United States Patent Office

3,088,875

Patented May 7, 1963

3,088,875
IMMUNOLOGICAL DIAGNOSTICS UTILIZING POLYSTYRENE LATEX PARTICLES OF 0.15 TO 0.25 MICRON

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No Drawing. Filed Oct. 27, 1959, Ser. No. 848,922
7 Claims. (Cl. 167—84.5)

This invention relates to diagnostic procedures for determining the presence or absence in blood and other body fluids of various substances which indicate an abnormal or pathological state or condition. More specifically, this invention is concerned with novel diagnostic reagents for immunological analysis of body substances.

A number of pathological states or conditions in both human beings and animals can be, and often are, diagnosed through the application of well-known immunological principles. It will be understood that the word, "animals" when employed herein includes "human beings," unless there is a specific statement to the contrary. Animals which are exposed to "foreign" proteins ((antigens) produce in their blood and tissue fluids certain soluble substances (antibodies). Such foreign proteins can be supplied by a microbiological or viral invader, in which case the antibodies serve a protective function. However, the presence of a microbiological or viral "infection" is not essential to the formation of antibodies. Any protein which is not normally present in a given animal can, when introduced into such an animal under the proper conditions, engender the formation of antibodies. All immunological testing procedures are based upon this so-called antigen-antibody reaction through the use of known diagnostic materials, either an antigen or an antibody, to determine the presence or absence of the corresponding antibody or antigen in the test animal.

When an antigen and its corresponding antibody come into contact under the proper conditions, they combine to form a complex which is less soluble than were either of the uncombined original components. This "insoluble" complex is discernible to the human eye in varying degrees. Certain antigens and antibodies will combine in a relatively short period of time to form large particles which are macroscopically visible. However, in many antigen-antibody systems the complex forms very slowly, and the particle size is so small that certain "carriers" must be employed to expedite the macroscopic visualization of the reaction. Among the carriers which have been employed are sheep and human erythrocytes, bacterial cells, bentonite and latex particles. Such carriers are usually characterized as "indicators" for each diagnostic test.

Each of the several immunological diagnostic procedures which is employed in the art is characterized by a number of drawbacks or disadvantages, among which are lack of sensitivity, lack of specificity, lack of stability, lack of avidity (speed and quality of reaction), and improper character of the resultant test mixture to provide an easily ascertainable visual evaluation. In the case of certain antigen-antibody reactions, the principal drawback is the lack of sensitivity and specificity which often results in false positive reactions, thereby necessitating coincident controls to avoid any misinterpretation. Another principal drawback is the inordinate length of time required for the tests. This time factor has in the past precluded many physicians from utilizing the tests when elaborate laboratory facilities were not immediately available.

It is an object of this invention to provide novel immunological diagnostic procedures and diagnostic reagents which are characterized by a high degree of sensitivity

and specificity and which can provide accurate results in substantially faster time than previously was thought possible. Another object of this invention is to provide novel diagnostic procedures and reagents which can be routinely employed by a medical practitioner without the need of elaborate and costly laboratory facilities. Other objects and advantages, both general and specific, will appear as this specification proceeds.

This invention involves the preparation and use of a polymerized styrene latex as a carrier for reactants (antigens or antibodies) which are used for diagnostic purposes. Optimum results are achieved when polystyrene latex is employed in a particle size in the range of from 0.15 to 0.25 micron. One such polystyrene latex suspension is available from Monsanto Chemical Company under the trademark "Lytron 615." The polymer particles of styrene in the dispersed phase of the polystyrene latex suspension average less than one micron in diameter (about 0.2 micron, with most of the particles being in the range of 0.15 to 0.25 micron) and are negatively charged. The polymer is of high molecular weight and is produced by polymerizing a styrene monomer in the presence of water to form lattices. Polystyrene latex is stable to repeated cycles of freezing and thawing and is infinitely dilutable in water. The material appears to be self-sterilizing. Another acceptable polystyrene latex suspension is obtainable from Koppers Company Inc., Pittsburgh, Pa., under the trademark Dylex K–31.

