Substrates for immunological tests and method of fabrication thereof

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Filed: Feb. 25, 1974
Appl. No.: 445,204

U.S. Cl. .............. 23/259; 23/230 B; 23/253 TP; 128/2; 204/192; 424/12; 427/250; 428/336; 428/434; 428/469; 428/474
Int. Cl. .............. G01N 21/06; G01N 33/16
Field of Search ........ 23/230 B, 253 R, 259, 23/253 TP; 204/192; 117/107; 128/2; 424/12; 427/250; 428/336, 434, 469, 474

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ABSTRACT
Substrates for providing contrast, visible to the unaided eye, between single and double layers of immunologically reactive biological particles are fabricated by depositing an alloy of indium and gold on glass slides, and then heating the slides in air at different temperatures or time intervals to cause various degrees of oxidation of the indium. The various degrees of oxidation produce different colored slides having different sensitivities for different thicknesses of the layers of the biological particles, and each differently colored slide is differently sensitive to single and double layers of particular biological particles.

26 Claims, 3 Drawing Figures
SUBSTRATE FOR IMMUNOLOGICAL TESTS AND METHOD OF FABRICATION THEREOF

My invention relates to a substrate utilized for detecting an immunological reaction between a first biological particle and a second biological particle specific to the first, and the method of fabrication thereof, and in particular, to a substrate which provides improved contrast, visible to the unaided eye, between monomolecular and biomolecular layers of immunologically reactive biological particles on the surface of the substrate.


Immunological reactions are highly specific bio-chemical reactions in which a first immunologically reactive biological particle (generally a protein) known as the antigen combines (links) with a second protein specific to the antigen, and known as the antibody, to form an immunologically complexed protein. Immunological reactions taking place within a biological system, such as an animal or human being, are vital in combatting disease. In a biological system, the entry of a foreign protein, i.e., the antigen, causes the biological system to produce the specific antibody proteins to the antigen in a process not fully understood at this time. The antibody protein molecules have available chemical combining or binding sites which complement those of the antigen molecule so that the antigen and antibody chemically link or bond to form an immunologically complexed protein.

Most antigens are proteins or contain proteins as an essential part, whereas all antibodies are proteins. Proteins are large molecules of high molecular weight, i.e., polymers consisting of chains of variable numbers of amino acids. The above-cited co-pending applications disclose that an arbitrary protein will adhere to a substrate in a monomolecular layer only, and that no other arbitrary protein will adhere to the protein layer. On the other hand, the specifically reacting protein to the first protein adsorbed onto the substrate will immunologically bond thereto. In accordance with the teachings of those applications, this discovery is exploited to provide medical diagnostic devices in which a slide having a monomolecular layer of one protein adsorbed thereon is used to test suspected solutions for the presence of the specifically reacting protein thereto. If the specifically reacting protein is present in the solution, the slide after exposure to the solution has a bimolecular protein layer thereon. If the specifically reacting protein be absent from the solution, the slide after exposure to the solution has only the original monomolecular protein thereon. Optical, electrical and chemical means for distinguishing between bimolecular and monomolecular protein layers are taught in the related co-pending applications and have different degrees of sensitivity and economy.

Because antibodies are produced by biological systems in response to invasions thereof by foreign proteins, the detection of antibodies in a biological system is of medical diagnostic value in determining the antigens to which the system has been exposed. A typical example of diagnostic detection of antibodies is the detection of antibodies to syphilis or gonorrhea in human serum. Conversely, the detection of certain antigens in a biological system also has medical diagnostic value; examples of diagnostic detection of antigens include detection of HCG-protein molecules in urine as a test for pregnancy, and detection of hepatitis-associated-antigen (HAA) molecules in the blood of prospective blood donors.

