

PIEZOELECTRIC IMMUNOSENSOR

FIELD OF THE INVENTION

The present invention is related to the apparatus and method for performing immunoassay. In particular, the present invention is related to a
5 diagnostic tool in allergy testing.

BACKGROUND OF THE INVENTION

The use of piezoelectric (Pz) oscillator, such as quartz crystal microbalances (QCM's) as a potential chemical sensor is based on the relationship of the frequency change and the mass loading on the surface of
10 the crystal described by the Sauerbrey equation :

$$\Delta F = \frac{-2F_0^2 \Delta m}{A(\rho_q \mu_q)^{1/2}}$$

where ΔF is the measured frequency shift, F_0 is the fundamental frequency of the uncoated Pz crystal, A is the area coated, ΔM is the mass change due to
15 surface deposition, ρ_q is the density of the quartz crystal (2.648gcm^{-3}) and μ_q is the shear modulus ($2.947 \times 10^{11} \text{gcm}^{-1} \text{s}^{-2}$ for AT-cut quartz crystals). The frequency which can be measured after mass change is known as the resonance frequencies. It has been suggested that biologically active materials such as antibody, antigen and enzymes may be used as a coating
20 on Pz crystals. The use of antigen as a Pz crystal coating was first demonstrated by Shons et al. (*J. Biomed. Mater. Res.* Vol. 6, pp565-70, 1972). In the experiments described in this prior art, the antigen-coated crystal was exposed to unknown amounts of antibody solution. The

immunoreaction between antibody and antigen-coated crystal resulted in the immunobinding of the antibody. Frequency shift caused by mass loading is proportional to the concentration of the specific antibody in solution.

U.S. Patent No. 4,242,096 discloses a competitive immunoassay for
5 detecting an antigen in a liquid sample, in which an antigen was adsorbed onto a crystal having a polymer monolayer such as poly(2-hydroxy-3-dimethylamino-1.4.butane). The immobilized antigen competes with the antigen in the test sample to react with a specific antibody which is added to the test sample. The amount of free antigen can then be determined by the
10 change of resonance frequency of the oscillation system by reference to a standard curve. The use of a polymer priming surface followed by adsorption of the antigen are also disclosed in U.S. Patent No. 4,236,893 and 4,314,821, among others.

US. Patent No. 4,735,906 discloses a method of protein immobilization
15 using a crystal with a surface modified to form a layer of siloxane polymer which in turn immobilizes proteins through amide linkages. The patent further suggested that protein adsorption on other polymers such as modified polyacrylamide polymers may also be used. Such methods, however, are based on randomly oriented polymers, which affects the coupling sites
20 available to the antibody/antigen used in the coating procedure. In addition, such polymers also interact with the crystal surface through physical adsorption, and therefore are also highly sensitive to washing and regeneration conditions.

Rickert et.al. in *Biosensors & Bioelectronic* 11:591, 1996 described a
25 method of covalently coupling a synthetic peptide onto the gold surface of an

oscillator using self-assembled thiols as selective monolayer coating for bioaffinity analysis, but they reported that even with covalent coupling, sensitivity was reduced to approximately 70% of the initial value after three regeneration cycles.

5 Immunoglobulin E (IgE) is a glycoprotein ($M_r=190000$, $S_{w,20^\circ\text{C}}=8.2$) containing a large amount of carbohydrate (11%). Its structural unit comprises two heavy chains and two light chains. IgE is present in human serum and has diagnostic functions in the detection of allergies. Several immunoassays have been reported for the determination of total IgE in
10 human serum. Immunoassay procedures such as radioimmunoassay, fluoroimmunoassay, enzyme linked immunosorbent assays and the use of ion-selective electrodes are the most widely used methods in immunochemistry. BioWhittaker, Inc. of Maryland, USA, has a Total IgE FAST test (Fluorescence Allergor-sorbent Test) which uses immobilized anti-IgE
15 antibody in a microtiter format, and is based on the conventional fluorescence enzyme immunoassay technique. Pharmacia of Sweden has a CAP-fluorescence system which uses an antibody-coated 3-dimensional porous matrix as a reaction carrier. Its principle of detection is also based on the fluorescence reaction between the enzyme-labeled antibody its specific
20 substrate. These conventional diagnostic methods, however, suffer from drawbacks such as requiring skilled personnel and using potentially hazardous and expensive reagents. In addition, the test procedures and instrumentation are complex and time-consuming.

