(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 March 2006 (30.03.2006)

PCT

(10) International Publication Number
WO 2006/033130 A2

(51) International Patent Classification:
G01N 33/53 (2006.01) *H03H 9/19* (2006.01)
G01N 33/80 (2006.01)

(21) International Application Number:
PCT/IT2005/000539

(22) International Filing Date:
21 September 2005 (21.09.2005)

(25) Filing Language: Italian

(26) Publication Language: English

(30) Priority Data:
VR2004A000149
22 September 2004 (22.09.2004) IT

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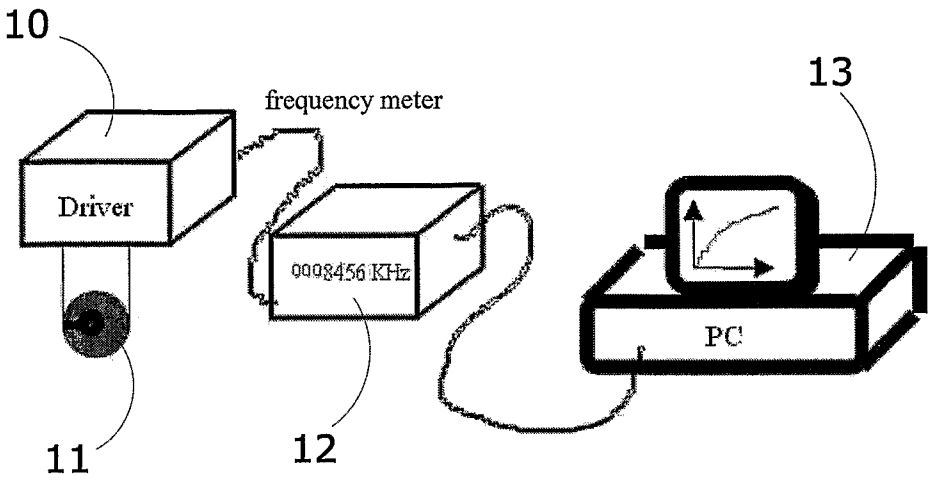
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: RAPID MONITORING SYSTEM FOR BLOOD GROUPS AND IMMUNOHEMATOLOGICAL REACTION DETECTION



(57) Abstract: A system for monitoring blood groups and for detecting immunohematological reactions uses a detection device consisting of a quartz crystal microbalance (QCM), a device able to measure very small variations in mass (down to fractions of a nanogram).

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"RAPID MONITORING SYSTEM FOR BLOOD GROUPS AND IMMUNOHEMATOLOGICAL REACTION DETECTION"

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TECHNICAL FIELD

This invention concerns a rapid monitoring system for blood groups and more in general for detecting immunohematological reactions by means of a detection device named quartz crystal microbalance (QCM).

More specifically, the QCM is used according to this invention for rapid monitoring of blood groups by performing direct and indirect tests.

For direct grouping, IgM type antibodies are selectively immobilized on the surface of the electrodes of an appropriately functionalized QCM transducer, starting from appropriate antiserum, so that red blood cells with the corresponding antigens on their surface can be specifically recognised.

For indirect grouping, the IgM antibodies present in the plasma are captured by the surface of the electrode which is functionalized to give it high specificity for IgM antibodies; the antibody status is then tested by exposure to blood tests.

Optimization of the accuracy of the antibody reaction by means of thermoregulation is a fundamentally important aspect for the success of the test. The method described below makes it possible to carry out tests very quickly (a few seconds for each test), cheaply and automatically.

This invention can be used in the field of diagnostic instruments and in particular in the sector of immunohematological reaction detectors.

BACKGROUND ART

It is known that immunodiagnostic methods for blood grouping are currently based on three different functioning principles: hemoagglutination, antibody-antigen bond, genetic analysis.

Hemoagglutination is the oldest method, dating back to the time of the first blood tests, when the donor's and the recipient's blood were simply placed

in contact on a glass slide to check for the presence or absence of the agglutination reaction that characterizes incompatibility between the two fluids: if the antibodies in the recipient's serum do not recognize the donor's red blood cells because they belong to a different blood phenotype, they attack them, causing a kind of coagulation (agglutination). Modern tests use monoclonal antibodies specifically created in the laboratory for a selective action against the various blood groups (direct method) or test red blood cells of a known group placed in contact with the patient's serum (indirect method), but the concept does not change.

The tests can be performed in liquid phase (the traditional manual method) or gel phase. The top of the range in this last category is the gel-test system patented by the Swiss company DiaMed. This method involves the use of special cards on which, on top of a layer of gel or glass microspheres, a dose of specific antiserum (anti-A, anti-B, anti-AB, and anti-D) has been applied in the factory.

The technician performing the test places the red blood cells obtained by centrifuging the patient's blood in each of the wells. The card is then centrifuged. If agglutination occurs, the size of the agglutinins does not allow them to pass through the gel. Non-agglutinated red blood cells, on the other hand, pass through the gel unaffected and are deposited at the bottom of the microtube.

By examining the card with the naked eye or with an automatic system, it is therefore easy to distinguish whether or not agglutination has occurred.

A similar card, but filled only with gel (no antiserum), is used for the indirect method: the technician adds test red blood cells of a known group and patient serum on top of the gel before centrifugation. The results are read according to the same logic as above.

The techniques based on hemoagglutination have the main disadvantage of requiring a sequence of controlled-temperature incubations, agitation, resuspension and centrifugation, reducing productivity and also requiring a certain number of devices in order to carry out the test. With respect to other techniques, there is also a certain lack of sensitivity.

A further means of blood group recognition is represented by the tests that exploit the antigen-antibody reaction. These tests used "marked" (i.e. bound) antibodies or antigens with an easily recognizable substance, using this substance to detect the amount of marked antibody or antigen that had bound with the surface antigens present on the red blood cells or antibodies present in the serum. All these methods are performed inside the microwell plates, the bottom of which is covered with unmarked antibodies specific for each test.

There are three main families of immunohematological methods: immunocompetitive assays, immunometric assays and immunoabsorbent assays.

