

of antibody moieties linked to each core particle is controlled. To prevent excessive biotinylated antibody from complexing to the avidin moieties linked to the core particles, a mixture of free biotinic acid and biotinylated antibody of a predetermined molar ratio is reacted with the core particle-bound avidin so that biotinic acid occupies a portion of the binding sites on the avidin which might otherwise be occupied by the biotinylated antibody.

The term "latex" herein is used broadly to include stable dispersions of particles of polymeric material. Suitable latexes include suspensions of minute polystyrene and polyacrylamide particles. To provide that a stable suspension of the diagnostic particle can be formed which will precipitate within a reasonable time upon exposure to the antigen, the starting polymeric core particles should be between about 0.2 and about 1.0 micron in diameter.

The polymeric core particles are carboxylate-derivatized to provide exposed carboxyl groups at their surfaces for attachment of avidin. Polyacrylamide particles may be derivatized by the method of John K. Inman, "Covalent Linkage of Functional Groups, Ligands, and Proteins to Polyacrylamide Beads", in *Methods in Enzymology*, Vol XXXIV, (ed. William B. Jakoby and Meir Wilchek, Academic Press, N.Y., 1974) pp. 30-58. Suitable carboxylate-derivatized latex particles are commercially available; for example, carboxylate-latex sold by Polysciences. It is found that particles carboxylated to between 0.1 and about 0.5 milliequivalents per gram are most suitable. This degree of carboxylation provides more surface carboxyl groups than are eventually used to bind avidin and, subsequently, biotinylated antibody. However, less successful results are achieved with particles carboxylated to a lesser degree. Therefore, it is not considered desirable to limit the amount of antibody bound on each particle through the number of carboxyl moieties on the polymeric core particles.

The high affinity of avidin for biotinic acid is well known, and the combination of avidin and biotin are found to provide a very effective means for linking controlled amounts of antibody to the latex. Biotin (hexahydro-2-oxo-1H-thieno [3,4] imidazole-4-pentanoic acid) is a growth factor present in very minute amounts of every living cell and is found mainly bound to proteins or polypeptides. Avidin is a glycoprotein containing four essentially identical peptide subunits, each having an attached carbohydrate moiety. Each subunit of avidin has a single biotin binding site. The combined molecular weight of the subunits is about 66,000. Avidin is most commonly isolated from raw egg whites but is probably found in the genital tract of all animals. Avidin is also produced by certain bacteria, such as *Streptomyces avidinii*, and avidin used herein is to be understood to refer to animal avidin as well as bacterial avidin, such as streptavidin. The high affinity of avidin for biotin has been demonstrated by the ability of large amounts of avidin to produce biotin deficiency in rats and chicks.

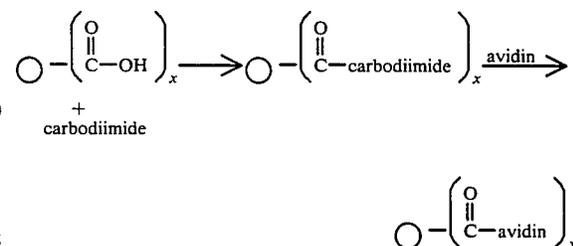
Because the core particles have a greater number of surface carboxyl groups than are to be eventually linked to antibody molecules, avidin is reacted with the core particles in amounts less than the stoichiometric concentration which would link to all core surface carboxyl groups. It is not preferred, however, to control the number of biotinylated antibody molecules that are subsequently linked to the cores by limiting the number of avidin molecules that are bound to the cores to the minimum that would be required to complex stoichio-

metrically to the desired number of biotinylated antibody molecules. It is found that best results are achieved when avidin is reacted with the latex particles to provide between about  $10^{10}$  and about  $10^{13}$  molecules of avidin per  $\text{cm}^2$  of estimated core particle surface area, and to this end, between about  $1.2 \times 10^{-3}$  and about  $1.2 \times 10^{-2}$  gm of avidin are reacted per gm of styrene core particles, and between about  $1.2 \times 10^{-3}$  and about  $1.2 \times 10^{-2}$  gm of avidin are reacted per gm of polyacrylamide core particles.

The antibody is selected according to the antigen to be detected. Any of the known types of immunoglobulins can be linked to latex core particles by the method of the present invention, including IgG, IgA and IgM. A general requirement is that the antibody be specific for an antigen having at least two antigenic determinants so that the antigen can bind to antibodies on different diagnostic particles and thereby cross-link the particles. Many large antigens of interest, such as the group carbohydrate antigen of Group A Streptococcus, have multiple, substantially identical, antigenic determinants. If the particular antigen does not have duplicate antigenic determinants, it may have spaced-apart distinct antigenic determinants, in which case a mixture of two or more antibodies, each reactive with one of the determinants, might be linked to the latex core particles to allow cross-linking between diagnostic particles to take place through the unique antigenic determinants.

It is preferred that, if available, monoclonal antibodies be used to detect antigens. Monoclonal antibodies, consisting of identical antibody molecules, are much more specific than conventionally obtained antibody fractions and provide for much greater reproducibility between lots of diagnostic particles. "Monoclonal antibodies" is used herein to refer to antibodies generated by hybridomas, as well as antibodies produced by other cell immortalization techniques, e.g., by infection with certain viruses. However, the invention is intended to encompass diagnostic particles incorporating conventional antibody fractions, particularly to detect antigens for which no monoclonal antibody is presently available.

The formation of the amide bond between the carboxyl groups on the latex core particles and an amine group of the avidin is preferably facilitated through an intermediate formed by reaction of a carbodiimide, such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, with the carboxyl groups on the latex particles. After the carboxyl groups have been activated through reaction with the carbodiimide, the avidin is introduced, whereupon primary amino groups on the avidin replace the carbodiimide linked to the carbonyl. These reactions are represented in equation 1 below:



A preferred method of forming the amide bond between the carboxyl group of biotin and an amino group