OBJECT OF THE INVENTION

It is therefore an object of the present invention to provide an apparatus and method for antibody testing which overcome the problems described above.

5 It is another object of the present invention to provide a simplified and shortened method for allergy testing.

It is another object to provide a method of allergy testing at reduced costs.

SUMMARY OF THE INVENTION

The present invention provides a method for the detection and quantitation of
10 antigen, and apparatus for performing the same. The apparatus according to one aspect includes an immunosensor which uses a metal-coated piezoelectric (Pz) crystal microbalance with a self-assembled monolayer (SAM). According to another aspect, an antigen-specific antibody is covalently coupled to the electrodes of the crystal. In one embodiment, the
15 antigen is human IgE which may be detected using the immunosensor having a Quartz Crystal Microbalance (QCM). The anti-IgE antibody is immobilized onto the crystal via the SAM. In one preferred embodiment, the gold-coated surface of the crystal is first modified with thioctic acid to form an SAM. Using the coupling reagent 1-ethyl-3-(3-dimethylaminopropyl)carbodi-
20 imid (EDC), anti-IgE antibody is then covalently coupled on the SAM. In another preferred embodiment, cysteamine is used to form the SAM, and glutaraldehyde (GA) is used to cross-link the antibody to the SAM. The highly ordered SAM results in very uniform amounts of antibody binding.

The IgE detection method includes incubation of the test sample with the antibody-coated crystal for indicated time. The frequency of the antibody-coated crystal is measured before and after incubation with antigen. The change of the frequency is related to the amount of antigen bound to the coated crystal, and the amount of IgE can be quantified by reference to a standard curve.

The QCM produced according to the present invention has a stable protein layer which is resistant to rigorous washing and stringent regeneration methods. As a result non-specific adsorption is minimized, and immunoassays provided according the present invention are highly reproducible. Furthermore, the assay method is simple, not only in respect of procedure and duration, but also in respect of reagents required. Since no secondary signaling molecules such as radiolabel or enzyme and substrates are necessary, costs can also be kept very low.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagram to show a QCM.

Figure 2A is a diagram to show the self-assembled monolayers of thioctic acid on the gold electrode surface of the crystal.

- 5 Figure 2B is a diagram to show the self-assembled monolayers of cysteamine on the gold electrode surface of the crystal.

Figure 3A diagram to show the antibody immobilization procedure using an SAM of thioctic acid.

- Figure 3B is a standard curve obtained by QCM analysis of various amounts
10 of human IgE using an crystal coated with monoclonal anti-IgE via an SAM of thioctic acid.

Figure 4A diagram to show the antibody immobilization procedure using an SAM of cysteamine.

- Figure 4B is a standard curve obtained by QCM analysis of various amounts
15 of human IgE using an crystal coated with polyclonal anti-IgE via an SAM of cysteamine.

Figure 5 is a graph to show the change in frequency at various steps of the immunoassay.

- Figure 6 is a graph to show the sensitivity of the assay after various cycles of
20 regeneration.

