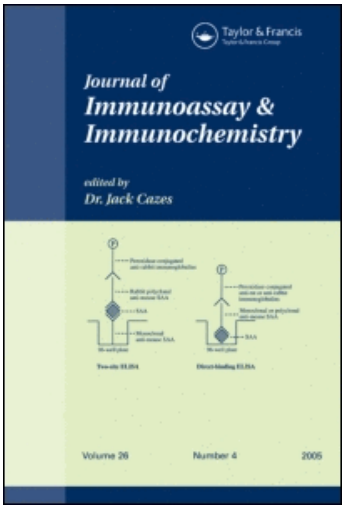

Journal of Immunoassay and Immunochemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

Coating of Polymeric Surfaces for Immunoassay by a Forced Adsorption Technique

Karim E. Hechemy[a]; Robert L. Anacker[b]
[a] Center for Laboratories and Research, New York State Department of Health, Albany, New York [b] National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratories, Hamilton, Montana

To cite this Article Hechemy, Karim E. and Anacker, Robert L.(1983) 'Coating of Polymeric Surfaces for Immunoassay by a Forced Adsorption Technique', Journal of Immunoassay and Immunochemistry, 4: 2, 147 — 157
To link to this Article: DOI: 10.1080/15321818308057007
URL: http://dx.doi.org/10.1080/15321818308057007

JOURNAL OF IMMUNOASSAY, 4(2), 147-157 (1983)

COATING OF POLYMERIC SURFACES FOR IMMUNOASSAY BY A FORCED ADSORPTION TECHNIQUE

Karim E. Hechemy* and Robert L. Anacker+

*Center for Laboratories and Research, New York State Department of Health, Albany, New York 12201; and +National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratories, Hamilton, Montana 59840

ABSTRACT

A forced adsorption technique for coating polymeric surfaces is described. This technique is simple and allows us to use (i) relatively impure ligands to be coated on latex surface and (ii) the minimum amount of ligands for preparation of large-volume lots of latex reagent. The serologic results are highly reproducible from lot to lot of the reagent, in contrast to the variability found with passively adsorbed reagents. Use of this technique could be extended to coat flat surfaces for solid-phase immunoassays. (KEY WORDS: forced adsorption, latex, Rocky Mountain spotted fever)

INTRODUCTION

We have previously (1) reported the development of a sensitive, specific latex-Rickettsia rickettsii test for detection of antibodies to Rocky Mountain spotted fever (RMSF). The reagent for that test was prepared by passive adsorption onto latex particles (1) of erythrocyte-sensitizing substance (ESS) prepared from R. rickettsii purified by sucrose density-gradient centrifugation (2).

 0197-1522/83/0402-0147$3.50/0

To undertake an extensive interlaboratory evaluation of this test, we prepared several lots of the latex-R. rickettsii reagent using several batches of ESS. Quality control of these lots indicated a lack of reproducibility from lot to lot and a decrease in the sensitivity of the test. This prompted our concern that the diagnostic value of the test might be impaired.

To overcome this problem, we developed a forced adsorption technique which has enabled us to prepare the latex-R. rickettsii reagent in large volumes and with consistent sensitivity and reproducibility from batch to batch of ESS antigen. This note details the forced adsorption method for coating ESS onto latex particles for use in immunodiagnosis of RMSF. The method may also be applicable to the coating of other ligands on particulates or flat surfaces.

MATERIALS AND METHODS

Preparation of ESS

Ten batches of R. rickettsii ESS were prepared, nine as described by Anacker (2) and the tenth, A255, according to the method of Chang (3).

Forced-Adsorption Technique

Latex polystyrene particles (0.81 μm diameter; Difco Laboratories, Detroit, MI) were adjusted with distilled water (DW) to contain 3.7×10^{10} particles per ml (4). One volume of the latex suspension was centrifuged at 16,000 x g for 30 min at 5°C.

The supernatant (labeled A), which included the manufacturer's stabilizers, was decanted, saved, and kept at 5°C for futher use to resuspend the latex pellet (4). The latex sediment was resuspended in 1/20th the volume of glycine-buffered saline (GBS; 4) pH 8.2, and stirred for 15 min with a magnetic bar until all clumps were broken.

