Phase-Separation Immunoassays


Solid-phase-based immunoassays have traditionally been plagued by nonspecific binding to the solid phase and by slow reaction kinetics relative to reactants that are free to diffuse in solution. We have developed two novel immunoassays in which the solid phase is generated in situ after the specific binding reaction has occurred, thereby enhancing reaction kinetics and minimizing the opportunities for nonspecific binding. In the first system, the capture antibody is conjugated to an organic monomer, polymerization of which to form insoluble polymer particles is initiated by a reaction involving free radicals. The amount of signal-labeled antibody incorporated into the resulting particles is directly proportional to the concentration of antigen. The principle is illustrated for the simultaneous assay of IgG and IgM in a single sample. In the second system, capture antibody is conjugated to a polymer, the solubility of which is a function of temperature. Specific binding is conducted below the critical solution temperature of the polymer, which is then separated from solution by increasing the temperature above the critical temperature. The incorporation of signal-labeled antibody into the precipitated polymer is directly proportional to the concentration of antigen. This principle is illustrated for the assay of hepatitis B surface antigen and Chlamydia trachomatis.

Additional Keyphrases: polymerization, immunoglobulins, hepatitis B surface antigen, bacteria, critical temperature, in situ generation of solid phase, fluoroimmunoassay, immunoenzymometric assay

Many different solid-phase-based immunoassays have been described in the literature (e.g., 1, 2, and reviewed in 3, 4). The solid phase can take the form of a microparticulate (e.g., latex beads), a molded surface (e.g., microtiter wells), or a filter membrane (e.g., cellulose acetate or polyvinylidene fluoride). Although versatile and easy to use, some such solid-phase immunoassays continue to be plagued by slow reaction kinetics and high nonspecific binding (5), necessitating multiple wash steps.

The kinetics of the binding of a macromolecular antigen to an immobilized antibody are influenced by the presence of an unstirred layer of solution at the surface of the solid phase, the Nernst layer (6, 7), which serves to limit the rate of diffusion of reactants from the bulk solution. Among the approaches to circumventing this problem are the use of ultrasound, to reduce the thickness of the unstirred layer (8), and the use of microporous membranes as the solid phase, to mimic solution-phase kinetics by keeping the diffusion distances within the matrix of the membrane short (9).

In this investigation, we describe two novel immunoassay methods in which the solid phase is generated in situ after the specific binding reaction between antigen and antibody has occurred. Thus, the kinetic properties of the reactions approach those of reactants that are free to diffuse in solution. And, because the solid phase is not present throughout the immunoreaction, the possibility of nonspecific binding is also minimized.

The first assay, which we call de novo polymerization, involves two antibodies—an antibody labeled with a polymerizable organic monomer, and a signal-labeled antibody (Figure 1). After the specific binding between antigen and antibody has taken place, polymerization of the monomer-labeled antibody is initiated by means of a reaction involving free radicals. This results in the formation of insoluble polymer particles, the signal content of which is directly proportional to the amount of antigen in the sample.

The second assay involves thermal precipitation, taking advantage of a class of water-soluble polymers that exhibit different solubilities as a function of temperature (10). Some of these polymers will reversibly precipitate upon heating above a characteristic temperature, the "lower critical solution temperature," for that polymer. In this assay we use an antibody labeled with such a reversibly soluble polymer and a signal-labeled antibody (Figure 2). After the specific binding reaction, we increase the temperature of the reac-

Genetic Systems Corporation, 3005 First Ave., Seattle, WA 98110.
1 Present address: Program for Appropriate Technology in Health, 4 Nickerson St., Seattle, WA 98109.
2 Present address: NeoRx Corporation, 410 W. Harrison, Seattle, WA 98119.
3 University of Washington, Center for Bioengineering, Seattle, WA 98195.
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tion milieu above the critical solution temperature of the polymer, such that insoluble polymer particles are formed. Again, the signal content of the particles is directly proportional to the amount of antigen in the sample.

Here we describe several examples in which these techniques are used in enzyme and fluorescence immunosays of such diverse analytes as IgG, hepatitis B surface antigen (HBs Ag), and Chlamydia trachomatis.4

Materials and Methods

Materials

All chemicals were obtained from Sigma Chemical Co., St. Louis, MO, unless otherwise noted. Monoclonal antibodies were purified from ascites fluid by ammonium sulfate precipitation, followed by ion-exchange chromatography on diethylaminoethyl-cellulose or affinity chromatography on Protein A–Sephadex (Pharmacia Fine Chemicals, Piscataway, NJ), depending on subclass.

