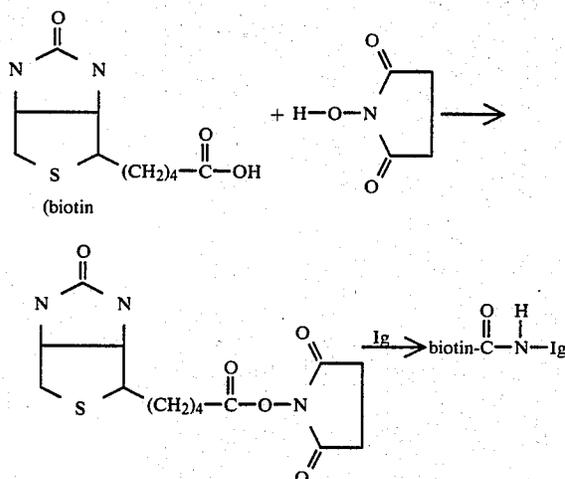


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of the antibody molecule is by initially forming an ester between an N-hydroxy imide, such as N-hydroxy-succinimide and biotin and then reacting the N-hydroxy imide-biotin with the immunoglobulin, whereupon an amino group of the immunoglobulin replaces the N-hydroxyimide linked to the carbonyl. This reaction is carried out at slightly alkaline conditions, preferably at a pH of between about 7.5 and about 9.0. These reactions are represented in equation 2 below:

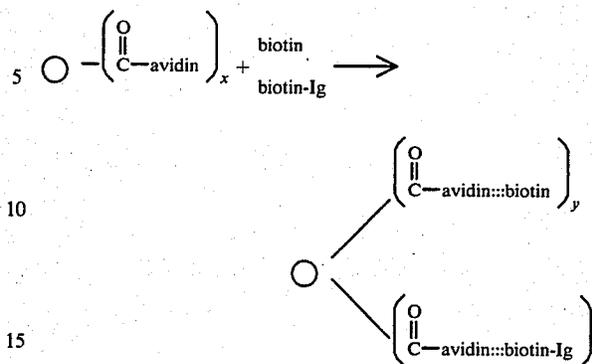


Because avidin is reacted in less than stoichiometric amounts, unbound surface carboxyl groups remain on the latex core particles. These unbound carboxyl groups are preferably neutralized, e.g., with an amine, such as ethanol amine.

It has been found that diagnostic particles have greater stability if, subsequent to neutralization, non-immunogenic proteinaceous material, such as bovine serum albumin (BSA), is adsorbed onto the surfaces of the avidin-bound latex core particle.

Control of the amount of antibody on the surface of the diagnostic particle, which is accomplished by mixing free biotinic acid with biotinylated antibody so that they compete for the excess avidin binding sites, is considered an important aspect of the invention. Too few antibody molecules may not afford agglutination at a suitable rate, whereas too many antibody molecules may result in premature precipitation. Generally, it is preferred that between 2×10^{11} and about 2×10^{12} antibody molecules be bound per cm^2 of estimated surface area of the latex core particles, although this may vary somewhat depending upon whether the antibody is small, e.g., IgG, or large, e.g., IgM. The molar ratio of biotinic acid to biotinylated antibody ratio is dependent on the number of avidin binding sites. It must also be taken into account that free biotinic acid reacts somewhat more rapidly with the avidin than does the antibody-bound avidin. Providing a desired amount of antibody on the diagnostic particles generally requires that a mixture of biotinic acid and biotinylated antibody in a molar ratio of between about 1:1 and about 10:1 be reacted with particles having avidin, in the above-mentioned preferred amounts, bound to the core particles. The reaction between a mixture of biotinylated antibody and biotinic acid with core particle-linked avidin is represented in equation 3 below:

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The liquid medium in which the diagnostic particles are suspended is generally aqueous as is consistent with the natural environment of antibody molecules. A slightly basic pH, e.g., between about 7.5 and about 8.5, contributes to stability of the diagnostic particles. Antimicrobial agents, such as NaN_3 may also be added to the medium. Generally latex suspensions for use in agglutination tests contain between about 5 and about 10 gm. of particles per liter of suspension.

The invention will now be described in greater detail by way of example.

EXAMPLE

4.0 ml of carboxylate-latex at 2.5 weight percent solids, obtained from Polysciences Inc. Warrington, Pa., is washed three times with distilled water, and after the final wash, the particles are resuspended in 4.0 ml of distilled water. 1.0 ml of 0.05 M KH_2PO_4 , pH 4.5 is added. The suspension is placed on a magnetic stirrer and maintained at 22°C ., and 5 ml. of a solution of 2 weight percent 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (obtained from Sigma Chem. Co., St. Louis, Mo.) is added and allowed to react with the latex for 3.5 hours. The carbodiimide-activated carboxylate-latex is then washed once in saline and resuspended in 5 ml of saline.

1.2 mg of avidin (all procedures are performed in duplicate, once using egg white avidin and once using streptavidin) is dissolved in 5 ml of 0.2 M borate, pH 8.5, and the 5 ml. latex suspension is added. The carbodiimide-activated carboxylate-latex and avidin are allowed to react for 20 hrs. at 22°C . To neutralize surface carboxyl groups that are not bound to avidin, 5 mM ethanolamine is added, and then BSA is added to a concentration of 2 weight percent. The avidin-latex is washed and taken up in 0.1 M glycine-saline, pH 8.2 containing 0.2% NaN_3 , 0.2% BSA and 0.05% Tween-20 and stored at 4°C .

Antibodies are bound to biotin as follows:

(A) 1 mg of polyvalent rabbit IgG (anti-group A streptococcal antigen) in 1.0 ml. of 0.2 M NaHCO_3 is reacted with 50 μl of a solution of d-biotin-N-hydroxy-succinimide ester in DMSO (1.0 mg/ml). The reaction 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 0.91 M tris-saline buffer, pH 8.0 containing 0.2% NaN_3 .

(B) 1.0 mg of mouse monoclonal IgG₃ (anti-group A streptococcal antigen) in 1.0 ml of 0.2 M NaHCO_3 is reacted with 8 μl of a solution of d-biotin-N-hydroxy-succinimide ester in DMSO (1.0 mg/ml). The reaction