An insertable orifice tube for *in situ* contamination counts of solutions in opened ampoules and vials using the Coulter Counter

SIR,—Attention has recently been focussed upon contamination by particles in injection solutions (Garvan & Gunner, 1963, 1964; Groves & Major, 1964; Groves, 1965, 1966; Vessey, Kendall & Peters, 1966; Vessey & Kendall, 1966). The Australian Department of Health has now (1966) issued a draft standard for the measurement of particulate matter in such solutions, using a Coulter Counter. To date little emphasis has been placed on the problems associated with the detection of contamination in glass ampoules and vials containing small volume solutions.

Large volume solutions can be transferred readily into the counting chamber by pouring, after adequate washing and rinsing of the chamber, with no increase in contamination level. The average count above 3·5 μ, and standard deviation, of twelve saline samples from a 1 litre Polyfusor container was 108 $\pm$ 11.

One well cleaned and dried 2 ml ampoule was sealed and opened in the normal way, and the fracture surface touched onto 6 ml of the "counted" saline; particles above 3·5 μ numbered 1460. Then the fracture surfaces from six cleaned ampoules were added to a fresh 6 ml of the saline when the count was 4690.

It is easily calculated that the surface of the one ampoule contributed approximately 8,100 particles above 3·5 μ and the six gave an average of about 4,850 each. Thus transfer of solution from ampoules by pouring or shaking into the counting chamber, or both, must increase contamination levels.

Transfer by a hypodermic syringe would be expected to prevent this. Commercially available disposable sterile syringes and well cleaned re-useable syringes were obtained, and filled to 2 ml 6 times from a supply of the counted Polyfusor saline. Each time the syringe was emptied into a well-cleaned and rinsed vial and the new contamination levels determined from the sum of two 0·5 ml sample counts above 3·5 μ. The numbers of particles above 3·5 μ for successive fillings for both types of syringe were 1043, 2073, 846, 723, 302, 741 for the reusable syringe and 270, 212, 209, 372, 370, 895 for the disposable syringe. Thus further filling and dispensing did not quickly reduce the new counts to the original "clean" level showing that additional contamination was continually coming from within the syringes, presumably by friction of the plunger and barrel.

To count small volumes of solutions of this sort, which cannot be easily transferred, a narrow version of the standard orifice tube which fits any Coulter Counter has been developed. This may be inserted directly into the ampoule itself. The present specification requires that the tube, with external electrode wrapped closely around it, should conveniently pass a 4·5 mm aperture. This is adequate for most British ampoules of 2 ml volume or more, and most American ampoules down to 1 ml or sometimes 0·5 ml. It has been verified experimentally that these narrow orifice tubes do not contribute to an increased noise level, and thus artificial counts, and do not reduce the performance of the Coulter Counter in any way. Care is necessary in using these tubes to avoid artificial counts from air bubbles; the tubes themselves are less robust than standard tubes. They have been manufactured with orifices of 50, 70 and 100 μ diameter to cover the needs in this field of study, that with a orifice of 100 μ being used in this present work.

The effect of transfer by a well cleaned re-useable syringe was again observed with solutions of drugs in 0·7% sodium chloride, from 2 ml and 5 ml all-glass ampoules, and compared with counts by the "direct" technique using the new orifice: transfer by syringe 710 $\pm$ 47; counted *in situ* 166 $\pm$ 10. The syringed

samples were again more contaminated. The direct technique gives a more meaningful result and seems therefore a significant advance in measuring true contamination levels in solutions in small ampoules. It is evident also that no major contamination is created by a glass shower entering the ampoule on fracture; such glass fragments as are present, however, would also be removed by the syringe.

All counts were made with a Coulter Counter Model B. This instrument has the advantage that changes in conductivity of the counting solution (e.g. the dissolved salt concentration) do not affect calibration over a wide range. Calibration was with Dow polyvinyltoluene latex (Dow Chemical Company, Midland, Michigan, U.S.A.) with a quoted mean diameter of 3·49 μ. Cleaning of equipment was by soaking for 24 hr in Decon 75 detergent solution (R. W. Jennings, Ltd., Nottingham), rinsing and ultrasonically vibrating in filtered de-ionized water (triply passed through 0·45 μ Millipore membranes) and rinsing in filtered saline (Polyfusor, with 108 ± 11 particles/ml above 3·5 μ). The equipment was shaken in clean air to remove surplus moisture, then used immediately. Drying, where necessary, was aided by rinsing in filtered acetone.

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