In order to perform such diagnostic tests, the appropriate protein of the immunologically reacting pair must be obtained. The only known source of an antibody protein is a living biological system. More particularly, only vertebrates are known at this time to exhibit immunological reactions to the introduction of a foreign protein. For example, many antibodies are found in the blood serum of animals and human beings which have been exposed to the corresponding antigens. Many antigens, however, may be controllably produced in laboratory cultures. However, some antigens, for example, hepatitis-associated-antigens, are at present, like antibodies, only obtainable from the higher living biological systems.

It is known in the immunological art that antibody molecules function as antigens when introduced into the system of a vertebrate to whom they are foreign proteins. Accordingly, specifically reacting antibodies to a given antibody may be readily produced in such vertebrate system.

Although the substrates (slides) described in the hereinafore referenced patent applications are satisfactory in their performance with many of the immunologically reactive proteins, for certain diseases such as hepatitis, my previous slides have not provided the desired high degree of contrast between single and double layers of the hepatitis (antigen and antibody) molecules. Also, the anodized tantalum slide described in the hereinafore-referenced articles "Interactions Among Human Blood Proteins at Interfaces" and "Three Simple Ways to Detect Antibody-Antigen Complex on Flat Surfaces" has been found to be less sensitive than my slide to be hereinafter described, especially in the detection of hepatitis.

Therefore, a principal object of my invention is to provide a simple and improved device for detecting immunological reactions occurring at a solid surface by direct visual observation.

Another object of my invention is to provide an improved substrate which obtains improved contrast between single and double layers of immunologically reactive biological particles.

A further object of my invention is to provide a relative simple method for fabricating the improved substrate.
Briefly, and in accordance with the objects of my invention, I fabricate my improved slide by evaporating small globules of indium on the surface of a suitable substrate which may conveniently be a glass slide. Alternatively, the indium may be evaporated onto the surface of the slide as a thin film of constant thickness. Subsequently, a thin film of gold is evaporated over the indium and some alloying of the indium and gold occurs in this step. Finally, the coated slide is heated in air sufficiently to complete the alloying and to obtain some oxidation of the indium and thereby form an indium oxide film on the outer surface of the substrate coating. The degree of oxidation of the indium determines the color of such oxide film which is significantly differentially sensitive to single and double layers of particular immunologically reactive biological particles, more specifically proteins, being detected such that the contrast is readily visible to the unaided eye.

The features of my invention which I desire to protect herein are pointed out with particularity in the appended claims. The invention itself, however, both as to its organization and method of operation together with further objects and advantages thereof, may best be understood by reference to the following description taken in connection with the accompanying drawings wherein:

FIG. 1a is an elevation view of an intermediate structure of the preferred embodiment of my improved substrate;
FIG. 1b is an elevation view of the preferred substrate after final fabrication; and
FIG. 2 is an elevation view of a second embodiment of my improved substrate.

Referring now to FIG. 1a, there is shown an elevation view of a substrate 10 having a substantially flat top surface and being fabricated of a suitable material which may be a metal, glass, plastic or similar material. Substrate 10 is preferably in the form of a glass slide such as a conventional microscope cover glass 25 millimeters square and 10 mils thick, the glass slide being preferred primarily due to its low cost and ready commercial availability. After selection of the substrate, the top surface thereof is coated with a plurality of metal globules 11 by evaporating a metal, for example, indium, onto the substrate. Typically, the indium is evaporated slowly from a tantalum boat in the evaporator onto the glass substrate in an ordinary vacuum of about 5 \times 10^{-5} \text{ mm of mercury. Because the indium atoms have high mobility on the surface of the substrate and do not wet the glass substrate significantly, the indium evaporated onto the glass slide agglomerates into small unequal size particles. Some other metals, such as tin, having similar characteristics so that they will also form globules on the substrate when evaporated thereon, and are oxidizable, can be used, and the particular metal used is dependent on the particular immunologically reactive biological solution (including its pH) being investigated. The slow evaporation of the indium is necessary in order to obtain the globules, the evaporating process taking approximately 3 to 5 minutes. The indium globules have average diameters on the order of 3,000 A and are closely spaced together, having an average maximum spacing of approximately 1/4 inch diameter. A significantly faster evaporation of the indium (i.e., in the order of 30 seconds) results in a deposit on the substrate of a film of relatively constant thickness, as illustrated in FIG. 2.