The class of immunocompetitive assays (EIA/RIA), the first in the immunohematology family from a historical point of view, foresees the use of a microplate divided into a number of wells, at the bottom of which antibodies specific for the antigen to be detected are chemically bound. The sample to be analyzed and a very precise amount of the same antigen, but "marked" by replacing one of its component atoms with a radioactive isotope – usually tritium, iodine 125 or carbon 14 (RIA, Radioactive Immuno Assay) or by chemically binding it to an enzyme, typically alkaline phosphatase (EIA, Enzymatic Immuno Assay), are placed inside the well.

The greater the amount of "natural" antigen in the sample, the lesser the amount of marked antigen that will be bound by the antibodies fixed to the bottom of the well. After washing to eliminate anything not bound to these antibodies, the binding percentage is assessed in the case of RIA by a radiation counter. In the other cases, again after washing, a substance that reacts with the marker enzyme is placed in the well, causing a change in colour (usually tending towards yellow) or creating a fluorescent or phosphorescent substance.

The colour change can be assessed visually or by photocells, while fluorescence or phosphorescence are evaluated by automatic devices equipped with light amplifiers, which give a greater precision in assessing the results.

The RIAs were the first immunohematological tests, dating back to the 70s. However, the lability of the marked molecules and the strict regulation of

the radioactive isotopes decreed the obsolescence of these tests some time ago in favour of EIAs which are still widely used.

According to the immunometric assay method (Sandwich), the sample containing the antigen to be tested is placed in the well, where it is bound by the antibodies bound to the bottom of the well. An antibody marked with an enzyme (for example alkaline phosphatase) is then added. This antibody binds to the immobilized antigen. Washing is carried out to remove any unbound substance, and a reagent is added which reacts with the enzyme and, in this case, causes a change in colour, phosphorescence or fluorescence, this time directly (rather than inversely) proportional to the amount of antigen present in the fluid being tested.

This change in colour, phosphorescence or fluorescence is assessed in the same way as above. This type of test has very rapid kinetics and a greater sensitivity compared to the EIAs.

The immunoabsorbent assay method, named ELISA (Enzyme-Linked Immunosorbent Assay), is nowadays widely used not only for blood grouping but also, and above all, because of the high specificity and very high sensitivity, for determining the presence of antibodies that respond to pathogens (e.g. HIV, HCV). Unlike the previous tests, the substance bound to the bottom of the microplate does not consist of antibodies but is a solid phase consisting of an inactivated virus or of synthesis peptide fragments the same as those present on the surface of the antigen whose presence is being assessed – in the case of blood grouping, the surface antigens of the various red blood cell phenotypes.

The patient's biological fluid is placed in this well: if the patient has been exposed to the virus, the antibodies against the virus will be present in the fluid and will bind to the solid phase. In the case of blood grouping, the patient's serum will instead contain antibodies against the other red blood cell phenotypes, which will attack the surface antigens present at the bottom of the well. The plate is then washed to remove any non-adsorbed substance and an anti IgG/IgM antibody, i.e. a non-human marked antibody specifically targeted against human immunoglobulins, is added to the well, binding to the patient's

antibodies bound in turn to the solid phase. Further washing eliminates the unbound substances: the well now contains only the solid phase, to which the patient's antibodies are bound and which are covered with the marked anti-IgG/IgM antibodies. An appropriate substrate is then added, reacting with the marker enzyme (e.g. 5-aminosalicylic acid or O-phenyldiamine in the case of peroxidase; 4-nitrophenylphosphate in the case of alkaline phosphatase), allowing the results to be read.

The ELISA tests are characterized by a very high sensitivity, since a large quantity of marked antibodies binds to a small amount of patient antibody. In blood grouping, these tests are therefore mainly used to detect irregular antibodies, as well as for screening against infections that can be transmitted via the blood (for example, the various forms of hepatitis).

There are numerous devices on the market that can perform analyses with all the above-mentioned methods - EIA, Sandwich and ELISA – for example the various Immucor Galileo, Prism and many other devices.

Among the main disadvantages of these techniques, however, is the need to use reagents (marked antibodies/antigens) that are very expensive and the need for great precision in dosing the sample and the reagents. All the techniques also require at least one washing phase, if not two separate washing phases as in the case of the ELISA technique, to eliminate anything which is not part of the antigen-antibody bond. This causes microfluid or manipulation problems, as well as making the test less portable for use in the field.

The methods based on genetic analysis are very recent techniques, based on the analysis of the points of the human genome relative to the encoding of the superficial peptides of the red blood cells – for example the point of chromosome 9 which identifies the ABO blood group, but also the points which encode other surface peptides, such as the Rh factor, or the rare phenotypes. This is a complex process, involving DNA amplification by means of processes such as polymerase chain reaction (PCR) and numerous other processes.

It should be noted that the use of genetic tests presents notable

advantages, not least a heuristic approach to diagnostics, i.e. a more overall vision compared to a single test, and the availability of DNA suitable for analysis in tissues other than the blood, such as for example epithelial tissues.

Genetic diagnostics is still in the early stages of development, the methods being mainly experimental at present, applied more in the sector of forensic medicine than in the transfusion field.

This sector is, however, rapidly expanding and many companies are investing considerable resources. Genetic tests are already raising ethical questions linked to the possible incorrect use of the information gathered. Even if any legal impediments related to these questions are ignored, experts nevertheless foresee that it will be at least ten years before reliable and affordable genetic tests are available.

DESCRIPTION OF THE INVENTION

This invention proposes to provide a system for monitoring immunohematological reactions that is able to eliminate or at least reduce the drawbacks described above.

The invention also proposes to provide a system for monitoring immunohematological reactions based on the use of quartz crystal microbalances (QCM), which are sensitive transducers that offer the possibility of studying immunological reactions without the need to mark molecules. This technique can be used both to measure reaction kinetics and the concentration of various (bio)analytes in solution.

This is achieved by means of a system for monitoring immunohematological reactions whose features are described in the main claim.

The dependent claims of the solution in question describe advantageous embodiments of the invention.

The main advantages of this solution concern the possibility of implementing techniques for rapid blood group determination, with direct and indirect analysis (red blood cells), and hepatitis screening, by use of the QCM.