The appropriate volume of antigen, determined by checkerboard titration, was added dropwise to the continually stirring latex mixture. The optimum volumes ranged from 15 to 50 µl/ml. After stirring for an additional 15 min at 25°C, 8 to 10 volumes of ethanol-acetate (95% ethanol containing 0.5% sodium acetate) was added. The mixture was stirred for another 30 min at 25°C and left overnight at 5°C. The latex suspension was then centrifuged at 16,000 x g for 30 min at 5°C, and the ethanolic supernatant was discarded.

The latex sediment was resuspended in 1/10th the volume of supernatant A, and the mixture was stirred at 25°C until all clumps were broken. Supplementary ESS (¼ to ½ the volume of ESS used in coating the particles) was gradually added, and the mixture was continually stirred for an additional 30 min. The remainder of supernatant A was then added, followed by GBS, so that the combined volume of GBS and supplemental ESS was equal to the original volume of the latex suspension. Then, for every 1 volume of this suspension, 0.125 volume of GBS containing 0.1% fatty acid-free bovine albumin (GBS-BAF; Sigma Chemical Co., St. Louis, MO) was added.

The latex-R. rickettsii suspension was dispensed in 2-ml aliquots. Each aliquot was sonicated for 15 s at a setting of 35, using the microprobe of a Fisher Sonic Dismembrator Model 300.

Test Sera

Individual or pooled human sera which were authenticated to contain anti-R. rickettsii antibodies by the microimmunofluorescence test of Philip et al. (5) were used to test the forced-adsorption technique. Each test serum or pool was first diluted at 1:16 in GBS-BAF and inactivated for 30 min at 56°C. The titration was performed exactly as previously described (1).

RESULTS

ESS antigen batches were used to prepare the latex-R. rickettsii reagent by both the passive and forced adsorption techniques. The variation in titer of a standard reactive serum assayed with reagents prepared by both techniques is shown in Table 1. Results with the passively adsorbed reagent were inconsistent. With the forced-adsorption reagents the standard serum was consistently reactive and at higher titers.

Eleven large-volume lots and seven pilot lots of latex-R. rickettsii reagent were prepared from 10 batches of ESS antigen by the forced adsorption technique and were titrated against four standard sera over a period of 3 years. The results are summarized in Table 2. For each standard the endpoints were within a single dilution with all latex-R. rickettsii lots tested, regardless of volume. Similarly all results with a given antigen

TABLE 1

Dependence of latex-R. rickettsii test reactivity on choice of passive or forced adsorption of ESS on latex particles[a]

ESS batch no.	Serum titer	
	Passive adsorption	Forced adsorption
A255	256	512
ZR195 - 1	32	512
- 2	NR[b]	1024
ZR197/198	NR	1024

[a] A standard serum with a microimmunofluorescence titer of 2048 was used to test these preparations.

[b] NR, nonreactive.

batch were within one twofold titer difference. These findings show the reproducibility of results with the forced-adsorption reagents and the applicability of this technique for preparing large volumes (up to 680 ml) of latex-R. rickettsii reagent. (The 0.6-ml lots were pilot lots for the checkerboard titration. They were used to establish the optimum antigen concentration for each ESS batch, which was then used to prepare the large-volume lots).

DISCUSSION

Man-made polymers such as latex have been used as carriers in immunodiagnosis to detect antigens (4) or antibodies (6). The

TABLE 2

Reproducibility of latex-*R. rickettsii* test with various lots of forced-adsorption antigen and four standard sera over a 3-year period.

