

[54] **METHOD AND APPARATUS FOR IMMUNOLOGICAL DETECTION OF BIOLOGICAL PARTICLES**

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[58] Field of Search **23/230 B, 253 R, 253 TP; 195/103.5 R; 424/12; 117/71 R**

[56] **References Cited**

UNITED STATES PATENTS

3,250,596	5/1966	Grafe	23/230 B
3,492,396	1/1970	Dalton	424/12
3,646,346	2/1972	Catt	424/12 X
3,770,380	11/1973	Smith	424/12 X

OTHER PUBLICATIONS

Chemical Abstracts, 63:3532d (1965).
Chemical Abstracts, 63:10483b (1965).
Chemical Abstracts, 65:2821g (1966).
Chemical Abstracts, 73:48541m (1970).

G. M. Edelman et al., Proc. Nat. Acad. Sci., 68, (9), 2153-2157 (Sept. 1971).

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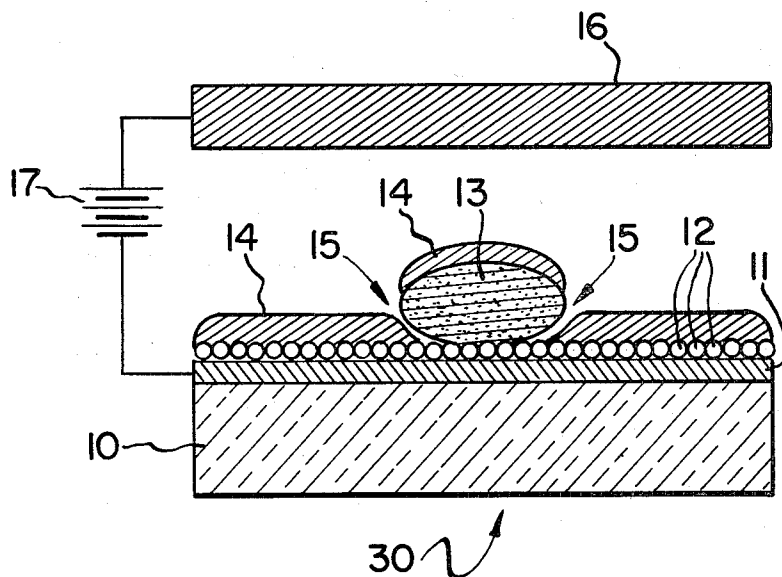
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[57] **ABSTRACT**

Method and apparatus for the detection of biological particles such as viruses, bacteria, and other cells by detection of the occurrence of an immunological reaction between the particle to be detected and its antibody. In one embodiment a substrate having an etchable metal surface is coated with a layer of antibodies to the biological particle to be detected. The antibody coated substrate is exposed to a fluid suspected of containing the biological particles to be detected. After such exposure, the structure is coated with a layer of a non-etchable metal of sufficient thickness to cover the antibody layer but of insufficient thickness to completely coat the biological particles and form a continuous film. The structure is then exposed to an etchant. After etching, positions at which biological particles had been immunologically bonded to their antibodies are observable as voids in the structure.