The polymer particles in the dispersed phase of this polystyrene latex and the appropriate reactant are combined in an aqueous buffered solution. Normally the pH is above 8.0, and excellent results are obtained at a pH of 8.2. A preferred buffered solution includes glycine and saline. Particularly advantageous results are obtained when the latex reactant mixture is heated for a period of time. This heating step seems to condition the reagent in such a manner that the appropriate diagnostic response is appreciably accelerated. A temperature of 57° C. for 15 to 30 minutes has been found to be satisfactory.

In order to facilitate visualization of the formation of particles, a dye is incorporated into the diagnostic reagent. For these purposes, brilliant green, added as a sterile 1% solution in the proportion of 1:500 in the final product, has been found to be satisfactory. The latex-reactant preparation is stable for an indefinite period of time. The final step in the preparation of the diagnostic reagent is the dilution of the concentrated latex-reactant mixture with an appropriate buffer. One volume of latex-reactant mixture can be combined with about 4.5 volumes of sterile glycine-saline buffer at pH 8.2 and containing 1% sodium azide to form a satisfactory reagent. Alternatively, the concentrated latex-reactant mixture can be diluted with a buffer prior to the heating step which would then be the final step.

In the ultimate reagent mixture excellent results have been obtained with the latex polymer present in an amount from about 0.2% to about 0.5% and with the reactant present in an amount from about 0.05% to about 0.1%. Normally, stock preparations will contain the latex polymer in more concentrated amount, for example, the latex polymer will comprise 5% or more by volume of the preparation.

The diagnostic reagents of this invention comprise complexes of polystyrene latex with either antigens or antibodies or substances which have the immunological characteristics of antigens or antibodies. Highly selective and satisfactory latex-antibody reagents have been prepared for the determination and analysis of various blood plasma proteins, among which are gamma globulin, fibrinogen, serum albumin and C-reactive protein. Equally satisfactory latex-antigen reagents have been prepared

for the diagnosis of rheumatoid factor in the serum of patients with rheumatoid arthritis and for the identification of the presence of auto-antibodies which appear in the serum of individuals afflicted with a thyroid disorder called Hashimoto's disease. Each of these diagnostic procedures will be discussed hereinafter in greater detail.

There are a variety of gamma globulin abnormalities. A gammaglobulinemia is a disease characterized by extremely low levels of serum gamma globulin, a marked deficiency of circulating antibodies and unusual susceptibility to repeated severe bacterial infections. Major acute infections associated with a deficiency of gamma globulin are: pneumonia, hepatitis, pyelonephritis, septic arthritis and meningitis. An abnormally high level of gamma globulin with little or no antibody content is usually found in the myeloma group of malignant blood disorders and may accompany hepatic disease. Gamma globulin deficiency, or disfunction may occur at any age in either sex. Most workers in the field recommend, therefore, an examination for the possible existence of one of these conditions whenever a patient shows unusual susceptibility to repeated bacterial infections.

The tests which have been employed for the diagnosis of gamma globulin abnormalties are of two general groups. The first group includes the so-called screening tests which, although not definitive, are helpful in arriving at a preliminary diagnosis. The second group comprises the definitive tests which include electrophoresis, Coombs hemagglutination inhibition test, preprecipitin reaction, gel diffusion and immunoelectrophoresis. In general, the screening tests have lacked either specificity or universal applicability. The definitive tests are more difficult and time consuming than the screening tests, require special equipment or reagents, and demand a high degree of technical skill for their proper performance and interpretation.

A latex-antibody reagent has been successfully prepared and employed for the determination of fibrinogen levels in blood. Hypofibrinogenemia, a deficiency in the fibrinogen blood level, is not uncommon. Such abnormally low fibrinogen levels may occur during and following certain surgical procedures and in cases of premature separation of the placenta. In such situations, fibrinogen falls below the critical level of about 100 mg. percent and may virtually disappear from the blood, a condition which can lead to uncontrollable hemorrhage. Through the use of the reagents of this invention, it is possible in minutes to determine whether a hemorrhagic condition is attributable to fibrinogen deficiency, thereby facilitating proper corrective therapy.

An imbalance of the various serum proteins is indicative of certain abnormal states. According to this invention it has been possible to prepare a latex-antibody reagent which can be employed for the quantitative determination of serum albumin.