ESS batch no.	Latex-*R. rickettsii* lot no.	Volume (ml)	Titer of standard serum control			
			A	B	C	D
A255	011179	144	512			
ZR195	011279	59	1024			
ZR197/198	011679	72	1024			
	061779	0.6	1024			
	062979	325	1024	256		
ZR214/215	012180	0.6		512		
	012280	680		256		
ZR220/221	042180	558			64	
ZR225/226	071880	0.6			64	
	072180	537			64	128
ZR205	060181	0.6				64
	060281	269				64
ZR228	083181	0.6				128
	090181	124				64
ZR238/228	022582	0.6				128
	030282	234				128
ZR239	050582	0.6				128
	051782	306				64

common methods to attach these ligands to the carriers are passive adsorption, which is based on hydrophobic bonding (7), and coupling, which is based on covalent bonding (8). Both procedures require highly purified components because contaminants will compete for the limited surface area on the particle or for the active sites on activated particles. In the coupling procedure, excessive amounts of ligands are usually required to complete the reaction. This may be wasteful of ligands which are difficult to prepare. Also, the coupling procedure requires involved protocols (9) and long preparation times, which may compromise the usefulness of these reagents in test systems.

Passive adsorption is a function of the affinity (7) and relative initial concentrations (10) of the components in solution. High concentrations of impurities with high affinities for the particle surface might compete too successfully for sites on the particle and result in decreased test sensitivity. With forced adsorption the main factor is the efficiency of the precipitating agent, rather than the affinity of the components for the surface. This efficiency can be seen in the results for the ESS system (Table 1).

Erythrocyte-sensitizing substance is a product of sodium hydroxide treatment of rickettsiae without further purification. It appears to be a protein-carbohydrate complex with a protein-to-carbohydrate ratio of 2:1 (11). The carbohydrate moiety of ESS is responsible for serologic activity (11). We theorize that in passive adsorption the protein competes with the poly-

saccharide for the surface of the latex particles, with a resulting loss in sensitivity. In forced adsorption, however, both components are precipitated without loss of sensitivity (Table 1). Futhermore the forced adsorption method is simple, and the time required to prepare the reagent is only that needed to precipitate the ligands onto the surface.

Selective use of precipitating agents is important and is governed by several factors. The precipitating agent should precipitate the desired ligand without destroying its biologic activity or the polymeric surface to be coated. Moreover the precipitating agent should not irreversibly aggregate the particles. We have found that trichloroacetic acid, for example, cannot be used to precipitate globulin on latex because the latex pellet cannot be resuspended without drastic measures. For particulate surfaces low aliphatic alcohols, alone or mixed with low concentrations of salt (e.g. ethanol and sodium acetate), can be used successfully to precipitate proteins and carbohydrates. For ESS ethanol meets all of the essential criteria.

The quantity of antigen that can be force-adsorbed is limited by the saturation point of the particle surface. In general, once the surface is coated with ligand, there is no point in trying to force on more. Layering ligand over ligand on the particles by increasing its concentration will not optimize the sensitivity of the reagent (6, 10) because antigenic determinants in the inner layers may not be available to react. However, since the concentration of antigen adsorbed on the surface of the particle is not

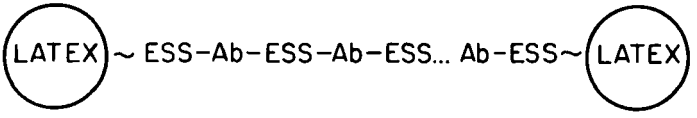

FIGURE 1. Simplified model of latex-R. rickettsii aggregation in the presence of supplementary souble ESS. ~~ESS represents ESS bound to latex particles.

usually optimum for a maximum antigen-antibody reaction (6), we add supplementary soluble ligand at a later step in the preparation of the reagent to maximize the sensitivity (6). This free ligand, e.g. R. rickettsii ESS, forms crosslinks between the newly formed latex-antigen/antibody complexes and free antibody to produce larger aggregates (Fig. 1), which result in higher sensitivity.

The sensitivity of the latex-R. rickettsii was much improved by the forced adsorption technique. Specificity was not adversely affected. During the development stages, when the reagent was prepared by direct adsorption, the specificity was 93.9% for single sera and approached 100% for paired sera (1). During the evaluation, when the reagent was prepared by forced adsorption, the overall specificity was 98.93% (12). The reagent prepared by forced adsorption was stable over a 17-month period with no appreciable loss of reactivity (12).

