

### On-board control for analytical elements

The invention concerns a control reagent system for an analytical element, for example in the form of a test strip and in particular a test strip for determining a coagulation parameter which allows a differentiation between functioning analytical elements and non-functioning analytical elements. The invention also concerns corresponding analytical elements and methods for their control.

So-called carrier-bound tests are being used to an increasing extent for the qualitative and quantitative analysis of components of a liquid sample in particular a body fluid from humans or animals. Analytical elements (also referred to as test elements) are used for this where at least one reagent is embedded in a test field consisting of one or more layers, which is brought into contact with the sample. The reaction of sample and reagent results in a change in the analytical element that can be evaluated visually or with the aid of an instrument (usually by reflection photometry or electrochemically). After a test has been carried out the used analytical element is disposed of.

Numerous different types of analytical element are known which differ in their measuring principle (e.g. optical or electrochemical) and the reagents that are used and in their construction and in particular with regard to the arrangement and attachment of the test layers. Strip-shaped analytical elements are of particular practical importance. These analytical elements that are also referred to as test strips are essentially composed of an elongate support layer made of a plastic material on which one or more test fields are attached.

The analytical elements are packaged in primary packaging in the interior of which they are stored until use i.e. until they are removed by the user and a test has been carried out with subsequent disposal. The analytical elements may be packaged individually in their own primary packaging. Analytical element packaging units are commonly used in which a plurality of analytical elements are located in the interior

of a common primary packaging. The primary packaging usually contains a desiccant.

The interior of the primary packaging is usually substantially hermetically sealed. Hence the storage conditions are essentially determined by the environmental conditions in the primary packaging during storage.

Many analytical elements contain reagents which can be damaged by certain storage conditions e.g. temperature, humidity, oxygen, light etc. which makes them unusable for carrying out a reliable test. Hence in order to avoid damage it is necessary to store the analytical element packaging unit under certain appropriate conditions recommended by the manufacturer. On the part of the manufacturer, the storage life of the analytical element is guaranteed for a certain period when stored properly.

The use of an analytical element with a damaged reagent can lead to a false test result which may result in a serious misinterpretation of for example the state of health of a person. Hence in the past various attempts have been made to reduce the risk of using analytical elements with storage damage.

For example test reagents have been developed that are relatively insensitive to external effects. The aim of another development is to use elaborate primary packaging to minimize external effects on the reagents of the analytical elements. Both solutions are associated with substantially increased manufacturing costs. For safety reasons a relatively short shelf life is stated. As a result analytical elements can no longer be used after the shelf life date has expired although it is not possible to check whether in fact there have been conditions which could have resulted in damage to the reagents.

Test strips for diagnostic blood examinations are subject to an extensive quality control before sale. Suitable shipping and storage conditions are intensively examined before they are launched on the market and are for example described on the packaging or in the package insert. Nevertheless, it cannot be completely excluded that strips are damaged before the expiry date during transport of the goods to the customer or due to incorrect storage by the customer and that there is a risk that false measurements are obtained when they are used.

Improper transport and/or storage conditions can be discovered by measurements using liquid controls which are distributed as additional system components besides the instrument and strips for most so-called point of care systems (PoC systems) that are used decentrally (i.e. outside of special laboratories i.e. for example in doctor's offices, pharmacies or by the patient at home). Disadvantages of using liquid controls for PoC systems (e.g. for coagulation measurement systems) are their somewhat complicated handling, the costs for using usually two test strips and two control liquids (level 1 & level 2 control) and the fact that although usually strips from the same production lot and packaging are measured with the controls they are, however, inevitably other strips than those with which the blood sample of a patient is in fact examined.

These disadvantages are avoided by an on-board control (abbreviated as OBC in the following) which is integrated into each test strip and does not require an additional test liquid. Systems with on-board controls require no control liquids but work with the same sample liquid from which the parameter to be determined is determined with the measurement system.

Such on-board controls are described for example in the patents US 5,504,011 (ITC), US 6,084,660 (Lifescan) and US 6,060,323 (Hemosense). A common feature of the control systems disclosed in the said documents is that a blood sample is taken up into an analytical element through a capillary channel and is transported by capillary forces to a site of examination within the analytical element. The sample is divided by one or more branches of the capillary system and conveyed into one or more side channels. Reagents are then located in these side channels which constitute the actual OBC.

