a lightly inoculated needle. Growth is determined by visual observation of the agar surface and is recorded as positive or negative after an incubation period sufficient for full development of positive cultures. However, the development of the culture on the disc can be followed conveniently with the microscope. The diameter of the growth area may have some quantitative significance with some organisms, since a standardized amount of media is employed with this technique and the cells can be conveniently resuspended if a turbidimetric check on the amount of growth is desired. The freshly inoculated plates are sealed in a plastic bag and placed on a level vibration-free shelf and incubated (upside down if elevated temperatures are used). The discs adhere well to the glass surface, their tendency to slide over the surface of the plate being eliminated if these incubation conditions are observed, dry plates are used, and the recommended 1.5% agar concentration is not significantly reduced. For extended incubation, an additional plate should be included containing a little water to help prevent dehydration. Contamination of the plates has been surprisingly infrequent when the procedure described here is followed, perhaps because of the small nutritive surface area. When it does occur, it is frequently recognized and often easily eliminated from the plate.

Disc auxanography is especially adaptable to and economical for the taxonomic investigation of large numbers of isolates. For example, by providing a large microbiology class with a single plate with each of 25 different media containing separate catabolites found to be of use in the classification of pseudomonads (reference 5, p. 181), auxanographic plates can be assembled similar to that of Fig. 1J for the evaluation of 100 fresh isolates. Appropriate arrangements can similarly be made for the taxonomic evaluation of other groups of organisms, the method permitting the inclusion of type cultures and media composition controls on the same plate used with unknown bacteria for careful comparisons and more rigorous determination. The auxanographic plate is ideal for the determination of the nutritional requirements of organisms capable of growing on defined media containing all of the commonly encountered microbial growth factors but unable to grow on simple media. The complex medium is separately formulated with individual components missing for each component, and an auxanographic plate is assembled with this variety of media. The growth responses of an organism to the individual deficiencies suggest the minimal nutritional requirements of that organism. Some preliminary results suggest that the disc technique might also be used to investigate the mineral metabolism of organisms on solid media. I have found that the agar discs may be extracted with (and sterilized by) chloroform containing 8-hydroxyquinoline in a manner similar to that used with liquid media to extract iron (6). Discs treated in this manner will not support the growth of many organisms in the collection of this laboratory unless iron is specifically introduced. The auxanographic plate should be advantageous in the extensive evaluation of the antibiotic sensitivities of organisms. Not only may several organisms be examined against several antibiotics on the same plate, but it becomes practical to include routinely serial dilutions of the antibiotic concentration by means of a row of agar discs and thereby to determine the degree of sensitivity of the organism to the antibiotic. Since the concentrations of the antibiotic are defined and the final result is not influenced by small differences in the growth rates of the organisms tested, several of the variables encountered in the more commonly employed methods of determining the degree of sensitivity by inhibition zone analysis (1, 2) are eliminated.

Although the agar disc auxanographic method presented here economizes in the use of media, it also encourages the use of a greater variety of media. Although this is desirable from the standpoint of obtaining a greater definition of microbial cultures on a more routine basis, the preparation of so many different original plates for the disc formation can be very tedious. I have found that this chore can be eliminated by preparing a single basal medium for disc formation and introducing variations by means of sterile paper discs impregnated with the various media components. The following information should be helpful to those wishing to try this approach. I have found that approximately 1 ml of a 20-fold concentration of a medium component will saturate a 9-cm Whatman 42 paper circle without dripping. If such a paper is dried and small paper discs (7 mm in diameter) are punched from it with a standard paper punch and sterilized, the discs so obtained when placed on the agar discs described here (Fig. 1J, top row) will reconstitute approximately the normal medium concentration of the component when the system reaches equilibrium. I have found that those components that are freely soluble at the 20-fold concentration will pass into (but will not equilibrate
throughout) the agar phase to the extent of 95% or better in 30 min. Those compounds which are not freely soluble at the 20-fold concentration should be included directly in the agar, or papers should be prepared by successive treatment with more dilute solutions or with suspensions of the compounds. However, the compounds do not pass rapidly into the agar from such papers, and such discs are best used by setting the agar discs on top of the papers and leaving them in place during the incubation. Once a large collection of appropriately impregnated paper discs has been prepared for the many applications of this technique and conveniently stored in small vials, the various complicated auxanographic plates described here can be elaborated without delay and the laboratory will have acquired a little facility of considerable potential.

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