Preparatory Procedures

Synthesis of monomers. We synthesized p-styrene sulfonyl chloride from sodium p-styrene sulfonate (Aldrich Chemical Co., Milwaukee, WI) and thionyl chloride. After extracting the mixture twice with benzene, drying it over magnesium sulfate, and concentrating by rotary evaporation, we analyzed the product by thin-layer chromatography, NMR, and mass spectrometry.

We synthesized the N-hydroxysuccinimide ester of 4-vinylbenzoic acid (Aldrich) from dicyclohexylcarbodiimide. We recrystallized the product once from methanol and analyzed it by NMR, mass spectrometry, and infrared spectroscopy.

We used isothiocyanatophenylacetylene (Aldrich) as received.

Conjugation of activated monomers to antibody. Styrene sulfonyl chloride, isothiocyanatophenylacetylene, and the ester of vinyl benzoate were conjugated to antibody via amino groups. We performed the conjugations with various ratios of monomer to protein in 0.29 mol/L sodium carbonate buffer (pH 9.3), incubating for 1 h at 37 °C. We desalted the conjugates on Sephadex G-25 (Pharmacia) equilibrated in phosphate-buffered saline, pH 7.4, containing 10 mmol of NaH2PO4, 150 mmol of NaCl, and 0.1 g of bovine serum albumin per liter (PBS/BSA).

Synthesis of antibody-conjugated poly-N-isopropyl acrylamide. To prepare antibody-conjugated poly-N-isopropyl acrylamide (pNIPAAm; Eastman Kodak, Rochester, NY), we co-polymerized NIPAAm monomers with monomer (vinyl benzoate)-conjugated antibody, prepared as described above. To 1.6 mL of 12.5 g/L NIPAAm solution in PBS, we added 200 μL of vinyl benzoate-conjugated antibody (8.4 mg/mL). To initiate polymerization, we added 100 μL of 100 mmol/L ammonium persulfate solution and 100 μL of 0.8 mol/L N,N,N′,N′-tetramethylethylene diamine solution (TMEDA; both from Eastman Kodak). The reaction mixture was incubated for 3 h at room temperature, after which we purified the resulting conjugate by one of the following methods:

- Chromatography on Sephacryl S-300 (Pharmacia). Free antibody was separated from antibody-conjugated polymer, which eluted in the void volume of the column.
- Serial thermal precipitation. The reaction mixture was diluted to 20 mL with PBS and heated to 37 °C for 10 min. The resulting suspension was centrifuged (4000 × g, 37 °C, 5 min) and the supernate discarded. The precipitate was redissolved to 20 mL in ice-cold PBS and the cycle repeated twice more. After the third precipitation, the precipitate was dissolved to 2 mL in PBS and stored at 4 °C until needed.
- Serial salt-induced precipitation. We added 12 mL of cold-saturated ammonium sulfate to the reaction mixture to bring the final concentration of ammonium sulfate to 14.3% of saturation. The suspension was then centrifuged (10 min, 2000 × g, 25 °C) and the supernate discarded. The precipitate was redissolved to 12 mL in PBS and the cycle was repeated twice more. The final precipitate was dissolved in 4 mL of PBS and desalted by chromatography on Sephadex G-25.

Synthesis of fluorescent conjugates. We synthesized fluorescein conjugates according to the method of Goding (11), using isomer II of fluorescein isothiocyanate (Molecular Probes, Junction City, OR). The fluorescein-to-protein molar ratio was typically between 4 and 5.

Phycoerythrin conjugates were synthesized by using Rhodophyceae purified from the red alga Porphyra yessoensis (American Sea Vegetable Co., Vashon Island, WA) (12). Thiol groups were introduced into the antibody by reaction with S-acetylmethylacetoxysuccinic anhydride (13), and reactive maleimide groups were introduced into the phycoerythrin by using succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (Pierce Chemical Co., Rockford, IL) (14). After coupling thiolated antibody to the maleimidoester of phycoerythrin via a sulfoether linkage, we separated the resulting conjugate from free phycoerythrin by gel-filtration chromatography on Sephacryl S-300, equilibrated in PBS containing 1 g of sodium azide per liter. The molar ratio of antibody to phycoerythrin was typically approximately 1:5:1.

