

# Detection and Characterization of Monoclonal Antibodies to Platelet Membrane Proteins

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**ABSTRACT** We have devised a solid-phase radioimmunoassay for the detection and characterization of monoclonal antibodies directed against platelet surface antigens. Platelet membrane proteins, solubilized with 0.1% Triton X-100, were covalently coupled to cyanogen bromide(CNBr)-activated filter paper disks that were then used as the support in antibody binding assays. SDS PAGE of solubilized membrane proteins taken immediately before and after incubation with activated disks indicated that representative amounts of each membrane protein were bound to the disks. Either monoclonal or heterologous anti-platelet antibody could be detected on disks that had been prepared using as little as 50  $\mu$ g of membrane protein per 100 disks. For the detection of antibody, disks were incubated with test sera for 2 h, washed, and incubated with  $^{125}$ I-labeled anti-immunoglobulin G, and the amount of bound radioactivity was determined. The sensitivity of the disk assay in detecting monoclonal antibodies was far greater than that of a corresponding radioimmunoassay that used whole platelets as the solid phase. By linking other proteins such as fibrinogen or anti-mouse subclass-specific antisera to CNBr-activated disks, the method was adapted for antibody characterization. The sensitivity and ease with which the assay can be performed make this technique most suitable for screening and characterizing monoclonal antibodies.

The development of a technique to produce murine hybridomas (11) has enabled investigators to obtain large quantities of highly specific monoclonal antibodies. These antibodies are well suited to serve as probes in studying the structure, orientation, and function of membrane proteins. In the process of preparing monoclonal antibodies against platelet membrane glycoproteins, we found it necessary to develop a rapid, sensitive screening technique to detect the presence of antibody in the many cell culture supernates generated during cloning.

Current methods for detecting anti-platelet antibody have a number of disadvantages. Techniques using whole platelets as the solid phase of the assay (2, 7, 12, 19) are faced with the inconvenience of preparing fresh cell panels on a regular basis. In addition, the platelet suspension must be washed before and at various points during the assay, a very tedious and time-consuming process necessitating repeated centrifugation and resuspension of many cell pellets.

Schneider and Eisenbarth (17) described a radioimmunoassay (RIA) that used cell monolayers bound to polyvinyl chloride microculture plates as the solid phase. Although this method overcomes many of the disadvantages of working with cell suspensions and is both rapid and sensitive, its use is restricted to cells that are able to grow on polyvinyl chloride plates as adherent, durable, and uniform monolayers. Another drawback of this technique, as well as those methods cited

above, is that it cannot be adapted for determining the specificity of the monoclonal antibodies produced.

As an alternative to an RIA using intact cells, several investigators (6, 14, 20) have coupled specific antigens to cyanogen bromide(CNBr)-activated filter paper disks that then served as the solid phase for the detection of their corresponding antibodies. In addition to obviating the need for working with cells, large numbers of homologous disks could be prepared and used for at least 6 mo (14). We have further characterized this method and modified it so that it may be used to detect anti-platelet antibody with much greater sensitivity than an RIA that tests for antibody binding to intact platelets. Moreover, we have enlarged the scope of the technique so that it may be used to characterize each monoclonal antibody produced.

## MATERIALS AND METHODS

### *Preparation of Platelet Membranes*

Human platelets were prepared by differential centrifugation (10) of units of whole blood collected from volunteer donors to the Missouri-Illinois Red Cross. All subsequent operations were carried out at 4°C unless otherwise specified. The platelets were washed three times in Phillips buffer (0.1 M NaCl, 0.085 M glucose, 1 mM Na<sub>2</sub>EDTA, 8.5 mM Tris, pH 7.4), and resuspended in 45 ml of 50 mM Tris, 1 mM EDTA, pH 7.4, containing 0.4 mM phenylmethylsulfonyl fluoride (PMSF). The cells were then disrupted by sonification using an Artek

Sonic 300 Dismembrator (Artek Systems Corp, Farmingdale, N. Y.) at a power setting of 50, and cellular debris was removed by centrifugation at 12,000 *g* for 10 min. The membrane-rich supernate, which presumably contains all cellular membranes, was spun at 48,000 *g* for 90 min, yielding a pellet containing 5–8 mg of membrane protein that was either solubilized immediately or stored at –20°C until use.

