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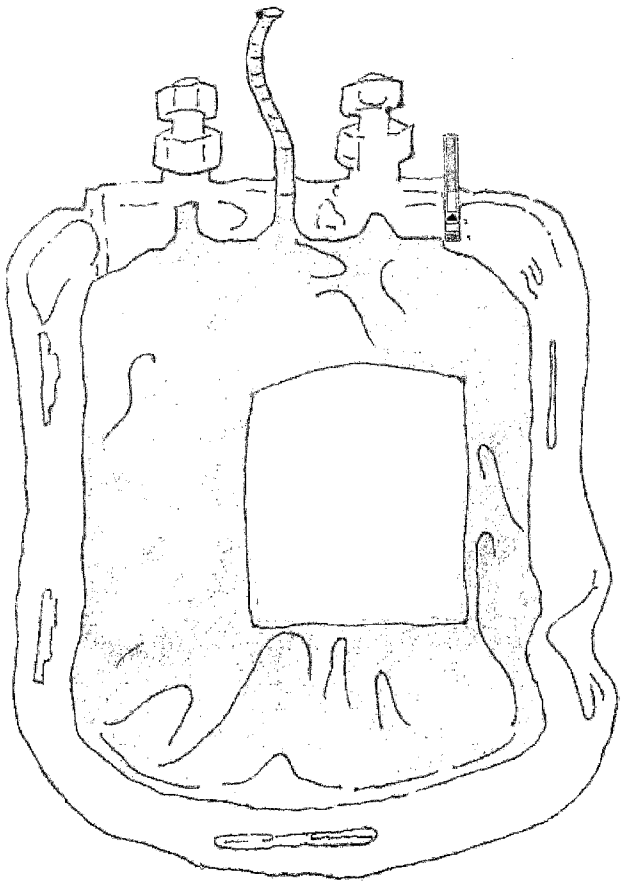

(57) Abstract: A method for quantitatively sensing, using an indicator system based on diffusion in space and time of a reaction front, for determining and reporting the prevailing concentration or exposure history of an analyte in health monitoring for animals including humans.

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TITLE OF THE INVENTION

Health indicator method and device

FIELD OF THE INVENTION

The invention relates to devices and methods for sensing changes in the concentration of an analyte or exposure history of an analyte that participates in a chemical reaction that affects, or indirectly is associated with, the control over health in animals. In particular, the invention relates to the measurement and reporting of respiration levels, homeostasis, disease and health status in animals, including humans. It also relates to the monitoring of the fitness and exertion of animals including humans.

The invention also relates to devices and methods for measuring prevailing and historical levels of the metabolism of micoroorganisms in health management, including monitoring permeable bags of blood products for bacterial contamination. In particular, the invention relates to the measurement and reporting the presence of microorganisms by changes in an indicator that responds to metabolism or respiration of micororganims, by enzymatic spoilage reactions or using immunological response to monitor blood products for contamination by microorganisms. It can also be used to monitor the evolution products from cellular apoptosis, such as from melonoma, a skin cancer.

BACKGROUND OF THE INVENTION

When blood is harvested from the skin of patients, bacteria can be collected with the blood inadvertently. Blood suppliers and health managers are interested to monitor stocks for microbial contaminations that threaten the health of patients. The metabolism of invasive organisms to blood products destined for transfusion into patients, such as bacteria and fungi, can be detected and metered via their consumption substrates or evolution products, or as surrogate molecules that report changes in the chemical environment to report presence and activity of microbial populations. Similarly, changes in the enzymes involved in spoilage process can be used as a detection method, and detector antibodies that form a detector antibody/antigen complex can also be used to report the presence of contaminating micoorganisms in blood products.

Synthetic membranes such as those used for blood bag containers, represent permeable membranes which may be crossed by respiration related molecules such as oxygen and carbon dioxide. The oxygen and carbon dioxide involved in respiration of microorgansims, which permeates these membranes, and the flow through passage-ways, presents an opportunity for the measurement of both equilibrium and cumulative levels of exposure to these molecules.

Wiping contaminated surfaces with a swab composed of the indicator device can detect microorgansims by subsequent placement in a test vessel.

Insertion or swallowing the indicator device can expose the indicator to micoorganisms in the body cavities and the alimentary canals of the digestive systems of animals, whilst placing a skin patch over suspected melanoma can scavenge volatile evolution products from cellular apoptosis.

The respiration status of cells is an important health indicator of homeostasis. In the case of humans and other mammals, the prevailing levels of oxygen and carbon dioxide in blood reflect cardiovascular and cardiopulmonary status. Transcutaneous measurement of oxygen and carbon dioxide has proved valuable in monitoring the health of hospital patients. Cumulative respiration, which can be measured as carbon dioxide evolution or oxygen consumption, is an indication of effort and correlates with energy expended, for example, in weight-watching and fitness monitoring programs of people and racing animals.

Natural membranes such as the skins and dermal layers of animals, as well as synthetic membranes such as those used for blood bag containers, represent permeable membranes which may be crossed by respiration related molecules such as oxygen and carbon dioxide. The oxygen and carbon dioxide in animals, which permeates these membranes, and the flow through passage-ways, presents an opportunity for the measurement of both equilibrium and cumulative levels of exposure to these molecules.

With the scavenging action of a sensing-indicator device, the exposure along a time continuum can be measured in an additive manner and reported to an observer as a moving colour-band. Cumulative evolution of these gases can be measured to indicate the respiratory history of the cells.

To provide meaningful information to assist in the decision making of health managers and the like, it is desirable to have a homeostasis sensor that has the capability to measure and report either prevailing levels of exposure or cumulative exposure history; and to report exposure as progressive change along either a discrete or continuous scale.

When an indicator device is connected by tubing to a bag of blood product, a sample can be extracted from the blood bag for the purposes of detection of microbiological contaminants.

With the scavenging action of a sensing-indicator device, the exposure along a time continuum can be measured in an additive manner and reported to an observer as a colour change.

There are several gas detection technologies incorporated into electronic instruments that employ coloured indicators, usually combined with luminescence, fluorescence, reflectance technologies. These instruments require the manual operation, calibration, and interpretation of trained technicians. Examples of patents that include such instruments include GB2102947, US5094955, WO0077242, WO9627796, US6908746, which can be used to detect spoilage products from bacteria in food and blood, and US2890177, US3068073, US3111610, US3754867, which can be described as gas detectors.

Visual readings are used to interpret values in sample tubes manufactured by Draeger ® and are used by technicians with suction pumping to extract gas samples and expose coloured indicators disposed in a sample tube to the target molecules to obtain a visual measurement by means of a moving coloured band. Similar technology, which manually

samples extracted spoilage gas in food containers and reports the attainment of a predetermined threshold value as a PASS/FAIL test, is disclosed in US 5,653,941.

Other indicators simulate real environments with analogue systems. Classic among these are the time-temperature indicators that report thermal exposure with reactants that share similar activation energy and rate constant as the system being thermally modelled, and the correlations drawn provide inference as to the condition of the real system. More recent indicators have been developed that meter exposure to an analyte directly responsible for changes in an environment. The metering, however is restricted to the attainment of a threshold value, and the communication, consequently, is limited to an ON / OFF or PASS / FAIL reading. Such an indicator is commercialised by Food Quality International for monitoring the quality of meats and fish, and by Ripesense for the ripeness of fruits. The limitation with these devices is that reliance is placed on a change in visible colour spectra to the observer, with reference to a colour chart to determine end-point. No numerical scale is obtainable for interpretation purposes with these devices, and the observer is left to judge colour spectra for the determination, which is problematic with resolution and accuracy.

No invention, however, has claimed application to include a measuring device that uses scavenging action to actively diffuse the target molecules of a chemical reaction responsible for quality changes, or markers associated with changes in the integrity of environments, through engineering structures in a direction that establishes a moving front, in synchrony with changes in the quality of an environment being studied. The present invention uses this moving reaction-front to create a sensor in an instrument that measures and reports either prevailing levels of target molecules (the analyte), or exposure history.

The reading provided by the novel device according to the present invention generates a point along a continuous numerical scale, with no upper limit, and consequently, caters for the demands for hard data in quality assurance for today's medical industry.

Whereas the prevailing level of the analyte provides information as to the acceptability of the analyte's concentration in the environment, the reported cumulative exposure is intended to result from the additive accumulations of reactions that occur with the analyte at various times during the deployment of the device.

Such an instrument, now disclosed, can be deployed in the confines of any closed or partially confined or steady-state condition of a real-environment containing the target molecules, or in a sample stream flowing into or out of such environment, gaseous or liquid, through which target molecules pass. Typical environments of interest to the present invention include chamber-like environments at the skin of animals, created by skin patches and implants, orifices in the body, the gastrointestinal tract, and specimens collected, for example pin-pricks to extract drops of blood for testing and surface wipes.
It would be a useful technological contribution if such technologies could be incorporated into passive indicator systems, *i.e.* systems that do not require human intervention, that run under expert design to meter exposure and report values interpretable by non-expert audiences, not just by technicians.

It would be a useful technological contribution if such technologies could be incorporated into passive indicator systems, *i.e.* systems that do not require human intervention, that run under expert design to meter exposure and report values interpretable by non-expert audiences, not just by technicians. There would be several industrial applications for such passive indicator devices, such as for reporting the vitality of red blood cells, contamination by bacteria of blood products in blood bags, the respiration of sleeping babies, the health of a dairy cow, the daily activity of children and many others.

Correlation schedules are provided in patents WO9837227 and US 6,908,746 for food quality between colour intensity arising from a change in a flat, colour indicator-surface measured against a colour chart based on colour spectra, and fluorescence respectively. No patented or published indicator system correlates movement of a colour-front with another variable in a correlation schedule , which is an objective of the present invention.

While many patents exist to provide intelligence with respect to the quality and expected shelf-life of perishable products, none are able to provide a simple and unambiguously heuristic indication of expected useful storage life or residual quality or meter respiration, and thereby show progress towards expiry of a biological product or report a level of physical activity.

Furthermore unlike all indicators in prior art measuring real environments, there is potentially no upper limit to the quantification of the quality parameter. Calibration is theoretically simpler so reliability and reproducibility should be much greater.

In the present invention, the indicator is restricted to an anisotropic environment and the diffusion of reactants is readily described mathematically enabling quantification of shelf-life and/or residual quality.

The technology is able to take advantage of many inventions and developments in the chemistry of intelligent packaging and related fields, which up to now have had limited commercial success because of their inherent difficulty in providing a quantitative and easily discernable indication of quality or remaining useful life.

A novel feature of the present invention, is a measuring device that uses scavenging action to actively diffuse the target molecules of a chemical reaction responsible for quality changes, or markers associated with changes in the integrity of environments, through engineering structures in a direction that establishes a moving front, in synchrony with changes in the quality of an environment of medical supplies being studied. The present invention uses this moving reaction-front to create a sensor in an instrument that measures and reports at the reaction-front, either prevailing levels of target molecules (the analyte), or exposure history.

The reading provided by the novel device according to the present invention generates a point along a continuous numerical scale, with no upper limit, and consequently, caters for

the demands for statistical data required for international quality assurance in today's health management and fitness industry.

Whereas indicating devices in prior art absorbed analytes onto a flat surface of thickness 100 microns or less, the present invention absorbs analytes along a column of length in excess of 100 microns, with 10 centimetres recorded in some useful applications. Whereas prior art provided a visual reading of a single point on a plot of analyte concentration *vs.* colour change, the present invention generates a regression relationship from the plot of analyte concentration or number of molecules generated of the analyte *vs.* displacement in space of the reaction front along a column, across a disc, or on a tangent through a 3-dimensional object, since any number of readings is achievable from the one sensor. The regression equation with displacement of the reaction front in the indicator in relation to space and time can be used to accurately correlate with the quality in the environment being studied. Everyday people with little technical education and training can undertake such readings, and people can set their own quality standard according to the continuous scale of the present invention.

Whereas the measure of prevailing level of the analyte with the present invention provides information as to the current acceptability of the analyte's concentration in the environment, the capability of the invention to report cumulative exposure results from the additive accumulations of reactions that occur with the analyte at various times during the deployment of the device.