18 Claims, 4 Drawing Figures



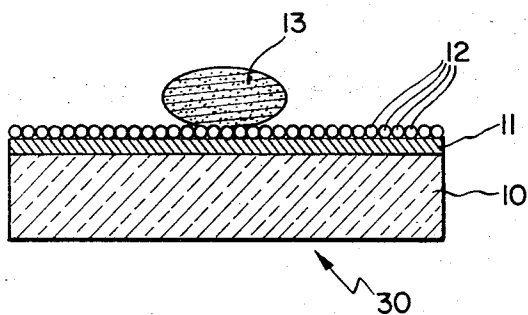


Fig. 1

Fig. 2

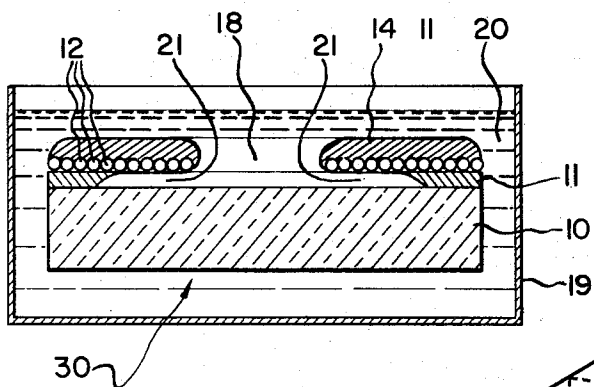
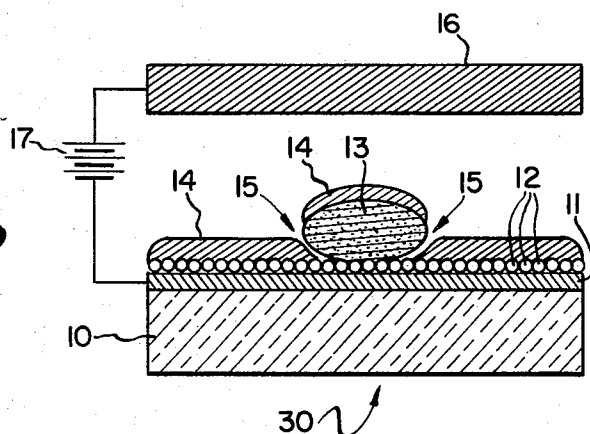


Fig. 3

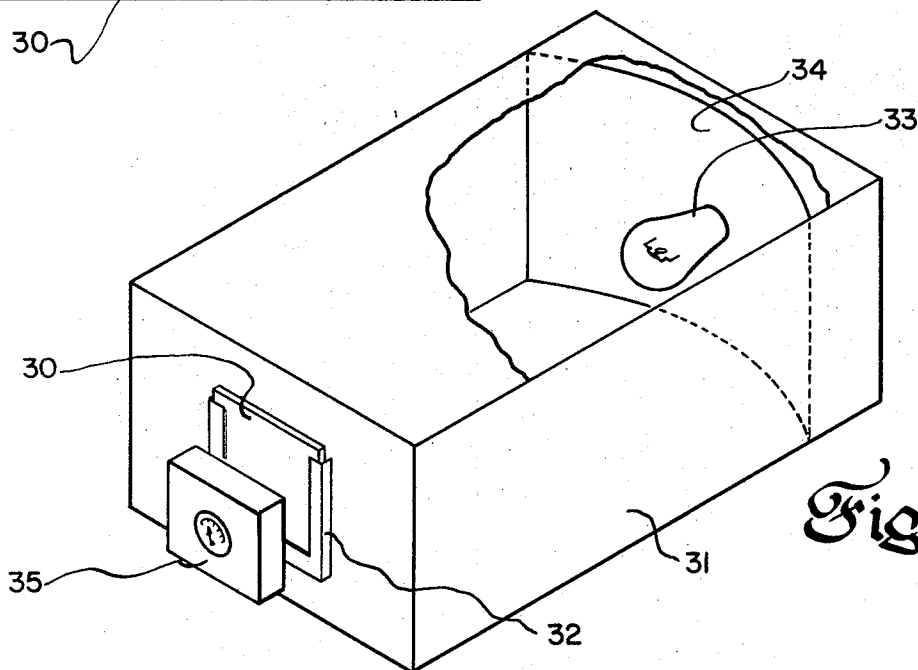


Fig. 4

METHOD AND APPARATUS FOR IMMUNOLOGICAL DETECTION OF BIOLOGICAL PARTICLES

This invention relates to immunological detection of biological particles. More particularly, this invention relates to immunological detection of biological particles such as viruses, bacteria, and other cells which are very much larger than molecules of their specifically reacting antibody proteins. This invention further has particular utility in the immunologic detection of particles whose presence in physiologic fluids in very low concentrations is of diagnostic interest, as for example, viruses.

This application is related to the copending application of Giaever, Ser. No. 266,278, filed June 26, 1972, and the copending application of Giaever, Ser. No. 384,113, filed July 30, 1973. These copending applications are commonly assigned with this application.