### *Solubilization of Membrane Proteins*

The membrane pellet was dissolved in 2 ml of coupling buffer (0.1% Triton X-100 in 0.1 M NaHCO<sub>3</sub>, pH 8.0, which contained 0.4 mM PMSF). Solubilization was carried out for 2 h. The mixture was then sonicated for 30 s, followed by centrifugation for 90 min at 48,000 *g* to remove unsolubilized membranes. A portion of the supernate was saved for electrophoretic analysis, and the remainder was used for coupling to activated filter paper disks.

### *Activation and Coupling to Filter Paper Disks*

Disks 6 mm in diameter were cut out of Whatman #542 filter paper (Whatman Inc., Clifton, N. J.) with a standard hole punch and activated with CNBr by a procedure modeled after that of Manson et al. (14). 2 g of disks were hydrated in 40 ml of deionized water for 1 h and then mixed with an additional 40 ml of 5% CNBr. Activation was carried out at 19°C with stirring, and a pH of 10 was maintained by the periodic addition of drops of 1M NaOH. After 3 min, the reaction was stopped by transferring the mixture to 300 ml of ice-cold 5 mM NaHCO<sub>3</sub>. The activated disks were then washed five times in 5 mM NaHCO<sub>3</sub>, followed by four washings in 100 ml of ice-cold acetone. The disks were allowed to dry at room temperature and then stored at –20°C.

Coupling of protein to the disks was achieved by allowing ~100 activated disks to react overnight with 2 ml of solubilized platelet membranes that had been adjusted with coupling buffer to a protein concentration of 1 mg/ml (except where otherwise indicated). Incubation was carried out in 12 × 75 mm glass test tubes that were continuously agitated on a test tube rocker. The reaction was stopped by removing the protein solution, and unreacted sites were blocked by incubation for 2 h with 10 ml of 1 M β-ethanolamine, pH 8.2. After inactivation, the disks were washed four times by alternatively rinsing with and then aspirating off 10 ml of PEB buffer (phosphate-buffered saline containing 1 mM EDTA and 1% bovine serum albumin [BSA], pH 7.2) and stored at 4°C in excess PEB for up to 6 mo. In some cases, proteins other than solubilized platelet membranes were coupled to disk by essentially the same methodology. For these experiments, human fibrinogen was purchased from Sigma Chemical Co., St. Louis, Mo., and anti-mouse subclass-specific antisera was obtained from Meloy Laboratories Inc., Springfield, Va.

### *Production of Mouse Anti-platelet Antisera*

10<sup>9</sup> washed human platelets pooled from four blood group O+ male donors were resuspended in 1 ml of Phillips buffer and emulsified in an equal volume of Freund's complete adjuvant. BALB/c mice were injected intraperitoneally with 0.2 ml of the cell suspension, and boosted 3 wk later with identical material emulsified in Freund's incomplete adjuvant. Antisera was collected from the retroorbital plexus 10 d after the last inoculation and stored at –80°C until use.

### *Mouse Anti-platelet Hybrid Cell Lines*

The immunized BALB/c mice that produced anti-platelet antisera were reboosted with platelets and sacrificed 3 d later. 10<sup>6</sup> spleen cells from an immunized mouse were fused with 10<sup>7</sup> Sp2/0-Ag14 myeloma cells, an azaguanine-resistant strain that neither synthesizes nor secretes its own immunoglobulin chains (18). The resultant hybridomas, FC201 A3/A1 and FC201 A3/A6, each secreted an anti-platelet antibody, large quantities of which were produced by growing these hybridomas as ascites tumors in BALB/c mice. For subclass identification of each monoclonal antibody, hybridomas were grown in tissue culture and allowed to secrete antibody directly into the cell culture media. Control ascites fluid was obtained from Bethesda Research Laboratories, Rockville, Md.

