A simplified method for reading hemagglutinations on a flat-bottom microtitration plate in the mouse H-2 assay[1]

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Summary. Kaliss' method for determining hemagglutination titer of mouse alloantiserum was modified to a simpler form. It became possible to read the hemagglutination directly on a flat-bottom microtitration plate using an inverted type microscope. Sensitivity of the modified method was almost the same as that of the original one. This method seems to be useful especially for the larger scale assay of H-2 specificities.

The original polyvinylpyrrolidone (PVP) method of Stimpfling[3] has been successfully modified to the more dependable form by Kaliss[4]. The principal point of his modification is a change in the specification of PVP. In Kaliss' method, moreover, the contents of each well are transferred to a glass plate to read the hemagglutinations precisely. As this process is considerably laborious in the larger scale assay, a simplified method for reading the hemagglutinations microscopically on a microtitration plate with flat-bottom wells, has been developed in our laboratory.

The recipe for working solutions followed previous papers[4,5] which suggested preparation of 1 l PBS (pH 7.2) with 0.725 g Na_2HPO_4 (anhydrous), 0.180 g $NaH_2PO_4 \cdot H_2O$, 9.0 g NaCl and distilled water. 5% (w/v) BSA solution in the PBS was made 1 day before use by using Bovine Albumin Fraction V powder (Armour Pharmaceutical Company, Arizona). PVP-K90 (GAF Corp, New York) was dissolved in the BSA-PBS at 1.2% (w/v) for the first well of each row and at 0.6% (w/v) for the rest just before use.

Fresh blood for target RBC was obtained from the orbital sinus, using a microhematocrit tube. As an average

1 Contribution from the National Institute of Genetics. Supported in part by grants-in-aid (No. 111510, 111504, 112105) from the Ministry of Education, Culture and Science, Japan.

2 Acknowledgments. The excellent technical assistance provided by Miss M. Kuranou, Mr K. Sakakibara and Miss M. Tsuyuki is gratefully acknowledged.

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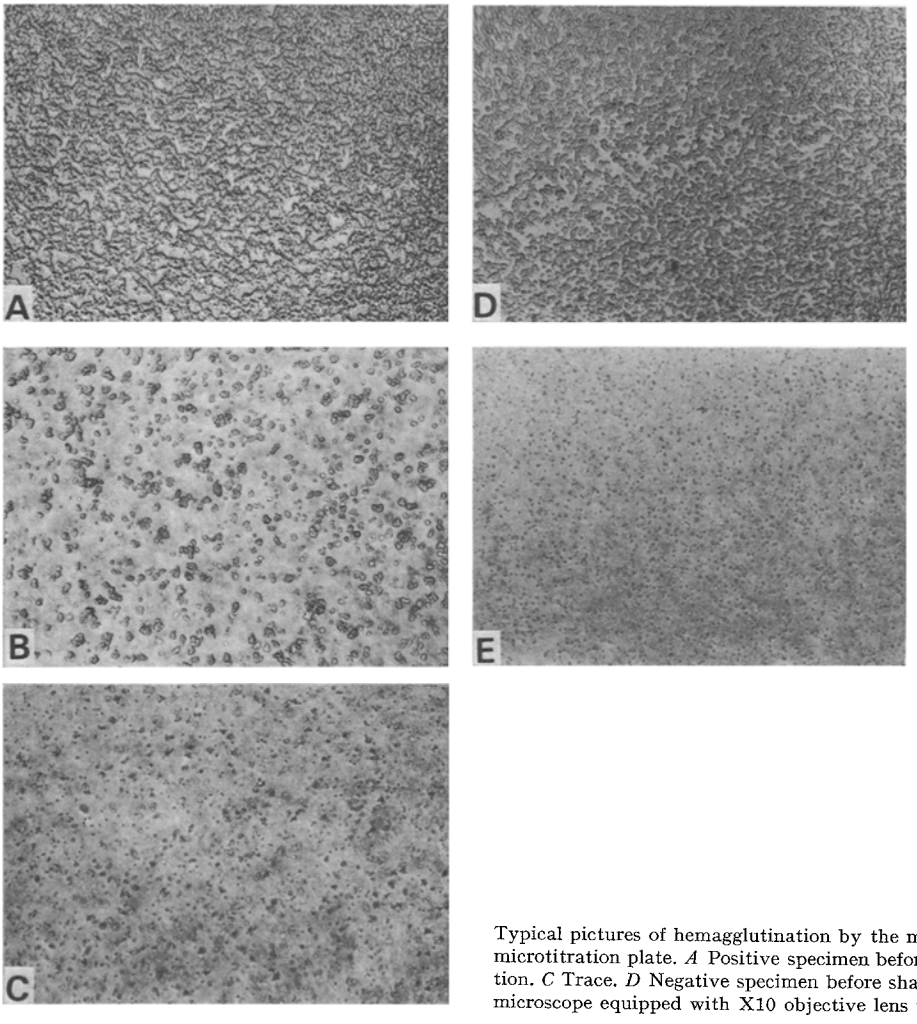

Typical pictures of hemagglutination by the mouse alloantiserum in a flat-bottom microtitration plate. *A* Positive specimen before shaking. *B* Grade 2 positive reaction. *C* Trace. *D* Negative specimen before shaking. *E* Negative. An inverted type microscope equipped with X10 objective lens was employed in this observation.

