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Piggy-Back Microtransfer Technique

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A new microtransfer technique is described which uses a modified, V-bottom, disposable, 96-cup plastic plate that permits serial dilutions and direct rapid transfer to a recipient micro cell culture plate.

The recent development of a sterile, plastic, tissue culture microplate with 96 cups which successfully supports the growth of various mammalian cell cultures (MicroTest II tissue culture plate, BBL) has proven to be an inexpensive, rapid, and reliable tool for virus research (1).

The well-established microtiter system employing spiral loops for rapid serial dilutions has been used for complement fixation, direct and indirect hemagglutination, and various other procedures (2, 3). Such a reliable method would also be desirable to dilute sera for use with microneutralization procedures. The spiral loops cannot be used in the plates containing tissue cultures, however, as they would destroy the cultures. Thus serial dilutions must be made in tubes or other plates and transferred individually and with accuracy. This laborious and delicate problem of transferring small volumes has been a decided hindrance to the use of the microplate for tissue cultures.

In an effort to increase the applicability and expedite the performance of viral microneutralization procedures, a rapid microtransfer system was designed and developed by our laboratory.

Preliminary studies have shown that a V-bottomed, soft-vinyl plastic plate containing 96 circular wells (Cooke Engineering Co., Alexandria, Va.) with slight modification can be used for a direct transfer of virus-serum mixtures without aerosol formation and with accuracy. The modification initially consisted of puncturing the tip of each V-shaped well with a 19-gauge sterile needle, producing uniform holes (Fig. 1). This method, which at times produced a rough lip on the undersurface of the well, was replaced with an improved procedure using a small bit in an electric hand drill. Undiluted or diluted serum was dropped into these modified wells and serial dilutions were made with the spiral loops (Microtiter; Cooke Engineering Co.) without loss of materials through the orifices (Fig. 1A). The liquids remained in the wells unless the bottom of a well contacted an absorbant surface (cotton towel, etc.). Such loss was prevented by working on a hard surface (table top) or by supporting the plate on a metal stand designed to hold the bottoms of the wells off the working surface. It was also found that each well of the modified plate could hold any desired volume for extended periods of time without loss through the hole.

To insure sterility, for tissue culture work, the V-bottomed plates were first exposed to ultraviolet light and covered with sterile plastic covers (MicroTest II lids, BBL).

Figure 1B illustrates a hard, flat-bottomed, plastic plate having 96 circular wells with each well containing a confluent cell sheet or "miniculture" (MicroTest II tissue culture plate, BBL). The planting concentrations and the growth and maintenance media used for any particular cell line have been previously described (1).

Immediately prior to using the transfer plate, growth medium was removed from each of the flat-bottomed wells of the cell culture plate (Fig. 1B) by aspiration with a sterile Pasteur pipette connected to a vacuum bottle. Maintenance medium was then dropped into each well (0.050 ml) by using calibrated sterile droppers (Microtiter, Cooke Engineering Co.).

To effect the microtransfer, the V-bottomed plate was placed "piggy-back" on top of the flat-bottomed plate (Fig. 1C) and the bottom of each well of the top plate was brought into contact with the fluid level in each well of the culture plate (Fig. 1C, wells I and II). As soon as contact is made between the two wet surfaces, the V-bottomed wells immediately empty (Fig. 1C, wells II and III). Almost total emptying of the upper wells occurs. Only slight wetting of the undersurfaces of the transfer wells takes place. No significant variation could be observed in the final volumes of fluid in each of the wells con-

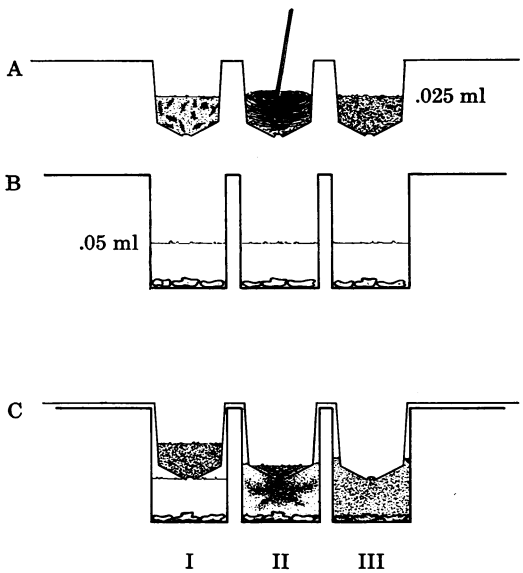

FIG. 1. *Tissue culture microplates. (A) Section of soft-vinyl plastic plate with modified, V-bottomed circular wells. Serial dilutions were made with spiral loops (well II). (B) Section of hard, flat-bottomed, plastic plate with circular wells containing micro cell cultures. (C) Section of the "piggy-back" plate; the V-bottomed plate (A) was placed "piggy-back" on top of the flat-bottomed plate (B).*

taining the cell cultures, nor were viral titration end points affected.

After completion of the transfer procedure, the "piggy-backed" plate was removed and the bottom plate was covered with plastic tape and sealed with pressure. The plates were then returned to a CO_2 incubator and handled as previously described (1).

Other methods of emptying the wells of the "piggy-backed" transfer plate were studied, including forcing the fluids through the holes with air pressure. This method, however, resulted in some aerosolization with viral contamination of adjacent wells.

The sole source of difficulty with the microtransfer method as presented has been with the design of the V-bottomed plate which is presently available. The diameter of the wells contained in these plates is slightly larger than desirable for transfer purposes and moderate pressure must be applied to force the transfer wells sufficiently deep into the cell culture wells to effect the contact of fluids and subsequent transfer.

We are making efforts to have plastic "piggy-back" plates produced commercially with optimal specifications to permit easy insertion in the recipient plates.

The microtransfer plate and technique would appear to have wide applicability to numerous methods which require accurate dilutions and rapid transfer for microneutralization procedures as well as immunoelectrophoresis, chromatography, antibiotic and antimetabolite assays, immunoglobulin quantitation, and other serological procedures.

With this additional methodology, the rapid performance of many microtissue culture procedures and similar transfers can be accomplished with a reduction in the amount of serum or other materials required for testing as well as significant savings in reagents, time, and cost.

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