Such an instrument, now disclosed, can be deployed in the confines of any closed or partially confined or steady-state condition of a real-environment containing the target molecules, or in a sample stream flowing into or out of such environment, gaseous or liquid, through which target molecules pass. Typical environments of interest to the present invention include chamber-like environments at the skin of animals, created by skin patches and implants, orifices in the body, the gastrointestinal tract, and specimens collected, for example pin-pricks to extract drops of blood for testing and surface wipes.

SUMMARY OF THE INVENTION

It is an object of the present invention to facilitate communication of quality status to a wide variety of audiences, including hospital pathologists, haematologists, general nursing staff, and those without technical training in the use of more elaborate instrumentation. It is another object to provide a device that passively meters exposure, of that device to an analyte, without manual operation. The communication over the extent of exposure to the analyte can report the attainment of a predetermined level of exposure or progress along an increasing scale of exposure, such as a visual quality scale, of for example, purity and integrity of blood in a blood-bag.

Accordingly, in one aspect, the invention provides a monitoring device using scavenging, and chemical reaction with an analyte, for measuring and reporting respiration levels, progress with metabolism and associated enzymatic reactions, or immunological response in blood products, as a passive and automated indicator of exposure which is incorporated within or affixed to the wall of a blood bag, placed in an separate monitoring compartment, or attached by tubing to a monitoring device that accompanies the blood bag during distribution from the blood bank to the hospital ward.

In another aspect, the invention provides a method for measuring and reporting respiration levels, enzymatic changes, or immunological response from contaminating microorganisms present in blood products, comprising the steps of:

locating a monitoring device on the external or internal surface of a permeable blood bag, within the wall of the blood bag, in a separate compartment of the blood bag through which some blood product is extracted aseptically, or attached by tubing to a separate monitoring device to an analyte associated with metabolism, respiration, enzymatic change or immunological response; and observing the level visually, receiving the electronic communication by radio signal, or other form of transpondence, or retrieving said device to record the level of said analyte.

It is another object of the present invention to facilitate communication of health or quality status through a chemical exposure history to a wide variety of audiences, including

general nursing staff, carers, and those without technical training in the use of more elaborate instrumentation. It is another object to provide a device that passively meters exposure, of that device to an analyte, without manual operation. The communication over the extent of exposure to the analyte can report the progress along an increasing scale of exposure, such as a visual numerical scale of quality, of for example, freshness of blood in a blood-bag, activity in exercising people and animals; transcutaneous measurement of the disease status of humans and animals though secretions from sweat and perspiration glands as disclosed in patent US 2002/0115921 A1, now incorporated in the present invention.

Accordingly, in one aspect, the invention provides a monitoring device using scavenging, and chemical reaction with an analyte, for measuring and reporting respiration levels, homeostasis and health status in animals, including humans, as a passive and automated indicator of exposure which is affixed to the skin, implanted into the skin, placed in an internal body cavity, or swallowed for passage through the digestive system.

The monitoring device may be placed in close contact with the skin of mammals to measure the prevailing level of respiratory gases in order to monitor health status. It may also measure the respiration history in order to monitor metabolic rate and physical exertion.

In another aspect, the invention provides a method for measuring and reporting respiration levels, homeostasis or health status in mammals, comprising the steps of:

locating a monitoring device on the skin of the animal, in an internal body cavity of the animal, or allowing the device to pass through the digestive system of the animal thereby exposing the monitoring device to an analyte associated with respiration, homeostasis or health status; and

observing the level visually, receiving the electronic communication by radio signal, or other form of transpondence, or retrieving said device to record the level of said analyte.

Suitably, the monitoring device otherwise known as an exposure indicator, reports prevailing level in an environment of the body, cumulative exposure to an analyte or target molecule, or as an integrated device reporting both prevailing and cumulative levels with more than one sensing-indicator of the present invention.

The monitoring device may report the concentration of the target molecule in a discrete scale as graduations along a continuum.

Suitably, the monitoring device reports concentration of the target molecule in a continuous scale, either linear or non linear.

The monitoring device may report either prevailing level or cumulative exposure in a readable scale whether by visual colour movement or separation in space possibly measured as the quantum of reflected light within a field of view of an instrument as an increasing or decreasing area of colour or colour intensity arising from an expanding of contracting coloured image, or with the aid of an instrument that measures colour development as wave length or frequency, reflectance, luminescence or fluorescence or other radiative technology, such as a bar-code scanner commonly used at supermarkets, or imaging devices used in digital photography, that result from either an progressively increasing or decreasing coloured area caused by a dynamic reaction front.

In certain embodiments, the exposure indicator indicates changes in concentrations or exposure history of analytes of homeostasis by communicating levels of carbon dioxide or oxygen levels, biological spoilage reactants or products such as carbon dioxide from metabolising bacteria which may be sensed by exposure reagents to pH or oxidation products measured by the device and possibly transponded by radio or other electromagnetic signal to a remote centre of coordination.

The exposure indicator may be deployed as stand-alone instrument for insertion into packages; as an adhesive label or print for deployment on the internal wall of packages, in the wall of a package, or the external wall of permeable packages; as a skin-patch, as a pill

for swallowing, retrievable for visual indication forms, potentially sacrificial for electrical readings; an insertion device into body cavities, or implant for the bodies or tissues of animals, or other means for location within close proximity to generating sources of analytes.

Suitably, the permeable substrate of the monitoring device has one or more chemical indicators disposed therewith which indicate the diffusion of a target molecule into the substrate.

Suitably, the target molecule induces a chemical transformation in the substrate such that the presence of the target molecule within the substrate is indicated. The chemical transformation may be an oxidation – reduction reaction or may an ionisation reaction such as induced by a change in pH. The chemical indicator may therefore be a pH indicator.

The chemico-physical properties of the permeable substrate, such as density and porosity, and/or size of aperture of the intake into the substrate, may be varied to increase or decrease the rate of diffusion of a target molecule through the substrate.

Suitably, the degree of diffusion of the target molecule through the substrate is metered by reaction of the target molecule with the chemical indicator.

In some embodiments, the degree of diffusion reports concentration of the target molecule in a continuous scale of moving linear colour band or moving colour ring.

Suitably, the monitoring device comprises a chamber wherein the substrate is disposed in the chamber, said chamber configured to ensure that the rate of colour change with distance in a continuous scale is achieved by ensuring that the reaction time at the front of the migration proceeds, in step with, the diffusion of the target molecule in the substrate.

The monitoring device may report the prevailing level of a target molecule or cumulative exposure to a target molecule, or as an integrated device it may report both the prevailing level and exposure history.

The monitoring device may be comprised of a reaction front, which is commensurate with the degree of diffusion of the target molecule within the substrate of the indicator device.

The indicating device may confine the indicator reaction front along a continuous scale by disposing the indicator medium in a narrow and elongated tube to confine the diffusion along the indicator in a progression along a plane to the observer.

The monitoring device may confine the indicator reaction front along a continuous scale by disposing the substrate in 2-dimensional form as a thin layered disc, with impermeable upper and lower surface, to confine the diffusion in a progression migrating from the outer edge to the inner centre to the observer, or alternatively, from the centre to the outer edge.

Suitably, the substrate is disposed in a 2-dimensional form such as a triangular shape or alternatively in a 3-dimensional form as wedge, cone or pyramidal form, or other tapered form or other form of variable thickness.

The monitoring device may be made to diffuse further along an increasing non-linear scale by varying the thickness of the substrate which comprises the indicator, along the length of a linear strip as in the case of the thermometer form of the invention to create a wedge; or increasing the thickness along the radian of an arc of a circle present in the disc form of the invention to create a hemispherical or hemi ovular shape in the case of the disc form of the invention. By making the intake end the tapered one, progressive diffusion becomes more non-linear with increasing distance of migration. Alternatively, the diffusion can be made more linear by diffusing from a thick end of the device to a thin one.

The monitoring device may be made to diffuse the analyte in successive layers from the surface toward the core of a sphere.

The monitoring device may report the concentration of a target molecule in a discrete scale by deployment of masking coloured print in stations over the moving colour band so that the arrival of the band at a station is observed by a colour change at the station, or where the colour of the band itself masks the appearance of a print below, and the progressive migration of the colour band alerts the observer to the attainment of new levels of exposure by colour loss in the previously masking band and appearance of the printed message below, previously masked by the indicator in its coloured state.

The monitoring device may report cumulative exposure to a target molecule such as carbon dioxide by the use of reactants within the substrate that produce semi-stable reaction products – reversible with mild heating in the range 50-80^{0}C, or with stable reaction products – reversible only at oven temperatures.

Suitably, the monitoring device reports the prevailing level of a target molecule through reactants – including buffers, deployed with a highly permeable substrate, that produce unstable reaction products at ambient temperatures making the reaction immediately reversible, so as to generate reports of prevailing levels of analytes.

The monitoring device may report either prevailing level or cumulative exposure in a readable scale whether by visual colour movement or separation in space possibly measured as the quantum of reflected light within a field of view of an instrument as an increasing or decreasing area of colour, or as colour spectrum or colour intensity, or with the aid of an instrument that measures colour development as wave length or frequency, reflectance, luminescence or fluorescence or other radiative technology, such as a bar-code scanner at a supermarket, or imaging devices used in digital photography, that result from either an progressively increasing or decreasing coloured area caused by a dynamic reaction front.

The monitoring device may report either prevailing level of cumulative exposure by changes in an electrical signal attached to a digital display or transponded by radiative

technology to a coordination centre and possibly relayed internationally by internet or satellite communications.

The monitoring device is comprised of colouring agents with the indicator substrate, or it may use masking or background layers of colour in order to alter the colour or legibility of the substrate as seen by the observer or by the reading obtained with an electronic scanning instrument.

The mode of communication to target different audiences, with respect to the monitoring device, may be varied in coded communications interpretable by only a targeted recipient class of people, to communicate the exposure of the device to the target molecules.

The monitoring device may be calibrated by: selection of an appropriate chemical reagent to indicate for the presence of a particular target molecule, the concentration of reagent; or rate of diffusion into an indicating medium by varying the permeability of the substrate.

The permeable substrate of the monitoring device may be disposed in micro-spheres in a linear configuration in a tube in order to establish a degree of tortuosity and thereby slow diffusion to ensure that the reaction time at the front proceeds at the diffusion rate, and to calibrate the rate of migration. The micro-spheres may be coated on the surface with reagent-indicator to accelerate the diffusion rate.

The monitoring device may measure cumulative exposure by mixing an indicator reagent with a scavenging reagent.

A protective filtering layer may be disposed over the monitoring device, or within close proximity, to scavenge non-target molecules from the environment being measured and so provide selectivity in the measurement as to target molecules and render the monitoring device solvent-proof.

Suitably, the exposure indicator is used for measuring the metabolism of cells, tissues and organs of animals, homeostasis, respiration and health in animals and human subjects.

Suitably, the exposure indicator is used to measure bacterial contamination in blood products, such as platelets.

Suitably, the exposure indicator is used to measure cumulative respiration in blood products, such as red blood cells and blood platelets.

Suitably, the exposure indicator is used to measure prevailing levels of oxygen and carbon dioxide in the blood by establishing equilibrium with blood vessels below the skin of an animal

Suitably, the exposure indicator is used to measure blood glucose in diabetes patients when the skin is pricked and blood is released onto the skin.

Suitably, the exposure indicator is used to measure exposure to markers of disease perfused through skin and sweat glands.

Suitably, the exposure indicator is used to measure exposure to alcohol perfused through skin.

Suitably, the exposure indicator is used to measure exposure to carbon dioxide perfused through skin as a measure of physical exertion.

Suitably, the exposure indicator is used to measure exposure to oxygen and carbon dioxide perfused through skin as a measure of cardiopulmonary status.

