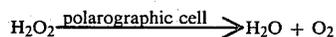


## 5. Detection Stage



In this embodiment, the detection stage is a three electrode electrochemical cell in which the hydrogen peroxide is quantitatively determined by generating a signal in the detection stage, which communicates the signal to readout means, typically a digital display panel calibrated in milligram percent to represent the quantitative amount of initial insulin antigen in a sample.

What is claimed is:

1. A method for quantitatively determining the amount of an antigen contained in a biochemical fluid comprising obtaining sample of said fluid and mixing it in a mixing zone with an aqueous solution, passing said sample-solution into a solubilization stage containing an immobilized antibody which is specific to said antigen, said immobilized antibody initially having an immunochemically equivalent amount of reversibly bound an antigen-enzyme complex wherein said antigen of said complex is the same as the antigen in the sample, maintaining said sample-solution in said solubilization stage for a period of time and under conditions sufficient for a competitive equilibrium reaction to take place between the said antigen and said bound antigen complex, thereby displacing a quantity of said bound antigen complex from said immobilized antibody into said sample-solution; passing said complex-containing sample-solution into a conversion stage containing a substrate capable of reacting with said enzyme of said complex to produce a measurable reaction product in said sample-solution; passing said measurable reaction product-containing sample-solution into a detection stage wherein said measurable reaction product is quantitatively measured to obtain a reading, and comparing said measured reading to the quantitative amount of antigen in said original sample.

2. The method of claim 1 wherein said original sample is diluted into an aqueous buffered solution prior to passing it into said solubilization stage.

3. The method of claim 1 wherein said antibody is immobilized on a solid support.

4. The method of claim 3 wherein said solid support is a refractory metal oxide.

5. The method of claim 4 wherein said refractory metal oxide is alumina.

6. The method of claim 1 wherein said antigen in said original sample is phenobarbital, said antigen-enzyme complex is a phenobarbital-glucose-6-phosphate dehydrogenase complex, and said antibody is anti-phenobarbital, and said substrate is glucose-6-phosphate.

7. The method of claim 1 wherein said conversion stage comprises an extended confined reaction zone through which said sample-solution is passed.

8. The method of claim 7 wherein said measurable reaction product in said detection stage is read spectrophotometrically.

9. The method of claim 1 wherein said antigen in said original sample is insulin, said antigen-enzyme complex is an insulin-glucoamylase complex, said antibody is anti-insulin, and said substrate is a starch.

10. The method of claim 1 wherein said original sample is contaminated with free glucose which includes flowing said sample through a scavenger stage prior to

the production of a measurable reaction product in said sample-solution.

11. The method of claim 1 wherein said original sample is contaminated with free glucose and said conversion stage includes a scavenger stage containing immobilized scavenger reagents capable of reacting with and removing said contaminating glucose, a substrate stage containing an immobilized starch reagent capable of reacting with said complex contained in said sample solution to produce oligosaccharides, a glucose generating stage containing immobilized glucose-generating reagent capable of reacting with said oligosaccharides to produce a glucose reaction product in said sample solution, and a hydrogen peroxide generating stage containing an immobilized reagent capable of oxidizing said glucose reaction product to generate hydrogen peroxide defining said measurable reaction product.

12. The method of claim 11 wherein said hydrogen peroxide is passed to and read in said detection stage in an electrochemical cell.

13. The method of claim 1 wherein said antigen is insulin.

14. The method of claim 1 wherein said antigen-enzyme complex is insulin-glucoamylase.

15. The method of claim 1 wherein said aqueous solution comprises a buffer and said buffered solution is passed as a flowing stream into a mixing zone wherein said original sample is introduced, and wherein said flowing stream containing said original sample is flowed sequentially through said solubilization, conversion, and detection stages.

16. An apparatus for the quantitative determination of an antigen contained in an aqueous biochemical sample, comprising in combination, a reservoir containing a buffer diluent solution, mixing means for said diluent solution and said sample after they are combined, solubilization means to receive the mixed diluent solution and sample containing immobilized antibody initially saturated with an immunochemically equivalent amount of antigen-enzyme complex reversibly bound thereon, conversion means to receive the stream from the solubilization means containing immobilized reagent capable of reacting with said complex to produce a measurable reaction product, detection means capable of monitoring said measurable reaction product to produce a reading relating to the amount of said antigen.

17. The apparatus of claim 16 wherein said antibody is anti-insulin and said antigen-enzyme complex is an insulin-glucoamylase.

18. The apparatus of claim 16 wherein said antibody is anti-phenobarbital and said antigen-enzyme complex is a phenobarbital-glucose-6-phosphate dehydrogenase.

19. The apparatus of claim 16 wherein said conversion stage comprises elongated, flexible, chemically resistant tubing.

20. The apparatus of claim 16 wherein said detection stage comprises a flow-through design spectrophotometric cell.

21. The apparatus of claim 16 wherein said sample is contaminated with glucose and said conversion stage comprises, in series, scavenger means comprising immobilized scavenger reagent capable of removing contaminating glucose from said sample, substrate means containing an immobilized starch reagent capable of reacting said complex to produce oligosaccharides, glucose generating means containing immobilized glucose generating reagent capable of reacting with said oligosaccharides to produce a glucose reaction product, and a