After the indium globules 11 have been evaporated on substrate 10, a thin substantially constant thickness film 12 of gold is evaporated over the indium globules, and during this evaporation step some alloying of the indium and gold occurs. Other metals than gold may be utilized, such as copper or silver when testing other type body fluids. However, if the immunological test to be conducted requires the use of human serum, as is often the case, it has been found that such other metals are attacked through some chemical reaction and therefore are unsatisfactory. The gold deposition can be accomplished at a faster rate than the indium deposition step, and again may be accomplished by evaporating the gold from a tantalum boat in an ordinary vacuum of about 5 \times 10^{-5} \text{ mm of mercury. The gold film 12 is of thickness in the order of 1,000 A for the best contrast between a monomolecular layer of hepatitis B antigen (HBAg) and a bimolecular layer of such antigen and its specific antibody (HBAb) for an indium layer of average thickness of 2,000 A. Thus, the average thickness of the indium layer (i.e., a constant thickness layer that would be obtained from globules 11) is approximately twice the thickness of the gold film 12.

The intermediate structure of the coated substrate after evaporation of the gold film thereon is similar to that illustrated in FIG. 1a with the gold film 12 forming an irregular or uneven undulating pattern (due to the indium globules) that diffractions incident light, and with the understanding that some alloying of the indium and gold has occurred as described in my referenced application Ser. No. 384,113. The evaporation of the indium and gold onto the substrate can be accomplished in any suitable evaporator, a typical evaporator being model type CV-18 manufactured by Consolidated Vacuum Corp., Rochester, N.Y. A Deposit Thickness Monitor, model DTM-3 and Deposit Rate Control, model DRC, both manufactured by Sloan Instrument Corp., Santa Barbara, California were utilized with the aforementioned evaporator for obtaining the desired thickness and rate of deposition of the indium and gold metals being evaporated. Due to the maximum current limitation in the aforementioned evaporator, the gold could not be evaporated as rapidly as it could be in a higher power evaporator, and such evaporation process therefore took about 3 minutes.

After the gold has been evaporated on the indium globule-coated substrate, the coated substrate is removed from the evaporator and placed in a suitable electric furnace for heating in an air atmosphere sufficiently to obtain some oxidation of the indium and thereby form an indium oxide film 13 on the outer surface of the substrate coating having the irregular pattern of the gold film in FIG. 1a. This oxidation step also completes the alloying of the indium and gold. The degree of oxidation of the indium determines the color of such oxide film. The various degrees of oxidation produce different colored slabs having different sensitivities for different thicknesses of the layers of the biological particles, and each differently colored slab is differently sensitive to single and double layers of particular biological particles. The oxidation accomplished by heating for approximately 150 minutes at 325°C yields an indium oxide film having greenish-gold or bronze color. A lesser degree of oxidation produced by heating 325°C for 100 minutes produces a reddish-gold color. A greater oxidation of the indium produced by heating at 325°C for 30–45 minutes produces a blue color. The greenish-gold (bronze) color is presently the
preferred color for HAA as will be described hereinafter, and such oxide film has a thickness in the order of several hundred Angstrom.

In the case of only indium globules evaporated on the substrate, as taught in my hereinabove referenced patent application Ser. No. 384,113, the detection of a single or double layer of immunologically reactive proteins thereon is obtained by light transmission, that is, the test is accomplished by direct visual observation of the light transmitted through the slide. In the case of my present gold-indium alloy and indium oxide coated substrate, biological particle layers are detected by reflected light since the irregular surface of the indium oxide diffractions the incident light. The coated substrate, after the indium oxidation process, appears as in FIG. 1b. The 25 millimeter square coated slides may then be cut into approximately four equal squares for use in the immunological tests to be described hereinafter. Obviously, a commercial production of my slides would probably begin with a much larger size glass, and could be cut to any desired size slides. The cutting process may utilize conventional glass cutting techniques such as scoring the glass with a diamond scribe, and then breaking the glass along the scored lines.