Suitably, the exposure indicator is used to monitor the respiration history of animals in a live export trade from harvest to sale, such as marine food products sold alive.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described on the basis of non-limiting examples shown in the drawings:

Figure 1: illustrates an aerial view of the moving colour-band indicator;

Figure 2: illustrates a section view of a linear indicator device;

Figure 3: illustrates an indicator device sandwiched to obtain planar diffusion;

Figure 4: illustrates an aerial view of a disc form of an indicator that applies planar migration during operation;

Figure 5: illustrates an indicator device in a tapered form such as a wedge, pyramid, cone or other tapered shape, so that colour change will progress with increasing exposure from the fine tip to the thick base;

Figure 6: illustrates an electrical device disposed as a sphere

Figure 7: illustrates a moving colour band migrating from left to right communicating coded communication to a target audience;

Figure 8 illustrates a monitoring device applied to the skin of a person;

Figure 9a details a monitoring device disposed over the skin of a person metering carbon dioxide in a section view;

Figure 10a illustrates a monitoring device placed over a blood bag,

Figure 10b illustrates a monitoring device placed in a monitoring compartment of a blood bag,

Figure 11 illustrates a how the invention correlates changes in a health parameter with the displacement of a moving colour-front resulting from an analyte diffusing along the indicator device.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Two types of measurement are possible in the present invention: the prevailing level and cumulative exposure. The first measures the level of an analyte recorded at the time of measurement, whilst the second meters accumulated units of exposure in an additive manner and reports the history of exposure. In the case of cumulative exposure, the reporting can be the attainment of some predetermined threshold value, or progressive metering along either a discrete and graduated scale, or along a continuous scale.

The monitoring device is typically comprised of an inert carrier medium, which may be composed of an inert water soluble carbonaceous polymer such as polyvinylalcohol. In order to ensure an aqueous chemical reaction, the carbon polymer may be polyvinylalcohol, polyvinylpyrrolidone or some other water-soluble polymer, or other transparent or translucent packaging material used in food and biological product distribution.

Plasticisers to establish a required permeation rate though the carrier medium may include propylene glycol, tetra methylene glycol, penta-methylene glycol or any glycol or polyhydroxyl material.

Exemplary pH indicators for reporting acid vapour presence or absence as colour change may be phenolphthalein, universal indicator, or other indicators changing colour around pH 8.0-10.0 range, or any other pH indicator, or combinations of different indicators to widen the colour possibilities or combinations of different indicators to widen the colour possibilities; and may be first dissolved in alcohol, or an appropriate polymeric solution.

The alkaline scavenging material may be potassium carbonate, sodium carbonate, calcium carbonate, or other carbonate of a strong organic or inorganic cation or an hydroxides or oxide of other strong organic or inorganic cations that is water-soluble; or any alkaline material. Examples include carbonates, hydroxides, or oxides of alkali metals or strong organic bases, which undergo a neutralisation process with acid vapours.

The acidic scavenging material may be acetic, tartaric acid, citric acid, and other weak organic acids.

pH buffers may be a carbonate or phosphate based one, an amino acid to undergo carbo-amino reaction, or any buffer to resist pH change.

Reagents that indicate the presence of ethylene include potassium permanganate, (colour change from purple to colourless or brown) and tetrazine derivatives (colour change from violet to colourless).

Reagents that indicate the presence of oxygen include leucomethylene blue, which can be considered a classic example for scavenging and indicating, together with many other leucodyes. The ones most similar to leucoMB [leuco thionine dyes] are generally colourless and oxidised to blue, green or violet dyes in the presence of oxygen. Another indicator dye is rubrene, bright orange in colour, which becomes colourless in the presence of both light and oxygen.

Barrier films to impede gaseous migration into indicator below may be composed of thin permeable plastic films such as polyolefins or polyvinylchloride.

Examples of water-proofing material and material that stop migration of reagents from the indicator device to food, whilst permitting gases such as carbon dioxide to permeate quickly include silanes like silicone.

Selective permeation of the target molecules such as carbon dioxide can be achieved by coating the carrier medium of the indicator with an encasing material like silicone or polyethylene.

Examples of suitable indicators, polymers and other appropriate reactive chemistries are disclosed in WO9209870 and extract is made of these disclosures.

A large number of reactions are associated with colour changes. In each type of colour changing reaction there are several classes of compounds and each such class has several compounds which undergo a colour change. Below are some type of reactions and classes of compounds, which can be used as indicators and activators in the invention device.

Colour changing reactions and indicators are used for detection and monitoring of organic, inorganic and organometallic compounds. Such colour changing reactions and compounds are listed in a large number of books, reviews and publications, including those listed in the following references: Justus G. Kirchner, "Detection of colourless compounds", Thin Layer Chromatography, John Wiley & Sons, New York, 1976; E. Jungreis and L. Ben. Dor., "Organic Spot Test Analysis", Comprehensive Analytical Chemistry, Vol. X, 1980; B.S. Furniss, A.J. Hannaford, V. Rogers, P.W. Smith and A.R. Tatchell, Vogel's Textbook of Practical Organic Chemistry, Longman London and New York, p. 1063-1087, 1986; Nicholas D. Cheronis, Techniques of Organic Chemistry, Micro and Semimicm Methods, Interscience Publishers, Inc. NewYork, 1954, Vol. VI,p. 447-478; Henry Freiser, Treatise on Analytical Chemistry, John Wiley and Sons, New York-Chinchester-Brisbane-Toronto- Singapore, 1983, Vol.3,- p.397-568; Indicators, E. Bishop (Ed.), Pergamon Press, Oxford, U.K., 1972. These reactions and compounds can be used in the monitoring devices to record exposure history.

Oxidising agents can oxidise reduced dyes and introduce a colour change. Similarly, reducing agents can reduce oxidised dyes and introduce a colour change. For example, ammonium persulfate can oxidise colourless leucocrystal violet to violet coloured crystal violet. Reducing agents such as sodium sulfite can reduce crystal violet to leucocrystal violet. Thus oxidising and reducing agents can be used as indicator reagents. Representative common oxidants (oxidising agents) include: ammonium persulfate, potassium permanganate, potassium dichromate, potassium chlorate, potassium bromate, potassium iodate, sodium hypochlorite, nitric acid, chlorine, bromine, iodine, cerium(IV) sulfate, iron(III) chloride, hydrogen peroxide, manganese dioxide, sodium bismuthate, sodium peroxide, and oxygen. Representative common reducing agents include: Sodium sulfite, sodium arsenate, sodium thiosulfate, sulphurous acid, sodium thiosulphate, hydrogen sulfide, hydrogen iodide, stannous chloride, certain metals e.g. zinc, hydrogen, ferrous(II) sulfate or any iron(II) salt, titanium(II) sulphate, tin(II) chloride and oxalic acid.

Acid-base reactions are colourless, but can be monitored with pH sensitive dyes. For example, bromophenol blue when exposed to a base such as sodium hydroxide turns blue.

When blue-coloured bromophenol blue is exposed to acids such as acetic acid it will undergo a series of colour changes such as blue to green to green-yellow to yellow. Thus, acids and bases can be used in conjunction with pH dependent dyes as indicators systems. The following are representative examples of dyes that can be used for detection of bases: Acid Blue 92; Acid Red 1, Acid Red 88, Acid Red 151, Alizarin yellow R, Alizarin red %, Acid violet 7, Azure A, Brilliant yellow, Brilliant Green, Brilliant Blue G, Bromocresol purple, Bromo thymol blue, Cresol Red, m-Cresol Purple, o-cresolphthalein complexone, o-Cresolphthalein, Curcumin, Crystal Violet, 1,5 Diphenylcarbazide, Ethyl Red, Ethyl violet, Fast Black K-salt, Indigocarmine, Malachite green base, Malachite green hydrochloride, Malachite green oxalate, Methyl green, Methyl Violet (base), Methylthymol blue, Murexide, Naphtholphthalein, Neutral Red, Nile Blue, alpha-Naphthol-benzein, Pyrocatechol Violet, 4-Phenylazophenol, 1(2Pyridyl-azo)-2-naphthol, 4(2-Pyridylazo) resorcinol Na salt, auinizarin, Quinalidine Red, Thymol Blue, Tetrabromophenol blue, Thionin and Xylenol Orange.

The following are representative examples of dyes that can be used for detection of acids: Acridine orange, Bromocresol green Na salt, Bromocresol purple Na salt, Bromophenol blue Na salt, Congo Red, Cresol Red, Chrysophenine, Chlorophenol Red, 2,6-dichloroindophenol Na salt, Eosin Bluish, Erythrosin B, Malachite green base, Malachite green hydrochloride, Methyl violet base, Murexide, Metanil yellow, Methyl Orange, Methyl violet base, Murexide, Metanil yellow, Methyl Orange, methyl Red Sodium salt, Naphtho-chrome green, Naphthol Green base, Phenol Red,4-Phenylazo-aniline, Rose Bengal, Resazurin and 2,2'4,4' ,4"-Pentamethoxytriphenylmethanol.

Organic chemicals can be detected by the presence of their functional groups. Organic functional group tests are well known and have been developed for the detection of most organic functional groups, and can be used as the basis for the indicator-activator combination. For example, ceric nitrate undergoes a yellow to red colour change when it reacts with an organic compound having aliphatic alcohol (-OH) as functional group. Organic compounds having one or more of the following representative functional groups can be used in the device as activators: alcohols, aldehydes, allyl compounds, amides,

amines1 amino acids, anydrides, azo compounds, carbonyl compounds, carboxylic acids, esters, ethoxy, hydrazines, hydroxamic acids1 imides, ketones, nitrates, nitro compounds, oximes, phenols, phenol esters, sulfinic acids, sulfonamides, sulfones, sulfonic acids, and thiols. There are thousands of compounds under each functional group class listed above. For example, the following is a representative list of aminoacids that can be used as activators in the device: alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, hydroxylysine, lysine, methionine, phenylalanine, serine, tryptophan, tyrosine, alpha-aminoadipic acid, alpha, gamma-diaminobutyric acid, ornithine and sarcosine. All alpha-amino acids undergo a colourless to purple-violet colour when reacted with ninhydrin. In addition, the following are some specific amino acid tests: 1) Diazonium salts couple with aromatic rings of tyrosine and histidine residues to produce coloured compounds. 2) Dimethylaminobenzaldehyde condenses with the indole ring of tryptophan under acid conditions to form coloured products. 3) alpha Naphthol and hypochlorite react with guanidine functions (arginine) to give red products. The following is a representative list of alpha-amino acids that can be used as solid amines: Lysine, hydroxylysine, alpha, gamma- diaminobutyric acid and ornithine. The following are some further selected examples of organic compounds that undergo a colour change in the presence of a functional group test reagent: Primary, secondary and tertiary aliphatic and aromatic amino bases can be detected with 2,4-dinitro chlorobenzene. The observed colour change is from colourless to yellow-brown. Aliphatic amines, primary aromatic amines, secondary aromatic amines and amino acids react with furfural in glacial acetic acid to give violet Schiff bases. A variety of triphenylmethane dyes react with sulfurous acid to produce a colourless leucosulfonic acid derivative. When this derivative is allowed to react with an aliphatic or aromatic aldehyde, coloured products are obtained. Fuchsin, decolourised with sulfite when exposed to aliphatic and aromatic aldehydes, gives a violet blue colour. Malachite green, decolourised with sulfite when exposed to aliphatic and aromatic aldehydes, gives a green colour.

A large number of reactions are associated with a change in fluorescence rather than a colour change in the visible region. Several fluorescent indicators are known (Vogel's Textbook of Quantitative Inorganic Analysis, Fourth Edition, Longman, p. 776.).

The device and its modifications are not limited to chemical indicator combinations, which are associated with chemical reactions for producing a colour change. Also included are any two or more compounds, which can undergo a noticeable or measurable physical change, which can be monitored by appropriate analytical equipment. Such changes include particle size, transparency, electric conductivity, magnetism and dissolution. For example, a change in conductivity can be monitored by an electrometer." (WO9209870).

The range of measurements and communication combinations possible with passive sensing-indicators in the present is articulated in Table 1.

Table 1 – Range or metering possibilities

Measure monitored	**Measurement taken**	**Visual communication**	**Electronic communication**
Prevailing level of an analyte OR – Cumulative exposure (exposure history)	Exposure as one-dimensional diffusion comprising a moving colour-band along a linear strip	Visual reading by a sensor showing moving colour change	Instrument reading of a sensor's colorimetry as wavelength, frequency, reflectance, luminescence, fluorescence, or quanta of light reflected over space in a field of view resulting from scavenging and reaction with an analyte in a moving band, and passed to the observer by electrical current, potential difference or resistance; potentially communicated by radio or other communications signal from remote location to a centre of coordination and relayed further by telecommunications. Instrument reading of the changed electrical conductance, resistance, or potential difference within an electrical circuit, possibly printed, due to a changed electrical property of a sensor that scavenges and reacts with target molecules in a moving reaction front along an electrode configured as a strip, column or spherical sensor, potentially communicated by radio signal from remote location to a centre of coordination and relayed further by telecommunications.