A common disadvantage of these on-board controls is that the test strips – in addition to the actual measurement channel for the patient sample – require several (usually two) additional channels. After being filled with the same patient blood, measurements are carried out in these additional channels which should give information about the integrity of the strip. This concept results in high manufacturing costs since the individual channels have to be separately provided with different reagents. Furthermore, such control systems require comparatively

large sample volumes since, in addition to the actual measurement channel, at least one and usually even several control channels have to be filled with sample. Large sample volumes are regarded as being a particular disadvantage where patients themselves have to regularly obtain the sample i.e. for example in so-called home monitoring especially in the case of diabetics or patients which have to monitor their own coagulation values since the collection of blood samples by puncturing the skin is painful and even more painful the more blood sample is required. Moreover, multichannel on-board control systems suffer from difficult filling mechanisms since the sample has to automatically penetrate into several channels and fill them.

It is known from DE-A 198 31 519 that irreversible moisture or temperature indicator elements can be attached to test elements or their packaging which should indicate damage to test elements for example by excessive temperatures or air humidity, light or the effects of oxygen.

The object of the invention was to develop an on-board control system which does not have the said disadvantages especially with regard to manufacturing costs and sample volume but nevertheless reliably indicates the potential unusability of test elements especially as a result of improper storage or transport conditions. In particular damaging humidity and/or temperature stress on the test elements should be detected with the aid of the OBC according to the invention. Furthermore, the intended OBC should not react too early in order to enable a long storage of the product at room temperature and should enable a good discrimination between intact and defective strips with a high precision.

This object is achieved by the subject matter of the invention.

The invention concerns a control reagent system as claimed in claim 1, its use as claimed in claim 8, a corresponding analytical element as claimed in claim 9 and a method for controlling analytical elements as claimed in claim 12. Advantageous embodiments of the invention are a subject matter of the subclaims.

The invention is further elucidated by the following detailed description, the figures and examples.

The concept according to the invention which can also be referred to as a 1-channel on-board control, provides that a reagent zone on a test element which can preferably also be used for the actual measurement of the patient sample, contains a substance which changes when stressed. The change in this substance preferably results in a degradation product which can either be detected visually or by the instrument provided to measure the test strip. A strip that has been recognized to be defective can for example not be released by the measuring instrument for measuring the patient sample.

The reagent system according to the invention is suitable for the on-board control of analytical elements. The reagent system contains at least one chemical substance, but preferably a mixture of chemical substances. At least one of these substances is suitable for directly or indirectly indicating environmental conditions which could impair the reliability of an analytical element. This property is referred to as an on-board control property. For this purpose damaging environmental effects such as temperature, humidity, light, oxygen etc. are utilized to carry out a preferably irreversible change in the said substance or with the said substance which enables a subsequent detection of the damaging effects. In this connection it is usually irrelevant to determine the exact type of damaging effects; it is usually sufficient to establish the fact that a damaging effect has taken place.

The reagent system for on-board control can contain other auxiliary substances in addition to the substance which has the actual on-board control function. These may be buffer substances, fillers, film formers and such like which are known to a person skilled in the art in numerous embodiments in connection with reagent formulations for analytical test elements.

In the sense of the invention analytical elements (also referred to as test elements, analytical test elements, test strips, test chips, test devices) are preferably suitable for determining analytes or other parameters in liquid samples and especially in liquid samples of human origin such as blood, serum, plasma, urine and such like.

Analytical elements in the sense of the invention always contain a reagent or reagent system on a support material which generates a detectable signal that is dependent on the analyte to be examined in the sample or on the property of the sample to be

examined. Such analytical elements are known to a person skilled in the art in numerous embodiments. Examples are optical or electrochemical test strips for detecting metabolites in blood or sample liquids derived therefrom and in particular for determining glucose, cholesterol and such like. Furthermore, test strips are known which can be used to determine coagulation parameters in a blood sample.

The reagent system that is located on the analytical element and which is responsible for a detectable detection signal for an analyte in the sample or for a property of the sample can preferably result in an optically detectable and evaluable measurement signal or a signal that can be detected by electrochemistry. Both variants are known to a person skilled in the art in numerous embodiments.

The reagent system for on-board control according to the invention is preferably integrated into the reagent system for the detection reaction. However, it is also conceivable and possible to accommodate reagent systems for the detection reaction and reagent systems for the on-board control in spatially separated zones on an analytical element.