My finished substrate, as illustrated in FIG. 1b or 2 is placed on a suitable support and a monomolecular layer of a first immunologically reactive biological particle is adhered onto the coated surface of the substrate. The adherence of the first biological particles may be accomplished by depositing a single drop of a first solution of the first biological particle on the substrate coated surface. The first biological particle is selected on the basis of its being specific to particular second biological particles which will form the second layer on the substrate surface if they are present in a solution to be tested. The first particles may be produced in laboratory cultures or obtained from the higher living biological systems as described hereinabove, and are generally commercially available in highly purified form, and if not available commercially, may be purified chemically. The solution of the first biological particles may be a salt solution of water or other liquid appropriate to, and not reactive with, the first biological particles. The substrate is preferably stored in a moist chamber for a time interval sufficient so that the first biological particles in the drop of the first solution are adsorbed onto the coated surface of substrate 10 and form a substantially complete monomolecular layer in the pattern of the drop in accordance with the teachings of the aforementioned co-pending U.S. patent applications of Giaever. The time interval (generally up to 1 hour) for the formation of the monomolecular layer on substrate 10 is an inverse function of the concentration of the first particle in the solution. The area size of the monomolecular layer on the substrate coated surface is preferably as small as practicable, and is generally in the range of 1 square millimeter to 1 square centimeter in order to conserve the amount of biological material used in the process. A rinsing of the coated surface of substrate 10 is often recommended after the formation of the monomolecular layer thereon in order to minimize nonspecific adsorption. The monomolecular layer coated substrate is then dried, if the slide is to be shipped commercially or stored, preferably by blowing air at room temperature across the substrate in order to speed the drying process. If the slide is to be used immediately, there is no need to dry it after the rinsing. For commercial use by others, the metallized slide could be sold by the slide manufacturer with or without the first monomolecular layer thereon. The spot on the substrate coated surface caused by the monomolecular layer pattern is generally barely, if at all, visible to the unaided eye.

The monomolecular layer coated substrate is then exposed to a second solution suspected of containing second immunologically reactive biological particles specific to the first in a direct test for such second particles. This exposure is generally accomplished by immersing the monomolecular layer coated substrate in the second solution for a time interval which is again an inverse function of the concentration of the second biological particles in the second solution. Since the concentration of the second particles is generally much less than the concentration of the first particle in the first solution, the immersent step generally takes much longer than the time interval for forming the monomolecular layer, and may take up to 24 hours. Presence of the second biological particles in the second solution results in the formation of a second substantially complete monomolecular layer on the pattern (generally a round spot) established on the coated substrate by the first monomolecular layer as a result of the immunological reaction. Chemical interaction of the second particles become bound to the first particles.

After the coated substrate has been sufficiently exposed to the second solution, the substrate is removed therefrom and may be immediately visually examined. Alternatively, and more generally, the substrate after removal from the second solution is again rinsed with a suitable solution which, in many cases, may be water or salt solution thereof, and the slide is then dried. The direct visual observation of the coated substrate is made by detecting the reflection off the coated surface of the substrate due to the light diffraction occurring at the oxidized surface, rather than be detecting the light transmitted therethrough. Absence of the second biological particles in the second solution results in only the presence of the monomolecular layer on the coated substrate surface and, as noted hereinabove, such single layer of biological particles is barely, if at all, visible on my improved slides. However, presence of the second particles in the second solution develops the second layer described hereinabove and produces a surprisingly different colored spot on the substrate so that the contrast between single and double layers of immunologically reactive biological particles is very pronounced.