Following the practice of this invention, it has been possible to prepare a latex-antibody reagent which can be employed either as a screening procedure to detect the presense of C-reactive protein or as a quantitative test to determine the level of C-reactive protein in an individual's serum. C-reactive protein is an abnormal protein, which is commonly found in the serum of persons with active inflammatory or tissue-destroying disease. C-reactive protein is not normally present in human serum. It is an acute phase protein, which usually occurs in patients with myocardial infarction, active rheumatic fever, advanced malignancy, rheumatoid arthritis, tuberculosis, pneumonia and other inflammatory diseases. As inflammation lessens, C-reactive protein decreases; when the inflammatory process is suppressed, the abnormal protein disappears from the blood. The presence of C-reactive protein is established by testing the patient's serum against an anti-serum prepared by hyperimmunizing rabbits to C-reactive protein. By combining the C-reactive protein antibody thereby obtained with latex particles, a reagent has been produced which provides clear-cut test results within two minutes or less.

A reagent for the diagnosis of rheumatoid arthritis is exemplary of the combination of polystyrene latex and an antigen-like substance. Rheumatoid arthritis is a statistically incapacitating disease with a prevalence estimated to be about 2.5% among persons over 14 years of age. Approximately 4,500,000 people in this country suffer from some manifestation of the disease. The cause of rheumatoid arthritis is as yet unknown, and there is no specific cure. However, much has been done in the management of the disease to minimize pain and control its crippling effects. Exceedingly helpful in the treatment is the differentiation of rheumatoid arthritis from several other arthritides and connective tissue diseases. To achieve this distinction physicians have resorted to clinical criteria in addition to laboratory tests.

The laboratory tests for rheumatoid arthritis have been serologic tests for the detection of an antibody-like or an agglutinating factor commonly called the rheumatic factor (often abbreviated RAF or RF). Present evidence indicates that RF is probably a gamma globulin of high molecular weight. Its presence in rheumatoid serum has been demonstrated as early as the day of onset of acute symptoms. Testing for the presence of RF has been based on the fact that it reacts with human gamma globulin or some other reactant present in Cohn Fraction II. Cohn Fraction II is a material obtained from human plasma or serum, using the fractionation method 6 and 9 of Cohn. The fraction is composed chiefly, although not exclusively, of gamma globulin.

In certain disease states of the thyroid gland, auto-antibodies are formed in response to thyroid protein which is released from this gland during the course of the pathology. One such thyroid disorder is characterized as Hashimoto's disease. A diagnostic reagent has been prepared by combining polystyrene latex and antigen obtained from either normal or pathologic thyroid glands. This latex-antigen reagent has been shown to be highly specific for the diagnosis of Hashimoto's disease. Such a diagnostic reagent is extremely useful, since surgical procedures are helpful only with respect to certain thyroid disorders and may be either unnecessary or harmful in the case of other thyroid disorders. Therefore, through the use of the diagnostic reagent of this invention, it is now possible to avoid unnecessary surgery.

When the polystyrene latex carriers which are employed in the practice of this invention are combined with a suitable reactant, it is possible to perform a quick (a matter of seconds) immunological diagnostic determination. In the case of a test for the presence of rheumatoid factor, the diagnostic reagents of this invention have dramatically lessened the time and work which has heretofore been required in the use of prior procedures. Ordinarily, 30 minutes' to 2 hours' incubation of a large number of test tubes has been required before a reading could be made. Reference can be made, for example, to the involved and time-consuming procedures described in the article by Singer and Plotz in the December 1956 issue of the American Journal of Medicine and the article by Rheins et al., in the July 1957 issue of the Journal of Laboratory and Clinical Medicine.

The following examples illustrate in greater detail the preparation and use of reagents which embody the teachings of this invention.

EXAMPLE I

A polystyrene latex suspension known as Lytron 615, available from Monsanto Chemical Company, is employed in sterile form. The latex polymer which in its commercial form ordinarily contains 40–50% solids is transferred aseptically to bottles of sterile distilled water to make a 1:10 stock suspension.