DESCRIPTION OF THE INVENTION

Figure 1 shows a conventional QCM design with a piezoelectric crystal **20** having a gold electrode **22** deposited on each of the two sides of the wafer. These two electrodes are connected to an oscillation circuit **24** and a frequency counter **25** for generating and detecting resonance frequencies respectively. For ease of description, the combined oscillation circuit, the frequency counter and power supply will be referred to as the oscillation system. Standard methods, such as described in Shons et al. *J Biomed mater. Res.* 565-570, 1997, uses a thin disk of AT-cut quartz crystal containing two plated gold electrodes on both sides. Other metals may also be used for the electrodes, such as silver, platinum, chromium and stainless steel. Due to the piezoelectric properties and crystalline orientation of the quartz, the application of the voltage between these electrodes results in a shear deformation of the crystal. The two electrodes are used to induce an oscillating electric field perpendicular to the surface of the quartz wafer. The oscillating field produces a mechanical oscillation or standing wave in the bulk of the quartz wafer. A resonance oscillation is achieved by including the crystal in an oscillation circuit **24**, where the electric and mechanical oscillations are close to the fundamental frequency of the crystal. The oscillation circuit and frequency counter (such as HP5213A from Hewlett packard, USA) are well known in the art, and are described in detail by Bruckenstein and Shay in *Electronchim. Acta*, 30:1295, 1985. The fundamental frequency (F_0) depends on the thickness of the wafer, its chemical structure, shape and mass, and can be determined using a universal counter. In the preferred embodiment, the crystal is an AT-cut

10MHz Quartz wafer of 14mm diameter and 0.2mm thickness. The gold electrode is 51mm in diameter and 100nm thick. The deposition of the electrode is also well-known in the art, such as the use of high vacuum deposition, vapor deposition and electrochemical plating of nickel, gold, chromium, titanium and silver. It would be appreciated by one skilled in the art that the present invention may be practised using other types of piezoelectric microbalances, such as surface acoustic wave devices.

The molecules useful for producing the SAM include alkanethiols which contain functional terminal groups for immobilization purposes. Examples include carboxylic acid- and amine-terminated alkanethiol molecules. Other examples, such as hydroxyl-terminated alkanethiols are described by Mittler-Neher et al in *Biosensors and Bioelectronics* 10 (1995), 903-916. Thioctic acid and cysteamines are the specific molecules used in the examples described below.

Figures 2A and 3A show the coupling of thioctic acid **26** and cysteamine **28** respectively to the gold electrode **22**. Due to the terminal position of the thiol group on the aliphatic chain, the resultant structure is an ordered array of a self-assemble monolayer, with the carboxyl or amino reactive group exposed on the surface of the monolayer and available for cross-linking.

In order to cross-link a desired protein to the SAM, cross-linkers which can react with the appropriate functional groups is provided. Useful linkers include glutaraldehyde (GA), N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide EDC, N-hydroxysuccinimide (NHS) and partially oxidized dextrin. Hermanson et al. in *Immobilized Affinity Ligand Techniques*, Academic Press, 1992 describe other available crosslinkers and their uses such as for

the specific application of antibody immobilization. Figure 3A shows one embodiment in which EDC is used in an intermediate step for the coupling between the thioctic acid-coupled crystal **30** and an antibody **34**. EDC reacts with the carboxyl group of the thioctic acid to form an intermediate **32**,
5 which in turn reacts with the amino group of the antibody **34**, resulting in the immobilization of the antibody **36** and the release of the reduced EDC.

A preferred linker between cysteamine and a protein is glutaraldehyde (GA). Figure 4A shows another embodiment of one aspect of the present invention in which GA reacts with the amino group of immobilized
10 cysteamine **40** to form intermediate **42**. The amino group of the antibody **44** is then cross-linked to the immobilized cysteamine monolayer **46**.

An optimal amount of antibody is required for immobilization in order to give the maximum cross-linking while minimizing costs. A useful range of antibody concentration for cross-linking is 0.5-5mg/mL. A preferred range is
15 0.5-1.0mg/mL. To evaluate the progress of immobilization, the resonance frequency of dried crystal is determined after all reaction steps and the amount of bound material calculated according to Sauerbrey's equation.

After antibody immobilization, the gold surface is preferably further incubated with a blocking agent such as a non-reactive protein or peptide to
20 block any residual non-specific adsorption sites. A useful blocking agent is bovine serum albumin (BSA). The antibody-immobilized crystal is then used for the analysis of the specific reactive antigen.