As noted above, the different degrees of oxidation of the indium produce different colored slides, and it has been found that different colored slides have different sensitivities for different thicknesses of particular biological particle layers. Thus, a particular colored slide is selected for the particular biological particle being investigated since such particular colored slide has the best sensitivity for such biological particle system. The greenshish-gold (bronze) color slide has been found to have the highest sensitivity for detecting the difference between single and double layers of many types of immunologically reactive biological particle systems, that is, provides the highest degree of contrast for detecting the double layer which appears as a purplish spot on the slide. Although this particular color of the indium oxide film has been described as being obtained by heating the slide in air for 150 minutes at 325°C, it should be obvious that an oxide film of comparable color may be obtained by heating the slide at a higher
temperature for a shorter time, or at a lower temperature for a longer time.

FIG. 2 illustrates a second embodiment of my improved substrate. This embodiment is fabricated in the same manner as the first embodiment except for the first step. In the first step, a thick continuous (as opposed to the noncontinuous film of globules in Fig. 1a) film of indium of substantially constant thickness is evaporated on the top surface of substrate 10. This thick film, in the order of 3,000 Å thickness, is obtained by evaporating the indium at a much faster rate than in the case of the first embodiment, the evaporation interval being approximately 30 seconds. Alternatively, the gold can be evaporated on the substrate before the indium. The finished structure of the FIG. 2 embodiment thus includes substrate 10, a layer 30 of indium-gold alloy of substantially constant thickness and an indium oxide film 13 which is flat. The light diffraction produced from the indium oxide film 13 in the FIG. 2 embodiment results from gold particles dispersed therein during the oxidation process. Such gold particles contribute to a high dielectric constant and therefore promote very transparent interference color. Such gold particles are probably present in the indium oxide film in FIG. 1b.

A specific application of my improved substrate for the detection of hepatitis B antigen (HBAg) and antibody (HBAb) will now be described. This particular biological system requires a very sensitive test since the HBAg is a large size protein (molecular weight of 5 x 10^6), whereas the HBAb is small (M.W. of 1.6 x 10^4) so that the added thickness of an HBAb monomolecular layer to one of HBAg does not produce a large change in thickness. Experiments involving single and double layers of the HBAb and HBAg molecules on the anodized tantalum slides described in the hereinafore referenced Rothen article were not very satisfactory since a sufficient contrast between the single and double layers for a sensitive test was not obtained. However, the use of an improved substrate described herein provided a high degree of contrast between the single and double layers so that the layers were detected with at least the same sensitivity as in standard radioimmunoassay tests.

In the tests for detecting the hepatitis B antigen and antibody, the first step requires the adsorption of a monomolecular layer of HBAg onto the coated surface of my substrate. Since the HBAg is readily available in purified form, a drop of a common solution thereof (such as a salt solution) is applied onto the coated surface of the slide. The drop of this first solution is maintained on the substrate surface for a time interval sufficient to obtain a substantially complete monomolecular layer over the area of the drop, and may be in the order of 15 to 30 minutes with the slide being stored in a moist chamber to prevent evaporation of the drop. The slide is then placed in a distilled water and may subsequently be gently blown dry with compressed air, although this drying step is not essential. The HBAg has now been adsorbed from the drop of solution in a monomolecular layer on the coated slide surface and is barely, if at all, visible to the unaided eye.

The HBAg monomolecular layered slide is next exposed to a second solution suspected of containing the HBAb. This second solution is generally a human serum sample. The exposure is generally accomplished by immersing the slide in the second solution for an interval up to about 24 hours due to the concentration of the specific antibody in the serum sample being low, it at all present. The slide is then again rinsed and dried and visually examined with the naked eye. A purplish spot on the slide indicates presence of a second monomolecular layer (i.e., the HBAb layer) whereas absence of the spot indicates absence of HBAb in the human serum sample in a direct test therefor.

The direct test for HBAb can be accomplished in a similar manner as described above, with HBAb being adsorbed on the coated slide surface as a small spot forming the first monomolecular layer, and HBAg in the second solution (human serum sample), if present, forming the second monomolecular layer.