The human gamma globulin reactant employed is Cohn Fraction II. This reactant is dissolved in a glycine-

saline buffer at pH 8.2 and containing 0.1% sodium azide to make a 1% solution which is sterilized by filtration through a bacteria-excluding filter. Equal volumes of the 1% Cohn Fraction II solution and the 1:10 stock latex suspension are mixed with sterile precautions, and this latex-globulin mixture is heated at 57° C. for fifteen minutes. The preparation is then strained with brilliant green which is added as a sterile 1% solution in the proportion of 1:90. The concentrated latex-globulin mixture is then diluted with 4.5 volumes of sterile glycine-saline buffer at pH 8.2 and containing 0.1% sodium azide.

In the use of the reagent, a 1:20 dilution of the serum under test in a glycine-saline buffer diluent is employed. This dilution reduces the chances of false positive reaction which may occur as the result of the interaction of certain components of non-rheumatoid serums with the latex-globulin reagent. At the same time, rheumatoid serums with rheumatoid factor possess sufficient activity to withstand at least this amount of dilution. The test using the reagent provides positive and negative control serums for use in controlling the technique of running the test, as well as a check on the performance of the reagent and diluent.

The technique of carrying out the test using the prepared reagent involves the following steps:

(1) Prepare a 1:20 dilution of the serum under test by adding one drop of serum to 1 ml. of the diluent (sterile glycine-saline buffer at pH 8.2).

(2) Place one drop of diluted serum in a section of a divided glass slide.

(3) Add one drop of the test reagent, mix with an applicator or toothpick, and spread over an area of approximately 20 x 25 mm.

(4) Prepare positive and negative controls, each with one drop of known positive and negative serums (diluted 1:20 as above under (1) and one drop of the test reagent. Use separate applicators or toothpicks for each mixture. Alternatively, commercially available controls of the same character may be employed.

(5) Tilt the slide from side to side for 1 minute and observe for macroscopic clumping.

(6) Serums containing rheumatoid factor produce visible fluocculation of the test reagent whereas a smooth suspension is observed in a negative reaction. In positive tests, visible flocculation usually occurs in a few seconds.

(7) Recommended method of reading the test is as follows:

Negative—Smooth suspension with no visible flocculation.
Weakly reactive—Visible flocculation but with small aggregates or partial clumping.
Reactive—Visible flocculation with large aggregates and complete clumping (clear background).

Another advantage of the reagent of this invention is that it may also be used for the titration of rheumatoid serums. The density and sensitivity of the reagent is such that one drop added to one milliliter of a buffer diluent gives a stable, smooth, good reading mixture and one drop added to one milliliter of a buffer diluent which contains rheumatoid factor gives visible flocculation to the titer of the serum specimen.

The technique involved in carrying out the serum titration using the reagent involves the following steps:

(1) Serially number 10 test tubes (size approximately 12 x 75 mm.). Pipette 1.9 ml. of the diluent (sterile glycine-saline buffer at ph 8.2) into tube 1 and 1.0 ml. into the other tubes. Add 0.1 ml. of the patient's serum to tube 1, mix and transfer 1.0 ml. to tube 2. Mix and continue to prepare serial dilutions through tube 9, discarding 1.0 ml. from this tube (tube 10, the reagent control, contains no serum). Tubes 1 to 9 represent, respectively, serum solutions of 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280, 1:2560 and 1:5120.

(2) Add one drop of the test reagent to each tube. Shake the rack of tubes to insure thorough mixing and incubate at 37° C. for 15 minutes.

(3) Centrifuge tubes at 2500 r.p.m. for 2 minutes. Shake tubes gently to dislodge buttons of sediment and observe for macroscopic clumping.

(4) The reagent control (tube 10) should form a smooth suspension, whereas flocculation will be observed in a positive reaction. The titer of the serum is the reciprocal of the highest dilution showing definite flocculation.

Shelf-life tests of various reagents embodying combinations of latex-globulin mixtures have shown them to withstand storage without visible spontaneous flocculation under conditions varying from 4° C. to 37° C. for at least twelve months. Further, reagents of the character described can be freeze-dried as by lyophilization (i.e., subjecting the reagent to reduced pressures at reduced temperatures, such as a vacuum of 75 microns at −40° C.) if further preservation is necessary.