For the preferred case that the reagent system for the on-board control is integrated into the reagent system for the detection reaction care must be taken that the reagents do not negatively affect one another.

The reagent system for the on-board control and the reagent system for the detection reaction can be based on the same or on different detection principles. Thus according to the invention it is possible to optically or electrochemically evaluate the on-board control. It is also possible to optically or electrochemically detect the actual detection reaction. It is particularly preferred to electrochemically detect the on-board control as well as the detection reaction. In this connection an optical detection can be carried out visually or by means of an apparatus with the aid of a photometer in which case any methods familiar to a person skilled in the art such as reflection measurement, absorption measurement, transmission measurement, luminescence measurement and such like can be used. In turn for the electrochemical detection methods such as potentiometry, amperometry, coulometry, chrono-amperometry and such like are suitable.

In particular an optical change in the on-board control field or detection reagent field can be evaluated with the naked eye without using a measuring instrument. However, it is preferable to evaluate the on-board control and the detection field with the aid of a measuring instrument, for example a photometer or an electrochemical measuring instrument. It is also possible to evaluate the on-board control without a measuring instrument but to evaluate the detection reaction with a measuring instrument.

The reagent formulations for the on-board control and the detection field can be applied to a support of an analytical element using any methods known to a person skilled in the art. In this connection the various reagents can be applied using the same or different methods. Some of the possible methods are for example: application in a liquid form and subsequent drying on or into a support; application as a coating mass by knife coating, slot nozzle coating and the like; printing processes such as ink-jet printing, screen printing, dispersing etc. It is also possible to mount reagents already applied to a first support together with this first support on the actual second support of the analytical element and to join them e.g. by gluing, welding etc.

The on-board control according to the invention should be able to reliably detect stressed test elements. In this connection stress is understood to mean that test elements have been exposed to environmental conditions which could lead to damage or impairment of the reagents for the actual detection reaction. In the case of analytical elements which are intended to be stored at room temperature in closed cans provided with desiccants or provided with moisture-impermeable foils, stress is for example understood as exposure to high temperatures in the closed cans or foils (e.g. during transport to the customer or behind a pane of glass in sunlight) or damp storage (in the case of improperly closed cans after removing test strips or defective foil packaging). Of course combinations of stress events such as concurrent humidity or elevated temperature can result in damage. In this case it is only important that a stress finally results in an impairment or failure of the detection reagent system. These can indeed be different specific conditions for the various analytical elements. A person skilled in the art knows how he can identify such damaging events for the respective reagent system.

According to the invention a stress of the analytical element or of the reagent system for the on-board control results in a – preferably irreversible – change in the reagent system of the on-board control. This ensures that damaging environmental effects are also detected when the environmental conditions have in the meantime changed into favourable conditions. For example a single damaging temperature elevation or a single brief effect of humidity which has resulted in damage can thus be reliably identified.

Surprisingly it was found that reagents which contain N-oxides or nitroso compounds, in particular in combination with reducing agents such as sugars, polyalcohols, cysteine-containing proteins, glycine etc. can be reduced under the same conditions and environmental effects under which an OBC should indicate undesired effects and negative changes of a test strip. The degradation products formed by reduction from the N-oxides or nitroso compounds can be detected in the test strip by suitable, preferably electrochemical or optical methods. In particular if sugars are used as the reducing agents, the redox properties and hence the kinetics of the redox reaction can be adjusted by means of the pH of the reagent.

According to the invention the N-oxide resazurin has turned out to be a particularly suitable molecule for the described indicator reaction and is thus preferred.

The blue resazurin is reduced to the red resorufin (cf. fig. 1) when stressed under conditions that could damage a test strip (elevated temperatures, humidity and light) where the reduction takes place especially in the presence of a suitable redox partner, preferably those from the reagent formulation. The change i.e. the decrease in the resazurin concentration with a simultaneous increase in the resorufin concentration can be determined visually by an optical detector in a measuring instrument or by an electrochemical sensor.

In the case of resazurin, glycine was surprisingly found to be a very specific reducing agent. A combination of suitable amounts of resazurin and glycine in an OBC reagent is particularly preferred according to the invention. A minimum concentration of ca. 0.01 g/l resazurin has turned out to be preferred since detection of resazurin is practically very difficult below this concentration. The maximum amount of

resazurin should not exceed 20 mmol/l since otherwise solubility problems can occur. As described above glycine is not absolutely necessary for the function of the OBC containing resazurin; a concentration of about 250 g/l has proven to be the maximum amount of glycine since solubility problems occur above this amount and glycine may crystallize from the solution which in turn can cause problems when coating the reagent mass and may lead to inhomogeneities in the coated mass.