An indirect or inhibition test for the detection of HBAb may also be demonstrated using my improved slide. The principle of the inhibition test is that HBAb particles, if present in sufficient quantity, will neutralize free HBAb in solution. This reaction will prevent the antibodies from forming observable complexes (i.e., a bimolecular layer) when the slide with the antigen spot (first monomolecular layer) is exposed to the solution.

The inhibition test is accomplished as follows: A monomolecular layer spot on HBAg is adsorbed on the coated slide surface as in the direct test described hereinabove. The second solution is prepared by adding the human serum sample to be tested to a solution of HBAb in a vial or other suitable container. The vial is then stored for a time interval sufficient for the HBAb to complex with HBAg in the human sample, if the antigen is present therein. The vial is preferably agitated to increase the rate of complexing. Finally, the HBAg monomolecular spot covered slide is immersed in the second solution, and after a suitable period of time (again up to 24 hours), the slide is removed, rinsed, dried and visually examined. The results of this inhibition test are the opposite of the direct test, that is, presence of the HBAg in the human serum sample produces no purplish spot on the slide, i.e., produces no second monomolecular layer on the slide, whereas presence of such purplish spot indicates absence of the HBAg in the human serum sample.

The inhibition test for the detection of HBAb is performed similarly to the inhibition test for HBAg with the obvious substitution of the antigen for antibody and antibody for antigen in each of the steps.

In all of the above tests, the HBAb may be obtained from human serum of a patient known to have hepatitis B, or it may be developed in a goat, rabbit or other suitable animal by injection thereof with the HBAg, waiting a suitable incubation period such as two weeks, and then drawing blood containing the specific antibody from the animal and separating the antibody from the remaining blood particles.

The same significant visual contrast of a purplish spot against a bronze color background was found for a CEA (cancer embryonic antigen) and anti-CEA double layer and for a BSA (bovine serum albumin) and anti-BSA double layer as compared to a barely, if at all, visible spot for a single layer of the CEA or BSA particles. In the case of a blue colored slide, the double layer spot was white.

From the foregoing description, it can be appreciated that my invention makes available an improved substrate for detecting immunological reactions occurring at the surface thereof by direct visual observation with the unaided eye, as well as a method for fabricating the improved substrate. The improved substrate has a metallized coating including an alloy of two metals and an
oxide of one of such metals. Thus, the two metals can be identified as a base metal and noble metal since only the base metal can be oxidized, and is overcoated by the second (noble) metal. Also, there is no reason to limit the coating to two metals, one of the materials can be of the semiconductor type, and the materials are not necessarily limited to only two, there may be more. In the case of the metallized coating being an indium-gold alloy and indium oxide, such coated substrate provides significantly improved contrast between single and double monomolecular layers of immunologically reactive biological particles including a single layer of hepatitis B antigen and a second single layer of its specific hepatitis B antibody. Different degrees of oxidation of the indium may be found more useful with other types of pairs of immunologically reactive biological particles for increasing the contrast thereof between single and double layers. The coated substrate of my invention is a simple, relatively inexpensive device which is easily fabricated in accordance with another aspect of my invention. The major advantage of my invention is the significantly improved contrast between single and double layers of immunologically reactive biological particles which thereby permits detection of such immunological reaction by direct observation visible to the unaided eye.

Having described my invention with reference to two particular embodiments, it is believed obvious that modification and variation of my invention is possible in the light of the above teachings. Thus, the coating on the substrate may be formed of an alloy of two or more metals (or metal and semiconductor other than the indium and gold) including a base metal, with the base metal forming the outer oxidized surface. Such other alloy-oxide coated substrate may be found to obtain better contrast between single and double layers of some immunologically reactive biological particles other than the hepatitis type than if the indium-gold slide was used. That is, each pair of immunologically reactive biological particles are detected with the greatest contrast between single and double layers thereof with a specific substrate fabricated in accordance with my invention. Finally, the irregular surfaced slide constituting my first embodiment could obviously also be fabricated by starting with an irregular surfaced substrate and evaporating constant thickness layers of indium and gold thereon. It is, therefore, to be understood that changes may be made in the particular embodiment of my invention as described which are within the full intended scope of the invention as defined by the following claims.

