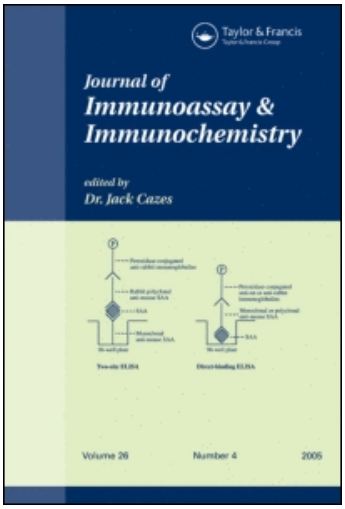

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A SIMPLE AND RAPID METHOD OF WASHING AND DRYING MICRO-TITRE PLATES USED IN ELISA

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ABSTRACT

A simple device constructed out of perspex sheeting and capable of washing 96-well micro-titre plates used in ELISA is described. It consists of three parts: (a) the 'shower' - a tank with 96 stainless steel syringe needles through which washing fluid runs into the wells of the micro-titre plate, (b) a sump into which washing fluid is collected and (c) a sliding base plate allowing the accurate positioning of the micro-titre plate below the shower. The efficiency of this washing device was established by comparing the results from several specific ELISAs with that obtained after washing duplicate plates by hand. The use of a specially constructed centrifuge to spin-dry plates is also described.

INTRODUCTION

In Enzyme-linked-immunosorbent-assay (ELISA) unbound sample and antibody-enzyme conjugate are removed from the solid phase by washing with buffer solution containing Tween 20. Efficient washing is very important if reliable results and good duplicate values are required. In this laboratory between 4000 and 5000 serum samples are examined daily using ELISA and automation of procedures is therefore highly desirable. Several automatic micro-titre plate washers are commercially available and have been experimentally used by us but in our experience these

instruments are relatively slow and leave the operator with the problem of drying plates properly after the washing procedure.

We have developed a 'shower', described here, which efficiently washes micro-titre plates in less than one minute. It is easily constructed and can be made at a fraction of the cost of commercially available plate-washing machines. This machine is based on a similar system initially developed in the laboratories of the Dutch National Institute of Health (1). Drying of washed plates is accomplished by spin-drying, i.e., centrifugation of the plates in a centrifuge specially built for this purpose. Details are given in this communication.

MATERIALS AND METHODS

General ELISA Methodology

Details of the general ELISA methodology have been described (2). Polystyrene flatbottom micro-titre plates (Dynatech 129b) were used in all experiments. Appropriately diluted samples were individually applied to the wells of micro-titre plates but antibody-enzyme (horse radish peroxidase (HRP)) conjugate, chromogenic substrate (H_2O_2/OPD.2HCl) and acid stop (1.5 N HCl) were introduced by means of a semi-automatic, 8-channel, syringe-type applicator (Flow, Titertek Autodrop). Different application heads were used for each reagent to avoid the possibility of a non-ELISA colour development. The absorbancy (492nm) in ELISA plates were measured by means of a dual wave-length, through-the-plate reader (Dynatech MR 580 Microelisa Auto Reader) or a Vitatron DCP photometer fitted with an 80 µl flow-through cell connected to a vacuum source.

Construction of Plate Washer (Shower)

The shower consists of a tank through the bottom of which are inserted 96 squared-off No. 16 hypodermic needles cut to 30 mm length; these are arranged to align with the position of the wells

in a micro-titre plate placed in the guide rack below the tank. The tank is supported above the plate by two cross members. The whole is supported above a sump which collects the washing fluid overflow from the plate. All parts are constructed from perspex (Lucite).

Construction of Drying Centrifuge

Centrifugal removal of fluid remaining in the wells after washing is done in a specially constructed centrifuge. The rotor of this centrifuge consists of a carefully balanced, sturdily constructed steel cage, 95 mm wide, 255 mm long and 140 mm high and open at the top. Up to 18 micro-titre plates (9 at each side) can be placed in this rotor. A 0.25 Kw electric motor running at 1400 rpm is the drive unit and a hand operated friction brake facilitates rapid stopping of the rotor.