The remaining resazurin and/or the resorufin that is formed can be quantified for the electrochemical detection of the OBC reaction.

A quantification of resazurin is preferred for large changes in concentration and can for example be carried out by electrochemical reduction of the resazurin at a potential of -700 mV against Ag/AgCl. At this potential the resorufin that is formed is reduced further to dihydro-resorufin (cf. fig. 1).

If mainly resazurin is present in the OBC reagent (and hence the reagent is not stressed or hardly stressed) a 4-electron transition is provoked which results in a higher current than when mainly resorufin is present whose reduction only leads to a conversion of 2 electrons. The current or charge measured at a predetermined potential and in particular at a preferred potential of -700 mV against Ag/AgCl thus allows deductions to be made about the extent of the temperature and/or humidity stress of the reagent.

Resorufin cannot be quantified by electrochemical (re)oxidation of resorufin to resazurin because the reduction of resazurin to resorufin is irreversible. A reductive detection in the described OBC system only requires that the reduction of resorufin is specifically detected but not the reduction of resazurin which may be present in the reagent. This can for example be accomplished by using a specific reduction potential which is preferably in the range of -450 to -550 mV against Ag/AgCl.

It was surprisingly found that the quantification of resorufin in the described OBC system can be achieved especially well when in a first step the resorufin present in the test strip is converted electrochemically into dihydro-resorufin (referred to as OBC-prepare) and the in situ generated dihydro-resorufin is electrochemically oxidized back to resorufin in a second step (referred to as OBC test in the following). This

oxidation reaction runs particularly specifically preferably at a potential of -100 mV against Ag/AgCl.

The intensity and specificity of the OBC test signal can be controlled by the length of the OBC prepare phase.

In addition to the particularly preferred resazurin/resorufin system, nitroso compounds and in particular p-nitrosoanilines are another preferred example for a class of substances which can be converted into products which can indicate a possible damage of the test strip under conditions that can damage test strips. p-Nitrosoanilines can for example be reduced under conditions which can also damage reagents in a detection reagent. The products (such as phenylenediamines) produced by reduction can also be detected optically or electrochemically. Other nitroso compounds which can be used according to the invention are described in US 5,206,147, US 5,334,508, US 5,122,244 and US 5,286,362. A combination of the nitroso compounds disclosed in these 4 US patents with heteropoly acids, in particular heteropoly acids in a precipitated form according to US 5,240,860 which when stressed formed readily visible heteropoly blue in the presence of reducing agents is particularly preferred.

#### Short description of the figures

Figure 1 shows the structural formulae of resazurin 1 which is converted by reduction into resorufin 2 and by further reduction into dihydro-resorufin 3.

Figure 2 shows absorption spectra (relative absorption A plotted versus the wavelength  $\lambda$  (in nm)) of resazurin 1 and resorufin 2.

Figure 3 shows the reflectance spectra (relative reflectance R (in %) plotted versus the wavelength  $\lambda$  (in nm)) of the reagent fields of test elements that have been stressed to different extents (1 unstressed, 2 slightly stressed, 3 strongly stressed).

Figure 4 shows the increase in degradation product (resorufin) in a resazurin-containing reagent film based on the increase of the relative reflectance R (in %) (measured at a wavelength of 620 nm) over the stress time t (in h).

Figure 5 shows the change of the signal that can be measured electrochemically during a first measuring phase lasting for 3 seconds at -700 mV versus Ag/AgCl ( $Q_{700}$ ) (lower two curves) and a second measuring phase lasting for 1.5 seconds at -100 mV versus Ag/AgCl ( $Q_{100}$ ) (upper two curves) on the basis of the dependence of the measured charge  $Q$  on the stress time  $t$  (in h).

The invention is characterized in more detail by the following examples which describe the advantages and properties of the OBC according to the invention using test strips for coagulation measurements as an example (prothrombin time test or PT test). It is clear to a person skilled in the art that the statements made on the basis of the coagulation test strip example also apply to other types of test strip and in particular to those used for the optical or electrochemical determination of blood glucose, lipids such as cholesterol and HDL cholesterol, triglycerides etc., for the determination of other coagulation parameters than PT such as aPTT, ACT, ECT, anti-factor Xa tests and also for immunological test elements, in particular chromatography test strips that can be evaluated optically and can be applied to them.