What I claim as new and desire to secure by Letters Patent of the United States is:

1. A medical diagnostic device comprising:
   a substrate member and
   a composite metallized coating attached to the surface of said substrate member, said coating containing an alloy having a noble metal constituent and an oxidizable metallic constituent and having present in the outer surface thereof oxide content derived from said oxidizable metallic constituent, said coating being free of oxide of said noble metal constituent.

2. The device set forth in claim 1 wherein the noble metal constituent is gold.

3. The device set forth in claim 1 wherein the oxidizable metallic constituent is indium, the noble metal constituent is gold, and the oxide content is of indium oxide.

4. The device set forth in claim 1 wherein the surface of the substrate receiving the coating is flat.

5. The device set forth in claim 1 wherein the substrate is a glass material.

6. The device set forth in claim 1 wherein the oxidizable metallic component is tin.

7. The device set forth in claim 1 wherein the noble metal constituent is silver.

8. The device set forth in claim 1 wherein the oxidizable metallic constituent is indium.

9. The device set forth in claim 8 wherein the color of the coating is bronze.

10. The device set forth in claim 1 wherein the outer surface of the coating is slightly irregular.

11. The device set forth in claim 10 wherein the oxide portion of the coating is about several hundred Angstrom thick.

12. The device set forth in claim 1 wherein the outer surface of the coating is flat.

13. The device set forth in claim 12 wherein unoxidized particles of the noble metal constituent are dispersed within the oxide portion of the coating.

14. A medical diagnostic device comprising:
   a substrate member,
   a composite metallized coating attached to the surface of said substrate member, said coating containing an alloy having a noble metal constituent and an oxidizable metallic constituent and having present in the outer surface thereof oxide content derived from said oxidizable metallic constituent, said coating being free of oxide of said noble metal constituent and
   a monomolecular layer of immunologically reactive protein overlying at least a portion of said coating.

15. The device set forth in claim 14 wherein the protein is an antigen.

16. The device set forth in claim 14 wherein the protein is an antibody.

17. The device set forth in claim 14 and further comprising
   a layer of second immunologically reactive protein bound to the first monomolecular layer wherein the second protein is specific to the first protein, the double layer of first and second proteins being visible to the unaided eye as a spot of color distinct from the color of the outer surface of the coating.

18. The method of making a diagnostic device for determining the presence or absence of a specific protein in a biological sample comprising the steps of:
   coating surface area of a substrate with noble metal and oxidizable metal,
   heating said coated substrate in an oxidizing atmosphere to convert to the oxidized state a portion of said oxidizable metal at the surface of the coating and to simultaneously cause substantial alloying between oxidizable metal and noble metal and
   contacting said surface with a solution of a specifically reactive protein to said specific protein to apply a monomolecular layer of said specifically reacting protein to at least a portion of said surface.

19. The method set forth in claim 18 wherein the noble metal and oxidizable metal are applied in layers with the thickness of the oxidizable metal being approximately twice the thickness of the noble metal when initially deposited.
20. The method set forth in claim 18 wherein the oxidizable metal is applied as small globules on at least a portion of the surface of the substrate.

21. The method set forth in claim 20 wherein the noble metal is applied in a substantially constant thickness film over the oxidizable metal globules.

22. The method set forth in claim 18 wherein indium is applied by evaporation as the oxidizable metal.

23. The method set forth in claim 22 wherein the step of evaporating indium consists of evaporating the indium in a vacuum of approximately $5 \times 10^{-3}$ mm of mercury for a time interval of 3 to 5 minutes.

24. The method set forth in claim 22 wherein gold is applied by evaporation over the indium as the noble metal.

25. The method set forth in claim 24 wherein the thickness of the indium is approximately 2,000 A, and the thickness of the gold is approximately 1,000 A.

26. The method set forth in claim 24 wherein the step of heating the metal coated substrate consists of heating in air for approximately 150 minutes at 325°C.