Plate Washing and Drying Procedures

After the micro-titre plate has been positioned below the shower, 500 ml of washing fluid (0.05 M Tris-0.1 M NaCl buffer, pH 8.0 containing 0.05% Tween 20 (TST)) is decanted rapidly into the reservoir. The fluid runs into the wells in about 15 seconds and the plate is then removed and allowed to stand for another 15 seconds or longer. Washing fluid is then shaken off and the plate transferred to the centrifuge for drying.

In order to soak up the small amounts of washing fluid released during centrifugation, plates are stacked with alternate sheets of absorbent material (eg. Webtex) between them. Plates are centrifuged for 15 to 20 seconds before the machine is switched off and the mechanical brake applied.

Manual Washing Procedure

This method has been fully described before (2) but is briefly outlined again for reasons of comparison.

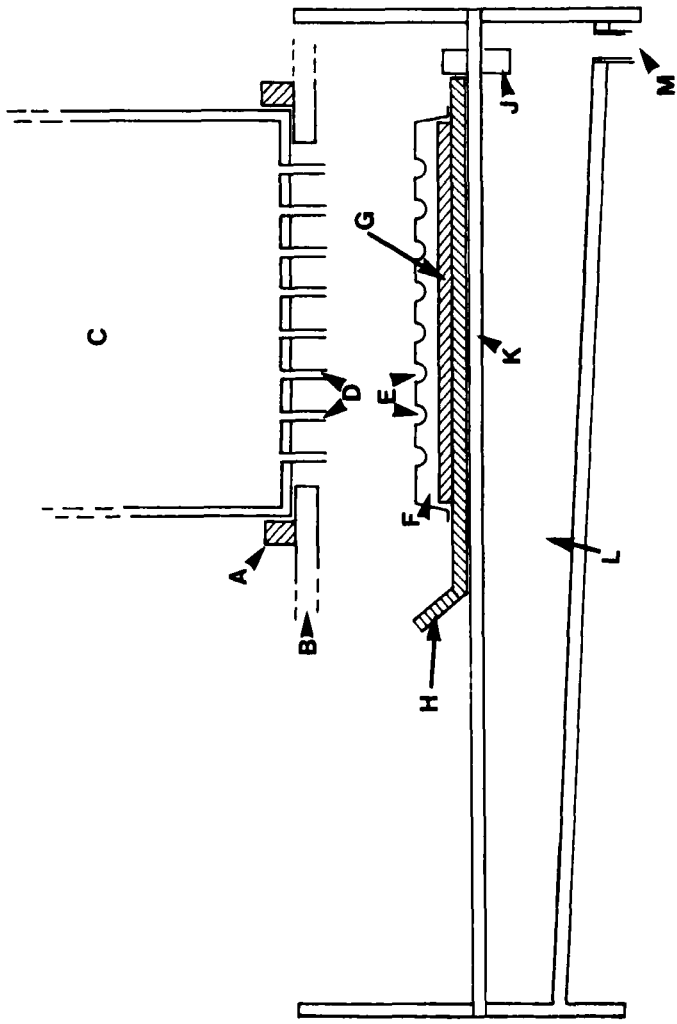

Fig. 1 Diagramatic section of the shower (not to scale). Stops (A) on the platform (B) locate the tank (C) so that the stainless steel tubes (D) are positioned over the wells (E) of a micro-titre plate (F), which is located by a former (G) on the carrying plate (H). Alignment of the plate can be corrected by moving the collars (J) along the stainless steel bearers (K). Overflow from the wells is caught in the sump (L) and led away through the drain (M).