**Example 1: Two reagent formulations containing different reducing agents for an on-board control that can be evaluated optically**

Table 1

Formulation for an OBC containing glycine as a reducing agent that can be evaluated optically

| Chemicals                     | Source         | Concentration |
|-------------------------------|----------------|---------------|
| Sucrose                       | Sigma          | 3.2 g/dl      |
| Mowiol 4/86                   | Clariant GmbH  | 1.3 g/dl      |
| Keltrol F                     | Kelco          | 2.98 g/ml     |
| Glycine                       | Sigma          | 1.35 g/dl     |
| HEPES <sup>1</sup>            | Sigma          | 0.33 mg/ml    |
| polyethylene glycol PEG 3,350 | Sigma          | 1.33 g/dl     |
| bovine serum albumin          | Sigma          | 0.4 g/dl      |
| mega 8                        | Sigma          | 0.67 mg/ml    |
| Resazurin                     | Riedel de Haen | 0.96 mg/ml    |

<sup>1</sup>HEPES: [4-(2-hydroxyethyl)-piperazino]-ethane sulfonic acid

The substances listed in table 1 are mixed homogeneously and adjusted to a pH of 7.4 with NaOH. The reaction mass obtained in this manner was coated onto a test strip that can be measured by reflection photometry as a reagent tape of 20 mm width and ca. 10  $\mu$ m thickness.

Table 2

Formulation for an OBC containing glucose as a reducing agent that can be evaluated optically

| pH of the formulation                      | 7.5                     | 9     | 10    | 11    |
|--------------------------------------------|-------------------------|-------|-------|-------|
| Chemical                                   | Amount weighed out in g |       |       |       |
| polyvinylpyrrolidone solution <sup>1</sup> | 7.09                    | 7.09  | 7.09  | 7.09  |
| glucose                                    | 1.2                     | 1.2   | 1.2   | 1.2   |
| HEPES <sup>2</sup>                         | 1.25                    | --    | --    | --    |
| CHES <sup>3</sup>                          | --                      | 1.04  | 1.04  | --    |
| CAPS <sup>4</sup>                          | --                      | --    | --    | 1.11  |
| bidistilled water                          | 7.72                    | 7.93  | 7.93  | 7.86  |
| Keltrol F solution <sup>5</sup>            | 17.04                   | 17.04 | 17.04 | 17.04 |
| BM propiofan 70 D                          | 5.69                    | 5.69  | 5.69  | 5.69  |
| titanium dioxide slurry <sup>6</sup>       | 55.32                   | 55.32 | 55.32 | 55.32 |
| resazurin                                  | 0.28                    | 0.28  | 0.28  | 0.27  |
| hexanol                                    | 0.17                    | 0.17  | 0.17  | 0.17  |
| methoxy-2-propanol                         | 4.25                    | 4.25  | 4.25  | 4.25  |

<sup>1</sup>40 g/l polyvinylpyrrolidone (PVP) is scattered into water and restirred for about 30 min.

<sup>2</sup>HEPES: [4-(2-hydroxyethyl)-piperazino]-ethanesulfonic acid

<sup>3</sup>CHES: 2-(cyclohexylamino)-ethanesulfonic acid

<sup>4</sup>CAPS: 3-(cyclohexylamino)-1-propanesulfonic acid

<sup>5</sup>6.8 g/l Keltrol is scattered into water while stirring and restirred for several hours. In order to ensure a complete swelling, the preparation is allowed to stand overnight at room temperature before further use.

<sup>6</sup>15 g titanium dioxide is scattered into 38 ml water while stirring and then homogenized in a dissolver stirrer at a high stirring rate.

The pH of the reagent mass is in each case adjusted to the stated value with sodium hydroxide solution.

The substances listed in table 2 are mixed homogeneously. The reaction mass obtained in this manner was coated onto a test strip that can be measured by reflection photometry as a reagent tape of 20 mm width and ca. 10  $\mu$ m thickness.

The reaction rate of the OBC reaction i.e. the conversion of resazurin into resorufin which depends on the environmental conditions can be adjusted by the pH. The more alkaline the pH the more rapid is this conversion and hence the more sensitive is the OBC.