The quartz crystal microbalance (QCM) is a device that can measure very small variations in mass (down to fractions of a nanogram). The extreme sensitivity to the mass is due to the use of small piezoelectric crystals oscillated at resonance frequency. This resonance frequency greatly depends on the mass present on the surface of the quartz and, by monitoring the trend of the resonance frequency, this makes it possible to follow the adsorption of more complex molecules or structures, for example cells, on the surface of the quartz. The equations describing the relationship between resonance frequency and adsorbed mass for an AT-cut crystal establish a direct proportion between frequency variation Δf and mass variation Δm:

$$\Delta f = - C \Delta m$$

The constant C represents the calibration coefficient and can be determined by placing known masses on the surface of the crystal.

A driver connected to a quartz crystal oscillator guides the transducer to oscillate at the correct resonance frequency. The output signal of the driver is sent to a frequency meter which measures the frequency. The entire system can be guided by a computer that can records the temporal variations of the resonance frequency.

The microbalance can function even when one of the two surfaces covered by the electrodes is immersed in liquid. In this last case the previous relationship between adsorbed mass and frequency variation is no longer valid and must be replaced by more complex equations that take into account other physical parameters of the liquid layer adsorbed on the surface (density, share viscosity). After removing the microbalance from the liquid it is however always possible to measure the resonance frequency and recover information on the adsorbed mass.

By functionalizing one of the two faces of the crystal with certain molecules it is possible to follow *in-situ* or to determine *ex-situ* the presence of specific recognition events between the molecules immobilized on the surface of the quartz and other molecules present in solution.

The quartz microbalance has been used to study various protein-protein

interactions, including in particular the antibody-antigen interaction.

In general the use of the QCM in these cases is designed to exploit the sensoristic properties of the device. The antibody being tested is immobilized with appropriate techniques on one of the two electrodes of the microbalance in such a way as to preserve its biological functionality.

The presence of the corresponding antigen in solution is determined by monitoring the variations of resonance frequency of the crystal following any specific bond between the antibody and the antigen and consequent increase in actual mass on the electrode.

The microbalance technique has also been applied in the study of more complex systems such as viruses, bacteriophages and cells. In the first two cases the approach foresees the immobilization of specific antibodies on the microbalance electrode and detection of the specific bond between the virus or bacteriophage and the antibody.

When studying cells, on the other hand, interest is focussed on the viscoelastic type modifications that a cell population adhered to the balance electrode undergoes following pharmacological treatment. This is based on the ability of the microbalance to detect not only variations in adhered mass, but also variations in the viscoelastic properties of the mass.

DESCRIPTION OF THE DRAWINGS

Other features and advantages of the invention will become evident on reading the following description of one embodiment of the invention, given as a non-binding example, with the help of the accompanying drawings in which:

- figure 1 represents the setup diagram of the quartz microbalance;
- figure 2 represents a schematic view of the immobilization of the antibodies on the balance electrode;
- figure 3 shows an atomic force microscope image of IgM molecules immobilized on a surface exhibiting SH groups;
- figure 4 represents optical microscope analysis of red blood cell immobilization;

- figure 5 shows red blood cell immobilization on a functionalized glass slide;
- figure 6 represents QCM frequency variation monitoring;
- figure 7 is the first table with the series of tests on the QCM for direct grouping;
- figure 8 is the second table with the series of tests on the QCM for indirect grouping;
- figure 9 shows the wiring diagram of a possible form of driver for the QCM.

DESCRIPTION OF ONE EMBODIMENT OF THE INVENTION

The quartz microbalance (QCM) is a device that can measure very small variations in mass (down to fractions of a nanogram).

The extreme sensitivity to mass is due to the use of small piezoelectric crystals oscillated at their resonance frequency.

This resonance frequency greatly depends on the mass present on the surface of the quartz and, by monitoring the trend of the resonance frequency, this makes it possible to follow the adsorption of more complex molecules or structures, for example cells, on the surface of the quartz. The equations describing the relationship between resonance frequency and adsorbed mass for an AT-cut crystal establish a direct proportion between frequency variation Δf and mass variation Δm:

$$\Delta f = - C \Delta m$$

The constant C represents the calibration coefficient and can be determined by placing known masses on the surface of the crystal.

Figure 1 shows a diagram of the experimental setup used.

A driver 10 connected to a quartz crystal oscillator 11 guides the transducer to oscillate at the correct resonance frequency.

The output signal of the driver 10 is sent to a frequency meter 12 which measures the frequency. The entire system can be guided by a computer 13 that can record the temporal variations of the resonance frequency.

The microbalance can function even when one of the two surfaces covered by the electrodes is immersed in liquid. In this last case the previous relationship between adsorbed mass and frequency variation is no longer valid and must be replaced by more complex equations that take into account other physical parameters of the liquid layer adsorbed on the surface (density, share viscosity). After removing the microbalance from the liquid it is however always possible to measure the resonance frequency and recover information on the adsorbed mass.

By functionalizing one of the two faces of the crystal with certain molecules it is possible to follow *in-situ* or to determine *ex-situ* the presence of specific recognition events between the molecules immobilized on the surface of the quartz and other molecules present in solution.

The quartz microbalance has been used to study various protein-protein interactions, including in particular the antibody-antigen interaction.

In general the use of the QCM in these cases is designed to exploit the sensoristic properties of the device.

Figure 9 shows the wiring diagram of the possible form of a driver for the QCM.

The antibody being tested is immobilized with appropriate techniques on one of the two electrodes of the microbalance in such a way as to preserve its biological functionality.

The presence of the corresponding antigen in solution is determined by monitoring the variations of resonance frequency of the crystal following any specific bond between the antibody and the antigen and consequent increase in actual mass on the electrode.

The microbalance technique is also applied in the study of more complex systems such as viruses, bacteriophages and cells. In the first two cases the approach foresees the immobilization of specific antibodies on the microbalance electrode and detection of the specific bond between the virus or bacteriophage and the antibody.

When studying cells, on the other hand, interest is focussed on the

viscoelastic type modifications that a cell population adhered to the balance electrode undergoes following pharmacological treatment. This is based on the ability of the microbalance to detect not only variations in adhered mass, but also variations in the viscoelastic properties of the mass.

The quartz microbalance has also been used to determine agglutination phenomena in solution between spheres of latex covered with antibodies induced by the presence of specific proteins. These agglutination phenomena cause a notable variation in the viscosity of the liquid in which the balance is immersed and this variation in viscosity is reflected in a variation in the resonance frequency.

