MECHANICAL SPINNER FOR ESMARCH CULTURES

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Shortly after Koch's plating methods were introduced, there was developed by Esmarch (1886) a procedure for spreading the solid films inside tubes. The Esmarch roll tube culture is familiar to bacteriologists, although it does not appear to have found general favor. In recent years the method was placed on a strictly quantitative basis by Wilson (1922), who found it advantageous in elaborate counts for statistical work.

Roll-tube cultures are more convenient than plate cultures for many purposes. It is possible that they would be more widely used if they required less manual dexterity and practice in preparation, and were suitable for the variety of media which can be used in the regulation plating method. In order to be incubated, examined, and counted satisfactorily, the film must be hard, adhering to the tube and free from wrinkles; it should not be thick at the base of the tube, and should not splash on the cotton plug. Esmarch developed the technic primarily for gelatin. Wilson used a hard, 2.5 per cent agar, which involves a high inoculating temperature.

For critical studies of marine organisms at this laboratory it was necessary to make viable counts at sea, preferably by a procedure permitting subsequent transfer of colonies. Petri dish cultures cannot be handled conveniently on board ship. Some have used tables hung on gimbals, similar to a compass mounting, for the solidification, but this arrangement is a make-shift.

The roll tube looked like a logical solution of this difficulty. It is possible that Esmarch had the same problem in mind when
he introduced the method. After describing the preparation of the roll tubes in detail, he remarks that he used it to advantage while on an eight-day cruise of the North Sea.

This author, however, has not been satisfied with different attempts to prepare hand-rolled tubes. The usual causes of failure are wrinkling of the films at the moment of solidification, and subsequent sliding of the medium down the tube during incubation. It is possible that either the higher summer temperatures of this continent or the difficulty of controlling artificial heating during the winter prejudices the chances for success.

It was found that most of the difficulties can be eliminated by preparing the tubes in a centrifugal machine. The device described in this paper turns out perfect films which can be solidified promptly and inverted in the incubator. It worked satisfactorily on a small boat rolling and pitching on the Pacific swells. The essentials of the machine are set forth here for the special information of bacteriologists interested in hydrobiology and for the general comment of the science. Wider applications suggest themselves to which reference will be made later.

APPARATUS

The mechanical arrangements are shown diagrammatically in figure 1. A culture tube containing liquified and inoculated medium is spun vertically in a water bath by means of a motor. As soon as the film has climbed the tube, ice water is run into the bath, freezing the medium in a few moments. The tube can then be removed and incubated.

The sizes given were found convenient for the present work on board ship involving counting and transfer of the colonies. The instrument may be considered as still in the developmental stage and subject to change and improvement. Thin-walled Pyrex tubes 25 by 200 mm. were used for the cultures. The water bath was a cylinder 10 by 20 cm., attached to a baseboard which could be moved vertically and clamped in any position. A small ball-bearing was cemented horizontally in the center of the bottom of the water bath with plaster of Paris. The bearing used for the 25 mm. tubes was taken from an automobile
Fig. 1. Spinner Showing Distribution of Agar Inside Tube at the Instant the Film is Congealed
generator with $\frac{5}{8}$-inch shaft. The water bath was fitted with a wide syphon extending to the bottom and bent close to the wall in order to be clear of the revolving tube. The spinning was accomplished with an automobile horn motor. No gear reduction was necessary. The motor was mounted so that it could be centered over the ball step bearing. The tubes were driven by means of a rubber stopper attached to the shaft of the motor. The stopper was fastened to a collar on the shaft with pins. When in place it was filed true to the axis of rotation with the motor in operation. The motor current was supplied by a 6-volt storage battery. This was placed in series with a sliding resistance made of six strands of 22 gauge nichrome wire in parallel about 3 feet long and twisted together. The 6-volt outfit was found convenient for work in the laboratory also. It was found to be more substantial and to possess a higher starting torque than small 110-volt motors which were tried out. An ordinary automobile battery can supply current for preparing several hundred tubes. The syphon in the water bath was connected with an overhead reservoir of ice water and also to a drain.