	Exposure as planar diffusion comprising an expanding or contracting concentric colour-ring	Visual reading by a sensor showing moving colour change	As above
	Exposure as an increasing non-linear measure into a 3-dimensional shape such as a wedge	Visual reading by a sensor showing moving colour change	As above

Table 1 shows combinations and permutations as follows:

Measurement taken X Level reported X Communication mode

The use of the appearance or disappearance of colour, as can be obtained with phenolphthalein composition in a carbon dioxide indicator, is a favoured method, as there is no wavelength change as the reaction proceeds, but an absorbance change occurs, which provides greater accuracy in visual detection and interpretation of the progress in metering.

In Table 1 it can be seen that the prevailing level of an analyte or the cumulative exposure to an analyte can be monitored and reported with an automated and passive device according to the present invention. It is also possible to combine both applications into the one device in order to report both prevailing and cumulative levels simultaneously.

In the present invention, prevailing concentrations and cumulative exposure to acid-base, or oxidation-reduction reactants or products are metered in six ways.

In the first, the saturation of colour intensity according to Beer's Law is used to meter levels, by relating colour intensity to the concentration of reaction products formed in the sensing-indicator. This may be undertaken with the ability of the naked eye to discriminate between the development of colour intensity as the analyte progressively diffuses as a migration front into the sensing-indicator and the consequent reaction proceeds. The resulting colour intensity is proportional to the concentration of a prevailing

molecule, or mass of reaction products in the case of cumulative exposure, and hence the exposure history.

This form of the present invention is best viewed in the same plane as the migration of the reaction front into deeper layers of reagents, and may involve an instrument capable of measuring the strength of signal or wave length or frequency, from colorimetry, reflectance, luminescence or fluorescence.

In the second, the rate of reaction according to Fick's law is used to meter levels by relating the level of the analyte to the rate of colour movement and/or distance of colour movement along a reaction front established by the special architecture of the sensing-indicator device, that confines the diffusion in a line or a plane. This form of the present invention is best viewed in the perpendicular plane to the migration of the reaction front.

To illustrate the second form, if the substance(s) of a detector film is sealed over its upper and lower surfaces by a barrier film, with its edges exposed, the access of an active analyte-reagent can be restricted to the edges of a laminate. A colour fringe moves from the exposed edge or area, the distance of colour migration being proportional to the time squared in accordance with Fick's Law. Thus if 1 mm of colour migration is apparent in one day, 1.4 mm will appear in two days, under exposure of a constant concentration of target molecules. The same indicator film only needs to be calibrated once for any particular application.

A sensing-indicator of the second from can alternatively be obtained by sealing all edges of a thin disc of the sensing-indicator described above, but now sealed at the edge, and later puncturing its middle so that the migration of colour change is from the centre to the edge. Sealing an elongated linear strip and exposing one end to an analyte can create a similar effect for a linear colour migration. This second form of the present invention is illustrative of metering along a continuous scale for visual readings by persons untrained in the intricacies of elaborate instruments, for example handlers of medical supplies being monitored during storage, transport, distribution, sale and usage.

In a third form of the invention, indication of a change in the electrical conductance, potential difference, or resistance of the sensor of the present invention can be detected.

In a fourth form, the change in coloured area of the indicator device described in the first, second and third forms is imaged using a light emitting diode and a light absorbing diode to an electrical circuit.

The third and fourth forms may be integrated into communications technologies that transpond signals using radio frequency or other electromagnetic waves to remote centres, and in this way the present invention can be monitored remotely of the monitoring station across the globe.

When powered by a detached power source, such as a battery or solar cell, the electrical reading may be conveyed by radio frequency identification devices now available as printed circuitry on food packages. The signal can be communicated by a transponder of radio signals to a remote centre. There are technologies available in industry for such communication. Inclusive amongst these are Radio Frequency Identification (RFID) tags for packages during distribution, and GSM-based General Packet Radio Service (GPRS); and a description of a container sensor unit that takes readings of temperature and reports them to a base station unit on board a ship for relay by satellite link for viewing over the internet by interested parties is provided by Morris *et al.* (2003). Whereas these commonly report temperature measured by a thermister sensor, the migrating reaction-front sensor of the present invention can be similarly linked with such circuitry.

Applications of the present invention to report current status will generally involve reporting rising or falling concentrations of a target molecule within semi-confined spaces.

The level of carbon dioxide within fresh produce packages is reported on a discrete scale with a plurality of individual sensors in patent EP0627363. The objective of the present invention, in contrast, is to adapt one single sensor to generate multiple readings along a continuous scale.

Reporting the prevailing status of chemical equilibria

A meter can be manufactured that reports the prevailing level of the target molecules in an environment by using reversible reactions, such as mixing a buffer with an indicator and a calibrating reagent in an indicating medium.

In the present invention of a moving reaction-front, a rapid response to environmental change is obtained by ensuring a high degree of permeability in the device to forward and backward diffusion of target molecules along a column or a plane, as reactants inputted into or products evolved from, a chemical reaction of dynamic equilibrium within the sensing medium. This way a rapid adjustment is achieved to the new level within the instrument in response to small changes in the concentration of target molecules in the outside environment, and is reported in a timely manner. The effect may be obtained by the use of a capillary-tube like environment and limited filling of a tube with material to create tortuosity.

High permeability in the indicator medium may be achieved selecting permeable materials for indicator composition and by abutting porous micro-spheres of high volume to mass ratio as an indicating medium in the confines of an elongated vessel; or manufacturing an indicator medium using crystalisation, plasticisation, perforation, polymer expansion, or other means known in the polymer-manufacturing industry to produce enhanced permeability or porosity.

Sensitivity enhancement

A first method to enhance the sensitivity of the device in detecting small pH changes to an analyte, pH buffers may be used. The buffers should desirably have a pK value close to the pK range of the typified environment being measured and produce a substantial colour change in response to very small changes in the analyte. To illustrate with carbon dioxide metering, enhanced sensitivity may be achieved by the use of amino acids or borate as buffers. The carboamino reaction may be adjusted with combinations of amino acid reactants like lysine or glycine, with or without borate. Desirably, pH buffers should have a pK value close to the pK range of the typified environment being measured and produce

a substantial colour change in response to very small changes in hydrogen concentration. Similar methods may be used to measure small changes in oxidation status with, for example, oxygen metering or other gases or liquids of interest.

A second method uses the scavenging action of an indicator to enhance sensitivity of the metering device. When low prevailing levels of a targeted chemical ion are measured, the response to a sensor based upon reversible reactions can be poor, as the low level is beyond the sensitivity range of the instrument. By scavenging low levels of target molecules into a sensor that accumulates molecules in an additive manner, detectable readings may be exhibited in a colour-changing trend.

Reversibility of indicator devices

The form of the invention that reports cumulative exposure can be manufactured with reagents that are either relatively semi-stable or stable at normal operating temperatures. A recharge capability can be obtained for the device if reagents are chosen that will form semi-stable reaction products within an operating temperature range of approximately 0-60^{0}C, but will reverse within a temperature range of approximately 60-80^{0}C that can be imposed on the device to reverse the reaction by mild heating to recharge it back to the zero value. One such reagent, which fulfils this requirement, is potassium carbonate, a reagent that can be used to measure exposure to acid vapours.

A related application can be applied to the problem with alkaline scavenging reagents used to measure exposure to acidic analytes during manufacture and storage, as they are reactive with carbon dioxide present in the atmosphere, and may be triggered to work prematurely. During manufacture of polymer packaging films, it is desirable to purge carbon dioxide absorbed during storage and handling with mild heating for example by passing film through an oven environment. The reporting device may be commissioned by mild heating to approximately 60-80^{0}C prior to packing the product, to bring the reported measurement back to zero or close to it.

In accordance with this inventive principle, reversibility in metering alkaline exposure may be achieved by heating acidic scavenging reagents such as acetic and tartaric acid, although the temperature range to achieve a reversal may differ.

In application, the recharge capability may be utilized in the manufacture of a rechargeable instrument to measure exposure to target molecules. The instrument could be re-charged by heating it at temperatures above room temperature, but below a temperature which will detrimentally affect the chemical composition of the reagents or the melting point of materials used in its manufacture.

Coded communication to different classes of recipients, commensurate with levels of exposure

In the management of quality, blood suppliers may wish to restrict the information about the incidence of contamination to those medical specialists immediately involved in blood distribution, and not to have that information shared with transport companies and the like.

In the present invention, the metering can be achieved by deployments that target communications at different audiences, wherein some interested parties are alerted in an early-warning, when the level of exposure is low, whilst others in a disparate class of recipients receive the communication when the reaction has progressed to an advanced stage, when the level of exposure is higher.

This may combine various modes of metering disclosed in the following section on colour possibilities. The coded message may be received by blood-supply staff or quality-control staff using special instrumentation, such as a bar-code scanner and take the form of a missing or additional bar-code using indicators that appear or disappear. It may also be a measurement taken, such as colour intensity or quantum reflected over a given space into a measuring instrument.

Extension to the possibilities within the colour range of the sensing-indicator device

Indicators can be mixed to provide an expanded spectrum of colour change to choose from, for example changes from acid to neutral and onto alkaline environments are widely

reported in chemical technology with universal indicator. The resulting colour changes can be correlated with varying levels of exposure to achieve a scale.

One method according to the present invention, to convert a single colour indicator to another, for example from pink to black, as with an application where an electronic bar-code scanning is required in the distribution of perishable, packaged chopped and diced vegetables' to a retail store, is to contrast it against a green coloured transparent layer placed above or green coloured background material below it. Upon exposure, if the colour change in the indicator is from pink to colour-less, then the effect of the green contrast layer is to alter the colour change to one where black turns to green.

Alternatively, the indicator may be mixed with a colouring reagent that does not participate in the exposure reaction, which will convert the colour change into one more desirable for communication purposes.

Humididty control

Many chemical reactions that result in an indicator changing colour depend upon the presence of water for colour change to occur; this dependence can involve the processes of migration of the target molecules into the indicating medium, solubilisation and ionization. Efficacious indicating materials therefore are selected for affinity with water for such applications and a humectant may be mixed with the sensing-indicator. A problem exists under humid operating conditions, as moisture uptake can cause the reaction front to be dissipated and the measure to be lost. This effect can be controlled by either adjusting the concentration of the humectant, or establishing a selective permeation of the target molecules through an encasing material like silicone or polyethylene which will limit moisture migration into the sensing-indicator, or by selecting plasticisers for indicator composition that prevent excessive moisture uptake, or by deploying with the indicator various salts that are known to regulate humidity within a particular range, or a combination of these methods.

Range of analytes and selectivity in sensing

It is possible that the invention could be used to measure acid or alkaline analytes, or oxidation or reduction analytes. This form of the invention is therefore versatile across a range of applications that are indicative of animal health.

Bagged blood is a sensitive material to ionic disturbance, and ionic leakage and migration into the sensing material through the wall of the blood bag is to be avoided, otherwise blood quality and safety may be impaired. Selective transmission of non-ionic molecules would be advantageous, and this can be achieved by a separation layer that is selective in transmission, for example it may be composed of a silane like silicone that transmits only non-charged molecules like carbon dioxide.

Another method is to select a polymer layer as a membrane between the blood and the sensor with micropores of diameters sufficiently narrow to permit diffusion of smaller target molecules, whilst excluding larger non-target molecules.

Still another method is to use filtering layers or scrubbers to remove confusing molecules from the sampling stream between the generating source and the indicating device. An example is where molecules are present of confusing, opposing chemical species to the crude measures of pH or oxidation state. An illustration is where volatile bases present in deodorants are present on skin whilst carbon dioxide from bacterial metabolism is being measured with an alkali mixed with an indicator. Deployment of filtering layers or scrubbers should remove confusing molecules of the deodorant from the sample stream.

Calibration

To relate readings to prevailing concentrations or cumulative exposure, it is important to calibrate the indicator response to exposure. In some applications, when measuring exposure to low concentrations for short periods of time, a high degree of sensitivity will be required, for example where indicators are used to report prevailing levels in blood platelets contaminated with lower counts of bacteria. To the contrary, for monitoring respiration from both a large mass of animal cells with a high respiration rate over a long

period, together with a high population of bacteria, the scavenging device will be exposed to high levels of respiration.