The antibodies of the immunohematological system, in particular the IgMs, present in appropriate animal antiserum, are immobilized on the surface of an electrode of a quartz microbalance (resonance frequency around 10 MHz) to determine the specific recognition of red blood cells provided with complementary antigens on their cellular membrane.

The description reported below considers the AB0 system, but the method and the technique described can also be applied to other systems.

Immobilization of the IgM type antibodies on the surface of the QCM transducers is achieved by first forming a self-assembled layer of molecules able to selectively bind the IgMs present in sheep antisera.

In the case in question, the self-assembled layer consists of molecules of 1-4 benzenedimethanetiol, which bind to the silver of the electrode present on the surface of the QCM transducer by using one of their two thiols and at the same time exposing the other thiol to form a covalent bond capable of immobilizing the antibody.

The bond with the antibody occurs by means of the thiol-disulfide exchange reaction. The disulfide bridges which the IgM are rich in are reduced close to the thiolated surfaces and the free thiols in turn form disulfide bridges with the thiols exposed by the self-assembled layer (figure 2).

The actual immobilization of the antibodies on a surface exhibiting SH groups was verified with atomic force microscopy (AFM), which clearly shows

the immobilization and the high specificity of the process, figure 3.

To check that the immobilized antibodies maintain their biological functionality, an optical microscopy study was performed on functionalized glass slides to expose the SH type functional groups. The results are shown in figure 3.

Two slides were functionalized with silanes (3-MPTS, 3-Mecaptopropyltrimethoxisilane) able to covalently bind on one side to the slide and to expose SH groups. It is important to point out that the chemistry of the first functionalization layer on the surface of the slide should be considered similar to the chemistry performed on the electrode of the quartz balance.

Even if the bond between the molecules and the solid surface is different, the exposed functional group is the same. The two slides were then exposed to the serum containing anti A type IgMs.

One of the two slides was then incubated with group A whole blood (figure 4a) while the remaining slide was incubated with group B whole blood (figure 4b). Figures 4a and 4b show slides incubated with blood before washing.

Figures 4c and 4d are the images of the slides in figures 4a and 4b respectively, after washing in saline solution. It is clear that only in the case of blood group A has a specific bond occurred between the antibodies present on the surface and the antigens present on the surface of the red blood cells (figure 4). The optical microscopy analysis confirms that the immobilized antibodies are biologically active, maintaining specificity for the corresponding antigens.

For monitoring of the specific recognition with the quartz microbalance, each functionalization process is checked by measuring the resonance frequency value of the crystal ex-situ and comparing it with the corresponding value of the previous phase.

The first value acquired corresponds to the resonance frequency value of the quartz without any treatment. The quartz, equipped with silver electrodes, is functionalized by exposure to a 1mg/ml solution of 1-4 benzenedimethanetiol in toluene for 2 minutes followed by washing with abundant toluene to remove the molecules that have not bound to the electrode. The resonance frequency of the

functionalized quartz is then measured. A reduction in frequency confirms functionalization of the electrode. The quartz is then exposed to the serum containing the IgM antibodies for 5 minutes and rinsed with saline solution. The resonance frequency of the quartz is measured again. A further reduction in frequency confirms immobilization of the antibodies. In the final stage, the quartz functionalized with the antibodies is exposed for 5 minutes to the whole blood undergoing direct grouping, appropriately diluted in saline solution up to a typical concentration of 3 10^4 rbc/μl.

The temperature at which the system should be maintained during incubation is an absolutely critical parameter for optimization of the reaction accuracy.

The surface of the quartz is then washed, using an appropriate washing cell which avoids direct flow of the solution on the surface and prevents problems of surface voltage connected with the repeated crossing of the surface of the solution by the electrode.

Repeated checks have shown that the temperature interval of the IgM-antigen reaction is 4° - 22°C with an optimum value of 10°±3°C. This temperature must also be maintained during the subsequent washing stage of the non-specific adsorption compounds.

The tests carried out used blood grouping performed with traditional techniques on the same sample as a reference. The reading of the final resonance frequency of the quartz makes it possible to establish whether specific recognition has occurred between the antibodies immobilized on the surface and the membrane antigens of the red blood cells.

A significant reduction in resonance frequency (more than a thousand Hz) is considered significant evidence that the blood group of the blood to which the crystal has been exposed specifically recognizes the antibodies on the quartz. In the case of anti-A antibodies, the specific recognition will take place for group A red blood cells.

In the case of anti-A antibodies and group B blood, the expected variation in frequency is less than a thousand Hz.

Figure 6 shows two examples of resonance frequency variation, starting from the immobilization phase of the antibodies, in both cases anti-A, and known blood group A (fig. 6a) and B and then A (fig. 6b). In both cases there is an evident decrease in resonance frequency following immobilization of the antibodies, while the decrease in resonance frequency following exposure to blood is present only in the case of group A blood, for which there is specific recognition of the immobilized antibodies.

In figure 6b, after being exposed to group B blood, the quartz is exposed to group A blood, for which specific recognition is expected. In fact, following exposure to group A blood, a significant decrease in resonance frequency can be measured once again.

The table in fig. 7 shows an example of results obtained with various combinations immobilized antibodies and different blood groups. The variation in frequency in the final stage is indicated with the symbol << when significant (above the threshold of 1000 Hz) and with the symbole ≅ when the variation is below the threshold of 1000 Hz.

Up to this point, the method described allows direct blood group determination, but a blood group test can only be considered valid if indirect tests are also carried out.

Indirect tests mean determination of the presence, in the plasma, of the natural antibodies relative to the particular blood group. For indirect determination of the blood group, a procedure similar to the case of direct grouping was used (in particular it is fundamentally important that the IgM-antigen reaction and the subsequent washing for removal of the aspecific adsorption compounds take place at a temperature in the range of 4-22°C, with optimum performance at 10°±3°C), the difference being that the blood group of the red blood cells used is known (test erythrocytes), the aim being to determine the type of IgM antibodies present in the plasma, which is separated from the whole blood by centrifugation.

The quartz is functionalized in the same way as the previous case, using

1-4 benzenedimethanetiol. The functionalized quartz is exposed to the plasma for 15 minutes to capture and immobilize the IgM antibodies present in the plasma. In this case too, measurement of the resonance frequency of the quartz after each functionalization phase makes it possible to check that immobilization has in fact taken place.