This forced adsorption technique might also be applied to prepare flat polymeric surfaces, for solid-phase immunoassay such as radioimmunoassay and enzyme immunoassay (EIA), so as to eliminate variation in test results. Kricks et al. (13) have shown substantial variation (5.2 to 29.5%) in the amount of protein adsorbed on micro-

titer plates for EIA by the passive adsorption method. They concluded that the variability in the amount of protein adsorbed onto the surfaces of individual wells on the same plate seriously affects the reliability and interpretation of EIA results.

In conclusion, we are presenting an alternative to the passive adsorption and coupling methods, which coat ligand onto particles in a random fashion. The forced adsorption technique enables the preparation of reproducible, stable, sensitive, and specific reagents for diagnostic test systems.

ACKNOWLEDGMENTS

We acknowledge Sandra Sasowski and Edith Michaelson for their excellent assistance.

Reprint requests to: Dr. Karim E. Hechemy, Center for Laboratories and Research, New York State Department of Health, Empire State Plaza, Albany, New York 12201.

REFERENCES

1. Hechemy, K. E., Anacker, R. L., Philip, R. N., et al. Detection of Rocky Mountain Spotted Fever Antibodies by a Latex Agglutination Test. J. Clin. Microbiol. 1980;12:144-150.

2. Anacker, R. L., Philip, R. N., Thomas, L. A., and Casper, E. A. Indirect Hemagglutination Test for Detection of Antibody to Rickettsia rickettsii in Sera From Human and Common Laboratory Animals. J. Clin. Microbiol. 1979;10:677-684.

3. Chang, S-M. A Serologically-Active Erythrocyte-Sensitizing Substance from Typhus Rickettsiae. I. Isolation and Titration. J. Immunol. 1953;70:212-214.

4. Hechemy, K. E., Stevens, R. W., and Gaafar, H. A. Detection of Escherichia coli Antigens by a Latex Agglutination Test. Appl. Microbiol. 1974;28:306-311.

5. Philip, R., Casper, E. A., Ormsbee, R. A., Peacock, M. G. and Burgdorfer, W. Microimmunofluorescence Test for the Serological Study of Rocky Mountain Spotted Fever and Typhus. J. Clin. Microbiol. 1976;3:51-61.

6. Hechemy, K., Stevens, R. W., and Gaafar, H. A. Antigen Distribution in a Latex Suspension and Its Relationship to Test Sensitivity. J. Clin. Microbiol. 1976;4:82-86.

7. Cantarero, L. A., Butler, J. E., and Osborne, J. W. The Adsorptive Characteristics of Proteins and Their Significance in Solid-Phase Immunoassays. Anal. Biochem. 1980;105:375-382.

8. Quash, G., Roche, A-M., Grange, J., et al. The Preparation of Latex Particles with Covalently Bound Polyamines, IgG and Measles Agglutinins and Their Use in Visual Agglutination Tests. J. Immunol. Methods 1978;22:165-174.

9. Cleveland, P. H., Wickham, G. M., Goldbaum, M. H., Ryan, A. F., and Worthen, D. M. Rapid and Efficient Immobilization of Soluble and Small Particulate Antigens for Solid Phase Radioimmunoassay. J. of Immunoassay 1981;2:117.

10. Herman, J. E. and Collins, M. F. Quantitation of Immunoglobulin Adsorption to Plastics. J. Immunol. Methods 1976;10:363-366.

11. Osterman, J. V. and Eisemann, C. S. Rickettsial Indirect Hemagglutination Test: Isolation of Erythrocyte-Sensitizing Substance. J. Clin. Microbiol. 1978;8:189-196.

12. Hechemy, K. E., Sasowski, S. J., Michaelson, E. E., et al. Rocky Mountain Spotted Fever: First-Year Evaluation of a Latex Agglutination Test for Antibodies to Rickettsia rickettsii, In W. Burgdorfer and R. L. Anacker (ed.), Rickettsia and Rickettsial Diseases. New York:Academic Press, 1981:159-169.

13. Kricks, L. J., Carter, T. J. N., Burt, S. M., et al. Variability in the Adsorption Properties of Microtiter Plates Used as Solid Supports in Enzyme Immunoassay. Clin. Chem. 1980;26:741-744.