A method for detection of low prevailing levels is to set a small differential between the indicator and the target level, and to use buffers known in science to resist only a small change in pH, so that minor changes in chemical equilibria will trigger a response in the sensor.

One method to calibrate between high and low exposures is by metering a proportion of the molecules generated by a chemical process, rather than all molecules. This can be achieved by restricting access to the sensing-indicator by narrowing access pores or creating tortuous access routes in apertures between the source of generation of the target molecules and the sensing-indicator device.

Variable permeability of the sensing-indicator material and that of encasing material such as barrier film, by material selection or varying plasticiser composition or the degree of crystallisation in manufacture, can be similarly used to calibrate response to exposure. Perforations can also be used to increase the surface area exposed to target molecules, relative to the volume of indicator, to accentuate colour change in certain regions of the indicator and so refine interpretations of the level of exposure attained.

Another calibration method is to vary the reaction rate with buffers, whilst another alternative is to deploy varying doses of reagent and indicator, and to vary the reagent / indicator ratio, that will react with the target molecules until the desired equilibrium is reached and colour change will occur.

Still another, is to vary the thickness of the indicator to alter the effect of the reaction on change in the indicator as visible colour observed by the naked eye, or as colour measured by an electronic instrument. With increasing thickness of the indicator material, whether disposed in a tube or a film, progressive migration of target molecules through successive layers results in a migration of the reaction front toward unreacted colour reagent. When

viewed at the perpendicular to a film indicator, increasing thickness will enhance the sensitivity of the exposure-indicating meter as a useful instrument to higher exposures, since the colour intensity will be lost at a slower rate with increasing exposure. When viewed in the same direction as the migration front, as in a tubular disposition of the device, providing an interpretation as a band-reading like that provided by a conventional thermometer, the longer the tube or strip of film, the greater the scale provided for metering exposure.

The rate of migration of the reaction front, the velocity, can be used as a calibration method for interpretation purposes with application of the time dimension. The rate of progress in the development or loss of colour intensity as the front moves away from the observation post at an angle of 90^0 into deeper layers of the indicator can be used as a calibration method. Alternatively, calibration may be obtained from the rate of linear migration of a colour-band in the same direction as the observation post of linear colour-band devices, or radian migration in the case of colour-ring devices. In the case of electrical measurement of changes in the scavenging sensor, the gain or loss in time of an electrical property such as current, due to the migration of the reaction front, may be calibrated with changes in the surrounding environment.

The extent of migration of the reaction front, a measure of distance can also be used to meter exposure and obtain calibration against levels of exposure.

In the case of colour readings, application of the time dimension can be used as a calibration method, as either the rate of progress in the development of a colour change, or the rate of colour migration in colour-band devices. In the case of electrical measurement of changes in the scavenging sensor, the gain or loss in time of an electrical property such as current may be calibrated with changes in the surrounding environment.

The above calibration methods can be used solely or in combination to meter exposure to target molecules.

Scales for the interpretation of exposure levels

As outlined above, there are two types of scale that the cumulative exposure indicator can be measured by, a discrete and a continuous one.

One form is the progressive exposure and reaction of target molecules with a reagent to form products in a continuous scale to indicate the degree of deterioration in quality, and again calibration of the device is important.

Metering can be communicated in a continuous scale by confining diffusion of the reaction in one dimension, and can be calibrated according to exposure by adjusting the velocity of the reaction front according to the methods disclosed in this invention. One such method confines one-dimensional diffusion in an elongated vessel, permeable or porous at one end, as shown in **Figure 1.** Referring to **Figure 1**, it can be seen that a strip of printed indicator, or indicator film, or fluid-filled cylinder with indicator gel is disposed linearly (1) and is covered by a barrier layer (2) to confine diffusion in one dimension. The one-dimensional progression communicates metered exposure visually, reflectantly, luminescently, fluorescently; is scanned or otherwise imaged to reveal colour intensity arising from an increasing or decreasing area of coloured surface using any radiation technology. The device is commissioned by removal of a sealing layer (3), for example with scissors or peeling away a barrier film or puncturing action or releasing a blister or any means known in the packaging industry to remove a seal, and a linear or non-linear scale printed along the linear progression in colour (4), provides a reading and facilitates interpretation. The figure shows linear progression in colour change to Level 2 out of 4 levels on the scale as a result of exposure.

Figure 2 shows a view in section to illustrate how the diffusion is confined linearly in space with a narrow strip of indicator-film (1) sealed with encasing material, in this form by two laminates, which may similarly be achieved with tubes filled with gel indicator.

A second method uses planar diffusion in two dimensions from the edge of a film toward the centre, as shown in **Figure 3.** Referring to **Figure 3**, it can be seen that a disc of

indicator print or film (1), is covered by barrier layers like a sandwich, (2) to confine diffusion in a plane migrating from the edge toward the centre, and the progression communicates metered exposure visually, reflectantly, luminescently, or fluórescently; or by imaging technology.

An aerial view is illustrated in **Figure 4** of the disc form that applied planar migration during operation. Referring to Figure 4, it can be seen that a linear or non-linear scale is printed as concentric circles along the radial progression in colour onto the upper sealing layer. Colour migrates in this form from the edge towards the centre, because an edging seal is broken and exposure drives the reaction toward the centre. Colour change at each concentric circle represents an increasing level of exposure according to a scale of interpretation calibrated for the particular industrial application. In Figure 4, it can be seen that colour changes from coloured to colour-less with increasing exposure, from the edge toward the centre. It can be seen that exposure to target molecules has moved the colour change from the outer edge toward the centre by one level on the printed scale. The device can alternatively be sealed and a hole punched in its middle for the migration of colour change to radiate from a central position.

Figure 5 shows a third form that shapes the indicator into the tapered form of a wedge, pyramid, cone or other three dimensional shape so that colour change will progress with increasing exposure from the fine tip to the thick base. Referring to Figure 5, it can be seen that exposure has moved the front of the colour change, from the thin end of the wedge toward the thick base, to level 2 on the interpretation scale.

The progression of colour-band migration in the above embodiments can be made to communicate metered exposure visually, luminescently, fluorescently, reflectantly, or using imaging technology.

Figure 6 shows a fourth form that uses a moving reaction-front to meter exposure to an analyte electrically. Electrical connection is made at the core of the sphere (1) with one electrical charge, and at the surface (2) with the opposing charge. The device is composed

of reagents that scavenge, react with, and by virtue of the configuration of the device to confine diffusion, establish a moving reaction-front from the peripheral edge of the sphere towards the core. When disposed in a chamber environment, or sampling stream or atmosphere, the electrical property of the sphere changes in accordance with exposure to the analyte being monitored, as the reaction front moves in a radian from the surface, into crust, on through the mantle and eventually toward the central core of the sensor; taking the layers of the earth as an analogy.

One method to achieve an acceleration or deceleration whilst the colour band migrates on its journey from the intake position to the terminus, is to provide a further port of entry to the analyte at stations along the line in addition to the intake aperture. This may be achieved at stations along the line of colour migration by reducing the thickness of barrier film at that section of line, or the layers of barrier film, or the permeability of barrier film, including perforations or incisions made though the barrier film. Another is to join various separate lines of indicator into a continuous one; the composition of each section may vary in respect of permeability, doses of reagent, selection of buffer or levels of buffering.

In some industrial applications, a combination of readings in continuous and discrete scales may be required. An example of the use of coded communications directed at disparate parties is the distribution chain for health products to indicate the degree of exposure from increasing deterioration in quality of, for example blood products. This can be achieved by a special adaptation of the moving colour-band device to modify the continuous scale into a graduated scale.

The moving colour band can be modified to produce a graduated scale by the use of masking over sections of the line of moving colour band or the printing of alpha-numeric text or symbols under the band of indicator. The objective is to progressively mask or reveal colour change along a line of colour diffusion.

By way of example, a continuous scale of the moving colour-band is made to produce a graduated scale and codified reports to various parties in the distribution of blood about the

respiration history. In **Figure** 7 it is shown how this can be achieved, and in this illustration, the moving colour band migrates from left to right. The device uses purple masking as a layer in sections over the purple colour band below. If an analogy is drawn with a rail-train underground subway, then as the colour-band migrates along the line, it becomes visible like a rail car at stations along a subway.

In another adaptation, if the band of purple indicator overlies purple print below as a ceiling colour and the colour change migrates linearly, then the purple print below will be unveiled by the passing reaction front which turns colourless and the underlying print is made visible to the observer.

This application modifies the continuous scale of the moving colour-band to produce a graduated scale and codified reports to various parties in the distribution of medical and pharmaceutical products about the residual quality. In Figure 7, it can be seen that the moving colour band migrates from left to right. The device uses masking layers, in some applications there are layers over the moving colour band, in others the band of indicator overlies coloured print below. Stages A to E in the progression of the colour band are shown.

Area 1 is a printed colour that masks the progression of the progression of the front of colour change from the observer, the colour change occurs beneath these panels, which overlay the indicator below.

At stage A – The migration of the reaction front whilst under blood-bank inventory has caused no discernible product deterioration.

At Stage B – Still under blood-bank inventory, the migration of the reaction front, causing the Area 2 to change colour from pink to transparent, has now consumed the tolerable change in the indicator for this stage of distribution. The blood should now be removed from storage and should be forwarded by transport to the ordering hospital.

At Stage C - The migration of the reaction front causing the Area 3 to change colour from pink to transparent whilst under handling and transport of the blood has consumed the tolerable change in the indicator for this stage in distribution.

At Stage D - The migration of the reaction front, whilst under hospital storage, has consumed the tolerable change in the indicator, causing the Area 4, one of the 4 bar-codes, to change colour from pink to transparent, communicating a coded message interpretable only by hospital specialists concerned with blood quality, whilst patients are oblivious to the freshness condition.

At Stage E –Area 5 comprises is a coloured masking layer of the indicator overlaying a printed message in ink of the same colour of the indicator. As the reaction front migrates, the colour of the indicator changes from pink to colour-less, and the masking layer disappears, revealing a universal message printed in pink and previously blanketed underneath the formerly pink and now transparent colour band, advising nurses and patients that the blood product is unfit for purpose.

Figure 8 shows a skin-patch according to the present invention in deployment on the inner wrist for monitoring respiration. As shown, with more respiration, increasing amounts of carbon dioxide will be scavenged by the indicator and the colour band will move to reflect the new level.

A detailed view of the adhesive skin-patch is shown in **Figure 9**, which incorporates the invention of Figure 2 under a seal over a material being monitored for homeostasis or parameter associated with animal or human health. Figure 9a is a view in section, whereas Figure 9b is an aerial view. In Figure 9a, the material being monitored (1) might be the skin of a person whose respiration is elevated from exertion. The moving-reaction front sensor of Figure 2 is disposed in Figure 9a as a moving colour band (2) migrating from left to right as shown by the arrow. A barrier film (3) may be an adhesive tape, which can be affixed onto the surface of study material (1), with or without an adhesive (4) to create a chamber environment. To ensure solvent-proofing and selective migration from study

material (1) to the sensor (2), a separating layer (5) may be disposed, which may be composed of covalent material like silanes or polyethylene, microporous material, a chemically filtering layer or other means of ensuring selective diffusion of the targeted analyte.

In Figure 9b, the material being monitored (1), is overlaid by the moving reaction-front sensor (2) described in Figure 2. The device is itself overlaid by barrier film (3), which may be an adhesive patch. The analyte being measured diffuses from material (1) into the chamber established by barrier film (3), is scavenged into sensor (2), the reaction front consequently migrates from left to right and the arrow shows the level of the measure.

Referring to Figures 9a and 9b, it can be seen that the indicator system for monitoring the respiration of a person monitors a real system based on the carbon dioxide evolved from the skin and thereby indicates to the person wearing the patch how much exertion they have achieved as shown by the meter (1), in Figures 9a and 9b. This ability to monitor changes in a real system is a distinct advantage over prior art, since more than serving as a timer, as prior art does, the method monitors changes in a real system more accurately that an inferred system based on simulation studies. Exertion will vary with daily routine and fitness training. As such, this method requires no complex interpretation, as the calibration has been performed for the user by those manufacturing the device.