Synthesis of antibody/oxidase conjugates. We conjugated the enzyme horseradish peroxidase (EC 1.11.1.7) to antibody by the periodate method (15) and purified the resulting conjugates by ammonium sulfate precipitation and dialysis against PBS. They were stored in 500 g/L glycerol matrix until needed. Immediately before use, we diluted the conjugates to working strength in PBS, pH 7.4, containing 250 mL of normal mouse ascites fluid, 500 g of glycerol, 50 g of

4 Nonstandard abbreviations: HBs Ag, hepatitis B surface antigen; NMR, nuclear magnetic resonance; PBS, phosphate-buffered saline; BSA, bovine serum albumin; pNIPAAm, poly-N-isopropyl acrylamide; TMB, N,N,N′,N′-tetramethylethylene diamine; TMB, 3,3′,5,5′-tetramethylbenzidine; ELISA, enzyme-linked immunosay.
human serum albumin, 0.1 g of thimerosal, and 0.5 g of Tween 20 [polyoxyethylene (20) sorbitan monolaurate] per liter.

Assay Conditions

De novo polymerization assay for IgG. For this assay we used a monoclonal antibody to the kappa light chain of IgG, designated 2H1, conjugated to monomer, and a fluorescently labeled monoclonal antibody to the gamma heavy chain, designated 3F6. The final concentration of each antibody in the assay was 30 μg/mL. In some cases, we replaced the monoclonal antibodies with polycrystal goat antisera (IgG fraction) to either the kappa light chain or the gamma heavy chain (Miles Scientific). The assay procedure was as follows: mix 50 μL of a solution containing the two antibodies and the co-monomer hydroxyethylmethacrylate (final concentration 10 g/L; Polysciences, Warrington, PA) with 50 μL of mock serum (PBS/BSA) reconstituted with a human myeloma protein (IgG/kappa) to a series of known concentrations. Incubate for 10 min at 37 °C, then initiate polymerization by adding 25 μL of 30 mmol/L ammonium persulfate reagent and 25 μL of 240 mmol/L TEMED reagent. Let polymerization proceed for 20 min at 37 °C. For analysis by flow microfluorimeter, dilute 50 μL of the reaction mixture with 2 mL of PBS.

We used a FACS IV flow microfluorimeter (Becton Dickinson, Mountain View, CA) equipped with an argon ion laser, exciting both the fluorescein and phycoerythrin conjugates with a 488-nm laser line. Results were displayed graphically, with the Y-axis representing the number of particles analyzed and the X-axis, the log of fluorescence.

Biochromatic de novo polymerization assay for IgG and IgM. The assay was performed essentially as described above, except that we incorporated an additional phycoerythrin-conjugated monoclonal antibody, 2C3, to the mu chain of IgM (final concentration 45 μg/mL), and prepared standards from mock serum reconstituted to contain a series of known concentrations of two myeloma proteins, IgG/kappa and IgM/kappa. We analyzed the reaction mixture by multi-parameter flow microfluorimetry, using a FACS IV equipped with a 560-nm dichroic mirror to split the emission wavelengths. To further separate the signals, a 580-nm long-pass filter was placed in front of the red photomultiplier tube, and a 540-nm short-pass filter in front of the green photomultiplier tube. A two-color compensator was used to correct spillover between the channels. The results were displayed graphically on a 64 × 64 grid, with the vertical axis representing the number of particles analyzed, the left horizontal axis the log of green fluorescence, and the right horizontal axis the log of red fluorescence.

