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Machine for Printing Hydrophobic Grids on Membrane Filters

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An apparatus for printing wax grids on membrane filters is described. The machine produced more and better hydrophobic grid-membrane filters much more rapidly than by hand.

The hydrophobic grid-membrane filter (HGMF) is a conventional membrane filter (MF) on the surface of which has been laid a grid of hydrophobic material. This grid determines the positions and lateral growth limits of microbial colonies (1) and produces what is, in effect, a miniaturized most probable number counting apparatus, equivalent to a very large number (1,000 to 10,000) of tubes inoculated from a single dilution. A preliminary study of HGMFs revealed a number of interesting properties that could have important applications in quantitative microbiology. For example, an HGMF of 3,650 squares will yield linear values of the most probable number of growth units at inoculum densities approaching 30,000 growth units, i.e., very much higher than may be applied to a conventional MF (manuscript in preparation). This greatly reduces the need to make dilutions for a sample of unknown history. Other advantages include easier manual counting and greater suitability for automated counting than the normal MF.

Since larger numbers of HGMFs were required, it was decided to improve on the time-consuming hand manufacturing process, which used an etched zinc plate "inked" with a molten wax. The grid-printing apparatus described here was constructed from readily available materials and components.

MATERIALS AND METHODS

Printer. The components of the printer are shown in Fig. 1-3. With the exception of roller A (Fig. 1 and 2), the dimensions and materials are not critical, and construction details are left to the individual laboratory workshop. Brass roller A was carefully grooved by using a lathe tool that had been ground to the width of the desired HGMF squares. Thus, to obtain 50 line/inch (about 2.5 cm) grids (0.508-mm spacings) with barriers 0.005 inches (about 0.125 mm) wide, the lathe tool was ground to 0.015 inches (about 0.375 mm) (0.020 - 0.005 inches [about 0.500 to 0.375 mm]) width (tolerance 0.0005 inches). The grooves were then cleaned with 600-mesh carborundum paste applied with a sharpened wooden stick, and the fins were finely polished with brass polish.

Brass counter roller (Fig. 1, B) provided pressure for printing by virtue of its own weight and the spring action of cantilevered bearings (C). The force required to ensure perfect contact without crushing was determined by line width, number of lines, and the brand of MF. The roller gap was controlled by screws (D), so that wax was not transferred from A to B between prints. Filters were guided into the rollers by a flanged guide (E) made of polished stainless steel.

The hot-air blower (I) was mounted so as to maintain the whole printing area at a suitable temperature (e.g., 90 C for dental sticky waxes). Asbestos insulators (not shown) were placed around the lower half of the printer. Roller A was driven in the direction shown by a small induction motor (J), geared to produce a printing speed of about 2 inches (5.0 cm) per s. It was unnecessary to drive roller B. The quantity of wax on roller A was controlled by the pressure of the applicator (F) against it, which in turn was controlled by a screw (G). The reservoir (H) (Fig. 3) was of copper to ensure good heat transfer to the wax. Molten wax was fed from the reservoir to roller A by capillary action through the polishing cloth applicator (F) (polishing cloth, catalogue no. 12-282A, Fisher Scientific Corp.).

Preparation of HGMFs. The hot-air blower (I) was switched on about 30 min before the printer motor to melt residual wax. Guide E and roller B were wiped clean of wax with a cloth moistened in chloroform. An MF (e.g., Millipore type HAWP 04700) was placed, using forceps, in the center of a 2-inch square of blotting paper (static electricity always provided sufficient attraction) and fed, MF down, through the printer to produce a set of evenly spaced parallel wax lines. The emerging square was immediately rotated 90° and fed through the printer again, to produce the square pattern. The resulting HGMF was peeled from the blotting paper square, placed grid uppermost in a box, and covered with a parchment paper separator.