1. Aspirate contents of each well carefully, avoiding carry-over between wells. Add 100 µl TST and allow to stand for 3-5 minutes. Aspirate TST and wash 3 times by flooding with TST; this is flicked off after a short incubation period of 1-2 minutes before addition of conjugate.
2. Washing after conjugate application is done in the same manner as in 1 above except that no danger of carry-over exists at this stage and the flooding of plates followed by a short incubation period is sufficient.

RESULTS AND DISCUSSION

Details of the washing shower are given in Fig. 1 and 2 and that of the drying centrifuge in Fig. 3.

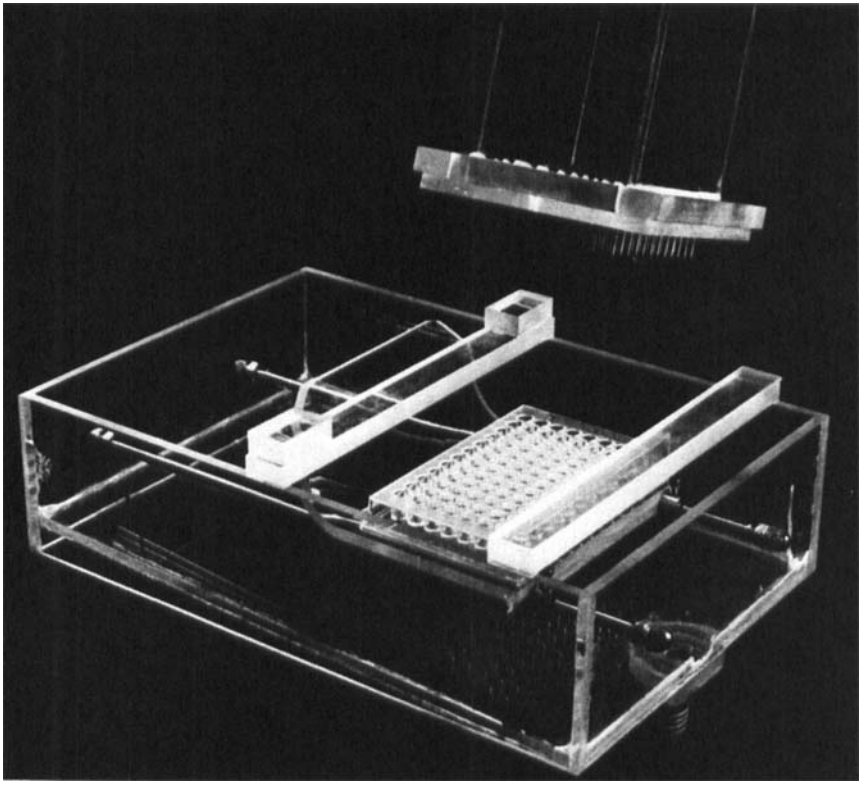

Fig. 2 The plate washer

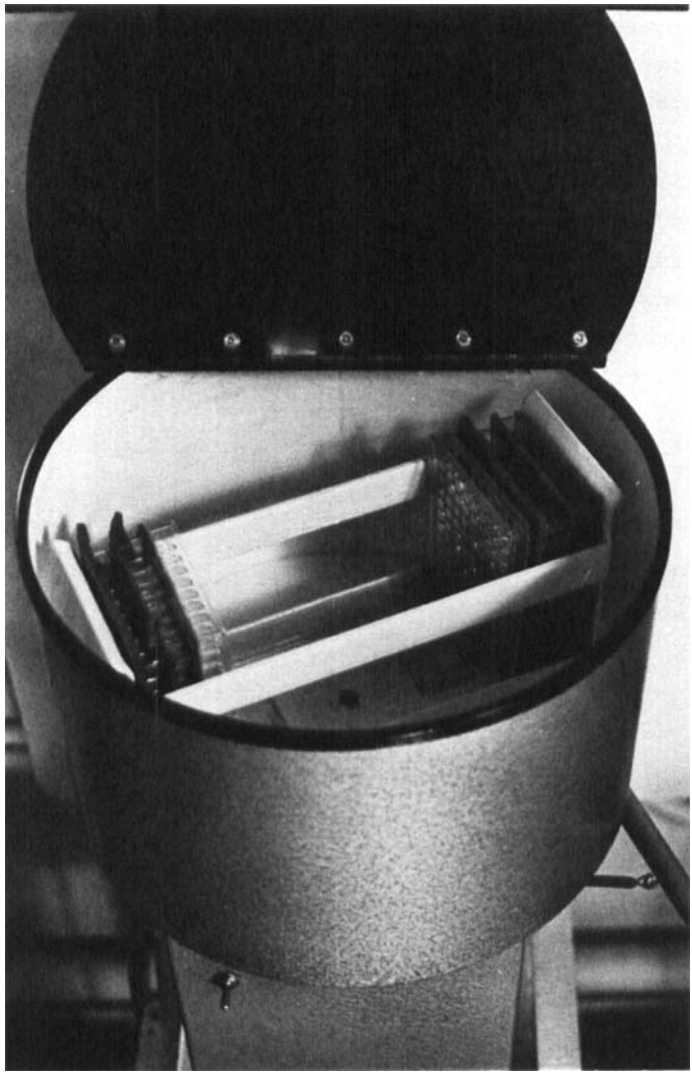

Fig. 3 The drying centrifuge

The manual and shower methods of washing were compared on pairs of equivalent plates where the only difference in procedure was the method of washing. This experiment included ELISAs for the quantitation of human antibodies of several specificities

and for serum ferritin. The results of these experiments are summarised in Table 1 and demonstrate that, on average, the shower method of washing yielded slightly improved coefficients of variation (C.V.). It appears that in the shower system the rapid flow of a relatively large volume of TST (approximately 5 ml/well) ensures a sufficiently fast dilution and removal of immuno-reagents to prevent unwanted reactions from taking place. With this system the usual period of soaking plates also appear to be unnecessary; this in itself saves a considerable amount of time.

The drying centrifuge, although not essential, was found to fit well into the general washing procedure and ensured that each

TABLE 1