For analysis, the test solution may contain known or unknown concentrations of the specific reactive antigen, which may be a patient's

serum, an unknown solution or a solution of known antigen concentrations. A method for allergy testing according to the present invention comprises the following steps :

- a) Obtaining the resonance frequency of the reactive crystal;
- 5 b) Incubating the test solution with the electrode of the reactive crystal;
- c) washing away unbound proteins; and
- d) checking the resonance frequency of the reactive crystal.

The amount of material bound to the electrode of the crystal can be determined using Sauerbrey's equation using the frequency shift determined
10 by steps (a) and (d). To determine the antigen concentration in an unknown test sample e.g. patient's serum, a standard curve is first constructed using solutions of known antigen concentrations. If the antigen is an antibody, it will be referred to as the second antibody, in order to distinguish it from the covalently immobilized antibody. For test solutions such as serum, the serum
15 may be incubated with the antibody-coated crystal without prior treatment. The incubation time can range from 30-120 minutes, but longer incubation periods may be used without affecting results. The preferred incubation time is 30-45 minutes in order to shorten the protocol time. Washing may be performed using many conventional buffers for biological samples at
20 physiological pH, for example phosphate-buffered saline, pH7.4. Low concentrations of mild detergents may also be used for washing to reduce non-specific adsorption, such as TritonX-100 and Tween 20.

The specific preferred embodiment for the apparatus and method for allergy testing using anti-IgE as the antibody and IgE as the antigen will be

described in the examples shown below. These examples illustrates the various features of the invention and the comparative results with other conventional methods. These examples, however, are meant for illustration only, and should not be taken as limitation to the present invention. It is contemplated that using the teachings described here, many variations and modifications can be made by one of ordinary skill in the art without departing from the spirit and the scope of the invention described.

EXAMPLE 1 : PREPARATION OF CRYSTALS COATED WITH ANTI-IgE VIA THIOCTIC ACID SAM

10 10Mhz AT-cut mounted crystals with gold electrodes (from International Crystal Manufacturing Co. Inc., Oklahoma, USA) were cleaned with hot Piranha solution (30% H_2O_2 : H_2SO_4 =1:3). The Piranha solution was spread on the gold surface for 5 minutes, after which the electrode surface was thoroughly rinsed with distilled water. This is followed by rinsing first with
15 95% ethanol and then with distilled water again. This procedure was repeated twice. The surface was then finally blown dry with a stream of nitrogen gas and the resonance frequency (F_0) determined.

To prepare the crystal for covalent binding of the antibody, the surface of gold electrode on the crystal was first modified by thioctic acid. This was
20 achieved by dipping the freshly cleaned crystal immediately into 0.2% solution of thiotic acid in ethanol for 24 hours at room temperature. After washing in ethanol and distilled water, the thioctic acid modified crystal can then be reacted with EDC by dipping in a solution of 2.1% EDC in ethanol for 1 hour. This is followed by rinsing with ethanol and distilled water again.
25 Then 5 μl of anti-IgE antibody was added on both sides of the crystal to cover

the entire surface of the gold electrode for 45 minutes at room temperature. The resonance frequency of the crystal was determined at every step of the preparation procedure to monitor progress.

For antibody immobilization, monoclonal anti-human IgE from Sigma
5 [catalog no. I-6150] (MW150,000) and polyclonal anti-human IgE with
peroxidase conjugate from rabbits [catalog no. A-9667] (MW 190,000) were
used. To ascertain the appropriate antibody concentration to use, an
experiment to evaluate the saturation amount of protein binding to crystal
was performed. This was done by coating diluted amounts of antibodies
10 (between 5mg/mL and 0.3mg/mL) onto different crystals until maximum
frequency shift was obtained. At each concentration, at least 5 replicates
were used to obtain the results. Separate Bio-Rad protein assays indicated
that the concentration of the undiluted monoclonal and polyclonal antibodies
had protein concentrations 5.0 and 4.5mg/mL respectively. In addition, a
15 standard inhibition test (Jones et al. Ann Allergy 222-225, 1986) was
performed to examine the amount of antibodies needed to inhibit the antigen
(IgE) at a concentration of 300 IU/mL, which is the highest concentration
needed to construct the standard curve. The inhibition experiments indicated
that concentrations of 0.1mg/mL of the monoclonal antibody (i.e. 1/50
20 dilution) and 0.45 mg/mL of the polyclonal antibody (i.e. 1/10 dilution) were
active enough to recognize at least 300 IU/mL of IgE antigen. The results
also suggested that crystals coated at the saturation point may be able to
read concentrations of IgE greater than 300IU/mL (results not shown).
Considering the two factors, 5 μ l of 1/10 and 1/5 dilutions of the monoclonal