After exposure to the plasma, the quartz is exposed to the test red blood cells. In the case of plasma from blood whose direct blood group has been identified as type A, the IgM antibodies which should have been immobilized by the functionalized quartz surface are the anti-B type, so that the test red blood cells that should be immobilized are group B.

The table in figure 8 shows a representative set of results obtained for indirect grouping. The variation in the final resonance frequency is shown in the table according to the same criteria used for the direct grouping table. As can be seen, capture of the test red blood cells of a certain group takes place only if the quartz was exposed to the plasma of blood containing complementary IgM antibodies.

With a similar approach and data, the functioning of the test was also checked for groups AB and 0 and for determining the Rh factor.

The use of a multiparametric QCM system integrated on a single crystal that can accommodate several sensors working in parallel or with multiplexer logic is envisaged.

These sensors are produced from a single AT-cut quartz crystal and are defined by means of lithographic techniques.

According to this system, each sensor can be addressed independently of the others and can be made specific for a certain antigen/antibody/antigenic determinant by means of selective functionalization, also assisted by microfluidics applied on the chip itself or on disposable polymer supports.

This system is therefore able to perform a complete series of analyses (for example direct grouping + hepatitis markers) by means of a single exposure to the biological fluid being tested, speeding up the time necessary for the tests and making the approach substantially easier and more automated.

The invention is described above with reference to a preferred embodiment. It is nevertheless clear that the invention is susceptible to numerous variations that lie within the framework of technical equivalents.

CLAIMS

1) A system for monitoring blood groups and for detecting immunohematological reactions characterised in that it uses a detection device consisting of a quartz crystal microbalance (QCM), a device able to measure very small variations in mass (down to fractions of a nanogram).

2) A monitoring system for detecting immunohematological reactions according to the foregoing claim, characterised in that the extreme sensitivity to the mass is due to the use of small piezoelectric quartz crystals oscillated at their resonance frequency.

3) A monitoring system for detecting immunohematological reactions according to either of the foregoing claims, characterised in that this resonance frequency strongly depends on the mass present on the surface of the quartz and, by monitoring the trend of the resonance frequency, this makes it possible to follow the adsorption of more complex structures or molecules, for example cells, on the surface of the quartz.

4) A monitoring system for detecting immunohematological reactions according to any of the foregoing claims, characterised in that the equations describing the relationship between the resonance frequency and the adsorbed mass for an AT-cut quartz crystal establish a direct proportion between frequency variation Δf and mass variation Δm: $\Delta f = - C \Delta m$, where the constant C represents the calibration coefficient and can be determined by placing known mass on the surface of the crystal.

5) A monitoring system for detecting immunohematological reactions according to any of the foregoing claims, characterised in that it comprises at least one driver (10) connected to a quartz crystal oscillator (11) (an AT-cut quartz crystal equipped with metal electrodes facing each other on two sides, with its own frequency of around several MHz) which guides the transducer to oscillate at the resonance frequency.

6) A monitoring system for detecting immunohematological reactions according to any of the foregoing claims, characterised in that the output

signal of the driver (10) is sent to a frequency meter (12) which measures the frequency.

7) A monitoring system for detecting immunohematological reactions according to any of the foregoing claims, characterised in that the entire system can be guided by a computer (13) which can record the temporal variations of the resonance frequency.

8) A monitoring system for detecting immunohematological reactions according to any of the foregoing claims, characterised in that by functionalizing one of the two sides of the crystal with certain molecules it is possible to follow *in-situ* or to determine *ex-situ* the presence of specific recognition events between the molecules immobilized on the surface of the quartz and other molecules present in solution.

9) A monitoring system for detecting immunohematological reactions according to any of the foregoing claims, characterised in that it allows detection of antibodies, where the antibody being tested is immobilized with appropriate techniques on one of the two microbalance electrodes in such a way as to preserve the biological functionality and in that the presence of the corresponding antigen in solution is determined by monitoring the variations in resonance frequency of the crystal following any bond between the antibody and antigen and a consequent increase in actual mass on the electrode.

10) A monitoring system for detecting immunohematological reactions according to any of the foregoing claims, characterised in that the immobilization of the IgM antibodies on the surface of the QCM transducers is achieved by prior formation of a self-assembled layer of molecules which can selectively bind the IgMs present in the antisera.

11) A monitoring system for detecting immunohematological reactions according to any of the foregoing claims, characterised in that the reaction interval for IgM-antigen antibodies is 4-22°C, with an optimum value of 10°±3°C.

12) A monitoring system for detecting immunohematological reactions according to any of the foregoing claims, characterised in that it foresees a multiparametyric QCM system integrated on a single crystal that can accommodate several sensors operating in parallel or with multiplexer logic.

13) A monitoring system for detecting immunohematological reactions according to any of the foregoing claims, characterised in that these sensors are produced starting from a single AT-cut quartz crystal and are defined by means of lithographic techniques.

14) A monitoring system for detecting immunohematological reactions according to any of the foregoing claims, characterised in that each sensor can be addressed independently of the others and can be made specific for a certain antigen/antibody/antigenic determinant by means of selective functionalization, also assisted by microfluidics applied on the chip itself or on disposable polymer supports.

15) A monitoring system for detecting immunohematological reactions according to any of the foregoing claims, characterised in that such a system is able to perform a complete series of analyses (for example direct grouping + hepatitis markers) by means of a single exposure to the biological fluid being tested, speeding up the time necessary for the tests and making the approach substantially easier and more automated.