In Figures 9a and 9b, the geometric configuration and impermeable barrier material to confine and route the diffusion of the analyte into the indicator system comprise barrier film (3) disposed along the measurable continuum of a permeable or porous carrier medium (2) loaded with scavenging reagent-indicator in Figures 9a and 9b, and the diffusion of the analyte, scavenged into the device, establishes a moving-reaction front so as to establish a moving colour-band of chemical change, shown by the arrow in Figures 9a and 9b, which generates numerical data for interpretation of exposure. The ability to generate numerical data from visual observation, as shown by the arrow along the strip in Figures 9a and 9b, is a distinct advantage over prior art that merely measures changes in a real environment by a traffic-light change in colour spectra simultaneously over a flat

surface when viewed from above. The intake into the device is located between the opening of the carrier medium (2) and the adhesive seal (4), and the skin patch is attached to the skin by the adhesive (4).

As disclosed in the present invention, there are a range of possible deployments of the sensors described. One such deployment is to monitor metabolism of bacteria that contaminate platelets in blood bags. **Figure 10a** shows the device connected to the contents of a blood bag, whilst Figure 10b is a magnification of the device shown in Figure 10a. In Figure 10a, the device is shown incorporated into the structure of the blood bag to withstand the rigors of handling and transport, however, it may be connected by tubing and ride with the blood bag until the product is to transfused, by means of an adhesive or physical attachment widely available in packaging industry. An alternative deployment may be as a sticker device placed over the contents of a bag of blood product for scavenging the evolution products or consumption of oxygen by the contents.

This form of the invention can be used to meter metabolism of bacteria, either by direct measurement, or by inference of the carbon dioxide or oxygen consumption sensed by the indicator, less that expected from platelets. Such a deployment may have fitted an indicator film that changes from one colour to another, or from coloured to colourless, to report prevailing levels or the attainment of a predetermined threshold level scavenged by the indicator film. It is also possible to deploy more than one film in the device, each calibrated as sensors to report different levels of exposure to report progressive exposure in a discrete scale.

A metering device according to the present invention is shown in Figure 10b, which incorporates the invention of Figure 2 or Figure 4 in a separate compartment to a material being monitored for evolution product or consumption substrate. In Figure 10b, the material being monitored (1) might be a blood bag, the skin of an animal, or other study material. The moving-reaction front sensor of Figure 2 is disposed in Figure 10b as a moving colour band (4) migrating from the point of attachment of the device (1), which may be a screw or bayonet fitting to the base of the device (3). A chamber environment is

established by covering material (3), which may be a barrier film or a transparent tube. Extraction of a certain volume of blood product may be achieved through a one-way valve (2), possibly by evacuating air from the chamber (3) to create a given vacuum. To ensure solvent-proofing and selective migration from the study material to the sensor, a separating layer may be disposed at (2), which may be composed of covalent material like silanes or polyethylene, microporous material, a chemically filtering layer or other means of ensuring selective diffusion of the targeted analyte.

The sensor device in Figure 10b, may be composed of indicator together with growth media which encourages bacteria to proliferate, whilst inhibiting the respiration of blood platelets. For screening purposes, deactivating platelets attributes the measurement of metabolites, such as carbon dioxide evolution to that from contaminating microorganisms by eliminating platelet metabolism from the calculation.

A correlation schedule is shown in **Figure 11** between the accumulated carbon dioxide scavenged by the patch in Figure 8 and migration of the reaction front. A similar correlation can be used to relate carbon dioxide scavenged by an indicating device placed on the exterior wall of a blood-bag. The migration of a colour-front will indicate relative expiry or contamination by bacteria.

Various applications of the invention for health monitoring

Blood distribution

Contamination of blood platelets

The harvest of blood through the skin of donors involves the insertion of an extraction tube into veins. As the skin is punctured, bacteria present on the skin surface can contaminate the blood harvested. There have been cases where bacteria have survived in blood platelets distributed from blood banks and go on to infect sensitive patients through the transfusion process in hospital. Furthermore, it is known in the food processing industry that even with the highest standards of good manufacturing process, some contamination can occasionally occur.

Blood suppliers and health managers are interested to monitor stocks for microbial contaminations that threaten the health of patients. The metabolism of invasive organisms to blood products destined for transfusion into patients, such as bacteria and fungi, can be detected and metered via their consumption substrates or evolution products, or as surrogate molecules that report changes in the chemical environment to report presence and activity of microbial populations. Similarly, changes in the enzymes involved in spoilage process can be used as a detection method, and detector antibodies that form a detector antibody/antigen complex can also be used to report the presence of contaminating micoorganisms in blood products.

Evolution products from bacterial growth are currently monitored at the stage of storage at the blood bank using detection vessels into which a sample is placed, but there is a need for continuous monitoring of platelets until the time of transfusion for greater reliability. This would also enable platelets to be distributed to hospitals earlier and so expand the platelet supply, as currently blood banks retain stocks for up to 3 days to ensure any bacteria present have overcome the lag-phase, have grown and are detected, whereas the 3-day examination could be preformed at the hospital's storage facility instead. The effect of current practice is to distribute platelets with a very limited life-span. By extending shelf life via application of the present invention, hospitals would be able to reduce the outdating of platelet inventories and use this limited medical resource more efficiently

The present invention solves this need by monitoring for bacterial contamination via the disposition with the blood bag, rather than testing in a laboratory at the blood bank, so that the hospital haematologist or nurse can monitor for contamination and reject the blood product if unfit for purpose. The present invention is advantageous over sampling at the blood bank before distribution, as it represents whole-of-population sampling, and continuous monitoring throughout processing, handling, distribution and hospital use of blood products. It is proposed to affix an indicating label or tag to the exterior wall of permeable blood bags, or within the wall, or as a device inserted into each and every blood bag for whole-of-population sampling, and so provide an early-warning system and a monitoring system to hospital haematologists on the condition of their blood stocks. A

separate compartment may be composed in the blood bag through which a sample is extracted, by for example, by squeezing a quantity through a one-way valve.

It is thus an object of the present invention to facilitate communication of quality status to a wide variety of audiences, including hospital pathologists, haematologists, general nursing staff, and those without technical training in the use of more elaborate instrumentation. It is another object to provide a device that passively meters exposure, of that device to an analyte, without manual operation. The communication over the extent of exposure to the analyte can report the attainment of a predetermined level of exposure or progress along an increasing scale of exposure, such as a visual quality scale, of for example, purity and integrity of blood in a blood-bag.

Accordingly, in one aspect, the invention provides a monitoring device using scavenging, and chemical reaction with an analyte, for measuring and reporting respiration levels, progress with metabolism and associated enzymatic reactions, or immunological response in blood products, as a passive and automated indicator of exposure which is incorporated within or affixed to the wall of a blood bag, placed in an separate monitoring compartment, or attached by tubing to a monitoring device that accompanies the blood bag during distribution from the blood bank to the hospital ward.

In another aspect, the invention provides a method for measuring and reporting respiration levels, enzymatic changes, or immunological response from contaminating microorganisms present in blood products, comprising the steps of:

locating a monitoring device on the external or internal surface of a permeable blood bag, within the wall of the blood bag, in a separate compartment of the blood bag through which some blood product is extracted aseptically, or attached by tubing to a separate monitoring device to an analyte associated with metabolism, respiration, enzymatic change or immunological response; and observing the level visually, receiving the electronic communication by radio signal, or other form of transpondence, or retrieving said device to record the level of said analyte.

Contamination of blood products, such as blood platelets, can be detected by metering the formation of spoilage products, such as carbon dioxide evolution from bacterial degradation or another analyte that is evolved from the metabolism or bacteria, or by-product which acts as a surrogate from the interaction between the bacteria and the blood product. Some of such analytes are covalent in composition and will cross the wall of a blood bag into a metering device placed on or in the wall of the blood bag. When an indicator device is connected by tubing to a bag of blood product, a sample can be extracted from the blood bag for the purposes of detection of microbiological contaminants. Alternatively, an immunological response may be detected by loading an antigen in a separate compartment of the blood bag, into which a small sample is extracted using tubing and a one-way valve and reacted with an immuno bead.

Metabolism of bacteria in a bag of platelets can be inferred from the total respiration recorded minus that expected from platelets, as for example carbon dioxide evolution or oxygen consumption reported to an observer via visual or electronic communication. An expected level of respiration from platelets can be made to trigger a sensor window at a first level of expansion of the moving colour-band, whilst contamination by bacteria of the platelet and growth media suspension and proliferation can be made to trigger a second sensor at a higher level of exposure to carbon dioxide, indicating to the observer that bacteria are present in the blood bag.

However, there are other analytes generated by the interaction between platelets and bacteria, which can be incorporated into suitable indicator systems to generate a report to the observer of the blood bag, or be devised to generate an electrical signal in the sensor for transpondence to a coordination centre. Such analytes are routinely monitored by food technologists for food undergoing spoilage by bacteria, and may include amines from degrading protein.

Should a blood bag be composed with a separate monitoring compartment, through which some of the blood product is squeezed in one-way flow, then chemicals known to selectively inhibit the respiration of platelets (Tsvetkova *et al.* 2000), can located in the

compartment to react with the incoming platelets, so that all respiration from that compartment can be attributed to contaminating bacteria. An indicator device, mounted on the wall of this sampling compartment of the blood bag, or incorporated within the bag wall, will report contamination to an observer.

Platelets may be deactivated by for example exposure to cold, the effect possibly accentuated with chemicals such as cholesterol extractors that accentuate cold-injury, or by inhibiting their activation with inhibitors like short-acting prostaglandins PGE-1 or PGE-2, Forskolin, another compound that raises intracellular levels of cyclic AMP; the phosphodiesterase inhibitors theophylline and caffeine (Tsvetkova *et al.* 2000); and asprin (Roberts *et al.* 1985). This method makes it possible to attribute all the respiratory carbon dioxide in the headspace of the sensor device to contaminating organisms. The hypotonic shock reaction may also be used to selectively inactivate platelets, whilst permitting bacteria to multiply and respire. The chemical inactivation process can be made passive by deploying the chemicals with the growth media in the indicator device. Exposure of platelets to respiration inhibitors may require detachment of the indicator device so that chemical contamination is precluded by virtue of a seal, with subsequent placement of the device with the blood bag to share the same temperature experience and act as chaperone to report contamination. The indicator device can be made to ride with the blood bag until the product is to transfused, by means of an adhesive or physical attachment widely available in packaging industry.

Growth media are known to encourage bacteria to proliferate in microbiology and may include: tryptic soy broth, possibly peptone-enriched; Middlebrook 7H9 broth; bovine serum albumin; glycerol, salts, oleic acid, saponin. These media can be incorporated into the indicator.

Various growth factors are known to selectively culture microorganisms in microbiology, and these can be incorporated in the growth media of the indicator device. Selectivity may be achieved with various antibiotics which may include amphotericin B, azlocillin, nalidixic acid, polymyxin B, trimethoprim, vancomycin.

When used as an indicator of immunological response, the indicator is composed of reagents required for the reaction including diluent, conjugate and substrate and the indicator device is coated with an antigen or antibody.

A culturing mixture of growth media, possibly combined with other additives that may provide selectivity in species composition, and growth factors may be loaded in the separate compartment, as is practiced widely in plate culturing in microbial enumeration procedures widely practiced in microbiology.

Temperature is important to the proliferation of bacteria, and samples of blood platelets are currently stored in the blood bank at temperature higher than room temperature to encourage growth. To encourage bacterial proliferation in the sample device that accompanies platelets on their journey from the blood bank to the hospital ward, the device can be encased in a layer of expanded polystyrene or other permeable insulating material, such as small plastic bubbles of trapped air in a pouch of transparent blood bag film.

Integration of the sensor within electrical circuitry and transpondence to a coordinator centre

Detection of oxygen tension and carbon dioxide evolution from bacteria proliferating and metabolising in blood products has been measured for several years in blood banks. Electrical instruments report levels sensed in sample vessels.

It is proposed to dispose a monitor for bacterial contamination, comprising a gas sensing-indicator in the package used to distribute blood to hospitals and clinics. Continuous monitoring by the sensor of the present invention, and transpondence by electromagnetic signals can alert a coordinator of blood stocks that contamination has occurred, despite being some distance from the blood product; for example whilst being trucked to a remote hospital.