and polyclonal antibodies in phosphate buffered saline (PBS, pH7.4) were selected for the immobilization procedure.

After allowing 5 μ l of antibody in PBS to air dry onto the electrodes, the electrodes were immediately washed three times with PBS and distilled water to remove any residual salt deposits or loosely adsorbed proteins. Table 1 below shows the frequency shift of the QCM after antibody coupling which is the quantitation of the amount of protein coupled.

Crystal No.	Frequency shift in Hz (ΔF)		
	Thioctic acid modified crystal	Monoclonal antibody coated crystal	Polyclonal antibody coated crystal
1	277	310	417
2	240	319	403
3	278	293	445
4	351	279	487
5	281	375	335
6	214	411	491
7	294	341	476
8	279	316	381
9	271	340	514
Average $\pm$ SD	276.1 $\pm$ 12.4	331.5 $\pm$ 13.7	438.8 $\pm$ 19.6
RSD (%)	4.5%	4.1%	4.5%

TABLE 1

10 IgE ASSAY PROCEDURE

To avoid non-specific adsorption, the antibody-coupled crystals were incubated with 5% bovine serum albumin (BSA) before exposure to antigen. For quantitation, a standard curve was obtained. To build the standard curve,

a series of standard IgE concentrations were obtained by dilutions from the Total IgE II FAST Test Kit from Biowhittaker, Inc. The varying concentrations of IgE were incubate with monoclonal antibody coated crystals for 45 minutes. The frequency changes of the coated crystal before and after incubating with antigen corresponding to the concentration of the IgE are listed in Table 2. To investigate the reproducibility of the method, at least 5 duplicate experiments were performed on 5 separate QCM crystals for each IgE concentration. Results in Table 2 show that the relative standard deviation of this method was less than 8%. The corresponding standard curve is shown in Figure 3B.

Concentration of Standard IgE(IU/mL)	ΔF (Hz) mean value	Standard deviation	Relative Standard Deviation (%)
0	0	0	0
50	65.0	± 2.8	4.3
100	130.1	± 7.5	5.8
150	177.0	± 9.4	5.3
200	249.0	± 18.3	7.3
300	381.3	± 27.5	7.2

TABLE 2

For the analysis of actual test samples, 5 μ l human serum was applied on both sides of the coated crystal for 45 minutes. After thoroughly rinsing with PBS and distilled water, the crystal was air dried and resonance frequencies were obtained. The concentration of total IgE in the human serum test samples can then be calculated according to the frequency change with reference to the standard curve. Results are shown in Table 3. These results

are in good agreement with values obtained using the FAST technique, as shown in column 3 of Table 3.

Serum	Concentration of IgE by QCM sensor (IU/mL)	Concentration of IgE by current FAST method (IU/mL)	Relative Standard Deviation (%)
1	113.3	116.1	1.3
2	219.2	211.0	1.9
3	104.0	102.5	0.7
4	211.3	206.1	1.3
5	240.0	244.3	0.8
6	227.0	204.5	5.3
7	323.3	299.1	3.9
8	115.5	102.5	0.5
9	231.5	206.1	5.5