Thermal precipitation immunoenzymometric assay of HBs Ag. We used pooled human serum reconstituted to a series of known concentrations with vaccine-grade HBs Ag (from the Institut Pasteur, Paris, France; or from Merck Sharpe and Dohme, Rahway, NJ). Mix 200 μL of serum standard with 50 μL of peroxidase-conjugated monoclonal antibody to HBs Ag (designated 6.16 and diluted 20 000-fold) and with 10 μL of pNIPAAm conjugated to a second monoclonal antibody directed to a different epitope on HBs Ag, designated 112 (final antibody concentration 1 μg/mL). The reaction mixture contains pNIPAAm (1 g/L) as carrier in a total volume of 0.5 mL of PBS/BSA. Incubate the assay mixture for 2 h at room temperature, dilute with an equal volume of PBS, and heat to 45 °C for 10 min to precipitate the polymer. Collect the precipitate by centrifugation and wash three times with hot (45 °C) PBS. Then resuspend the pellet in 100 μL of a color developer solution containing 80 μg of 3,3',5,5'-tetramethylbenzidine (TMB) and 1.5 μL of hydrogen peroxide. Stop the color development after 30 min by adding 100 μL of 3 mol/L HCl reagent and determine the absorbance at 450 nm. Alternatively, a fluorogenic substrate—p-hydroxyphenyl propionic acid, 2.5 mg/mL, and hydrogen peroxide, 0.06 mL/L, in phosphate buffer (0.1 mol/L, pH 7.0)—can be used, in which case the stopping solution consists of glycine, 0.1 mol/L, adjusted to pH 10.3 with concentrated NaOH. To measure fluorescence, we used a Perkin-Elmer LS-5 fluorimeter, with an excitation wavelength of 320 nm and an emission wavelength of 405 nm.

Thermal-precipitation immunoenzymometric assay of C. trachomatis. C. trachomatis LGV strain L2/434/BU was grown in HeLa 229 cells in Eagle's minimal essential medium containing, per liter, 100 mL of fetal calf serum, 100 mg of vancomycin, 100 mg of streptomycin, sodium pyruvate, and l-glutamine. Cells were harvested, washed by centrifugation, and sonicated on ice for 2 min. The sonicated material was centrifuged at 200 × g for 10 min and the supernate transferred to a clean tube. We then centrifuged the supernate for 30 min at 20 000 × g to pellet the elementary bodies, which are the infectious form of C. trachomatis, and fixed them in 0.2 mL/L formalin solution before use in assays. The number of elementary bodies per nanogram of protein was determined by direct immunofluorescence by using a fluorescein-labeled monoclonal antibody to the major outer membrane protein of C. trachomatis.

For assay purposes, pNIPAAm can be used either unconjugated (nonspecific capture) or conjugated to a monoclonal antibody to the lipopolysaccharide of C. trachomatis (specific capture). In both cases, a peroxidase-labeled monoclonal antibody to the major outer membrane protein of C. trachomatis is used as the detection reagent (final concentration 40 ng/mL). Mix antibody-conjugated or unconjugated pNIPAAm with peroxidase-labeled antibody and 200 μL of antigen standard in PBS containing 4 g of BSA per liter (0.5 mL final volume, with pNIPAAm at 1 g/L). Incubate for 20 min at room temperature, then heat to 45 °C to precipitate the polymer. Collect the precipitate by centrifugation, wash twice with hot (45 °C) PBS, and develop and measure color as described in the preceding assay.

Results and Discussion

De Novo Polymerization

The key reagent in the de novo polymerization assay is the monomer-conjugated antibody. We synthesized a variety of different monomers, conjugated them to antibody, and evaluated their performance in an assay for human IgG. A schematic representation of a typical conjugation is shown for the monomer vinyl benzene in Figure 3. The number of monomers per antibody molecule was determined in each case by liquid chromatography and (or) isolectric focusing of the immunoglobulin heavy and light chains, which had first been separated by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate. The latter method was particularly informative because it allowed us to determine which immunoglobulin chains were derivatized, as well as the extent of derivatization.

For each monomer tested, we carried out conjugations at several different ratios of monomer to antibody. The optimal ratio was determined on the basis of performance in the assay itself. The best conjugate was not always the most
highly derivatized. However, because the conjugates were assayed in use, we could not discriminate whether this difference was due to loss of antigen-binding ability by the antibody or to loss of activity by the monomer at higher loadings.

As Figure 4 shows, there was considerable difference in performance between different conjugated monomers. This difference is thought to be due to differences in the efficiency of incorporation of the monomer-conjugated antibody into polymer, iso-thiocyanatophenylacetylene presumably being incorporated least efficiently and vinyl benzoate most efficiently. In actual fact, the percentage of monomer-conjugated antibody incorporated into polymer is quite low in all cases. This could indicate that a high proportion of the conjugated monomer is inactivated by the conjugation procedure or that it is sterically hindered and thus unavailable to participate in the polymerization reaction. In an effort to render the monomer more accessible, we used an avidin-biotin bridge between the monomer and the antibody, but the efficiency of incorporation was not substantially increased (data not shown). Neither did increasing the time of polymerization beyond about 20 min significantly increase the extent of incorporation of monomer into polymer (data not shown).