The related copending applications cited above 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. This discovery is exploited for diagnostic purposes in accordance with the teachings of the aforementioned related copending applications by means disclosed therein for efficiently and economically distinguishing between a monomolecular protein layer and a bimolecular protein layer on a substrate.

The diagnostic methods disclosed in the aforementioned copending applications require the formation of a substantially complete biomolecular protein layer for detection. While in theory, any immunologically reactive particle will form a detectably complete bimolecular layer when exposed to a slide coated with a monomolecular layer of its specifically reacting protein, in cases in which the particle to be detected is present in very dilute concentration in a fluid, detection in accordance with the teachings of the referenced copending applications may be unacceptably time-consuming.

A number of biologic particles are of significant diagnostic interest in substantially dilute concentrations. A prime example of these are the viruses. It is important to be able to detect the presence of viruses in body fluids in concentrations on the order of several thousand virus particles per cubic centimeter. Viruses are uniformly immunologically reactive. Viruses are not detectable by means of optical microscopic examination.

It is accordingly an object of this invention to provide method and apparatus for the immunologic detection of large biological particles.

It is another object of this invention to provide such method and apparatus which is highly sensitive so that particles present in highly dilute concentrations may be detected within short periods of time.

A further object of this invention is to provide such method and apparatus which provides for both the detection of such biological particles and for a determination of the concentration thereof in a fluid specimen.

Yet another object of the invention is to provide such method and apparatus which is sufficiently sensitive to provide for the detection of a single biological particle.

Briefly, and in accordance with one embodiment of this invention, a wafer of substrate material has a film of etchable metal on one surface thereof. The metal film is coated with a monomolecular layer of antibodies to the biological particle to be detected. The substrate is then immersed in a fluid specimen to be tested for the presence of the particular species of biological particle of interest. Subsequently, a layer of etch-resistant metal is applied to the substrate over the layer of antibodies and any biological particles bonded thereto. The substrate is then immersed in an etchant bath which dissolves the biological particles, those antibodies immediately underlying the biological particles, and a portion of the etchable metal film. After etching, the substrate is examined and points at which the biological particles to be detected had been bonded thereto are observed as voids in the non-etchable metal layer on the substrate.

The novel features of this invention sought to be patented are set forth with particularity in the appended claims. The invention, together with further objects and advantages thereof, may be understood from a reading of the following specification and appended claims in view of the accompanying drawing in which:

FIG. 1 is a sectional elevation view of apparatus in accordance with this invention showing a substrate having an etchable film thereon, a monomolecular antibody layer over the metal film, and a biological particle immunologically bonded to the antibody layer.

FIG. 2 is a sectional elevation view of the apparatus in FIG. 1 having additionally a non-etchable metal layer thereover and further illustrating means for applying the non-etchable layer in accordance with one embodiment of this invention.

FIG. 3 illustrates the etching of the apparatus of FIG. 2.

FIG. 4 is an isometric view, partially cut away, of apparatus in accordance with one embodiment of this invention for examining the etched apparatus of FIG. 3.

FIG. 1 is a sectional elevation view of a diagnostic slide in accordance with this invention indicated generally at 30 and comprising a light-transmissive substrate member 10 which may be glass, mica, plastic, fused silica, quartz, or similar material, with glass being preferred, having a layer 11 of an etchable material which is preferably a film of an opaque etchable metal such as, for example, indium, and hereinafter, for convenience, referred to as "metal," attached to one surface thereof. Metal layer 11 may be conveniently applied to substrate member 10 by evaporation as one example. The metal coated slide is then immersed in a solution of antibodies to the biological particle to be detected to adsorb a monomolecular layer of antibodies 12 on the surface of etchable metal film 11; this completes the fabrication of a diagnostic slide. The diagnostic procedure utilizing this slide comprises immersing the slide into a physiologic fluid specimen to be tested for the presence of biological particles of the species of interest. If the specimen contains particles of the species of interest, some of the particles, as 13 in FIG. 1, become immunologically bonded to antibody molecules 12 on the slide. Only particles specifically reactive with the antibodies will immunologically complex therewith.