### *Radioimmunoassays for Detecting Anti-platelet Antibody*

**WHOLE PLATELET RIA:** Washed platelets were resuspended in PEB and adjusted to a concentration of 750,000/μl. The assay was performed in 12 × 75 mm polystyrene test tubes that had been coated with 5% BSA overnight. Antibody dilutions were made into PEB. To each tube containing 0.2 ml of platelet suspension, 0.1 ml of antibody was added and the mixture was allowed to

incubate for 30 min at 37°C. The cells were then washed three times with 1.0 ml of PEB followed by the addition of 0.1 ml of <sup>125</sup>I-labeled rabbit anti-mouse IgG containing 100,000 cpm. After a second 30-min incubation at 37°C, the cells were washed three times in PEB, and the radioactivity of the cell pellet was measured with a gamma counter (counting efficiency = 83%). Samples having more than twice the counts per minute of the negative control were considered positive.

**FILTER PAPER DISK RIA:** The filter paper disks to which antigen was bound were placed in 60-well microtiter trays (Abbott Laboratories, North Chicago, Ill.). Each well has a diameter of 10 mm and a capacity of 0.8 ml. 50 μl of test sera was added and allowed to incubate with the disks at 25°C for 2 h (see Results). This volume is sufficient to completely submerge the disk. After incubation, the disks were washed three times in PEB by repeatedly withdrawing and adding buffer to each well. Agitation of the disks during washing had no beneficial effect. 0.1 ml of <sup>125</sup>I-labeled rabbit anti-mouse IgG containing ~50,000 cpm was then added and allowed to incubate at 25°C for 2 h, after which time the disks were washed as before. The disks were then transferred to counting tubes and bound radioactivity was determined as described above.

### *Radioiodination of Proteins*

100 μg of the immunoglobulin fraction of rabbit anti-mouse IgG (Miles Laboratories, Elkhart, Ind.) was labeled with 1 mCi of pH 8–10 Na<sup>125</sup>I (New England Nuclear, Boston, Mass.), using insolubilized lactoperoxidase (Enzymobeads, Bio-Rad Laboratories, Richmond, Calif.) according to the manufacturer's directions. Unbound <sup>125</sup>I was removed from the reaction mixture by gel filtration through a 10-ml Sephadex G-25 column equilibrated in PEB. Fractions containing >98% bound radioactivity, as determined by TCA precipitation, were pooled and stored at –80°C for up to 3 mo. A specific activity of 2–4 μCi/μg was routinely obtained.

### *SDS PAGE*

Protein samples were solubilized in 2% SDS, 5% 2-mercaptoethanol by boiling for 5 min. Protein concentration was determined by the method of Bradford (4). 50–150 μg of sample was applied to a 5–13% linear gradient slab gel and electrophoresed for 3 h at 30 mA. The buffer system of Laemmli was used (13). Electrophoresis was performed in a Hoefer SE 600 vertical slab gel apparatus (Hoefer Scientific Instruments, San Francisco, Calif.), which was kept at 5°C during the run. After electrophoresis, gels were fixed for 1 h in 12% TCA, 3% sulfosalicylic acid, transferred to universal solvent (10% acetic acid, 25% methanol) for 30 min, and then stained in 0.2% Serva Blue R (Accurate Chemical Corp., Hicksville, N. Y.) dissolved in universal solvent (15). The gels were completely destained in universal solvent within 6–10 h and dried on a Bio-Rad Model 224 slab gel dryer.

## RESULTS

### *Extraction of Proteins from the Platelet Membrane*

Platelet membrane protein could be quantitatively extracted with Triton X-100. Various concentrations of this detergent were tried, with 0.1% giving optimal extraction (data not shown). Using this concentration of Triton X-100, we could routinely solubilize 75% of the protein from an 8-mg membrane pellet. Samples of the detergent extracts analyzed by SDS PAGE were qualitatively shown to contain all the major surface glycoproteins when compared with an SDS solubilized whole membrane fraction. The solubilized proteins were then coupled directly to CNBr-activated disks.

### *Ability of Activated Disks to Bind Membrane Proteins*

Because selective binding of individual membrane proteins to disks might bias the test results, it was important to determine whether representative amounts of each solubilized membrane protein had been bound. To answer this question, samples were taken immediately before and after the coupling procedure and subjected to SDS PAGE. The postcoupling extract contained ~20% of the precoupling protein. We found no significant

qualitative or quantitative differences in the one-dimensional electrophoretic patterns when samples used for electrophoresis had been adjusted for comparable amounts of total protein. As shown in Fig. 1, equal amounts of each protein appeared to have been linked to the disks.

