TECHNICAL METHODS

A Simple Apparatus for Shaking Bacterial Cultures

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The importance of shaken cultures as compared with stationary cultures is now well established. This paper describes a simple shaker which, it is believed, will be particularly valuable in routine laboratories. It is made of slotted angle "dexion" to fit a suitable water-bath. Owing to its rigid construction it may be removed from the water-bath freeing the latter for its original purpose. The shaker is provided with rubber feet and may be employed standing by itself as an agitator at room or refrigerator temperatures. The cultures may be grown in optical tubes, flasks, or bottles. A brief description of some typical results obtained with this machine is included.

Materials and Methods

The Shaker.—This is illustrated in Fig. 1. In essence it consists of a rigid framework of "dexion" which fits closely over the bath employed, but it is detachable and has rubber feet so that it will stand quite safely on the bench. At one end is a single-speed electric motor which drives an overhead rod. The number of swings per minute is controlled by means of the two pulleys and driving belt. The vessels to be shaken are either clipped on to a bar some 5–6 cm. below the rod or else on to a horizontal plate suspended in the water. This plate carries two rows of Terry clips which are rubber-covered to prevent them scratching the optical glass tubes.

In the machine illustrated the speed in complete cycles/min. is variable from 30 to 90. Above 90 cycles/min. the contents of the bath splash a great deal when large flasks are used. It was decided to use a motor (with a reduction gearing giving a fixed speed), with a condenser in the circuit rather than a variable speed motor (Klaxon, 49, Upper Brook Street, W.1, output torque 4 lb. in., 110 r.p.m.). This was done because it is not uncommon for the electric power to fail and then suddenly return with unfortunate effects on the second type of motor. With the fixed-speed motor one may safely leave the shaker running for days on end without attention.

In order to shake vessels of varying shape it is convenient to be able to vary the stroke. In Fig. 1

"view on arrow B" shows the connexion of the overhead rod with the gear wheel. This connexion is attached to the wheel by means of a milled-headed screw which, it will be seen, can be screwed into any one of eight positions. These alternative positions are dispersed on a curved line from the centre to the periphery.

Bacterial Cultures.—In the experiments described here two coliform organisms were employed, namely, motile coli K12 and non-motile coli NCTC 6064. These cultures were usually shaken in the optical glass tubes described elsewhere (Gorrill and Gray, 1956), but equally good results were obtained with conical flasks.

Media.—Good results were obtained with tryptic digest broth from the Southern Group Laboratory, Park Hospital, London, S.E.13. Buffer 56 (Monod, Cohen-Bazire, and Cohn, 1951) was used for making the dilutions.

Counting.—Serial dilutions were made in buffer using 0.1 ml. pipettes and 9.9 ml. volumes of buffer. The pipettes were made from "pyrex" glass tubing internal bore 1 mm. and overall diameter 5–8 mm. The tubing was drawn into blunt-nosed pipettes 20 cm. long. They were stored in copper cans 25 cm. long by 7 cm. diameter. The pipettes were calibrated to contain 0.1 ml. by the use of a weighed quantity of mercury. Tenfold steps were interposed where necessary by using 1 ml. pipettes and 9 ml. of buffer. Counts were made by adding 0.1 ml. of the dilution to 3 ml. of digest broth containing 0.7% agar held at 46° C. When mixed the agar was poured over an infusion agar plate and allowed to set. Only plates with between 40 and 200 colonies were counted. Duplicate dilutions were made and two plates poured from each dilution.

Results

This shaker has been in use for over a year and has shown itself to have considerable practical value. It is capable of producing higher densities of bacteria more quickly than the equivalent stationary culture. This is well shown in Fig. 2, which gives the results of shaking *E. coli* K12 at different speeds compared with a stationary culture. It will be seen that while the rate of shaking between 30 and 90 cycles/min. has little effect on the growth rate, the difference between shaken and unshaken cultures is considerable. Similar types of curves have been produced with a wide range of bacteria. In the experiment illustrated in Fig. 2 the inoculum was derived from an overnight broth culture, but equally good results can be obtained

FIG. 1.—The design of the shaker.

FIG. 2.—The effect of speed of shaking on the growth curves of *E. coli* K12.

when a bacterial colony picked off a plate is used as the inoculum. This is of value in producing broth cultures for subsequent antibiotic sensitivity testing. If the inoculum size is important as it is with some antibiotics, the cultures should be shaken to a standard turbidity before use.

It has been the custom to carry out dilutions and counts using cold (4° C.) buffer, as this prevents further multiplication. The buffer is without effect on the bacteria tested, an important point in view of Wilson's findings (1922, 1935) that many diluents were bactericidal. However, it was possible that during logarithmic growth the actively dividing cells would be susceptible to cold shock (Hegarty and Weeks, 1940), and killed when diluted into ice cold buffer. With the organisms tested the phenomenon of cold shock has not been seen, but it would be as well to test the susceptibility to dilution at 4° C. of any organism before using this method.

