A Simple Device for Semiautomatic Washing of Adhering Cells in Microtest Plates

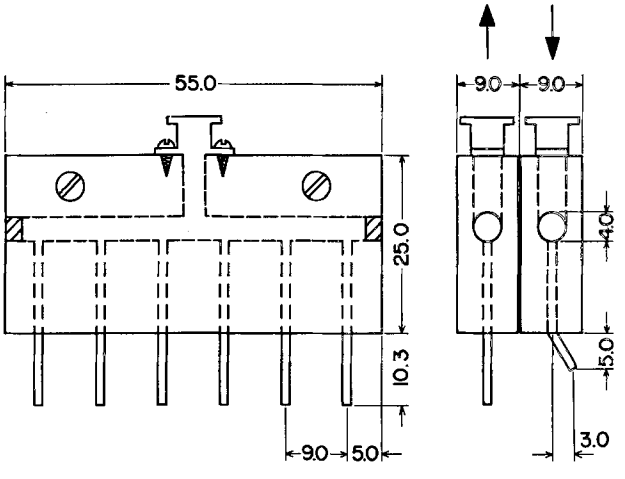
Since the introduction of Microtest plates more and more tissue culture work with a wide variety of cells has been performed on this small scale. When adhering cells have to be washed in a 3040 Falcon plastic Microtest plate II with flat-bottomed wells, the supernate in 96 wells per plate with a capacity of 250 µl has to be exchanged several times. Especially when the aim of the washing procedure is to remove excess isotope label after any labelling procedure of adhering cells, it may be essential for reasons of accuracy to do this in a precisely defined short period of time to have comparable conditions in the different wells (e.g. nonspecific spontaneous isotope release). For such a purpose[1] we have developed a semiautomatic 'dispenser-suction-device' that removes the supernate simultaneously in a row of 6 wells and at the same time adds a desired amount of medium to the neighbour row emptied a few seconds before.

The device is simple to make in any laboratory workshop (Figures 1 and 2). It consists of 2 flat blocks of synthetic material (plexiglass, teflon if it has to be sterilized) in which a larger horizontal channel is connected to 6 small channels fitted with a metal cannula (inner diameter 0.6 mm, outer diameter 1.2 mm). In one block these cannulae are perpendicular to the plate and just long enough to obtain total emptying of the wells without aspiration of adhering cells. In the other block, the cannulae are shorter and oblique in order to direct the jet of the tissue culture medium against the wall of the well and not directly against the cells at the bottom, and to obtain a better washing effect on the walls. In between 2 washing processes, the plate, sealed with tape, may be agitated on a vibrator thoroughly to remove non-adhering cells.

The 2 blocks are connected in order to allow aspiration of fluid in one row of a Microtest plate and simultaneously to deliver medium to the row just emptied. The aspiration has to be gentle to avoid detachment of cells. The medium is delivered by a Cornwall continuous pipetting seringe of a maximum volume of 2 ml (Becton-Dickinson). All parts of this device may be sterilized.

This device has been extensively used in cell mediated cytotoxicity (CMC) experiments where ^{51}Cr labelled rat peritoneal exudate cells harvested 2 days after i.p. injection of proteose peptone (Difco) have been used as target cells[1]. They were of (Lewis × Brown Norway) F_1 (LBN) or Brown Norway (BN) origin. These target cells were labelled during the process of adherence to the bottom of the wells of a 3040 Microtest II plate during a 3 h incubation period. Following this, the non-adhering cells and excess ^{51}Cr were removed by 3 washes with the device described here. Lewis rats were immunized by BN skin grafting. Regional lymph-nodes were harvested 8 to 9 days after grafting. Normal or immune Lewis lymphocytes were added to the labelled and washed adhering (LBN or BN) target cells and the ^{51}Cr released into the supernate counted after generally 4 h of joint incubation of effector and target cells. The nonspecific spontaneous release was measured in wells where only 200 µl of tissue culture medium was added to the target cells. Maximum ^{51}Cr release was obtained by addition of 200 µl of a 10% solution of Zaponin (Coulter Electronics). For more technical details, see[1]. Figure 3 shows a typical experiment in which LBN and BN target cells were incubated 3 h for adherence and labelling, washed and subsequently incubated for 2, 4, 6 and 8 h with normal or immune Lewis lymphnode cells, 4×10^6 or 1×10^6 cells per well. Each point represents the mean ^{51}Cr release of 3 wells expressed as precentage of maximum release ± standard deviation. The ^{51}Cr release increases in a linear fashion over an 8 h period. It starts very soon after immune lymph node cells

[1] F. Harder, M. Demarchi, G. Trinchieri, G. Thiel, Transplantation, *17*, 551 (1974).