### Effect of Varying the Concentration of Membrane Protein Coupled to Disks

Because many antigens of interest are obtainable only in small quantities, we determined the minimum amount of antigen necessary to perform the assay. 0.1%-Triton-X-100-solubilized membrane proteins were diluted with coupling buffer to various concentrations. As shown in Fig. 2, antibody-containing culture supernates could still be discriminated from negative supernates using as little as 50  $\mu$ g of membrane protein per 100 disks. Heterologous anti-platelet antibody could be detected on disks containing much less protein.

### Length of Incubation

To minimize the length of time necessary to perform the assay, we examined the effect of varying the first and second

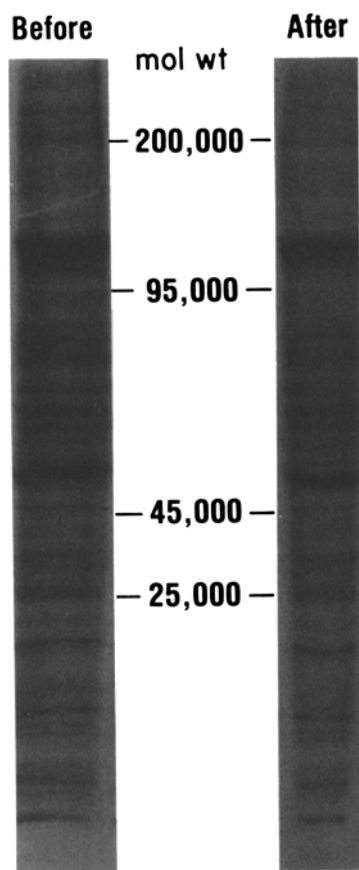


FIGURE 1 Ability of activated disks to bind detergent-solubilized membrane proteins. Electrophoretic analysis of Triton-X-100-solubilized platelet membrane proteins was performed to determine whether the disks would bind representative amounts of each protein. Samples taken immediately before and after coupling were solubilized in 2% SDS, 5% 2-mercaptoethanol and boiled for 5 min. The protein concentration of the two samples was 1.0 and 0.2 mg/ml, respectively. 50  $\mu$ g of each was applied to a 5–13% linear gradient slab gel. The buffer system of Laemmli (13) was used. Gels were stained with 0.2% Serva Blue R dissolved in universal solvent (15).

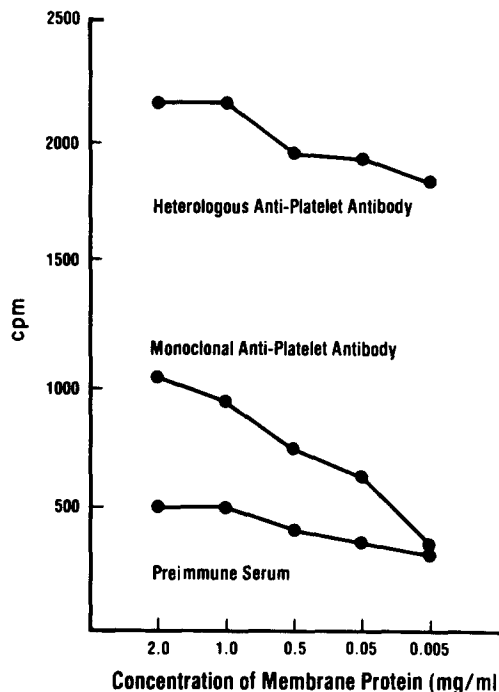


FIGURE 2 The effect of varying the concentration of membrane protein for coupling to activated disks. 100 filter paper disks were incubated overnight at 4°C with 2 ml of solubilized platelet membrane protein diluted to various concentrations with 0.1 M NaHCO<sub>3</sub>, 0.1% Triton X-100, pH 8.0. Assays were performed using both immune mouse serum and monoclonal antibody directed against platelet membrane protein. Both preimmune serum and negative hybridoma culture supernates were used as negative controls and gave similar results.