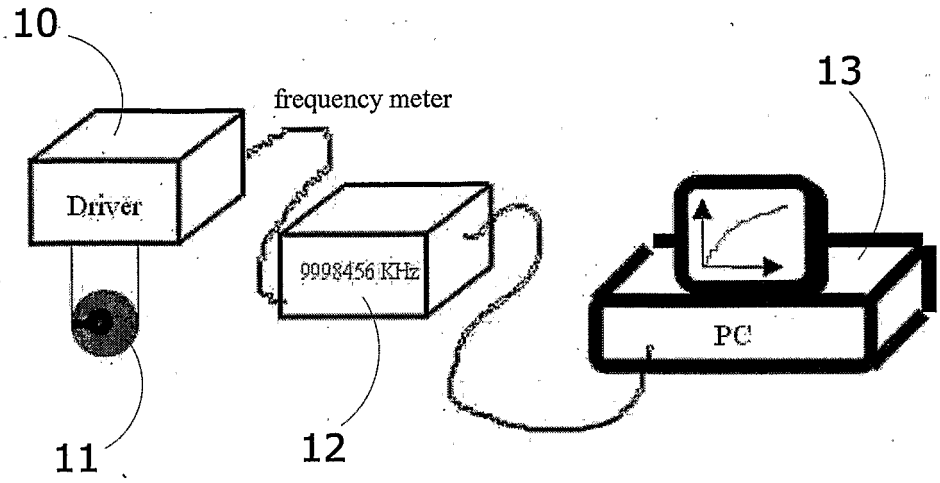

10
frequency meter
13
Driver
9998456 KHz
PC
11
12

Fig. 1

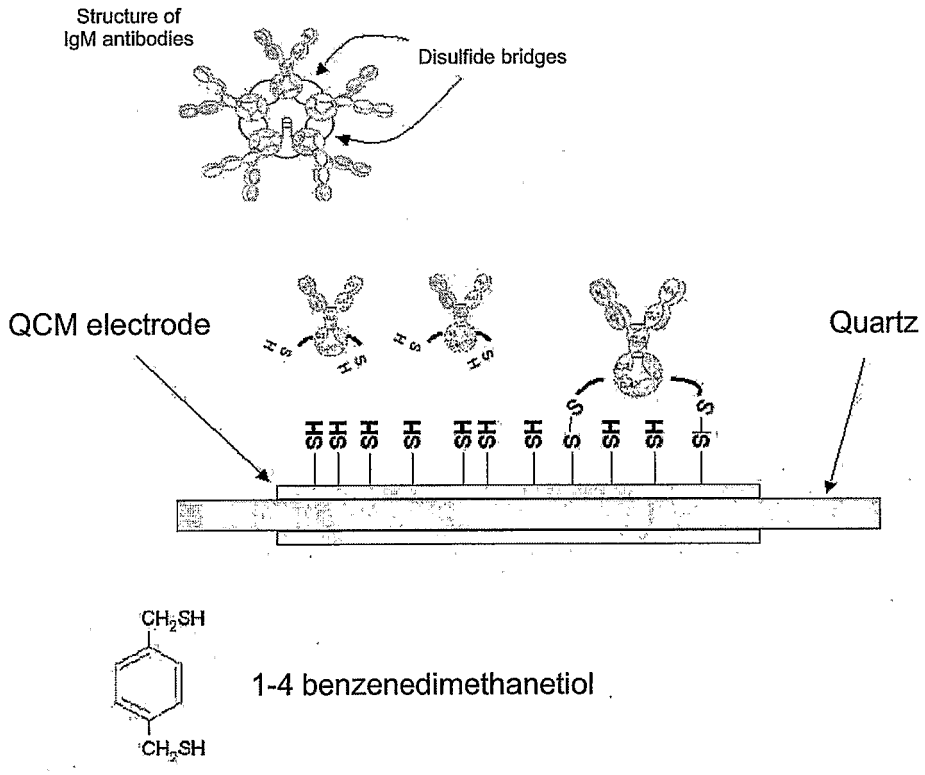

Structure of
IgM antibodies
Disulfide bridges
QCM electrode
Quartz
1-4 benzenedimethanetiol