**Example 2: Mass spectroscopic determination of the degradation product after stress in the reagent formulation according to table 1 from example 1**

In the case of test strips that are intended to be stored at room temperature in closed cans provided with desiccant or moisture-impermeable foils, the degradation reaction should not occur or only to a very slight extent under these storage conditions.

The on-board control should in particular detect the following as incorrect storage!

- high temperatures in closed cans / foils (e.g. during transport to the customer or behind a pane of glass in sunlight), and
- humid storage (cans that have not been properly closed after removing test strips or defective foil packaging).

These requirements were simulated by the following stress models:

- Storage for several weeks at 50°C in closed cans.
- Storage for hours and several days at 50°C and elevated (50 % / 75 %) air humidity.
- Storage for several days in an open or defective packaging under environmental conditions of climate zone 4 (30°C, 70 % air humidity).

The mass spectrum of a stressed reagent formulation which was prepared according to table 1 in example 1 shows that after stress (6 hours at 50°C and 75 % relative air humidity) a few percent of the resazurin was converted into resorufin (cf. also figure 1). The main peak of the resorufin spectrum (212.20 mass units) is present in addition to the main peak of the mass spectrum of resazurin at 228.17 mass units.

**Example 3: Absorption spectra of resazurin and resorufin (fig. 2)**

The absorption spectra shown in figure 2 of resazurin 1 (a blue dye) and resorufin 2 (a red dye) show that in principle it is possible to optically detect (e.g. visually or by reflection photometry) the conversion of resazurin 1 into resorufin 2 which occurs when a reagent formulation is stressed as shown in example 2.

**Example 4: Reflection spectra of unstressed and stressed test strips**

Test strips whose reagent films were prepared based on the formulation of table 1 from example 1 were stressed for 0 hours (unstressed; cf. curve 1 in figure 3), 6 hours (slightly stressed; cf. curve 2 in figure 3) and 12 hours (strongly stressed; cf. curve 3 in figure 3) at 50°C and 75 % air humidity. The change in the corresponding reflectance spectra was measured (fig. 3). With an increase in stress, an increase in the reflectance at a wavelength of 620 nm is seen which is associated with a decrease in the amount of resazurin.

**Example 5: Optical detection of the degradation product resorufin in test strips that were stressed to different extents**

Test strips whose reagent films were prepared on the basis of the formulation of table 1 from example 1 were stressed for different periods at 50°C and 75 % air humidity and then measured in a simple reflection photometer whose LED operated with light at a wavelength of 620 nm.

Fig. 4 shows the increase in the degradation product (recognizable by the increase in reflectance R) over the stress time t (in h).

The change in the test field can also be readily detected visually i.e. by the user with the naked eye. The reagent zone of the unstressed test strip which is firstly blue changes its colour into pink as the stress period increases.

**Example 6: Formulation for a PT coagulation test with integrated OBC and electrochemical detection**

Table 3

Formulation for an amperometric prothrombin time test with integrated OBC reagents

| Chemical                                            | Source                | Concentration |
|-----------------------------------------------------|-----------------------|---------------|
| relipidated recombinant human thromboplastin (rhTF) | Dade Behring, Marburg | 72 ng / ml    |
| Sucrose                                             | Sigma                 | 3.2 g / dl    |
| mowiol 4/86                                         | Clariant GmbH         | 1.3 g / dl    |
| Keltrol F                                           | Kelco                 | 2.98 g / ml   |
| Glycine                                             | Sigma                 | 1.35 g / dl   |
| Polybrene                                           | Sigma                 | 10 µg / ml    |
| HEPES <sup>1</sup>                                  | Sigma                 | 0.33 mg / ml  |
| polyethylene glycol PEG 3,350                       | Sigma                 | 1.33 g / dl   |
| bovine serum albumin                                | Sigma                 | 0.4 g / dl    |
| mega 8                                              | Sigma                 | 0.67 mg / ml  |
| Resazurin                                           | Riedel de Haen        | 0.96 mg / ml  |
| Electrozym TH                                       | Roche Diagnostics     | 1.2 mg / ml   |

<sup>1</sup>HEPES: [4-(2-hydroxyethyl)-piperazino]-ethanesulfonic acid

The substances listed in table 3 were homogeneously mixed and adjusted with NaOH to a pH of 7.4. The reaction mass obtained in this manner was coated in a width of 4 mm and a thickness of ca. 90 µm (wet) or ca. 10 µm (dry) on a test strip that is to be measured amperometrically such that the whole area of the working electrode was

covered with reagent. An Ag/AgCl electrode which also served as the counter-electrode was used as a reference electrode.