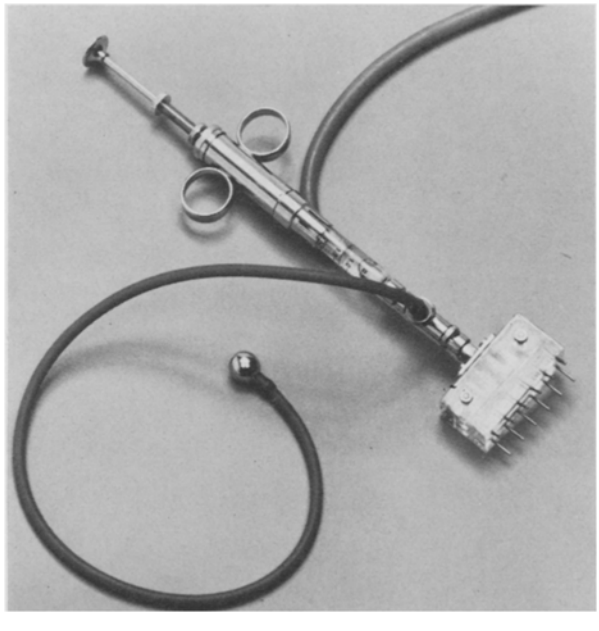


Fig. 1 and 2. Washing apparatus consisting of 2 identical blocks of synthetical material but of 2 different sets of cannulas. The size is indicated in mm.

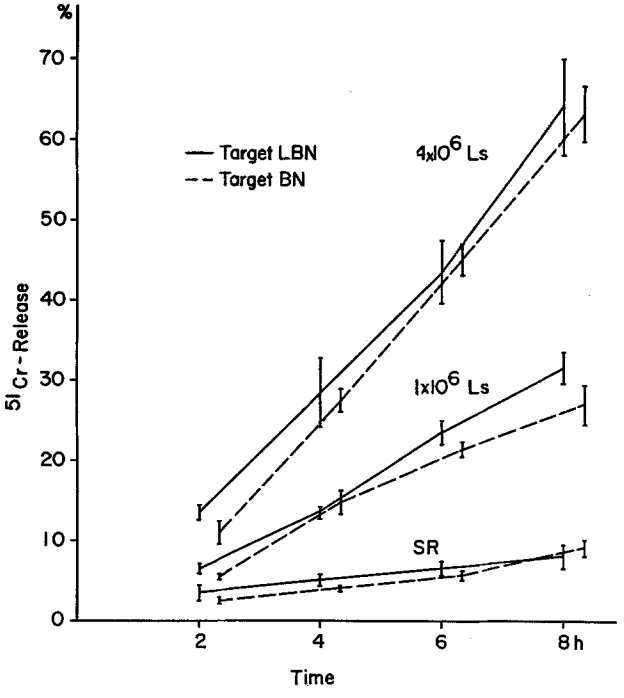


Fig. 3. Kintetics of CMC: ^{51}Cr release from Brown Norway (BN) and (Lewis × Brown Norway) F_1 (LBN) target cells. Incubation time of target macrophages with effector cells 2–8. h

Allogenic BN and semiallogenic LBN target macrophages, incubated for 3 h for adherence and labelling, were washed and subsequently incubated between 2 and 8 h with immune Lewis lymph node cells (Ls), normal Lewis lymph node cells (Ln) or with TC-medium alone for spontaneous release (SR). The ^{51}Cr released from target cells in triplicate wells is expressed as percentage of maximum ^{51}Cr release from target cells completely lysed with Zaponin (16 wells). Max. release: BN, 3167 $\pm$ 267, $n = 16$; LBN, 2143 $\pm$ 150, $n = 16$. Labelled adhering target cells per optical field: BN, 212 $\pm$ 25 ,$n = 4$; LBN, 221 $\pm$ 26, $n = 4$. (1 well = 45 optical fields).

have come into contact with the target cells and depends on the lymphocyte/target cell ratio being 400 :1 and 100 :1 for the 2 lymphocyte doses used. Normal L lymphocytes (not shown here) always elicits a ^{51}Cr release in the range of the nonspecific spontaneous release. There is no difference in the rate of specific target cell killing between BN and LBN macrophages, the latter carrying only the haploid gene dose.

Zusammenfassung. Es wird ein einfacher Apparat beschrieben, der schnelles und gleichmässiges Waschen von adhärenten Zellen in Microtest II 3040 Platten (Falcon) erlaubt. Eine Gruppe von 12 zylindrischen Vertiefungen mit flachem Boden wird gleichzeitig behandelt. Der Apparat ist einerseits an Sog und anderseits an eine automatische Spritze mit verstellbarem Volumen (Cornwall) angeschlossen. Er fand ausgiebig Verwendung in einem in vitro Testsystem für direkte zellvermittelte Zytotoxizität. Als Zielzellen dienten ^{51}Cr markierte peritoneale Macrophagen.

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