Fig. 2

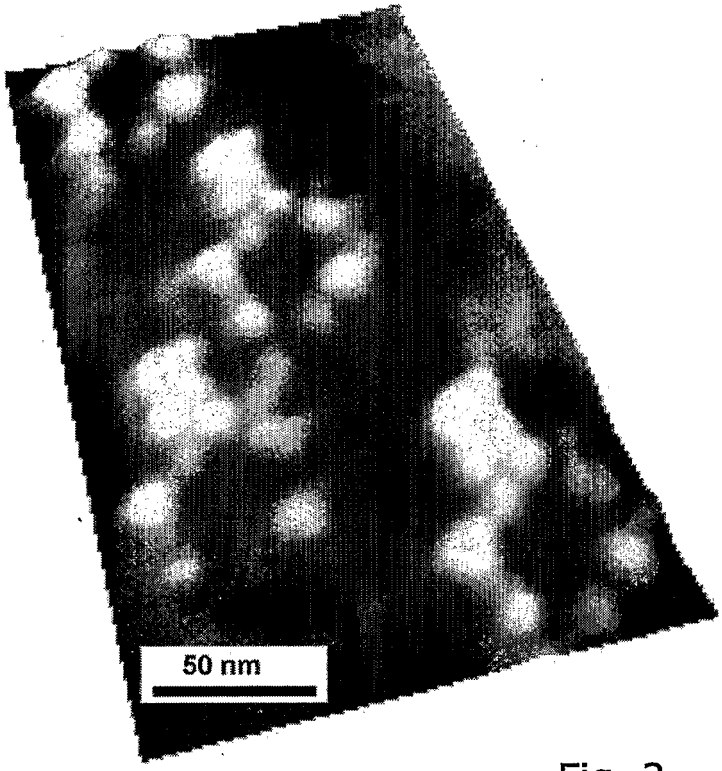

50 nm

Fig. 3

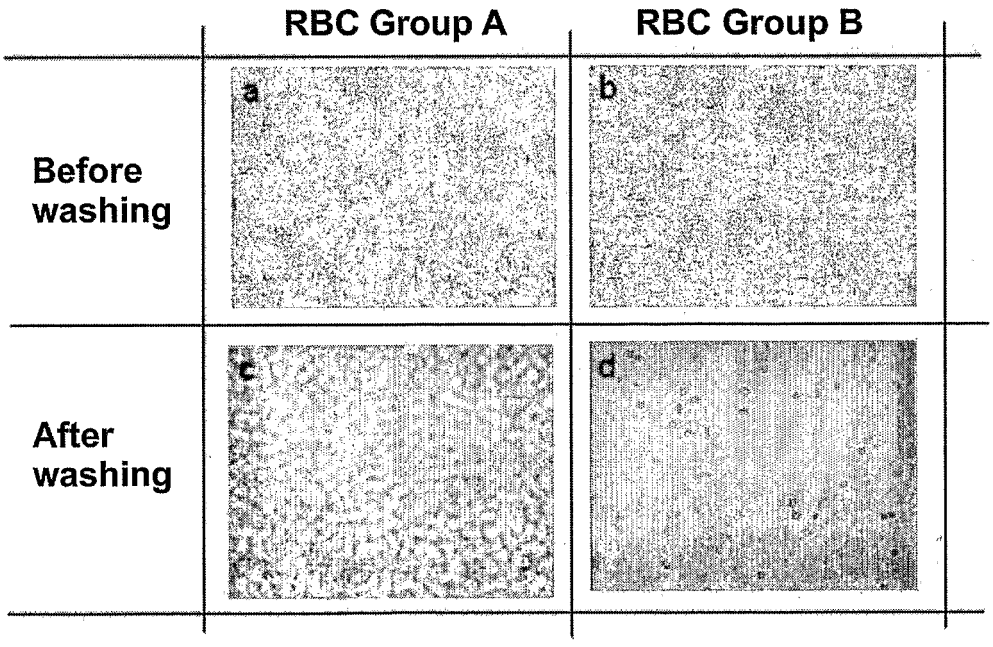

RBC Group A
RBC Group B
Before washing
After washing
a
b
c
d

Fig. 4

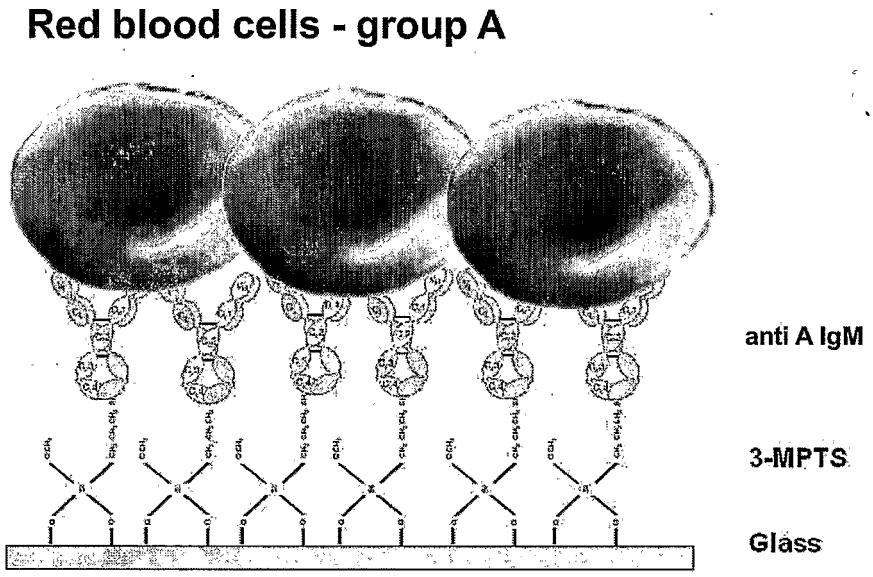
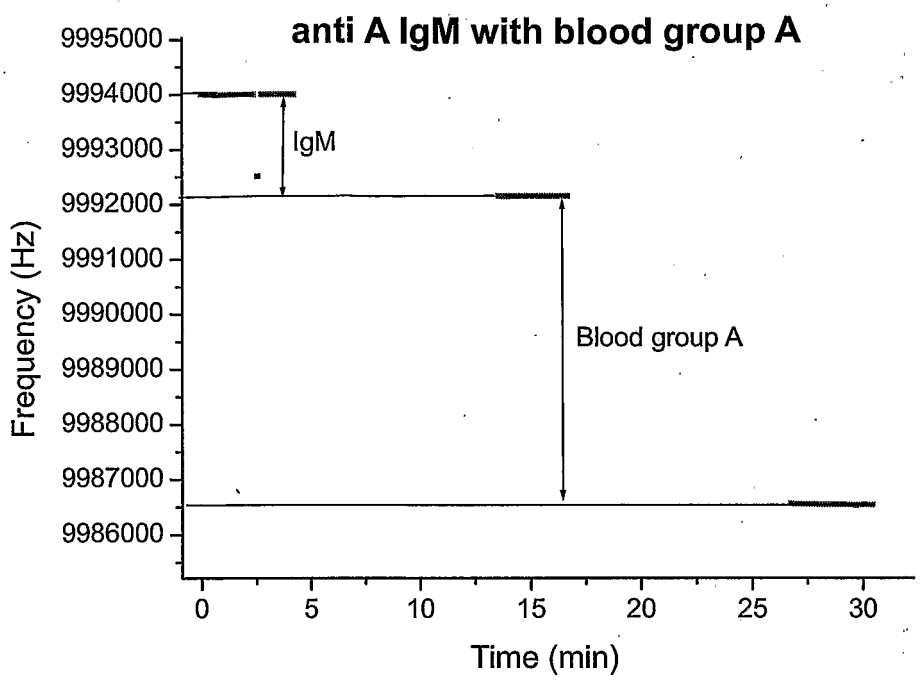


Fig. 5

A


anti A IgM with blood group A
9995000
9994000
9993000
9992000
9991000
9990000
9989000
9988000
9987000
9986000
Frequency (Hz)
IgM
Blood group A
0
5
10
15
20
25
30
Time (min)