**Example 7: Electrochemical detection of resazurin and the degradation product resorufin in test strips that were stressed to different extents**

The test strips prepared according to example 6 were stressed for different periods at 50°C and 75 % air humidity. Whole blood was then measured amperometrically with these test strips.

The following potentials were applied in order to quantify resazurin and resorufin:

-700 mV vs. Ag/AgCl for 3 seconds (so-called OBC prepare phase); subsequently  
-100 mV vs. Ag/AgCl for 1.5 seconds (so-called OBC test phase); subsequently  
+200 mV vs. Ag/AgCl for 90 seconds (for the actual coagulation measurement).

In order to quantify the "OBC prepare" and "OBC test" signals, the integrals under the current-time curves were calculated. Figure 5 shows how these integrals (referred to as  $Q_{700}$  and  $Q_{100}$ ) change with an increasing stress of the test strips.

Whereas there is a major change in the OBC signals, the clotting time measured with stressed test strips is almost stable up to a very long stress period (cf. table 4).

**Table 4**

**Effects of stress at 50°C and 75 % relative air humidity on the measurement of clotting time**

| Stress time (h) | Clotting time (s) |
|-----------------|-------------------|
| 0               | 12.78             |
| 0.33            | 12.38             |
| 0.66            | 12.72             |
| 1               | 13.14             |
| 2               | 12.52             |
| 4               | 13.53             |
| 6               | 13.26             |
| 12              | 12.78             |
| 24              | 13.16             |
| 36              | 14.00             |

This ensures that the OBC indicates improper storage of the test strips before false clotting times are generated.

**Example 8: Indication of the OBC with electrochemical detection when unpackaged strips are stored in climate zone IV**

The greatest risk of damaging test strips whose packaging is defective or has not been closed again is in the case of customers that live in climate zone IV (hot and humid). For this climate zone a humidity of 70 % and a temperature of 30°C have been described in the literature as “average climate conditions”.

Table 5 shows that the OBC with electrochemical detection indicates changes in the test strip due to storage under these conditions.

Table 5 shows on the basis of the measured charge  $Q$  (in nAs) versus the stress time  $t$  (in h) the change in the electrochemically measurable signal during a 3 second measuring phase at -700 mV vs. Ag/AgCl ( $Q_{700}$ ) and during a 1.5 second measuring

phase at -100 mM vs. Ag/AgCl ( $Q_{100}$ ) for different sample materials (normal blood (N) 1 and 2; blood from donors that had been treated with Marcumar (M) 1 and 2).

**Table 5**

**Effects of a stress at 30°C and 70 % relative air humidity on the electrochemical on-board control for different sample materials**

| t (h) | $Q_{100}$ (nAs) |       |       |       | $Q_{700}$ (nAs) |      |      |      |
|-------|-----------------|-------|-------|-------|-----------------|------|------|------|
|       | N 1             | N 2   | M 1   | M 2   | N 1             | N 2  | M 1  | M 2  |
| 0     | 58.42           | 57.3  | 51.7  | 56.8  | 2663            | 2512 | 2580 | 2735 |
| 2     | 58.62           | 57.9  | 56.0  | 58.0  | 2779            | 2551 | 2619 | 2748 |
| 6     | 62              | 62.0  | 62.3  | 63.6  | 2673            | 2567 | 2568 | 2728 |
| 9     | 62.21           | 61.4  | 61.0  | 63.8  | 2644            | 2466 | 2535 | 2713 |
| 12    | 65.16           | 63.4  | 61.7  | 65.8  | 2637            | 2488 | 2480 | 2723 |
| 24    | 77.07           | 76.0  | 75.6  | 78.8  | 2608            | 2455 | 2485 | 2722 |
| 48    | 94.56           | 89.0  | 84.4  | 89.2  | 2622            | 2493 | 2526 | 2665 |
| 72    | 102.3           | 104.3 | 100.1 | 105.2 | 2615            | 2455 | 2545 | 2705 |
| 96    | 119             | 123.7 | 122.1 | 127.5 | 2575            | 2466 | 2479 | 2722 |
| 120   | 151             | 141.8 | 145.0 | 167.2 | 2491            | 2434 | 2467 | 2535 |

Thus "stressed" analytical elements can be identified on the bases of measurement data which were obtained during the so-called OBC prepare phase (cf. example 7) as well as during the so-called OBC test phase (cf. example 7).