TABLE 3

5 EXAMPLE 2 : ANTI-IgE IMMOBILIZATION ON CRYSTAL VIA CYSTEAMINE SAM

As shown in Figure 2B, the thiol group strongly reacts with the gold surface to form a covalent bond. As a result, the amino-terminal groups of the cysteamine formed a highly ordered monolayer. In preparation for
 10 immobilization, 10 Mhz AT-cut crystals with gold electrodes were cleaned as described in example 1. After cleaning, the crystals were modified by soaking in 0.1% cysteamine solution in distilled water overnight (approx. 20 hrs) at room temperature to form a stable SAM on the surface of the gold electrode. Then the crystal was then washed in distilled water before being
 15 soaked in 2.5% GA in PBS, pH7.4 for 1 hour. The GA was then washed away

in PBS followed by washing in distilled water. The anti-IgE used for coating are monoclonal or polyclonal antibodies and the working concentrations are the same as described in Example 1. The antibodies was incubated with the GA-treated electrodes for 45-60 minutes before being washed away in PBS and distilled water. The change in frequency is measured for each step and shown in Table 4. The standard curve obtained is shown in Figure 4B.

Step	ΔF (Hz)	Relative Standard Deviation (%)	Binding Capacity (nmol/side)
Cysteamine	465 $\pm$ 24.5	5.3	2.143
GA	170.7 $\pm$ 14.5	8.5	0.888
Polyclonal Antibody	477.2 $\pm$ 27.6	5.8	0.00128
BSA blocking	34.2 $\pm$ 5.0	14.5	0.000261
Standard IgE 0 IU/mL	0	0	
Standard IgE 50 IU/mL	65.3 $\pm$ 3.5	5.4	
Standard IgE 100 IU/mL	120.8 $\pm$ 7.7	6.3	
Standard IgE 150 IU/mL	185.7 $\pm$ 10.7	5.8	
Standard IgE 200 IU/mL	250.1 $\pm$ 12.0	4.8	
Standard IgE 300 IU/mL	349.2 $\pm$ 11.5	3.3	

TABLE 4

After blocking with BSA, the coated crystal was exposed to human serum for 45 minutes. The frequency changes before and after incubating with human serum were measured. The concentration of total IgE in human serum can

be calculated with reference to the standard curve as shown in Table 5 below.

Serum	Concentration of total IgE by Pz biosensor (IU/mL)	Concentration of total IgE by FAST method (IU/mL)	Sr %
1	241.0	204.5	8.1
2	123.5	116.1	3.1
3	52.7	45.6	7.1
4	210.5	206.2	1.0

TABLE 5

EXAMPLE 3 : COMPARISON OF NON-SPECIFIC ADSORPTION BETWEEN
CONVENTIONAL METHODS of IMMOBILIZATION AND THE PRESENT
SAM METHOD

Besides reproducibility, the use of SAM to immobilize antibody offers other advantages such as minimal non-specific adsorption and ease of regeneration of the antibody coated crystal. To demonstrate the advantages of SAM with other methods, several anti-IgE immobilized crystals were prepared using the conventional γ -aminopropyltriethoxysilane (γ -APTES) or polyethylenimine (PEI), both of which form random polymer meshes adsorbed on the surface of the electrode rather than an orderly, covalently coupled SAM. In these methods, the cleaned crystals were first modified by dipping in 5% γ -APTES acetone solution or 2% PEI ethanol solution for 1 hour. Anti-IgE antibody was then cross-linked using 2.5% GA in PBS as described in Example 2. This was followed by blocking of non-specific sites with 5% BSA as described in Example 1. The resonance frequency of each

crystal was obtained before and after the BSA blocking step as described in Example 2.

In another experiment, 5 μ L of 1mg/mL protein A, which has a well-known affinity for certain classes of immunoglobulins was adsorbed onto the surface of the gold electrode of the crystal. The Protein A was dried in a 37°C oven for 40 minutes before anti-IgE was added for binding with the protein A. After antibody coating, 5% BSA was used to block non-specific residues. The changes in frequency caused by BSA adsorption are in Table 6, which also contains results of BSA adsorption using the various immobilization methods. These results show that prior art methods of immobilization onto the AT-crystal give approximately 3-5 times more non-specific binding sites than SAM methods. The data shown in Figure 5 are mean values from at least 5 different crystals.