The sensor, connected with electrical circuitry as a variable resistor, whose increasing or decreasing resistance, current or potential difference characteristic meters exposure, may be connected with electronic equipment that can perform alarm, data-logging and visual-display functions, and may relay radio or other telecommunications emanating from the sensor to a coordination centre. Alternatively, the sensor may communicate as an expanding or contracting area of reflected colour which is imaged by a scanning device and subsequently relayed electrically to a communicatrions device for transpondence to a coordination centre.

Vitality of red blood cells and blood platelets distributed in bags

For effective quality management of blood during distribution it is desirable to report on the progressive deterioration in freshness from donor harvest to transfusion into patients. Hence a metering system to show the degree of expiry in the product's life whilst in the hands of each party in storage, transport and hospital inventory is desirable. The present invention satisfies this need by communicating the respiration history of the blood cells.

In the case of the storage of red blood cells in blood-banks, transport and distribution to hospitals, and handling within hospitals, prevailing oxygen and carbon dioxide levels in blood bags reflect environmental conditions of temperature and gaseous atmosphere, whilst cumulative respiration, when related to age, is an indicator of compliance with protocols to ensure that the holding temperature and atmosphere are conserving of the metabolism of the sensitive cells.

Red blood cells and platelets, removed from the nourishment of the body's digestive system and the filtering of wastes by the kidneys, are imbued with a limited lifespan. By holding them at low temperature (2-4^{0}C) metabolism is slowed so that metabolite reserves are conserved, waste accumulation is limited and the structural integrity of cellular membranes is maintained. This is a physiological foundation for refrigerated storage of red blood cells at blood banks, cool handling during transport, and refrigerated storage as hospital inventory.

For some time mechanical temperature recorders and electronic data-loggers have been used to monitor temperature management during blood distribution.

The problems with these are:

a) they are costly to purchase and to replace due to loss.

b) the downloading of the temperature record is usually performed by the blood bank upon return of the data logger, where the logger is calibrated. The information on the trip is delayed until the blood bank forwards the report.

c) their use may be confined to a small sample within the populations of packages and shipments, however, temperature varies within the stow and between positions within the outer package and refrigerated container, consequently sampling error can be high.

'Respiration-life' modelling should assist in the prediction of the senescence and expiry of red blood cells in the chain of distribution to hospitals and transfusion within hospitals with the following possible benefits:

1. Blood bags with superior freshness may be identified, potentially for improved clinical outcomes
2. Blood stocks, held at optimum conditions for more than conventionally accepted 42 days storage-life (with in-built safety margin due to the vagaries of transport and distribution), might be confirmed as acceptable by the respiration indicator whereas the expiry date deemed them unacceptable, thereby expanding the available stocks in hospital inventories
3. Stocks held at damaging temperature above the optimum, can be identified from the early warning provided by a respiration sensing-indicator
4. Whole-of-population sampling can be performed as each and every blood bag can be monitored with an affixed sensing-indicator device, reducing the sampling error that occurs with electronic data loggers which are merely placed in proximity to blood bags

The technology is generally based on a model for respiration of a mass of blood in relation to temperature and atmosphere, and a 'Respiration-life' model of freshness for blood cells, defined as the threshold of respiratory history in relation to the life-span of cells and could report:

1. Respiration-life, i.e. freshness and remaining life-span [meter reading on a graduated or continuous scale, and / or attainment of threshold value 'expiry' for example, progressive movement along a colour-band scale, or change in colour to Colour indication 1]
2. Prevailing high temperature extreme, inferred from an excessive level of prevailing carbon dioxide level [e.g. Colour indication 2]
3. Prevailing low temperature extreme, inferred from a depleted level of prevailing carbon dioxide level [e.g. Colour indication 3]
4. Prevailing anoxic storage condition or exposure to inhibiting gas such as carbon monoxide [e.g. Colour indication 4]
5. Incorrect pH [e.g. Colour indication 5]

It is proposed to affix a label or tag to the exterior wall of permeable blood bags, or within the wall, or as a device inserted into each and every blood bag for whole-of-population sampling, and so provide an early-warning system and a monitoring system to hospital haematologists on the condition of their blood stocks. In the case of specimens of living cells in other non-permeable containers, such as transparent glass jars, attachment of a solvent-proof label to an interior wall enables metering and reporting functions to occur. Should a non-transparent container be used, a pin-hole may be punched into the vessel of for example, polyethylene or other polymer, and the label-device can then be applied as a sealing-patch in the same manner that a puncture in a bicycle tube is repaired. Alternatively, a bayonet fitting through a pin-hole punched in the package wall, or typical connecting fittings used in scientific measurement of gas streams may connect a tube to the intake of a metering tag. These methods enable monitoring biological specimens held in non-transparent vessels and containers.

The method rivals the expensive electronic dataloggers currently used to monitor the process of temperature management, and more accurately reports actual metabolic activity of blood cells. The improvement in control over blood quality is to more accurately apply a measure of the "utilisation of life-span" rather than "use-by-date" with its in-built risk allowance for the error associated with the vagaries of temperature management during blood distribution.

The low-cost nature of the technology and its simple application may assist in blood regulation, and be valuable in the more challenging blood distribution systems, for example to remote areas and in major catastrophes.

Haematologists can follow-up on the warning the sensing-indicator device provides with laboratory testing, of for example, examinations of cell volume, cell density and filterability. Thus, the technology provides increased reliability for existing quality assurance systems in blood supply, as an additional level of managerial control.

Cool storage conserves metabolism, and energy levels in red blood cells and platelets will be higher in those with less respiration history and higher conservation of reserves. An exterior label on blood bags that reports current respiration activity and respiration history would be of great benefit in indicating freshness. By extracting only a very small portion of the carbon dioxide evolved through the blood bag wall for measurement, the integrity of the contents should not be compromised and with the use of a selective polymer that will only pass covalent molecules, like carbon dioxide, between the wall of the blood bag and the scavenging sensing-indicator, the ionic balance of the contents should be maintained.

Coded communications to different classes of recipients, commensurate with levels of exposure

In the management of quality, blood suppliers may wish to restrict the information about the incidence of contamination to those medical specialists immediately involved in blood distribution, and not to have that information shared with transport companies and the like.

In the present invention, the metering can be achieved by deployments that target communications at different audiences, wherein some interested parties are alerted in an early-warning, when the level of exposure is low, whilst others in a disparate class of recipients receive the communication when the reaction has progressed to an advanced stage, when the level of exposure is higher.

This may combine various modes of metering disclosed in the following section on colour possibilities. The coded message may be received by blood-supply staff or quality-control staff using special instrumentation, such as a bar-code scanner and take the form of a missing or additional bar-code using indicators that appear or disappear. It may also be a measurement taken, such as colour intensity or quantum reflected into a measuring instrument.

Respiration from blood cells as a simulation of other systems

Respiration rate of cells usually has an Arrhenius function with temperature and can be modified with atmospheric concentrations of oxygen and carbon dioxide. Measures of prevailing levels of oxygen and carbon dioxide can therefore be used indicate conditions of temperature and atmosphere by inference. This can be communicated to process workers with indicators responsive to these gases. Process workers in the distribution of blood can be alerted to sub-optimal transport and storage conditions such as abusive temperature extremes, poorly ventilated outer-packaging causing relative anoxic conditions, and exposure to carbon monoxide from internal combustion engines and vehicle exhausts. If oxygen and carbon dioxide levels are not within the acceptable range that correlates with good handling, then an indication like a colour change on a device attached to the blood-bag can alert all observers.

Transcutaneous measurement of human physiological status

Permeation of carbon dioxide through the skin of patients and equilibration of arteriole oxygen at the skin have been measured for several years in the intensive care wards of hospitals. Electrical instruments report levels sensed on fingers, chest cavities and ear lobes.

It is proposed to dispose a homeostasis monitor comprising a gas sensing-indicator on the skin of patients. Monitoring the physiological status of humans is an important application of passive monitoring technology, particularly for low-care patients, for people in nursing homes, in first-aid stations and in developing countries where hospitals are poorly equipped. Health of subjects can be measured with the oxygen level in arterial blood, monitored through gas equilibration through the pores and the permeability of skin; and their respiration can be monitored through transcutaneous carbon dioxide permeation. Babies and young children, deemed to be at-risk, or recovering from episodes of poor health, can be monitored by parents with the electrical sensor form of the metering device, for example whilst sleeping in their homes.

The sensor, connected with electrical circuitry as a variable resistor, whose increasing or decreasing resistance characteristic meters exposure, may be connected with electronic equipment that can perform alarm, data-logging and visual-display functions, and may possibly relay radio communications emanating from the sensor to a coordination centre.

Out-patients are often encouraged to exercise to assist with recovery. In a similar scenario, air travelers on long-distance flights are at risk of deep leg thrombosis through inactivity. The low level of exercise within the population of developed countries is an international concern, particularly among children. Placement of a skin patch comprising the indication technology can report inadequate exercise and blood flow in a colour indication and so encourage exercise.

Respiration in humans and animals converts energy in cellular carbohydrates, body fat and body protein into metabolisable energy for homeostasis and work, and there are electronic instruments with carbon dioxide and oxygen sensors to measure prevailing levels through the skin of subjects (Carter and Barnam, 2000).

The present invention uses indicators to report prevailing and cumulative evolution of carbon dioxide along a scale from the skin of subjects, and by inference, the energy consumed in the process. It can provide information for fitness measurement and weight-

watching, and to monitor the health of out-patients and people under care. The present invention deploys the exposure sensing-indicator as a skin patch or passive extraction vessel, which establishes a connection with blood flow in arterioles through skin pores, permeable tissues and fat. The connection could be obtained with a suction cap, permeable wrist-band, ring for a finger, or other device that contacts with or is in close proximity to the skin. It is possible that an implant could be inserted under the skin of humans and animals, or in connection with a tube device into the body. It is also possible that the device could be placed into an internal body cavity or be swallowed as a device that passes through the digestive tract for monitoring purposes, where other analytes associated with health are studied.

Carbon dioxide evolved from respiration can be related to respiration rate and the level of exertion in fitness work-outs, so that the energy consumed or weight lost during the period can be monitored. The level of oxygen within a chamber environment of a skin patch or suction cap will equilibrate with arterial levels within the organ below, and so prevailing transcutanuous oxygen, perfused through the skin, can similarly be reported with sensing-indicators. When related to a scale, this can report the cardiovascular and respiratory physiology of humans in exercise testing, monitoring of out-patient physiology and the like.

Insertion device into body cavities or under the skin

Measurement of body function can be undertaken with the present invention. The device may be inserted into a body cavity to measure analytes associated with the metabolism of disease organisms, such as placement into the alimentary canal via a pill-form that is swallowed, placement of a sampling device in the mouth, nasal, ear passages, vagina or the rectum among others, and may be inserted as an implant under the skin, such as into the ear lobe of livestock.

The sensor can be disposed at contact points where the analyte can be readily monitored; one method is to incorporate the indicator into the composition of polymer components of a human health device which is in contact with the skin or placed in a body cavity when

the device is used. An example in application is to compose a mouth guard with the sensor in permeable transparent plastic material. As the mouth guard in close contact with the gums of the mouth, metabolic evolution products from bacterial infection can be metered and a visual indication can be obtained to report that the gums of the mouth are infected. A similar application is to place an ear plug comprising the sensor in the ear canal to detect ear infections.

Freshness / cleanliness of contact lenses, worn glasses, hearing aids, jewellery, dentures, mouth guards and other health devices in contact with the body

The freshness of contact lenses, hearing aids, baby dummies / pacifiers, mouth guards, jewellery, vaginal tampons, nappies, and other devices that are worn or inserted into body cavities to improve health can be monitored with the homeostasis meter. Similarly, freshness can be monitored in surgical gloves, face masks and other protective equipment worn in contact with the body. A related application is for monitoring communication devices such as ear-phones and the handsets of telephones for exposure to respiration, and by inference the use-pattern and consequent need for cleaning.

Cumulative carbon dioxide evolution and other gases and fluids, such as human perspiration associated with homeostasis indication, can be metered from air expired, transcutaneous perfusion, or membrane diffusion in order to monitor freshness, and a determination can be made as to when the health device should be removed and cleaned or replaced.