After approximately every 25 prints, an HGMF was floated on a bath or writing ink and examined

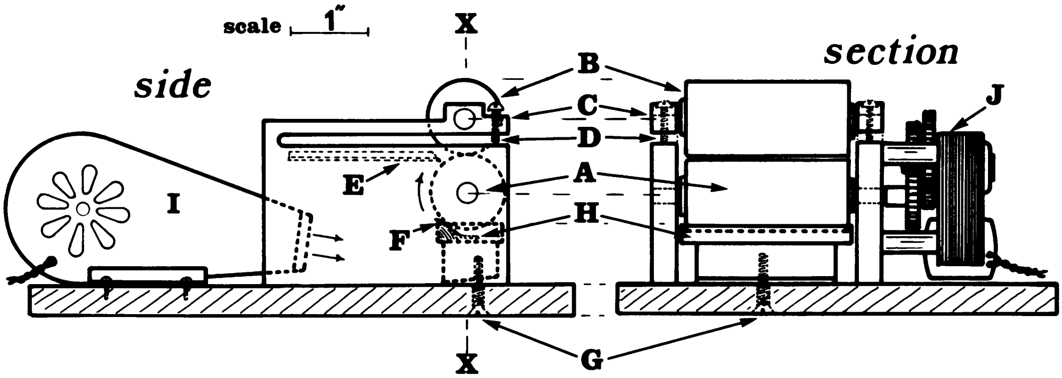

FIG. 1. *Side view and section through X- - -X (also showing screws [D]) of the printing machine. (A) Roller; (B) brass counter roller; (C) cantilever bearings; (E) flanged guide; (F) applicator; (G) screw; (H) reservoir; (I) hot-air blower; (J) induction motor.*

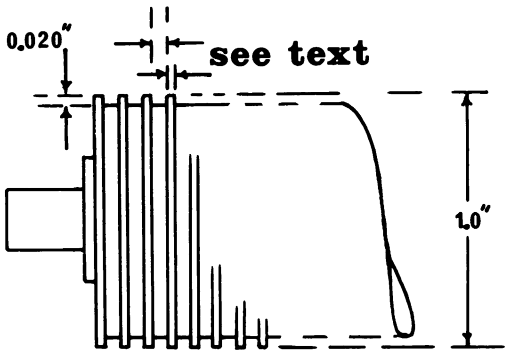

FIG. 2. *(Section) Detail of brass roller A, illustrating the cutting of fins to produce parallel wax lines of the desired spacing.*

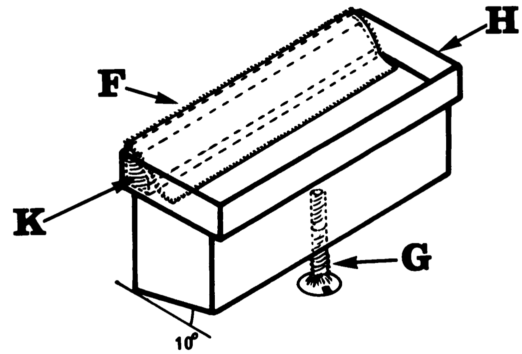

FIG. 3. *(Isometric) The reservoir and applicator. Contact adhesive is suitable for cementing wooden filler block (K) to the reservoir (H) and the applicator (F).*

with a stereomicroscope to check on the quality of production. A blotting paper square could be reused many times; its primary purpose was to ensure that the two printings were at right angles to each other.

RESULTS AND DISCUSSION

The machine produced well-defined grids, of a quality much higher than that of the hand process, in almost 100% yield, and at the rate of about 120 HGMF/h. Removing MFs from their boxes and separating them from the parchment paper separators was by far the most time-consuming step. Of the two brands of MF tested, Millipore HAWP were found to be most suitable for printing because of their stiffness. The more flexible Gelman GN6 Metricel MFs tended to flex and be picked up easily by the printing roller.

A variety of waxes was tested, including paraffin (m.p. 54 C), beeswax, carnauba wax, strearic acid, and dental sticky waxes. Ash model cement (Amalgamated Dental Trade Distributors Ltd., London, England) was preferred because of its ease of handling and the hydrophobic quality of the barriers it produced. Microscopic examination of ink-filled filter sections showed that the barriers were not raised above the filter surface, although they often appeared so to the naked eye or when rubbed by the finger. The wax normally penetrated about one-third to one-half of the filter thickness but did not appear to spread laterally away from the region of roller contact.

It would be possible to sterilize HGMFs by nonthermal methods such as ethylene oxide treatment. However, with reasonable care in handling, bacterial contamination was not found a problem, contributing only one colony in five or six HGMFs plated on selective media such as M-FC agar for fecal coliform analyses and none on HGMFs plated on Baird-Parker agar for *Staphylococcus aureus* analyses (manuscript in preparation). A possible improvement to the machine might be the use of a thermostatically controlled heating element rather than the hot-air blower, to minimize the chances of airborne contamination.

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LITERATURE CITED

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