Method	Frequency shift caused by BSA (Hz)	Binding capacities (pmol/side)
PEI	130	0.99
γ -APTES	164	1.25
Protein A	208	1.60
Thioctic acid	48	0.37
Cysteamine	34	0.26

TABLE 6

Tests were also performed to determine the effect of multiple wash cycles on the IgE assay using the conventional Adsorbed Protein A method and the SAM coupling method of the present invention. For the Protein A method, the 5 μ L of 1mg/mL Protein A was adsorbed onto the crystals and oven dried at

37°C for 40 minutes. The crystals were then washed thoroughly with PBS, pH7.4 before 5µL of polyclonal anti-IgE antibody (dilution 1/5) was applied on both sides. After 45 minutes incubation, the crystals were washed three times with PBS before 5%BSA was applied to block non-specific adsorption.

5 Finally, the anti-IgE coated crystals were exposed to human serum or IgE standards. For the thioctic acid- immobilized anti-IgE crystals, the immobilization protocol was the same as described in Example 1. The frequency change was monitored for each step of the two methods, and results are shown in Figure 5. For the thioctic acid method, no loss of

10 antibody was detectable after 3 washes, whereas the Protein A method showed 10-15% loss of antibody after 3 washes.

EXAMPLE 4 : COMPARISON OF ANALYSIS RESULTS BETWEEN THE SAM-QCM METHOD, FAST METHOD AND THE CAP METHOD

15 Twenty six human serum test samples were analyzed for total IgE using the SAM-QCM method and the conventional FAST (BioWhittiker) and CAP (Pharmacia) Tests. The FAST and CAP tests were performed according to manufacturer's instructions. For the SAM-QCM method, the human test serum were incubated with the crystal coated with anti-IgE via thioctic acid

20 SAM for 45 minutes. All tests were performed according to the method described in Examples 1. The frequency changes before and after incubating with the test serum were measured. The concentration of total IgE in human serum can be calculated with reference to the standard curve shown in Figure 3B. Results are listed in Table 7.

SERUM NO.	IgE Concentration (IU/mL)		
	QCM	FAST	CAP
1	278	299	345
2	43	49	58
3	230	271	256
4	235	240	215
5	166	196	224
6	185	149	175
7	53	24	16
8	107	123	158
9	206	202	194
10	247	218	220
11	146	148	207
12	130	158	113
13	105	89	95
14	126	136	125
15	302	281	339
16	127	176	238
17	240	225	257
18	122	116	not tested
19	225	205	not tested
20	238	244	not tested
21	104	102	not tested
22	217	205	not tested
23	85	50	not tested
24	224	206	not tested
25	72	103	not tested
26	123	98	not tested

TABLE 7

After analysis has been performed, the crystal may be regenerated by washing with denaturing agents to disrupt the antibody-antigen complex. Each regeneration cycle involves incubating the electrode of the crystal in 8M urea for 30 minutes to remove the antigen, and then rinsing three times with 0.2M glycine-HCl (pH2.2). Finally, the crystals were washed with distilled water and air dried. Total IgE testing was then repeated on the regenerated crystal. At least 5 replicate crystal was performed for each method. Results as shown in Figure 6 indicate that cross-linking via Thioctic

acid SAM (curve 50) provides superior activity after multiple regeneration cycles compared to IgE cross-linking to γ -APTES (curve 52) and adsorbed Protein A (curve 54). Anti-IgE immobilized according to the present invention retained more than 90% of its activity after 5 regeneration cycles, and still
5 retained 65 % of its activity after 6 cycles. For the APTES method and the protein A method, activity of the antibody was reduced to approximately 60% and 40% respectively of the original after 3 regeneration cycles.