Comparison of hemagglutination titer between Kaliss' method and the present 'plate' method

Antisera*	RBC	Method	Hemagglutination at $\log_2$ dilutions** 3	4	5	6	7	8	9	10	11
D-4	B10A	Kaliss	3	2	2	2	2	2	1	1	tr
		Plate	2	2	2	2	2	1	1	tr	—
D-5	B10A	Kaliss	1	1	2	2	2	1	1	1	tr
		Plate	1	2	2	2	2	2	1	tr	—
D-23	B10A	Kaliss	2	2	2	1	1	1	1	tr	—
		Plate	2	2	2	1	1	tr	tr	—	—

* Those alloantisera were given by the Transplantation Immunology Branch, NIAID, NIH. ** We adopted three grades of positive reaction, 3, 2 and 1. tr, trace; —, negative.

hematocrit value was 50.6 ± 3.5% for 12 specimens after 2700 × g for 1 min centrifugation in our case, a full capillary tube of blood (75 µl) contains about half the volume of RBC. We usually pour the full tube of blood into 10 ml Alsever solution immediately after bleeding, mix gently and wash 3 times with PBS by centrifugation at 540 × g for 3 min. Final volume was adjusted 5 ml to make 0.75% RBC suspension. By this procedure one can prepare RBC suspension at a given concentration very easily and fairly accurately. In the present study, we compared three concentrations of RBC suspension, 0.5%, 0.75% and 1.0%. A relatively clear result was obtained at 0.75%, though drastic effect of RBC concentration on the reliability of agglutination has not been observed within these ranges.

Titrations are performed in the Microtiter Plate for tissue culture (Cooke Laboratory Products, Virginia) made of rigid polystyrene, 12.7 × 9.5 cm and with 96 (8 × 12) flat-bottom wells. One drop (25 µl) of 1.2% or 0.6% PVP-BSA was dispensed to each well, then antisera were serially diluted by 25 µl loops. For this process, the microtitier plate should be kept somewhat declined to mix antiserum with diluent throughly. Finally 25 µl RBC suspension was dispensed to each well and shaken for about 10 sec by pressing down the microtitration plate on a voltex mixer. After incubation at 37 °C for 1 h and at 5 °C overnight, each plate was again shaken for 10 sec just before reading. As the specific hemagglutination seems to be fairly tight, one can read it repeatedly following preceding shaking. Without this, false positive agglutination is observed quite often even in the control. Microscopic observation of the hemagglutinations was conducted by the inverted type microscope for tissue culture equipped with X10 objective lens. The distance between the condenser lens and stage should be adjusted to give an appropriate contrast. The figure demonstrates the typical features of positive, trace and negative hemagglutination. Positive reaction is clearly discriminated from either trace or negative.

The sensitivity of the present method was compared with that of Kaliss' method using D-4, D-5 and D-23 alloantisera given by the Transplantation Immunology Branch, NIAID, NIH and B10A mouse RBC (table). Controls consisting of normal B10 mouse serum and B10A RBC have all shown negative reactions. Though the plate method seems to be slightly less sensitive than the original Kaliss' method, its operational simplicity could efficiently compensate the small defect.

Evaluation of humoral response with ragweed antigen in pregnant rats

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29 October 1976

Summary. No difference was observed between the homocytotropic (IgE) antibody titers in the sera of pregnant and non-pregnant rats immunized with DWSR, suggesting that there is no evidence of immunosuppressive factor in humoral response during pregnancy.

Recently, it has been reported that the large increases in the blood level of pregnancy-associated α-microglobulin or the whole pregnancy plasma have an inhibitory effect on several in vitro methods of immunological assessment[1]. This suppressive activity of plasma from pregnant women seemed to be levelled primarily at the cell-mediated immune response. Petrucco and his colleagues[2] reported that the cell-mediated response was significantly reduced in the second and third trimesters of pregnancy. Since, cellular hypersensitivity reactions are believed to be T-cell-mediated[3,4] these authors[2] gave an interpretation that T-cell activity is suppressed during pregnancy and this depression could be the result of immunosuppressive factors present in the lymphocytic environment.

Very little is known about the effects of pregnancy on humoral, i.e., immediate hypersensitivity reactions. The present study was designed to evaluate the humoral response with ragweed antigen in pregnant rats.

Experimental. Short ragweed 1:20 (Hollister-Stier, Mississauga, Ontario) was dialyzed against water at 4 °C and the nondialyzable part followed by lyophilization was used as an immunizing antigen, herein after designated as DWSR.

Nulliparous female outbred Wistar rats (225–250 g) (Woodlyn Farms, Guelph, Ontario), having free access to food and water, were used in this study. 2 females were paired overnight with one male proved fertile in breeding cages. Next morning, copulation was ascertained by finding sperms in vaginal smears or vaginal plug, and this was counted as day one pregnancy. On day 9 of pregnancy, the rats were randomly divided into 2 groups (10 animals per group). One group was immunized with a single i.p. injection of 1.0 ml of mixture containing

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