As shown in table 6, the clotting times are also much more stable under these conditions and hence stressed test strips can be reliably detected by the OBC before false coagulation measurement values would have possibly been generated.

**Table 6**

**Effects of stress at 30°C and 70 % relative air humidity on the measurement of clotting time**

| <b>Stress time (h)</b> | <b>Clotting time (s)</b> |
|------------------------|--------------------------|
| 0                      | 11.66                    |
| 2                      | 11.94                    |
| 6                      | 12.28                    |
| 9                      | 12.14                    |
| 12                     | 12.27                    |
| 24                     | 11.82                    |
| 48                     | 11.88                    |
| 72                     | 11.66                    |
| 96                     | 11.63                    |
| 120                    | 11.67                    |

**Example 9: Glycine as a specific redox partner for resazurin**

If test elements which contain the formulation from example 6 are stored for 48 hours at 25°C, the ratio of resazurin to resorufin changes because the former is converted into the latter due to the "OBC reaction". The following table 7 shows that the reaction rate of this reaction is influenced by the presence or absence of glycine. An OBC is also possible in the absence of glycine; however, the sensitivity of the OBC is considerably increased by the presence of glycine.

**Table 7**

**Relative amount of resorufin (%) in relation to the total amount of resorufin and resazurin versus the stress period**

| Stress period (h) | Resorufin percentage (%) |                      |
|-------------------|--------------------------|----------------------|
|                   | Reagent without glycine  | Reagent with glycine |
| 0                 | 5                        | 10                   |
| 0.5               | 6                        | 16                   |
| 1                 | 7                        | 21                   |
| 3                 | 8                        | 27                   |
| 6                 | 8                        | 41                   |
| 9                 | 10                       | 52                   |
| 18                | 12                       | 72                   |
| 24                | 14                       | 89                   |
| 48                | 24                       | 89                   |

### Claims

1. Reagent system for the on-board control of analytical elements containing an organic N-oxide or a nitroso compound.
2. Reagent system as claimed in claim 1, characterized in that it undergoes a change when stressed that can be detected optically or electrochemically.
3. Reagent system as claimed in claim 1 or 2, characterized in that it contains a reducing agent.
4. Reagent system as claimed in claim 3, characterized in that the reducing agent is selected from the group comprising sugars, polyalcohols, glycine, and cysteine-containing proteins.
5. Reagent system as claimed in claim 4, characterized in that the reducing agent is glycine or glucose.
6. Reagent system as claimed in one of the claims 1 to 5, characterized in that the N-oxide is resazurin.
7. Reagent system as claimed in one of the claims 1 to 5, characterized in that the nitroso compound is an optionally substituted p-nitrosoaniline.
8. Use of a reagent system as claimed in one of the claims 1 to 7 for the on-board control of analytical elements.
9. Analytical element containing a reagent system for a detection reaction and a reagent system for an on-board control as claimed in one of the claims 1 to 7.
10. Analytical element as claimed in claim 9, characterized in that the on-board control is integrated into the reagent system for the detection reaction.
11. Analytical element as claimed in claim 9 or 10, characterized in that the reagent system for the detection reaction contains reagents for determining coagulation parameters.

12. Method for the control of analytical elements as claimed in claim 9 to 11, in which the reagent system for the on-board control is examined optically or electrochemically with the aid of a measuring instrument for changes which can indicate a stress of the analytical element.
13. Method as claimed in claim 12, characterized in that analytical elements for which a stress has been detected are not released by the measuring instrument for measurement of a sample liquid with the aid of a reagent system for the detection reaction.
14. Method as claimed in claim 12 or 13, characterized in that the reagents for the control reaction as well as the reagents for the detection reaction are examined optically.
15. Method as claimed in claim 12 or 13, characterized in that the reagents for the control reaction and the reagents for the detection reaction are examined electrochemically.
16. Method as claimed in claim 12 or 13, characterized in that the reagents for the control reaction are examined optically and the reagents for the detection reaction are examined electrochemically.
17. Method as claimed in claim 12 or 13, characterized in that the reagents for the control reaction are examined electrochemically and the reagents for the detection reaction are examined optically.