The next step in accordance with this invention is illustrated in FIG. 2 and comprises the deposition of a non-etchable layer over the structure of FIG. 1. The non-etchable layer is preferably a light-transmissive, non-etchable material and may be glass or other suitable oxide or a metal such as gold. In accordance with one embodiment of this invention, diagnostic slide 30 is immersed in an electrolyte bath (not shown) and an electrical potential is applied by a source 17 thereof between metal film 11 and a quantity 16 of non-etchable metal which is also immersed in the electrolyte solution to plate a quantity 14 of non-etchable metal onto the structure shown in FIG. 1. For reasons more fully set forth hereinafter, metal 16 is preferably gold. It is important in practicing this invention to carefully control the deposition of non-etchable metal coating 14 to insure that the deposited metal is discontinuous about the periphery of particle 13 as shown in FIG. 2 by gap 15. In an alternative embodiment of this invention, non-etchable metal may be applied by evaporation, again preferably gold. In this case, also the deposition process is monitored to insure discontinuities of the non-etchable metal coating about the peripheries of each biological particle bonded to the slide.

FIG. 3 illustrates the next step in the practice of this invention and comprises the immersion of diagnostic slide 30 after the deposition of the non-etchable metal layer thereon into a container 19 of a quantity 20 of an etchant solution, such as an acid. Etchant 20 dissolves the biological particles bonded to the slide, the immediately underlying antibody which had been complexed therewith and the underlying portion of etchable metal film leaving a void 18 in the slide structure and exposing a portion of light-transmissive substrate 10. Etchant 20 further undercuts a portion of the structure surrounding void 18 by dissolving additional quantities of etchable metal 11 to produce an expansion of void 18 into area 21. This undercutting serves to provide amplification of the detectability of void 18 when the non-etchable metal is gold. This is the reason for preferring gold as the non-etchable metal and results from the fact that a thin gold layer such as is applied in accordance with this invention is quite light transmissive in the absence of an underlying layer of another metal but is substantially opaque when the underlying layer is present. Accordingly, after removal from the etchant bath, slide structure 30 exhibits light-transmissive regions corresponding to the points at which biological particles have been bonded thereto, which light-transmissive regions have dimensions determined by the diameter of regions 21 which are substantially greater than the dimensions of voids 18. At other regions diagnostic slide 30 remains opaque.

Following the etch procedure, slide 30 is examined to determine whether or not biological particles had been immunologically bonded thereto and consequently whether or not the test specimen contained the specific biological particles of interest. A virus particle, for example, is typically 1,000 Å in diameter. By the use of gold metal backed by etchable metal which is undercut in the etch process as discussed above, light-transmissive regions in excess of 10,000 Å in diameter are produced at sites at which biological particles had been immunologically bonded to their specific antibodies on the slide. This provides for reading of the slide to detect particles in the specimen by optical microscopy. Furthermore, if desired, a counting grid may be

superimposed over the microscopic image to thereby provide for determination of concentration of the particles of interest in the specimen.

FIG. 4 illustrates alternative examination procedures to optical microscopy in accordance with this invention. In FIG. 4 an enclosure 31 has a bracket 32 on one side thereof for supporting slide 30 adjacent an opening (not shown) in enclosure 31. An illumination source such as incandescent lamp 33 is positioned within enclosure 31 to direct light against slide 30. If desired, a reflector 34 may be employed to increase the light intensity impinging on slide 30. By use of such a light box, a diagnostic slide 30 processed as hereinbefore described may be examined by unaided visual observation in a slightly darkened room. The sites at which biological particles have been immunologically bonded to the slide appear as clearly distinct speckles of illumination when so viewed. Obviously, quantitative information regarding the concentration of particles in the specimen may be obtained by counting the speckles of illumination and this may be aided by placing a grid structure over the outer surfaces of slide 30 in retaining means 32. Alternatively, an integrating, indicating photoresponsive device 35 may be employed to provide quantitative information on the concentration of biological particles in the specimen. Device 35 may, for example, conveniently comprise a lens system for focusing light transmitted through slide 30 onto the photocathode of a photomultiplier tube operating in the current mode having a millimeter connected in series with the photomultiplier tube.