TABLE I  
Effect of Varying the Incubation Time

(A) Incubation of Test Sera with Disks		(B) Incubation of <sup>125</sup> I-labeled Anti-mouse IgG with Disks	
Time	P/N Ratio	Time	P/N Ratio
h		h	
1	4.0	0.5	5.2
2	4.2	1	6.0
5	3.6	2	6.3
10	3.1	5	6.3
18	3.3	10	6.1
24	3.1	24	7.1

Incubation times for both first and second antibodies were varied to determine the optimum conditions for the assay. (A) Test serum was incubated with disks containing platelet membrane protein for the times indicated. The disks were then washed and incubated for 2 h with 30,000 cpm of <sup>125</sup>I-labeled anti-IgG. (B) Test serum was allowed to incubate with disks for 2 h. The disks were then washed and incubated with <sup>125</sup>I-labeled anti-IgG for the times indicated. Data are expressed as the ratio of counts bound to disks from positive serum (P) compared with the negative control (N).

antibody incubation time. Although the absolute number of bound counts increased with time when disks were incubated with either preimmune or anti-platelet antisera (data not shown), the ratio of counts from positive and negative samples was maximal when test sera were incubated with the disks for 2 h (Table I). The optimal incubation time with the second, radiolabeled antibody was 24 h, but, because of the inconvenience of such a long incubation time without a substantial gain in sensitivity, we routinely used a 2-h incubation. Under these circumstances, 200 tests could easily be performed each day.

## Sensitivity of Filter Paper Disk RIA

Fig. 3 shows a typical titration curve of heterologous anti-platelet antisera assayed using both whole platelets and disks as the solid phase. Although the absolute number of counts bound to the disks was never as high as that bound to whole platelets, the disks were far superior in their ability to detect antibody present in very low concentrations. This may be the result of the greater availability of proteins on the disk as compared with that of proteins in the plasma membrane. When monoclonal antibodies FC201 A3/A1 and A3/A6 were used, the difference in sensitivity between the two tests was not as pronounced, but the disk RIA was still substantially more sensitive. For example, the titer of both monoclonals was 1:20,000 with disks vs. 1:4,000 in the whole-cell RIA.

## Characterization of Anti-platelet Antibodies by Use of the Disk RIA

Heterologous anti-platelet antisera often contain substantial amounts of anti-fibrinogen antibody. The presence of anti-fibrinogen can, in some cases, greatly decrease the usefulness of such an antiserum. To detect the presence of anti-fibrinogen activity, purified fibrinogen was coupled to activated filter paper disks. Using these disks, the presence of anti-fibrinogen antibodies in heterologous mouse anti-platelet antisera was easily detected. Hybridomas generated from this same mouse, however, secreted antibodies that were negative when tested with fibrinogen disks, indicating that the anti-fibrinogen activity had been cloned out (Table II).

When anti-mouse subclass specific antibody was linked to disks, the subclass of each monoclonal antibody could be readily identified (Table III). As expected, normal mouse serum contained all subclasses, whereas both FC 201 A3/A1 and A3/A6 were clearly shown to be IgG<sub>1</sub> antibodies. Ascites fluids were unsuitable for use in subclass identification, because the low levels of immunoglobulins normally present were easily detected by the disk assay, yielding ambiguous data.

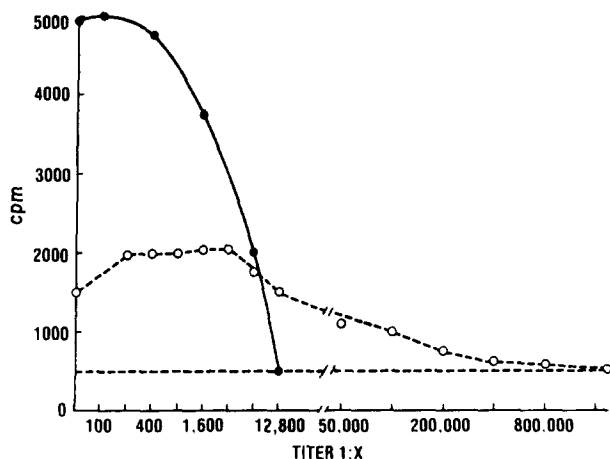


FIGURE 3 Titration of mouse anti-human platelet antibody. Preimmune or heterologous anti-platelet antiserum was serially diluted and then assayed, using as the solid phase either whole platelets (750,000/ $\mu$ l) or disks to which Triton-X-100-solubilized platelet membrane proteins were bound. Negative hybridoma culture supernates or control ascites fluid could also serve as the negative control. Negative control serum bound ~500 cpm regardless of which solid phase was used. (●) Whole platelet RIA; (○) disk RIA; (---) preimmune serum.