CLAIMS

- 1 1. A system for detecting and quantifying an antigen comprising :
 - 2 a) an immunoreactive piezoelectric crystal with electrodes on
 - 3 opposing sides, said electrodes coated with a self-assembled
 - 4 monolayer; said self assembled monolayer covalently linking
 - 5 an antibody specific for said antigen;
 - 6 b) an oscillation system, electrically coupled to said electrodes for
 - 7 generating and detecting resonance frequencies.
- 1 2. A system for detecting and quantifying antigen according to claim 1
- 2 wherein the piezoelectric crystal is a quartz crystal.
- 1 3. A system for detecting and quantifying antigen according to claim 1
- 2 wherein the piezoelectric crystal is an AT-cut quartz crystal with gold
- 3 electrodes.
- 1 4. A system for detecting and quantifying antigen according to claim 1
- 2 wherein the antigen is a second antibody.
- 1 5. A system for detecting and quantifying antigen according to claim 1
- 2 wherein the antigen is IgE.
- 1 6. A system for detecting and quantifying antigen according to claim 1
- 2 wherein the antigen is human IgE.
- 1 7. A system for detecting and quantifying antigen according to claim 1
- 2 wherein the antibody is polyclonal or monoclonal anti-IgE.



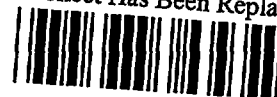
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- 1 8. A system for detecting and quantifying antigen according to claim 1
2 wherein the self-assembled monolayer comprises alkanethiols with a
3 functionally active terminal group; said functionally active terminal
4 group selected from the the group consisting of carboxylic acid,
5 amine, adehyde and alcohol.
- 1 9. A system for detecting and quantifying antigen according to claim 1
2 wherein the self assembled monolayer is thioctic acid.
- 1 10. A system for detecting and quantifying antigen according to claim 1
2 wherein the self assembled monolayer is cysteamine.
- 1 11. A system for detecting and quantifying antigen according to claim 10
2 wherein glutaraldehyde is the cross-linker for the covalent linking of
3 the cysteamine to the antibody.
- 1 12. A system for detecting and quantifying antigen according to any one of
2 the preceding claims wherein non-specific binding sites are blocked
3 by a non-reactive molecule.
- 1 13. A system for detecting and quantifying antigen according to claim 12
2 wherein said non-reactive molecule is bovine serum albumin.
- 1 14. A method for detecting and quantifying an antigen using a
2 piezoelectric crystal microbalance; said piezoelectric crystal
3 microbalance having a piezoelectric crystal with electrodes on
4 opposing sides, said electrodes electrically coupled to an oscillation
5 system and coated with a self-assembled monolayer; said monolayer



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6 covalently linking an antibody specific for said antigen, said method
7 comprising the steps of :

8 a) measuring the initial resonance frequency of said crystal;

9 b) incubating said crystal with a test solution containing said
10 antigen for a sufficient amount of time for the antigen to bind to
11 the antibody;

12 c) washing away the unbound test solution;

13 d) measuring the final resonance frequency of the crystal.

1 15. A method for detecting and quantifying an antigen according to claim
2 14 wherein an extra step of drying said crystal is performed after step
3 (c).

1 16. A method for detecting and quantifying an antigen according to claim
2 14 or 15 wherein the difference between the initial and final
3 resonance frequencies is compared to a standard curve.

1 17. A method for detecting and quantifying an antigen according to claim
2 14 or 15 wherein non-specific binding sites on the electrodes and the
3 monolayer are blocked by a non-reactive molecule.

1 18. A method for detecting and quantifying an antigen according to claim
2 17 wherein the non-reactive molecule is bovine serum albumin.

1 19. A method for detecting and quantifying an antigen according to claim
2 14 or 15 wherein the antigen is a second antibody.



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- 1 20. A method for detecting and quantifying an antigen according to claim
2 14 or 15 wherein the antibody is anti-IgE.
- 1 21. A method for detecting and quantifying an antigen according to claim
2 14 or 15 wherein the antigen is human IgE.
- 1 22. A method for detecting and quantifying an antigen according to claim
2 10 wherein the test solution is human serum.



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