While this invention has been described with reference to particular embodiments and examples, other modifications and variations will occur to those skilled in the art in view of the above teachings. Accordingly, it should be understood that within the scope of the appended claims, the invention may be practiced otherwise than is specifically described.

The invention claimed is:

1. Apparatus for detecting biological particles of a particular species in a fluid comprising:
 - a substrate;
 - a metal film on a surface of said substrate;
 - a layer of antibodies to said species of biological particle, said antibodies being adsorbed on said metal film;
 - means for applying a second metal layer over said layer of antibodies and any of said biological particles bonded thereto; and
 - means for selectively etching said biological particles, said metal film, and said antibodies.
2. The apparatus of claim 1 wherein:
 - said means for selectively etching comprises an acid;
 - said metal film comprises a metal which is etchable by said acid; and
 - said second metal layer comprises a metal which is resistant to etching by said acid.
3. The apparatus of claim 2 wherein said second metal layer has voids therein, said voids being disposed about the peripheries of said biological particles.
4. The apparatus of claim 3 wherein said substrate comprises a light-transmissive material which is resistant to etching by said acid.
5. The apparatus of claim 4 including additionally means for directing light onto a surface of said substrate.

6. The apparatus of claim 5 wherein said means for directing light onto a surface of said substrate comprises:

an enclosure having first and second end members, said second end member having an aperture therein;

a lamp within said enclosure adjacent said first end member; and

means attached to a surface of said second end member external to said enclosure for receiving said substrate and for positioning said substrate adjacent said aperture.

7. The apparatus of claim 6 further including means external to said enclosure for receiving light transmitted through said substrate and for indicating the quantity of light energy received.

8. A method for detecting biological particles of a particular species in a fluid comprising the steps of:

immersing a substrate having a metal surface into a solution of antibodies to said species of biological particle to adhere a layer of said antibodies thereon;

immersing said substrate having said antibodies thereon into said fluid whereby a plurality of said biological particles, if present in said fluid, immunologically bond to said antibodies;

applying a second metal layer over said layer of antibodies and any of said biological particles;

immersing said substrate in an etchant solution; and examining said substrate to determine whether any of said biological particles had bonded to said substrate.

9. The method of claim 8 further including the step of:

bonding a metal film to a wafer of light-transmissive material to form said substrate.

10. The method of claim 8 wherein said applying step more particularly comprises the steps of:

immersing said substrate in a solution of a salt of said second metal;

immersing a block of said second metal in said solution of salt; and

applying an electrical potential difference between said block and said metal surface to electroplate said second metal onto said substrate.

11. The method of claim 8 wherein said applying step

comprises evaporating said second metal onto said substrate.

12. The method of claim 8 wherein said applying step further includes the step of monitoring the deposition of said second metal to insure that said second metal layer is discontinuous about the peripheries of said biological particles.

13. The method of claim 8 wherein said examining step comprises microscopic examination of said substrate.

14. The method of claim 8 wherein said examining step more particularly comprises:

backlighting said substrate; and

visually observing as points of transmitted light locations on said substrate at which said biological particles had been bonded.

15. The method of claim 8 wherein said examining step more particularly comprises:

backlighting said substrate;

receiving and integrating light transmitted through said substrate; and

indicating the integrated quantity of said light to provide a measure of the concentration of said biological particles in said fluid.

16. Apparatus for detecting biological particles of a particular species in a fluid comprising:

a substrate;

an etchable film on a surface of said substrate;

a layer of antibodies to said species of biological particle, said antibodies being adsorbed on said etchable film;

means for applying a layer of non-etchable material over said layer of antibodies and any of said biological particles bonded thereto; and

means for selectively etching said biological particles, said etchable film, and said antibodies.

17. The apparatus of claim 16 wherein:

said means for selectively etching comprises an acid; said etchable film comprises a metal which is etchable by said acid; and

said layer of non-etchable material comprises a metal which is resistant to etching by said acid.

18. The apparatus of claim 16 wherein said etchable film is opaque and said non-etchable material is light transmissive.

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