TABLE II  
Ability of Disk Assay to Determine Antibody Monospecificity

Sera tested	<sup>125</sup> I-labeled anti-IgG bound cpm
Heterologous anti-platelet	2,300
Monoclonal	600
Preimmune	500

Purified fibrinogen was coupled to disks in order to detect the presence of anti-fibrinogen antibody. The disks were then incubated with the samples indicated. Binding was detected by a second incubation with <sup>125</sup>I-labeled anti-mouse IgG.

TABLE III  
Identification of Mouse Immunoglobulin Subclass by Use of Disk RIA

Antibody coupled to disk	Samples tested*		
	Normal mouse serum	Monoclonal A3/A1	Monoclonal A3/A6
Anti-IgG <sub>1</sub>	1,000	1,550	1,100
Anti-IgG <sub>2A</sub>	360	0	0
Anti-IgG <sub>2B</sub>	675	0	0
Anti-IgM	365	0	0

\* Numbers shown are in cpm of sample minus cpm of negative control. Subclass-specific anti-mouse immunoglobulins were covalently coupled to disks. Each disk was then incubated with the antibody sample indicated. Binding was detected by a second incubation with light-chain-specific <sup>125</sup>I-labeled anti-mouse IgG.

## DISCUSSION

Many procedures have been developed to detect the presence of antibody to membrane-associated antigens; virtually all of these techniques rely upon the binding of antibodies to intact cells. The major disadvantages of these methods are that they are time-consuming, cumbersome, and cannot readily be used to characterize the antibody in question. Other investigators (6, 14, 20) overcame these problems by linking purified antigen to CNBr-activated filter paper disks, which then served as the solid phase to detect the corresponding antibody. We have extended this method for use in the detection of anti-platelet antibodies. Moreover, it was possible to partially characterize the antibodies produced by coupling specific, purified antigens to disks.

The ability to covalently link any protein(s) to the disk is perhaps the most useful feature of the method. We took advantage of this in several ways. First, rather than working with whole cells, we linked solubilized membrane proteins to the disks. Treatment of platelet membranes with Triton X-100 extracted proteins that appeared to bind uniformly to disks (Fig. 1). Our membrane preparation presumably contains total cellular membrane, therefore both intracellular and plasma membranes can be linked to disks to test for antibodies. Of importance is the observation that the extraction and coupling procedure did not result in any loss of antigenicity to either heterologous or monoclonal antibody. It is likely, however, that some proteins will lose their antigenicity after extraction from the membrane, and thus the disk technique may be unsatisfactory under certain circumstances. On the other hand, there are many reports indicating that nonionic detergents by and large do not affect protein antigenicity (3, 8, 9). Moreover, solubilization of membrane proteins may be advantageous when detection of antibodies directed either against poorly expressed membrane proteins or against cryptic sites of a membrane protein (e.g., hydrophobic or cytoplasmic domains)

is desired. In studies we recently performed, membrane proteins were extracted from SDS polyacrylamide gels and then linked to disks. Heterologous antibody retained 90% of its reactivity to these disks compared with disks prepared with the same proteins before electrophoresis. These preliminary results indicate that individual membrane proteins might be isolated from gels and used to prepare disks of predefined specificities. Data so obtained might supplement and, in some instances, replace techniques such as immunofixation (16) and "western blot" analysis (21).

Second, we made use of the versatility of the technique by linking purified antigens to the disks for antibody characterization. For example, by coupling fibrinogen to the disks, we were able to identify clones that did not have anti-fibrinogen activity. Also, we were able to rapidly determine the subclass of our monoclonal antibodies by coupling anti-mouse subclass specific immunoglobulins to disks. Subclass identification by this method offers several advantages over traditional double-diffusion techniques, including speed and sensitivity.

Third, we could prepare hundreds of disks in a very short period of time using very little antigen, with the added convenience of being able to use the disks for at least 6 mo with no appreciable loss in activity. These advantages enabled us to achieve consistent and reproducible results. Moreover, the speed and sensitivity of the technique made it possible to screen hundreds of samples each day.