**MEDIA**

Either gelatin or agar may be used in the medium. Nutrient broth containing 10 per cent gelatin was found satisfactory. Gelatin sticks immediately to the inside of the tube, but it must be incubated well below its melting point to prevent softening and collapse. Broth containing 1.5 per cent agar was also found satisfactory where high incubation temperatures were necessary. The agar must be carefully handled in order to prevent sliding down the tube.

**OPERATION**

Plug the tubes for rolling tightly and dry sterilize them. To prepare a culture, melt sufficient medium for the work, and pour 15 ml. into each of the roll tubes. Hold them at 30°C. in the case of gelatin, and at 45°C. in the case of 1.5 per cent agar. It is advisable to immerse the tubes almost up to the neck and
to hold at the proper temperature for fifteen minutes. Have ice water ready for the water bath. Inoculate a tube prepared with medium by dropping in 1 ml. of culture, suspension or dilu-

![Fig. 2. Photograph of Mechanically Prepared Culture of Marine Organisms on an Agar Medium](image)

Shows uniform distribution of colonies, facilitating counting. Procedure removes medium and inoculant from the base of the tube.

Place the tube in the spinner, seating the stopper first and then raising the base so that the lower end rests firmly in the bearing but without introducing too much friction. The tube
should rotate freely but without wobble. Start the motor and increase its speed until the medium climbs near the plug. The rise of the medium can be watched through the outside. Fill the bath with ice water and maintain the speed of the motor for a minute. The motor can then be stopped and the tube removed. It should show a clear, uniform film, adhering to the glass and rising to within a safe distance of the cotton plug. The bottom of the tube should be empty.

Gelatin tubes may be incubated upside down immediately. It is advisable to lay agar tubes horizontal in the incubator for several hours before inverting.

No advantage was found in the use of agar stiffer than 1.5 per cent. The best results were obtained by holding agar medium of this concentration at 45°C. for fifteen minutes after melting and before spinning and inoculating. Stiffer agar is not an advantage in preventing the films from sliding, and it requires a warm water bath for the spinning operation unless the temperature of inoculation is made unreasonably high.

Esmarch used gum arabic and fish glue in his agar films. In the present work the agar was prepared in the conventional way, and it was found that the tubes could be inverted without collapse if they were held horizontally for a few hours after the rolling. It is possible that agar-agar will prove more satisfactory than purified Bacto-agar, since it contains a higher percentage of salts,—such as calcium which seems to be involved in the solidification.

The attempt was then made to incorporate both agar and gelatin in a single medium, but the mixture is less satisfactory than either constituent alone unless the proper physico-chemical treatment to prevent the formation of a mush is discovered. Recent research in photographic materials may furnish a satisfactory combination of the two substances.

APPLICATIONS

The 25 mm. tubes were used to facilitate picking of the colonies. A similar mechanical spinner could be designed for smaller tubes exclusively for counting purposes. It may be found convenient
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to use tubes 15 by 200 mm., thus cutting down the initial cost and incubator space but retaining sufficient length to prevent splashing of the medium on the plugs.

The apparatus as here described is admittedly crude. If additional uses for roll tubes occur to bacteriologists, there are several refinements which suggest themselves. The lower bearing could be held in a spring mounting to prevent occasional sticking and changes of speed. There could also be substituted for it a lower shaft operating through a stuffing gland at the same speed as the upper shaft. A tachometer on the motor would permit the spinning of the medium to a standard height without observation of the climbing film. The water bath could be provided with a copper coil joined to a two-way supply, with temperature indicated on a dial thermometer and controlled by a mixing fixture supplied with hot water and ice water. In this way the bath could be changed promptly from warm water for the spinning operation to cold for the freezing.

A fully mechanical arrangement could be devised for viable counts. This may be of interest in sterilizer control and in the milk and fermentation industries. Using a mechanical spinner operated in a sterile chamber, it is possible to visualize a mechanical device withdrawing samples at intervals, spinning tubes with or without dilution of the inoculant, and storing them in a sterile incubator for examination by a routine worker.

SUMMARY

A mechanical device is described for preparing Esmarch roll tubes.

REFERENCES