As we expected, the polymer particles formed by this reaction were polydisperse, with smaller particle sizes predominating. The size distribution of the particles was not static, but changed as a function of time after the initiation of polymerization. If left long enough, the particles would eventually coalesce into a single large bead that could not be readily dispersed.

Figure 5 shows a series of histograms corresponding to different concentrations of human IgG, as assayed by flow microfluorimetry. The sensitivity (detection limit) of this assay is approximately 5 ng/mL ($2 \times 10^{-11}$ mol/L). Although accuracy has not been rigorously evaluated in this system, we find that the results consistently correlate with those by ELISA (data not shown). Recovery experiments have not been performed.

As illustrated in Table 1, sensitivity is a function both of the label (fluorescein or phycoerythrin) and the antibody (monoclonal or polyclonal) used. For antibody 3F6, sensitivity can be increased approximately threefold by changing the label from fluorescein to phycoerythrin. We saw a fivefold improvement in changing from a phycoerythrin-labeled monoclonal antibody (3F6) to a phycoerythrin-labeled polyclonal (goat anti-gamma heavy chain) antibody. Changing from a monoclonal (2H1) to a polyclonal (goat anti-kappa light chain) monomer-conjugated antibody produced a smaller (only threefold) increase in sensitivity, 

![Schematic representation of the conjugation of a monomer, vinyl benzoate, to antibody](image)

**Fig. 3.** Schematic representation of the conjugation of a monomer, vinyl benzoate, to antibody.

DCC, dicyclohexylcarbodiimide

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regardless of which fluorophore was used to label 3F6.

We also used de novo polymerization to assay IgG and IgM in a single sample, using a phycoerythrin-labeled antibody to report IgM molecules and a fluoresceinated antibody to report IgG. In Figures 6 and 7, the data from two such assays are presented as contour plots on 64 × 64 grids, each 64 dots representing four logs of fluorescence. The log scale has the effect of amplifying small differences in the amplitude of the signal near the origin, while minimizing the larger differences in amplitude; hence, even unlabeled particles show a measurable autofluorescence (Figure 6a). In the presence of either IgM or IgG at 1 μg/mL, particles are formed that fluoresce red (Figure 6b) or green (Figure 6c) in proportion to their respective antigen content. When we physically mixed the two populations of particles, we obtained the profile shown in Figure 6d, namely, two discrete peaks, one corresponding to green IgG-containing particles and the other to red IgM-containing particles. In Figure 7, we held the IgM content of the assay constant at 1 μg/mL, while increasing the amount of IgG from 0 μg/mL (Figure 7a) to 1 μg/mL (Figure 7d). A single peak of copolymer particles was formed in each case, with red fluorescence in proportion to the IgM content of the particles and green in proportion to their IgG content.

To assess the effect of serum in this system, we added increasing amounts of pooled human serum, which had been stripped of IgG and IgM by affinity chromatography, to the assay at two concentrations of analyte (IgM, 0 and 1 μg/mL). We saw no effect on assay results even when serum represented as much as 20% of the total assay volume (data not shown). We do not have sufficient data at this time to say whether icteric, lipemic, or hemolyzed samples will be tolerated.

Thermal precipitation. Polymers are known that precipitate either above or below a certain critical temperature; the former are said to exhibit a lower critical solution temperature, and the latter, an upper critical solution temperature. Our efforts have focused primarily on the former polymers, chiefly acrylamide and N-alkyl acrylamide polymers or copolymers.

That polymers with a lower critical solution temperature are reversibly soluble provides a dual advantage: the efficiency of washing is maximized while dilution of the signal and sample is minimized. Ideally, the polymer is precipitated initially from a relatively small volume, to minimize dilution of the sample, and is redissolved in subsequent steps in a relatively large volume, to promote washing. Signal development can be carried out in either phase—when the polymer is precipitated or when it is in solution. The maximum concentration effect is achieved when signal development is carried out in the solid phase, which represents the smallest possible volume the polymer can occupy. However, if the signal is generated enzymatically, it may be desirable to carry out signal development in solution, because the rate of diffusion of the enzyme substrate into the precipitate may be rate-limiting.

