Continuous Dispenser for Multiple-Well Serological Plate

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Materials and methods are described for the construction of an inexpensive multiple-well dispensing apparatus for use in serological microtitration.

The desirability of being able to add a uniform but variable volume of aqueous buffer simultaneously to every well of a multiple-well plate for serological microtitration led us to devise the apparatus described here (Fig. 1). It consists of three units: a dispensing chamber, a continuous pipetting syringe, and a reservoir for the buffer. All of its component parts are readily available commercially, except the top of the dispensing chamber which has to be fashioned from a sheet of Plexiglas.

The dispensing chamber is constructed in three sections, A, B, and C (Fig. 2). The three sections of the dispensing chamber are then sealed together with a moderately viscous solution of polystyrene plastic in chloroform and allowed to harden overnight.

Section A consists of a Microtiter plate (Cooke Engineering Co., Alexandria, Va.) with as many stainless-steel 21-gauge tubes (Small Parts, Inc., Miami, Fla.) as there are wells in the plate. Each stainless-steel tube is 20 mm in length, and it is inserted through a hole drilled in the bottom of a well so that 1 cm of the stainless-steel tube extends below the bottom of a well. Enough viscous epoxy resin-catalyst mixture is distributed into each well around its stainless-steel tube to fill the well up to within about 1 mm from the top of the tube, and the resin is hardened at 60°C overnight.

Section B serves as the walls of the chamber and consists of a Microtiter plate with the interior entirely cut out, leaving only the frame.

Section C is the top of the chamber. It is specially made from a 0.5 inch (1.27 cm) thick plate of Plexiglas with a hole 3/8 inch (0.96 cm) in diameter in the center to allow insertion of a male connecting tube so bevelled as to accept the female part of a polyethylene double connector (no. 6150, size A, Nalge Co., Rochester, N.Y.). Attached to the polyethylene connector by means of a 0.5 inch length of tygon tubing [0.25 inch (0.63 cm) outer diameter, ½ inch (0.32 cm) inner diameter] is a three-way plastic valve (polypropylene stopcock, no. 5851, Ace Glass Inc.).

The dispensing chamber is filled with buffer by applying suction through the side arm of the three-way valve of section C with the stainless-steel tubes of section A immersed in buffer. Provided that reasonable care is taken to hold the chamber nearly horizontal at all times, it remains full. In any event, some bubbles at the top of the chamber do not interfere with the uniform dispensing of buffer into the wells of a test plate. For storage of the dispensing chamber, the tubes are submerged in buffer in the wells of a Microtiter plate and operated once before reuse.

Section C is connected to the continuous pipetting syringe by way of a plastic intravenous tubing (Pharmaseal no. 5420, Glendale, Calif.) attached to the three-way valve. A 0.25 inch (outer diameter) tygon tubing is employed to connect the pipetting syringe for aspiration of buffer from the reservoir through a bored silicone rubber stopper, which is also equipped with another hole for a tube containing glass wool to act as an air vent and to prevent dust from entering the reservoir.

The syringe (5- or 10-ml capacity) can be adjusted for delivery of volumes from 0.025 to 0.1 ml per well. The 0.1-ml volume per well has been employed for repeatedly washing red blood cells for the performance of antiglobulin (Coombs) tests, in which case a spacer, identical to section B above, was used to raise the tubes of the dispensing chamber above the level of the wells of the test plate.

With the dispensing syringe set to deliver a total volume of 2.4 ml, the smallest volume studied, the accuracy of the volume of buffer delivered to each well was determined as 0.025 ml with a standard deviation of 0.0014 ml or a coefficient of variation of ± 5.6%.
Several of these inexpensive apparatuses have been successfully employed in our laboratories for more than 1 year, with much saving of time and effort in the performance of various serological procedures. Other devices, similar in function to the one described here, have been previously described (1–3). Two of these (2, 3) are available commercially. However, they are more expensive and either are not so flexible in the range of volume of buffer which can be added to a microtitration plate or are more limited in the number of wells which can be serviced at one time.

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