is allowed to proceed for 4 hours at 22° C., and the reaction mixture is then dialyzed at 4° C. for 18 hours against a 500-fold excess of 0.01 M tris-saline buffer, pH 8.0 containing 0.2% NaN_3.

(C) 1.0 mg of mouse monoclonal IgM (anti-N meningitis B capsular polysaccharide) in 1.0 ml of 0.2 M $NaHCO_3$ is reacted with 8 to 10 μl of a solution of d-biotin-N-hydroxysuccinimide ester in DMSO (1.0 mg/ml) for 4 hours at 22° C., and the reaction mixture is then dialyzed against a 500-fold excess of 0.01 M tris-saline buffer, pH 8.0 containing 0.2% NaN_3.

Each of the biotinylated antibodies, formed above, is attached to the avidin-latex particles. 1.0 ml. of avidin latex is pelleted and the supernatant removed. The pellet is taken up in 1.0 ml of biotinylated antibody containing 5 μl of 10^{-3}M biotinic acid, and the mixture is stirred for 1 hour at 22° C. to react the biotin and biotinylated antibody. After 1 hour, a second 5 μl aliquot of 10^{-3}M biotinic acid is added to block potentially unoccupied biotin binding sites on the avidin moieties.

The diagnostic particles are centrifuged, washed and suspended at 0.6% solids in 0.1 M glycine-saline buffer, pH 8.2 containing 0.2% NaN_3, 0.2% BSA and 0.05% Tween-20.

To 15 ml of the diagnostic particle suspension formed from the polyvalent rabbit IgG, 50 ml containing 0.1 μg of Group A Streptoccous antigen are added. Agglutination of the suspension is noted after 4 minutes.

To 15 ml of the diagnostic particle suspension formed from the mouse monoclonal IgG_3 antibody, 50 ml containing 0.1 μg of Group A Streptococcus antigen are added. Agglutination of the suspension is noted after 10 minutes.

To 15 ml of the diagnostic particle suspension formed from the mouse monoclonal IgM antibody, 50 ml containing 0.1 μg of N mening B polysaccharide antigen are added. Agglutination of the suspension is noted after 10 minutes.

Suspensions of diagnostic, particles prepared according to the present invention, are stable if stored under refrigeration for periods of several months. The diagnostic particles are extremely sensitive, and using microtechniques, the suspensions can be used to detect as little as nanogram quantities of antigen.

Several advantages of the present invention can now be more fully appreciated. The invention provides for attachment of all types of immunoglobulins, including IgM. The amount of antibody attached to the latex is not dependent upon the adsorption characteristics of a particular lot of latex. Similarly, although the degree of carboxylation is preferably within a certain range, the amount of antibody attached is determined independently of the precise degree of carboxylation of the core particles, i.e., by the less than stoichiometric amount of avidin bound to the carboxyl groups and then by the selected molar ratios of biotinic acid and biotinylated antibody. Thus reproducability of manufacture is significantly enhanced relative to prior latex particle-to-antibody linking procedures, particularly with respect to IgM, which previously had to be bound to the latex by the highly variable adsorption technique.

While the invention has been described in terms of certain preferred embodiments, modifications obvious to one with ordinary skill in the art may be made without departing from the scope of the present invention. For example, while it is not preferred, the amount of antibody bound to the particles could be made through control of the number of avidin molecules bound to the latex core particles followed by saturation of the biotin binding sites with biotinylated antibody molecules. While the diagnostic particle suspensions generally require that the antigen be large, having at least two antigenic determinant sites, it is also contemplated that a small antigenically active molecule or hapten could agglutinate the particles if the particles were suspended in a liquid medium that contained a chemical which would link two or more of the hapten molecules.

Various features of the present invention are set forth in the following claims:

What is claimed is:

1. A diagnostic particle comprising

a carboxylate-derivatized polymeric core between about 0.2 and about 1.0 micron in diameter,

a plurality of avidin moieties linked through amide bonds to said core,

a plurality of biotin moieties complexed to said avidin moieties, and

a plurality of antibody molecules linked through amide bonds to a significant portion of said biotin moieties, the amount of antibody molecules on the surface of said particle being controlled so that said diagnostic particle may be stably suspended in aqueous medium in the presence of like particles without agglutination, but subsequently agglutinate in the presence of a substance with which said antibody molecules react.

2. A diagnostic composition comprising a plurality of particles, each particle comprising a carboxylate-derivatized polymeric core between about 0.2 and about 1.0 micron in diameter, a plurality of avidin moieties linked through amide bonds to said core, a plurality of biotin moieties complexed to said avidin moieties and a plurality of antibody molecules linked through amide bonds to a significant portion of said biotin moieties and an aqueous medium in which said particles are suspended, the amount of antibody molecules on each of said particles being controlled so that said particles remain in suspension in said aqueous medium without agglutination, but when a substance reactive with said antibody molecules is introduced into said aqueous medium, said particles agglutinate.

3. A composition according to claim **2** wherein said particles cores are formed of a polymer selected from the group consisting of polyacrylamide and polystyrene.

4. A composition according to claim **2** wherein said particles have between about 10^{10} and about 10^{13} avidin molecules per cm^2 of surface area of said cores.

5. A composition according to claim **2** wherein said particles have between about 2×10^{11} and about 2×10^{12} antibody molecules per cm^2 of surface area of said cores.

6. A composition according to claim **2** wherein said diagnostic particles have neutralizing moieties attached to core surface carboxyl groups that are not linked by amide bonds to avidin.

7. A composition according to claim **2** wherein said diagnostic particles also include non-immunogenic proteinaceous material adsorbed on surfaces of said cores.

8. A composition according to claim **2** wherein said antibody molecules are monoclonal antibody molecules.

9. A composition according to claim **2** wherein said cores are carboxylated to between about 0.1 to about 0.5 milliequivalents per gram of core particles.