Lastly, because surfactant effects of nonionic detergents could retard, if not prevent, protein binding to polystyrene microtiter wells, this commonly used support (1, 5) may be unsuitable when detergents used for membrane solubilization are retained in subsequent steps. The covalent binding of protein to CNBr-activated filter paper is unaffected by the presence of nonionic detergents, making the disk technique most suitable for identifying antibodies to solubilized membrane proteins.

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#### REFERENCES

1. Abraham, G. E. 1969. Solid phase radioimmunoassay of Estradiol-17B. *J. Clin. Endocrinol. Metab.* 29:866-870.
2. Aster, R. H., H. E. Cooper, and D. C. Singer. 1964. Simplified complement fixation test for the detection of platelet antibodies in human serum. *J. Lab. Clin. Med.* 63:161-172.
3. Bjerrum, O. J. 1977. Immunochemical investigation of membrane proteins: a methodological survey with emphasis placed on immunoprecipitation in gels. *Biochim. Biophys. Acta.* 472:135-195.
4. Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248-254.
5. Catt, K. J., and G. W. Tregear. 1967. Solid phase radioimmunoassay in antibody-coated tubes. *Science (Wash. D. C.)*. 158:1570-1572.
6. Ceska, M., R. Eriksson, and J. M. Varga. 1972. Radioimmunosorbent assay of allergens. *J. Allergy Clin. Immunol.* 49:1-9.
7. Cines, D. B., and A. D. Schreiber. 1979. Immune thrombocytopenia. Use of a Coombs antiglobulin test to detect IgG and C3 on platelets. *N. Engl. J. Med.* 300:106-111.
8. Colonna, G., S. S. Alexander, K. M. Yamada, K. Pastan, and H. Edelhofer. 1978. The stability of cell surface protein to surfactants and denaturants. *J. Biol. Chem.* 253:7787-7790.
9. Helenius, A., and K. Simons. 1975. Solubilization of membranes by detergents. *Biochim. Biophys. Acta.* 415:29-79.
10. Kahn, R. A., I. Cossette, and L. I. Friedman. 1976. Optimum centrifugation conditions for the preparation of platelet and plasma products. *Transfusion (Phila.)*. 16:162-165.
11. Kohler, G., and C. Milstein. 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature (Lond.)*. 256:495-497.
12. Kr. Von Dem Borne, A. E. G., F. W. A. Verheugt, F. Oosterhof, E. von Riesz, A. Brutel De La Riviere, and C. P. Engelfriet. 1978. A simple immunofluorescence test for the detection of platelet antibodies. *Br. J. Haematol.* 39:195-207.
13. Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature (Lond.)*. 227:680-685.
14. Manson, L. A., E. Verastegui-Cerdan, and R. Sporer. 1978. A quantitative disc radioimmunoassay for antibodies directed against membrane-associated antigens. *Curr. Top. Microbiol. Immunol.* 81:232-234.
15. Radola, B. J. 1980. Ultrathin-layer isoelectric focusing in 50-100  $\mu$ m polyacrylamide gels on silanized glass plates or polyester films. *Electrophoresis*. 1:43-56.
16. Ritchie, R. F., and R. Smith. 1976. Immunofixation. I. General principles and application to agarose gel electrophoresis. *Clin. Chem.* 22:497-499.
17. Schneider, M. D., and G. S. Eisenbarth. 1979. Transfer plate radioassay using cell monolayers to detect anti-cell surface antibodies synthesized by lymphocyte hybridomas. *J. Immunol. Methods.* 29:331-342.
18. Shulman, M., C. D. Wilde, and G. Kohler. 1978. A better cell line for making hybridomas secreting specific antibodies. *Nature (Lond.)*. 276:269-270.
19. Shulman, N. R., V. J. Marder, M. C. Hiller, and E. M. Collier. 1964. Platelet and leukocyte isoantigens and their antibodies: serologic, physiologic, and clinical studies. *Prog. Hematol.* 4:222-304.
20. Smith, H. V., R. Quinn, R. G. Bruce, and R. W. A. Girdwood. 1980. A paper radioimmunosorbent test (PRIST) for the detection of larva-specific antibodies to *Toxocara canis* in human sera. *J. Immunol. Methods.* 37:47-55.
21. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. U. S. A.* 76:4350-4354.