The reagent of this invention has application in the shorter laboratory procedures which are preferred by some practitioners. A method of preparing a reagent suitable for testing specimens of whole blood is set forth in Example II.

EXAMPLE II

A polystyrene latex of the type mentioned in Example I was employed and had a particle size in the range of 0.15 to 0.25 micron. The polystyrene latex was transferred aseptically to bottles of sterile distilled water to make a 1:10 stock suspension. Cohn Fraction II was employed as the human gamma globulin. This was dissolved in a glycine-saline buffer at pH 8.2 containing 0.1% sodium azide to make a 1% solution which was thereafter sterilized by filtration through a bacteria-excluding filter at room temperature. One part of the sterile latex 1:10 stock suspension was mixed with two parts of the sterile 1% Cohn Fraction II solution. The mixture was heated at 57° C. for 15 minutes. This latex-globulin preparation was then diluted with an equal volume of sterile buffer solution at pH 8.2 and containing citrate anticoagulant. The buffer-anticoagulant diluent was prepared by mixing two parts of glycine-saline solution at pH 8.2 with one part of 2% sodium citrate solution at pH 8.2. The latex-globulin reagent was then stained with neutral acriflavine which was added as a sterile 1% solution in the proportion of 1:240.

In the use of the reagent whose preparation was just described, one drop of whole blood specimen obtained by finger or ear prick can be added to two drops of the reagent on the surface of a flat glass slide. The blood and reagent are mixed and spread over an area of about 20 x 40 mm. The slide is then tilted back and forth while being observed for macroscopic clumping. Blood specimens containing rheumatoid factor produce visible flocculation of the latex-globulin particles in a matter of seconds. The agglutinated particles then accumulate at the periphery of the preparation which aids in visualizing a positive reaction.

The reagent whose preparation was just described can also be used by a capillary tube method. In this procedure, one drop of whole blood specimen obtained by finger or ear prick can be added to two drops of the reagent on a non-absorbent surface such as clean paper. The blood and reagent are mixed and a portion of the mixture is drawn into a capillary tube to a height of about 5 cm. The tip of the tube can then be forced into a small slab of modeling clay which serves to seal the capillary and to hold the tube erect. Blood specimens containing rheumatoid factor produce within five minutes visible flocculation of the latex-globulin reagent, a phenomenon which is characterized by a granular appearance. The yellow stained clumps of latex-globulin

reagent which cause the granular appearance of a positive reaction become larger upon standing for 10 or 15 minutes. During this time the blood cells tend to settle below the clumped reagent, leaving the supernatant quite clear and free of yellow stained latex-globulin. Negative reactions remain smooth and as the blood-cells settle during 10–15 minutes the supernatant remains turbid and contains the yellow stained latex-globulin reagent.

In the use of this invention in testing whole blood, the agglutination of latex-globulin particles by rheumatoid factor can be quickly achieved by employing a more dense suspension of latex particles than that which is optimum for serum tests. False positive reactions are prevented by using a larger amount of Cohn Fraction II solution than that required in diluted serum tests. The latex-globulin reagent can be stained a bright yellow to facilitate reading of the test in the presence of red blood cells.

EXAMPLE III

This example is concerned with the preparation and diagnostic use of a reagent of this invention for the determination of gamma globulin levels in blood.

The polystyrene latex suspension which is employed is that which was described in Example I. The antibody reactant is rabbit antibody to human serum gamma globulin prepared by conventional techniques. This reactant is dissolved in distilled water containing 0.1% sodium azide to make a 1% solution which is sterilized by filtration through a bacteria-excluding filter. One volume of the reactant solution is diluted with 9 volumes of sterile glycine-saline buffer at pH 8.2 and containing 0.1% sodium azide, and to this is added one volume of a 5% stock latex suspension. This latex reactant mixture is heated at 57° C. for 30 minutes, and the preparation is then stained with brilliant green, which is added as a sterile 1% solution in the proportion of 1:500. This reagent will provide rapid and clear-cut reactions with proper solutions of the serum under test to indicate a normal level, a clinically significant low level, or an abnormally high level of gamma globulin.