In Figure 8, the lower critical solution temperature of
Thus the nurture various agents, substances, and solutions of NIPAAm formed from different combinations, which might be used to solubilize the sample. We examined the effects of serum, urine, detergents (ionic, non-ionic, and zwitterionic), and a variety of other substances, including ions, anticoagulants, chelating agents, and N-acetylcycteine (a sputum-solubilizing agent) on the critical solution temperature of the pNIPAAm polymer. None of these compounds caused a significant change in the critical solution temperature of the polymer over the range of concentrations in which they might be expected to be found in biological samples (data not shown here; see 16). Furthermore, the critical solution temperature of the polymer was unaffected by the conjugation of antibody to the polymer backbone, although the size of the particles that formed above the critical solution temperature was dependent on the degree of antibody substitution.

To prepare antibody conjugates of pNIPAAm, we copolymerized vinyl benzoate-conjugated antibody with NIPAAm monomers in various ratios and separated the resulting conjugates from unreacted monomer and monomer-conjugated antibody by serial precipitation or by gel filtration. If desired, the mixture could be further purified by chromatography on hydroxyapatite. Under the appropriate conditions, antibody-conjugated polymer will bind to hydroxyapatite and can be eluted in a subsequent step by increasing the ionic strength (data not shown).

In addition to being thermally precipitable, pNIPAAm and copolymers thereof can be precipitated from solution by adding ammonium sulfate to 14%–20% of saturation, depending on the composition of the polymer. Because immunoglobulins do not precipitate at these salt concentrations, this is a convenient method of separating antibody-conjugated polymer from free antibody.

Independent of the method of purification, we find the resulting conjugates to have an apparent molecular mass >10^6 Da, based upon their elution profile from Sephacryl S-300. However, this is not likely to be an accurate representation of their molecular mass, given the dependence of such determinations on hydrodynamic properties of the polymer. We estimate the number of antibody molecules per polymer to be between one and three.

In Figure 9, we compare the amount of fluorescence in solution before and after thermal precipitation of pNIPAAm conjugated with a fluoresceinated monoclonal antibody. Approximately 90% of the fluorescence (and, by inference, of the polymer) is recovered after a single precipitation. In a parallel experiment, in which unconjugated polymer was precipitated in the presence of a fluoresceinated "bystander" antibody, <2% of the bystander antibody was precipitated, providing a measure of nonspecific binding prior to any washing of the polymer. This value compares favorably with the amount of nonspecific binding typically observed in a coated-tube RIA, which is usually at least 3% of the total counts (4). That the observed nonspecific binding in the thermal precipitation system is probably due to entrapment is suggested by the fact that the background goes to zero upon dissolution and reprecipitation of the polymer (data not shown).

Figure 10 represents a standard curve for the immunoenzymometric assay of HBs Ag. In the left hand panel we used TMB, a chromogenic substrate for horseradish peroxidase,
Fig. 10. Standard curve for the thermal precipitation immunoenzymometric assay of HBs Ag (left) with the chromogenic substrate TMB, and (right) with the fluorogenic substrate \( p \)-hydroxyphenylpropionic acid.

while in the right hand panel we used \( p \)-hydroxyphenylpropionic acid, a fluorogenic substrate. As shown, the sensitivity of the chromogenic assay was approximately 0.5 ng/mL and of the fluorogenic assay, approximately 0.25 ng/mL. These data compare favorably with those by ELISA-based methods, both in terms of sensitivity and performance time. Accuracy has not been rigorously evaluated with this system, but we find that the results correlate well with those by ELISA.

Figure 11 displays a standard curve for the immunoenzymometric assay of \textit{C. trachomatis}. Note that comparable lower limits of detection are observed, regardless of whether or not a capture antibody is conjugated to pNIPAAm. This unexpected observation presumably reflects binding of elementary bodies to the polymer via hydrophobic interactions, with the binding being enhanced relatively little by the conjugation of antibody to the polymer. These assays compare favorably with ELISA in terms of sensitivity and can be performed in much less time.

In summary, we have described two novel immunoassay systems that combine the convenience of a solid phase with the superior reaction kinetics of the solution phase. Although much work remains to be done before the clinical utility of these assays can be established, we have shown that these assays are applicable to a variety of analytes, including macromolecular antigens such as HBs Ag and \textit{Chlamydia}. We have described both immunoenzymometric and fluoroimmunoassays and shown that both assay systems can be adapted for use with existing laboratory instrumentation.

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References