B

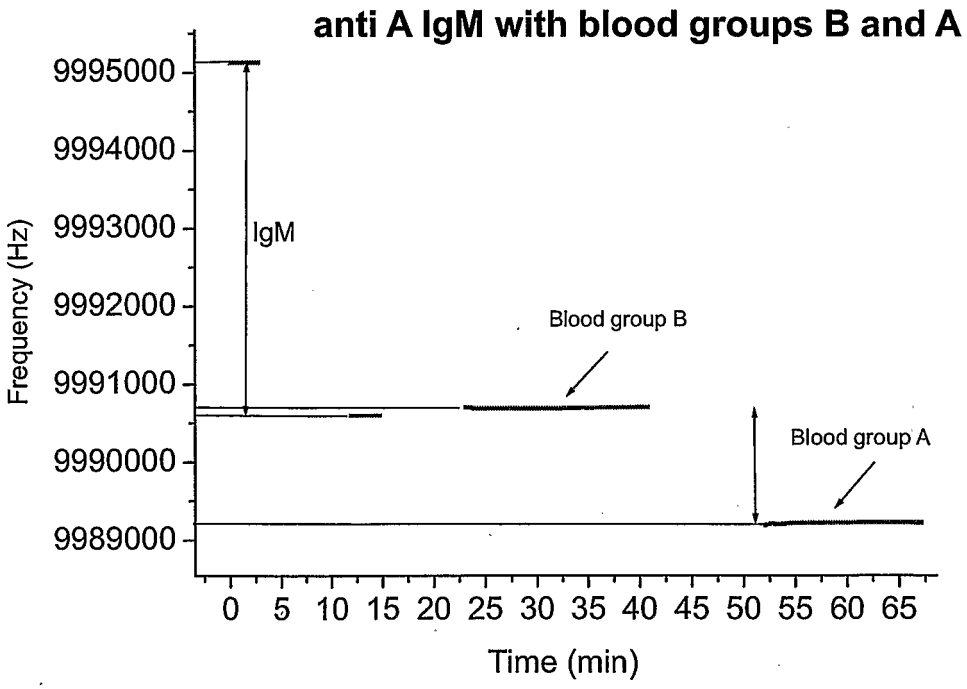

anti A IgM with blood groups B and A
9995000
9994000
9993000
9992000
9991000
9990000
9989000
Frequency (Hz)
IgM
Blood group B
Blood group A
0 5 10 15 20 25 30 35 40 45 50 55 60 65
Time (min)

Fig. 6

Type of immobilized IgM	Type of blood tested	Frequency variation after incubation with blood (Hz)
Anti A	Group B purified	≅
Anti A	Group B whole	≅
Anti B	Group A purified	≅
Anti B	Group B purified	<<
Anti B	Group A whole	≅
Anti B	Group B whole	<<
Anti B	Group A whole	≅
Anti B	Group A whole	≅
Anti B	Group B whole	<<
Anti B	Group A whole	<<
Anti A	Group A whole	<<
Anti A	Group B whole	<<

<< Significant decrease
≅ Value almost unchanged

Fig. 7

Type of plasma	Erythrocytes	Δf after incubation with erythrocytes
Group B	Erythrocytes group A	<<
Group B	Erythrocytes group B	≅
Group B	Erythrocytes group A	<<
Group B	Erythrocytes group B	≅
Group B	Erythrocytes group A	<<
Group B	Erythrocytes group B	≅

<< Significant decrease
≅ Value almost unchanged

Fig. 8

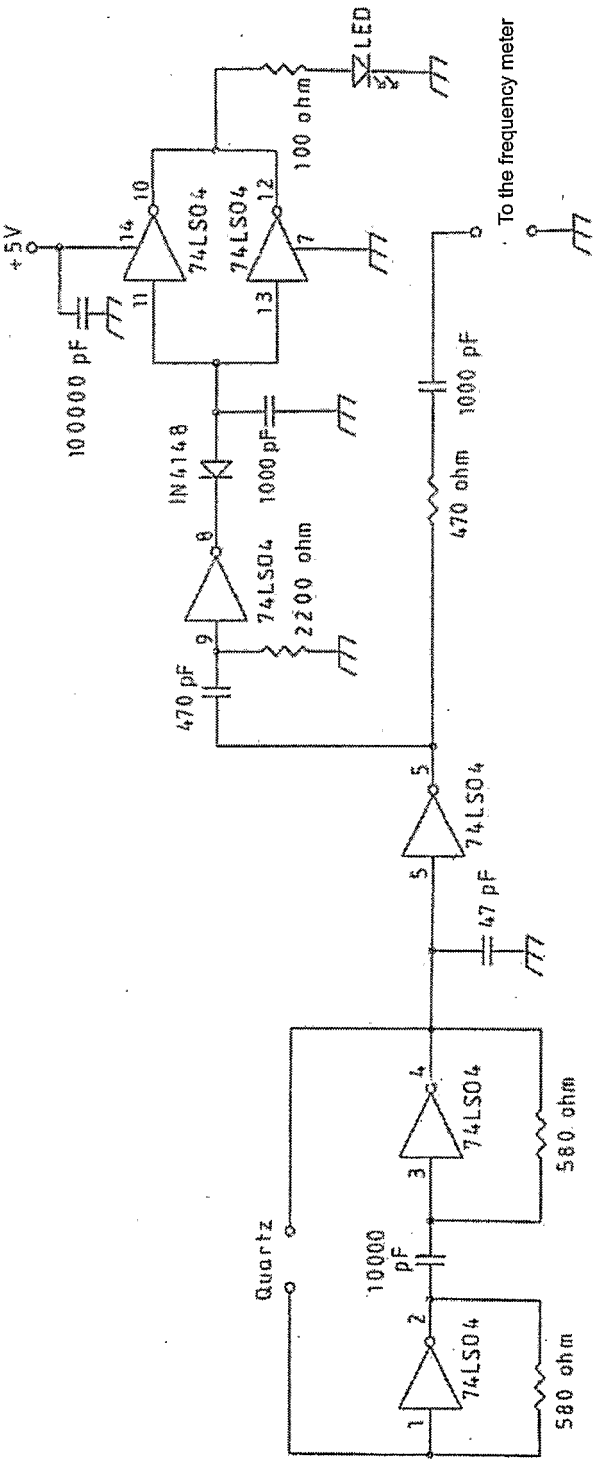

LED
100 ohm
+5V
74LS04
74LS04
100000 pF
1000 pF
IN4148
1000 pF
74LS04
2200 ohm
470 pF
470 ohm
1000 pF
To the frequency meter
74LS04
47 pF
74LS04
580 ohm
Quartz
10000 pF
74LS04
580 ohm

Fig. 9

专利名称(译)	血型快速监测系统和免疫血液学反应检测		
公开(公告)号	EP1797426A2	公开(公告)日	2007-06-20
申请号	EP2005794452	申请日	2005-09-21
[标]申请(专利权)人(译)	SANITARIA SCALIGERA		
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IPC分类号	G01N33/53 G01N33/80 H03H9/19		
CPC分类号	G01N33/80 G01N33/54373 G01N2291/014 G01N2291/02466		
优先权	102004901245584 2004-09-22 IT		
其他公开文献	EP1797426B1		
外部链接	Espacenet		

摘要(译)

用于监测血型和用于检测免疫血液学反应的系统使用由石英晶体微量天平（QCM）组成的检测装置，该装置能够测量非常小的质量变化（低至纳克的分数）。