The test is conducted as follows:

(1) To three small (10 x 70 mm.) test tubes pipette 2.5, 0.5 and 0.5 ml. of the glycine-saline buffer diluent for the serum under test.

(2) Prepare dilution 1 of the serium specimen by adding a drop of serum from the capillary pipette to the first tube. Discard that pipette.

(3) Mix the contents of the tube and with a fresh pipette transfer one drop of dilution 1 to the second tube and leave the pipette in the first tube.

(4) Mix the contents of the second tube containing serum dilution 2. With a fresh capillary pipette transfer one drop of serum dilution 2 to the third tube, and leave the pipette in the second tube.

(5) Mix the contents of the third tube containing serium dilution 3. Put a fresh pipette in that tube.

(6) Using the pipettes which are now in the respective tubes, transfer one drop from each tube to successive sections of a divided glass slide. Transfer one drop from each dropper bottle of normal human control serum, dilutions 1, 2 and 3 to the other sections of the glass slide. The three dilutions of the normal human control serum are prepared by transferring one drop of a standard solution which contains 1 gram of gamma globulin per 100 ml. to 2.5 ml. of glycine-saline buffer, thereby forming dilution 1. One drop of dilution 1 solution is transferred to 2.5 ml. of glycine-saline buffer to form dilution 2. One drop of dilution 2 solution is then transferred to 2.5 ml. of glycine-saline buffer to form dilution 3.

(7) Add one drop of the latex-anti-human gamma globulin reagent to each serum dilution. Using separate toothpicks or wooden applicators, mix each of the reaction mixtures on the slide and spread it over an area of approximately 20 x 25 mm.

(8) Tilt the slide slowly from side to side for two minutes and observe for macroscopic clumping. Reactions are complete in this time. Observations should be made before appreciable drying occurs.

As is the case with all antigen-antibody reactions, the clumping phenonemon observed in this test is most pronounced when the antigen and antibodies are present in the most nearly optimal ratio. Prozones sometimes occur and these may aid in interpreting test results. If N (negative) represents no clumping and P (positive) represents clumping in the areas containing, respectively, serum dilutions 1, 2 and 3, the following interpretations can be made:

NNN—Agammaglobulinemia, or less than about 25 mg. %.

PNN—Hypogammaglobulinemia, or approximately 50 to 200 mg. %.

PPN—Normal range, or approximately 600 to 1200 mg. %.

PPP or NPP—Hypergammaglobulinemia, or more than 3 mg. %.

EXAMPLE IV

This example is concerned with the preparation and diagnostic use of a reagent which is suitable for the detection and quantitation of C-reactive protein.

The polystyrene latex employed in the reagent of this example is identical to that set forth in Example III and is prepared in the same manner. The reactant which is combined with this latex preparation is rabbit antibody to C-reactive protein. This reactant is prepared by conventional techniques. The latex-anti-C-reactive protein reagent is prepared in the same manner as the reagent of Example III was prepared, the rabbit antibody to C-reactive protein replacing the rabbit antibody to human serum gamma globulin of Example III.

The test is conducted in the following manner:

Qualitative Procedure

(1) Using a capillary pipette, place one drop (approximately 0.02 ml.) of serum specimen in a section of a divided slide.

(2) Add two drops of latex-anti-C-reactive protein reagent. With a wooden applicator or toothpick mix the reaction mixture and spread it over the area of approximately 20 x 40 mm.

(3) Tilt the slide from side to side for one to two minutes and observe for macroscopic clumping. Visible flocculation indicates the presence of C-reactive protein. Serum devoid of this abnormal protein gives a smooth suspension with no visible flocculation.

Quantitative Procedure

(1) Prepare serum dilutions in a glycin-saline buffer diluent as hereinbefore described. Serum specimens are tested at dilutions of 1:2, 1:4, 1:8, 1:16, 1:32 and 1:64.

(2) Using a capillary pipette transfer one drop of each serum dilution to successive sections of a divided slide. The same capillary pipette can be used for a series of dilutions if the transfer is started with the highest dilution and continued toward the lowest dilution.

