

-continued

MIXTURE	ABSORBANCE AT 410 nm
Mixture containing heat-inactivated (normal) rabbit serum	.01
Test mixture containing antiserum to dinitrophenylated bovine serum albumin	1.2

EXAMPLE IV

In this Example, the immune specific lysis of liposomes is applied to the determination of relative concentrations of specific antibody. All conditions were identical to those of Example III but various dilutions of the DNP-BSA antiserum were employed in order to assess the effect of different antibody concentrations upon the extent of complement mediated lysis.

The extent of absorbance increase at 410 nm in 5 minutes was recorded at various dilutions.

Antiserum Dilution	410
1:50	1.5
1:75	1.4
1:100	1.15
1:200	.65
1:300	.30

Thus, this method can be employed to assess specific antibody levels.

EXAMPLE V

To test whether antigen would inhibit complement mediated lysis and whether such inhibition could be employed to quantitate antigen levels in a test sample, the protocol of Example III was modified to allow for inclusion of 50 μ l. of DNP-Lysine solutions at various concentrations in the initial incubation mixture. If the absorbance increase at 410 nm in the absence of free DNP-Lysine is taken as 100% lysis, then at various levels of free antigen the following percentages of lysis were recorded:

Free DNP-lysine(picomoles)	% Lysis
6	83
9	69
13.5	56
18	48
27	33
36	23

Over this range, there is a linear relationship between the percent lysis and the logarithm of the antigen concentration. Analyzed by linear least squares regression the linear relationship is characterized by the following parameters:

slope = -33.3
y intercept = 143
correlation coefficient = .998
50% occurs at 16.2 picomole.

EXAMPLE VI

Quantitative immunoassay is performed according to a one-step format, i.e. all reagents including enzyme substrate are mixed together at once so that lytic and enzymatic reactions occur coterminously. Such a single

step format is simple in practice and can be easily automated.

Twenty-five milligrams of L- α -Lecithin-Dipalmitoyl (Calbiochem-Behring Corp., LaJolla, California) 8.6 mg cholesterol (Sigma), 1.6 mg dicetyl phosphate (Sigma), and 1.5 mg of Dinitrophenyl aminocaproyl phosphatidyl ethanolamine (Avanti) were mixed in chloroform solvent. The solvent was removed under reduced pressure in a rotary evaporator, and a thin film of lipids was formed on the interior of a 50 ml. round bottomed flask. This film was then dispersed in an aqueous solution containing 5 milligrams of alkaline phosphatase (Sigma) in 3 ml. of PBS-Dextrose buffer. The liposomes were then harvested by centrifugation as in the previous examples.

To a single tube were added 2 microliter of these liposomes (20 nanomole of phospholipid), 100 microliter of guinea pig complement (diluted 1.8 in complement lysis buffer, 50 microliter of Rabbit antiserum to DNP, 100 microliter of buffer or standard solution and 1 ml. of phosphatase substrate. The reaction mixture was incubated at 37° C. for ten minutes whereupon 1 ml. of 0.5 N sodium hydroxide was added to terminate the enzyme reaction. The absorbance at 405 nm was obtained spectrophotometrically. The extent of reaction was dependent on the quantity of DNP-Lysine (Sigma) as follows:

Amount of DNP-Lysine(pmole)	Abs. at 405 nm
0	.95
2.0	.902
2.5	.811
4	.573
5	.430
6	.311
7	.257

In the absorbance at 405 nm is plotted against the logarithm of the amount of DNP-Lysine a straight line is derived with slope-66.8, intercept 143 and correlation coefficient 0.995. Fifty percent inhibition of lysis is achieved with 4 pmole of DNP-Lysine.

EXAMPLE VII

In an example of kinetic mode quantitation liposomes as described in the previous example are applied to quantitation of antigens by measuring the rate of the enzymatic reaction. In this case the reagents in quantities described in Example VI are mixed in a spectrophotometer cuvette. The time course of the enzymatic reaction may then be monitored directly. After a characteristic lag phase, the rate of increase in absorbance at 405 becomes a linear function of the free antibody concentration. Typically, one adds to a spectrophotometer cuvette 0.75 ml. of phosphatase substrate solution, 50 microliter of antibody, 0.1 ml. of complement and 5 microliter of liposomes. The cuvette is then a thermostatted spectrophotometer and the absorbance at 405 nm is recorded. A characteristic lag phase of 2-3 minutes occurs during which the absorbance changes slightly. After this lag, the absorbance increases rapidly. Beyond 5 minutes, the rate at increase is a function of the amount of antibody available.

Proteins and other macromolecules can be coupled to liposomes. Such liposomes with proteins attached to the outer surface are susceptible to complement mediated lysis in the presence of antibodies to the attached prote-