Apart from the obvious uses of the shaker there are others that have been developed. Only one will be described here, and that is the maintenance of cultures in the continuous log. phase with a known mean count. The method is illustrated in Fig. 3 using *E. coli* 6064

as the test organism. A series of tubes were set up in the bath containing 9.9 ml. of broth, and the first inoculated with 0.1 ml. of broth culture. Every two and a half hours 0.1 ml. was removed from the growing culture and transferred to the next broth tube. By this means log. cultures may be maintained throughout the working day. The optimum time of transfer and the volume transferred varied with the culture used. Only by using the correct proportions was it possible to maintain the mean count at a steady level.

Discussion

It is believed that the great merit of this shaker is its adaptability. While being built to the size of an available bath it may be easily modified to fit a new bath owing to the versatility of the "dexion" from which it is made. Similarly, it is strong enough to be lifted off the bath and stacked out of the way when not in use. Finally, it has a number of uses as a shaker at room and refrigerator temperatures.

Antibiotic sensitivity testing is an important part of every routine laboratory's work, and it is undoubtedly important to employ actively dividing cells at a reasonably constant viable count as the inoculum for either plate or tube tests. By the use of this shaker colonies may be picked into broth and the resulting culture tested in the afternoon.

The application of this machine to the production of a continuous log. phase culture has been included to show that it has considerable applications in research.

Summary

A simple, robust shaker designed to fit over an existing water-bath or to function standing alone is described. It is easy to make and may be adapted to individual needs. Some examples of its use have been briefly described.

FIG. 3.—*E. coli* 6064 maintained in the logarithmic phase of growth.

It is a pleasure to acknowledge the assistance of the workshop at Guy's Hospital Medical School where the shaker was built. Our thanks are due to the Medical Illustration Department who produced the figures.

REFERENCES

- Gorrill, R. H., and Gray, R. A. (1956). *J. gen. Microbiol.*, **14**, 167.
 Hegarty, C. P., and Weeks, O. B. (1940). *J. Bact.*, **39**, 475.
 Monod, J., Cohen-Bazire, G., and Cohn, M. (1951). *Biochim. biophys. Acta*, **7**, 585.
 Wilson, G. S. (1922). *J. Bact.*, **7**, 405.
 — (1935). *Spec. Rep. Ser. med. Res. Coun. (Lond.)* No. 206.

Perspex Jars for Pathological Museums

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During the past 10 years "perspex" has become the accepted material for constructing jars for the display of pathological specimens. While it is now possible to embed specimens in solid blocks of transparent plastic, the jar containing fluid continues to be the popular choice. The elasticity of the material allows the jar to be completely filled with fluid giving an effect of solidity, and the specimen can easily be removed for further histological examination at any time.

A plastic jar to be satisfactory should be easy to make, remain free from leakage, and be perfectly rectangular so as to take full advantage of the optical properties of the material.

Many of the early difficulties in construction have been due to failure of cemented joints, since the nature of the material makes it almost impossible to obtain machined surfaces of sufficient accuracy to employ simple butt joints. The method to be described has avoided these difficulties and the jars have stood rough usage for up to 10 years without evidence of breakage.

For permanent joints in large containers which are submitted to considerable stress, the makers recommend their cold welding process. In this process the joint is filled with a methacrylate dough which is then polymerized by exposure to ultra-violet light. The resulting join has similar properties to the parent material (Imperial Chemical Industries Ltd., Plastic Division, I.S. Notes, 343 and 141).

Method of Construction and Materials

The method depends on the use of rebated joints for the body of the jar. This type of joint enables the solvent to correct minor irregularities in the cut edges and checks any tendency for the plastic sheet to bend while the cement is hardening. In order to obtain sufficient accuracy, the pieces for the jar are first cut out roughly with a band saw and then trimmed accurately to size by removing a thin shaving with a trimming cutter. In this way heat distortion is reduced to a minimum.

Since perspex is a bad conductor of heat, the cutting tools should be sufficiently heavy to conduct the heat away from the cutting edges. The use of fine-toothed slitting saws should be avoided.

Perspex is supplied in convenient stock sheets measuring 3×4 ft., and three thicknesses cover most requirements for the usual museum jar size: 3/16 in. for the body of the jar, 1/4 in. for the top and bottom, and 1/16 in. for the specimen supporting panels, which may be clear, opaque, or coloured as required.

The milling equipment is mounted on a bench with a working top made of 1/4 in. duralumin plate 4×2 ft.