(3) Add one drop of latex-anti-C-reactive protein reagent to each drop of serum dilution. Using a wooden applicator or toothpick and starting with the highest serum dilution, mix each reaction mixture and spread it over an area of about 20 x 40 mm.

(4) Tilt the slide from side to side for one to two minutes and observe for macroscopic agglutination.

(5) Interpretation of quantitative test: The highest serium solution showing visible flocculation is the C-reactive protein titer of the serum.

EXAMPLE V

The example is concerned with the preparation and diagnostic use of a reagent which is suitable for the deter-

mination of fibrinogen levels in blood. A polystyrene latex suspension is employed in sterile form. The polymer particles of styrene in the dispersed phase average less than one micron in diameter (about 0.2 micron with most of the particles being in the range 0.15 to 0.25 micron). This latex polymer is transferred aseptically to bottles of sterile distilled water to make a 1:10 stock suspension.

The antibody reactant is rabbit antibody to human plasma fibrinogen and is prepared by conventional techniques. The latex-anti-human fibrinogen reagent is prepared in the same manner as the reagent of Example III was prepared, the rabbit antibody to human plasma fibrinogen replacing the rabbit antibody to human serum gamma globulin of Example III.

The test is conducted in the following manner:

(1) Using a capillary pipette, transfer one drop of the test blood to 3 ml. of a diluent (sterile glycine-saline buffer at pH 8.2). This provides a 1:20 dilution of the blood under test.

(2) Place one drop of the diluted blood specimen in a section of a divided glass slide. Transfer one drop of a normal fibrinogen control preparation to another section of the glass slide. This normal fibrinogen control is prepared from blood with a plasma fibrinogen level of 300 mg. percent by diluting one drop in 3 ml. of glycine-saline buffer as is done with the blood under test.

(3) Add two drops of latex-anti-human fibrinogen reagent to the test blood and to the normal fibrinogen control, mix with an applicator or toothpick and spread over the area of approximately 20 x 25 mm.

(4) Tilt the slide slowly from side to side for one minute and observe for clumping. Blood specimens with plasma fibrinogen levels of less than 100 mg. percent will fail to show agglutination in this antigen-antibody system. Clumping in a degree comparable to that shown by the normal fibrinogen control indicates a level in the normal range of 250–400 mg. percent.

This application is a continuation-in-part of my copending application, Serial No. 742,009, filed June 16, 1958, now abandoned.

While in the foregoing specification, a detailed description of embodiments of the invention has been set forth for the purpose of understanding, it will be apparent to those skilled in the art that many modifications in the details thereof may be made without departing from the spirit and principles of the invention.

What is claimed is:

1. An immunological diagnostic reagent comprising polymerized styrene latex having a particle size in the range of about 0.15 to 0.25 micron and an immunological reactant selected from the group consisting of specific, known antibodies and antigens.

2. The reagent of claim 1 in which the reactant is present in an amount by weight of from about 0.05% to about 1.0%.

3. The reagent of claim 1 in which the latex is present in an amount by weight of from about 0.2% to about 5%.

4. An immunological diagnostic composition comprising polymerized styrene latex having a particle size of 0.15 to 0.25 micron and Cohn Fraction II.

5. In the performance of an immunological test based upon the well known antigen-antibody reaction, which test comprises combining a reactant selected from the group consisting of specific, known antibodies and antigens, with a carrier and bringing the thus formed combination into intimate contact with a specimen suspected to contain the corresponding opposite reactant and observing the thus formed mixture for evidence of the formation of a macroscopic insoluble complex which indicates a positive reaction; the improvement which comprises employing as said carrier a polymerized styrene latex having a particle size of about 0.15 to 0.25 micron.

6. The method of preparing an immunological diagnostic reagent comprised of a reactant selected from the group consisting of specific, known antibodies and antigens, and a polymerized styrene latex carrier which comprises forming a mixture of an aqueous solution of a polymerized styrene latex having a particle size of about 0.15 to 0.25 micron, an aqueous buffered solution of about pH 8 and said reactant, and then heating the mixture at about 57° C. for about fifteen to thirty minutes.

7. The method of claim 6 in which after heating the mixture is stained with a dye.

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