Comparison of the Mean Coefficients of Variation (C.V.) of Absorbancies ($A_{492\ nm}$) Measured on ELISA's Washed Manually and by the Shower Method

	Mean coefficient of variation (%)[a]	
ELISA	Manual	Shower
Anti-Cytomegalo virus	8.9	5.6
Ferritin	5.9	6.0
Anti-Rubella	8.5	7.3
Anti-Tetanus	7.3	4.9
Anti-Tetanus	9.1	7.5
Ferritin	9.8	10.1
Ferritin	11.0	10.0
Anti-Cytomegalo virus	6.0	5.6
Anti-Cytomegalo virus	7.7	6.9
Anti-Rubella	6.0	11.5
Anti-Rubella	5.6	4.6
Anti-Rabies	5.9	6.7
Anti-Rabies	5.5	6.5
Anti-Varicella Zoster	4.9	4.3
Anti-Varicella Zoster	5.2	3.9

a. This is the unweighted mean of the C.V.s calculated for each of 8 points on the standard curve of each respective ELISA and includes the zero dosage point. Each individual C.V. was calculated from 12 replicates.

well of every plate was equally and sufficiently dried. This is of importance especially after the second washing and immediately before addition of chromogenic substrate where small amounts of residual washing fluid may contribute negatively to the overall C.V. of the ELISA.

CONCLUSION

We have concluded trials to determine optimum parameters for the washing shower using tubes of different diameters, altering the height of the shower above the plate, volume of washing fluid, and the time (i.e. volume) allowed for showering. Using the combined shower plus centrifugal drier, plates may be washed and dried very rapidly and at a fraction of the time and cost of commercially available washing machines. It must be pointed out that the details given above were established for polystyrene micro-titre plates and are empirical. Conditions for polyvinyl chloride or "soft" plates are different and would best be established for each individual ELISA.

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