The sensor can be disposed at contact points where the analyte can be readily monitored; one method is to incorporate the indicator into the composition of polymer components of the health device which are in contact with the skin or placed in a body cavity when the device is used. An example in application is to compose the attachment appendage 'the arms' of glasses worn to improve sight with the homeostasis meter disposed in permeable transparent plastic material. As the appendage is in contact with the ear, transcutaneous carbon dioxide can be metered and a visual indication can be obtained to report that the time to clean the appendage has come.

Deployment as a test-vessel

Attachment with tubing under the skin of animals can preclude the need to establish a sampling chamber, as can the use of tubing in connection with device into the generator of analytes such as sampling carbon dioxide in expired air for measurement of cumulative respiration. The device can be used in connection with tubing and other apparatus to obtain exposure to target molecules by methods commonly used in medicine and veterinary science to obtain a sampling stream.

The device can be used in connection with tubing and other apparatus to obtain exposure to target molecules by methods commonly used in medicine and veterinary science to obtain a sampling stream. One such application is to connect a sampling vessel comprising the indicator of the present invention and bacterial growth and platelet inhibition media to a blood bag through a one-way valve connector, and for the sampling vessel to ride with the blood bag as chaperone from the blood bank to the hospital ward.

Deployment as a swab for wiping over test surfaces

The indicator device can be composed into a carrier medium that can be wiped over a test surface, such as a kitchen bench or surfaces in operating theatre in a hospital to determine the level of bacterial contamination. It is common for health inspectors to monitor food preparation areas for bacterial contamination. In current practice, surfaces are wiped with a swab which is subsequently wiped onto growth media in culturing vessels, such as petri dishes to transfer bacteria to the culturing medium. The advantage of the present invention in this application, is that the swab and culturing vessel are combined into one device, together with an indicator to report presence of microorganisms passively, rather than the manual sampling method used by microbiologists. The swab of the present invention, comprising the indicator device composed with growth media can be placed into a test vessel, such as a transparent plastic bottle, to establish a chamber that establishes a gascous concentration from metabolising microorganisms. The time at given temperature for bacteria or fungi to proliferate and produce detectable gas, is an indication of the population of bacteria present on the surface being tested, as a high population of starter culture will produce more gas sooner.

Similarly, common kitchen wipes that are used to wipe down sinks and benches can be composed of the indicator. They may be returned to a transparent sealing vessel after use, which may have the indicator device affixed or incorporated into its wall, so as to report bacterial contamination of the wipe to a householder so that a decision to sterilise or replace it can be made.

Deployment as a detecting skin-patch over suspected melanoma

Volatile organic compounds are evolved from cells undergoing apoptosis from skin cancer. The present invention can be placed over a suspected skin spot or mole and if a positive indication is found, then more thorough testing, for example a biopsy can be invoked.

Detection and monitoring of disease

It is now known that certain volatile compound secreted from sweat glands are markers of disease within the body of an animal and indicator systems for skin patches are proposed. Enumeration of the readings obtained by skin patches and surface wipes for disease is now made possible by the diffusion methods of the present invention that establish a moving reaction-front and generate numerical values along a continuous scale, possibly assisting in monitoring the onset of disease and the ongoing health status of the animal as the body fights the disease.

Apparel and foot-wear application

Freshness of clothing, shoes and other body coverings may be monitored with the device and persons can be made aware that it is time for replacing them when no longer fresh due to accumulated exposure of the device to a body odour monitored by the device.

Distribution of living food

Live marine animals, such as lobsters, crabs, prawns, oysters, mussels, fish are transported from ocean and fish-farm harvests in packages, often in water, to markets and restaurants. There is a live export trade in these animals. The monitoring device can be placed as a freshness monitor to accompany the animals on the journey, reporting levels of oxygen and

carbon dioxide, cumulative respiratory history and the levels of other analytes from metabolism that report animal health.

Poultry eggs are known to lose quality as a result of thermal exposure, and changes in the metabolism of the egg or infection with spoilage organisms can be reported by a freshness indicating sticker placed onto the egg shell to detect prevailing levels or cumulative exposure to analytes that measure the health of the egg inside. Such analytes may include amines from protein degradation in the albumen, water loss, hydrogen sulphide and carbon dioxide evolution.

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Claims:

1. A method for quantitatively sensing, using an indicator system based on diffusion in space and time of a reaction front, for determining and reporting the prevailing concentration or exposure history of an analyte in health monitoring for animals including humans, the device comprising:
    a. An inert carrier medium that will host the chemical reaction and provide for controlled diffusion of the analyte
    b. Geometric configuration and impermeable barrier material to confine and route the diffusion of the analyte into the indicator system along a measurable continuum of a permeable or porous carrier medium established by varying density, porosity, permeability, crystallization, or disposing a column of microspheres
    c. Reagents loaded into the carrier medium that scavenge the analyte into the device and react with the analyte to provide a determination in either chemically stable, semi-stable or unstable reactions
    d. An indicator system that reports the attainment of determination of the progressive end-point at the reaction-front of a diffusing analyte's interaction with a reagent
    e. A quantitative scale for measurement of exposure, either as graduations along a metric continuum for visual readings, or as signal of the intensity of changed electrochemical or electromagnetic property
    f. A window for visually monitoring the progress of the migrating reaction-front generated by diffusion of the analyte along the measurable continuum
    g. An aperture for intake and absorption of the analyte into the monitoring device
    h. An attachment means for positioning the device in relation to a sample stream emanating from the source of generation of the analyte, or within the semi-confines of a chamber over the generating source
    i. A reference scale for interpretation of the movement of the reaction-front, either numerical graduations in scale (quantitative) or ratings prepared by scientists or expert judges of quality (qualitative)

.. whereby the measurable active diffusion of the analyte along a metric continuum in space and time correlates in an mathematical manner with changes in the surrounding environment with respect to the analyte being measured, by comparing the detection time to reach a displacement of the moving colour-front, or the extent of the moving colour-front, to a correlation schedule with the concentration or number of molecules of the analyte generated, so establishing a severity scale for the change in quality of the analyte in the environment being measured and thereby reporting the corresponding state of the medical material or equipment

2. The method of Claim 1, wherein the correlation schedule relates oxygen or carbon dioxide ingress into an aseptically packaged medical package with the aperture of a rupture in the package seal, to report loss of package integrity by a moving colour-front
3. The method of Claim 1, wherein the correlation schedule relates the carbon dioxide evolution under a adhesive wound dressing with wound healing to report wound status by a moving colour-front
4. The method of Claim 1, wherein the correlation schedule relates the concentration of a chemical residue in a medication skin-patch or skin-implant by a moving colour-front
5. The method of Claim 1, wherein the carrier medium is composed of water-soluble carbonaceous polymer or any polymer with chemico-physical properties to calibrate the migration of the reaction front such as density and porosity, crystalisation, plasticisation, perforation, and polymer expansion
6. The method of Claim 1, wherein the carrier medium and surrounding barrier material is geometrically configured variably to calibrate the migration of the reaction front as a column of micro-spheres, or a strip or disc of film with potentially variable thicknesses, or tortuosity in intake and pathway of diffusion, or size of a single aperture at the intake, or number of intakes, or a combination of these methods

7. The method of Claim 1, wherein the reagents loaded into the carrier medium that scavenge the analyte into the device and react with the analyte to provide a determination include titration reagents and oxidation-reduction reagents commonly used to achieve a chemical determination, or when used as an indicator of immunological response, the indicator is composed of reagents required for the reaction including diluent, conjugate and substrate and the indicator device is coated with an antigen or antibody.
8. The method of Claim 1, wherein the indicator system reports the attainment of determination of the progressive end-point at the reaction-front by a moving colour-band indication viewed by the observation post
9. The method of Claim 1, wherein the indicator system reports the attainment of determination of the progressive end-point at the reaction-front by changed electrical property arising from integrating the device into an electrical circuit
10. The method of Claim 1, wherein the quantitative scale for measurement of exposure is achieved by graduations along a metric continuum for visual readings by placing alpha-numeric text alongside the migrating colour-front for visual reading, or by generating a signal of the intensity of changed electrochemical or electromagnetic property to a receiving station in electrical circuitry
11. The method of Claim 1, wherein a window for visually monitoring the progress of the migrating reaction-front is achieved by the use of transparent or translucent materials over the moving colour-front
12. The method of Claim 1, wherein an aperture for intake and absorption of the analyte into the monitoring device is provided by covering an exposed entry-point with selectively permeable material which may be exposed to the scavenging action of the indicator-device to molecules of the analyte upon the removal of a seal, such as a peel-off, cut-away, tear-away, bubble-burst or other means; or by placing the monitoring device into a designed environment which is to be tested for its integrity of seal, whereby the commissioning of the device commences as the packaging and sealing of the outer packaging over the medical contents occurs
13. The method of Claim 1, wherein an attachment means for positioning the device in relation to a sample stream of molecules of the analyte emanating from the source

of generation of the analyte, or within the semi-confines of a chamber over the generating source includes covering the monitoring device so that it may be deployed as a stand-alone instrument for insertion into packages; composing an adhesive on one side so that it may be affixed as a label or print for deployment on the internal wall of packages, disposing it as an adhesive skin patch or wound-dressing and / or on the external wall of permeable packages or adhesive skin patches or wound-dressings; or composing the monitoring device as a laminate in packaging material, skin patches or adhesive wound dressings, protected with solvent-proof material

14. The method of Claim 1, wherein the reference scale for interpreting readings on or near the instrument is alpha-numeric or symbolic for quantitative readings along a continuous scale so that determination of movement in space can be a measurable distance

15. The method of Claim 1, wherein the reference scale for interpreting readings on or near the instrument is made into a graduated scale using masking in sections over the colour front to, in some sections of the journey hide from view, and in other sections reveal the moving colour front, at certain stations along the line or tangent so that determination of movement in space can be a measurable distance

16. The method of Claim 12 wherein masking colours present in transparent overlay, or background colours below the moving colour-band are used to generate a traffic-light colour change at the station / graduation when contrasted with the moving colour of the indicator

17. The method of Claim 1, wherein the reference scale for interpretation of readings on or near the instrument is disposed so that the first reading is obtained by a movement of a colour fringe moving in space by greater than 100 microns from the surface of the indicator medium where the analyte was first absorbed

18. The method of Claim 1, wherein a multitude of visual readings may be taken from the one sensor, relating analyte concentration or number of molecules generated of the analyte *vs.* displacement in space of the reaction front, and generating a regression relationship from this *x-y* plot to assess changes in the environment of the sensor

19. The method of Claim 13, wherein readings are taken by electronic means, possibly but not restricted to, sensing light emitted from the indicator, relaying this to a communications device, and transponding the data generated to a remote centre of coordination

20. The method of Claim 1, wherein one or more devices are deployed simultaneously to meter exposure as a means of achieving coarse and fine tuning

21. The invention also resides in any alternative combination of features which are indicated in this specification. All equivalents of these features are deemed to be included.

Figure 1

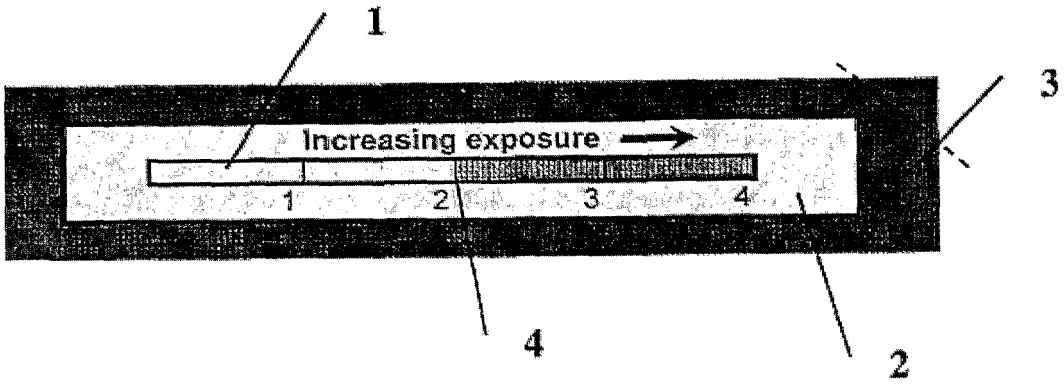


Figure 2

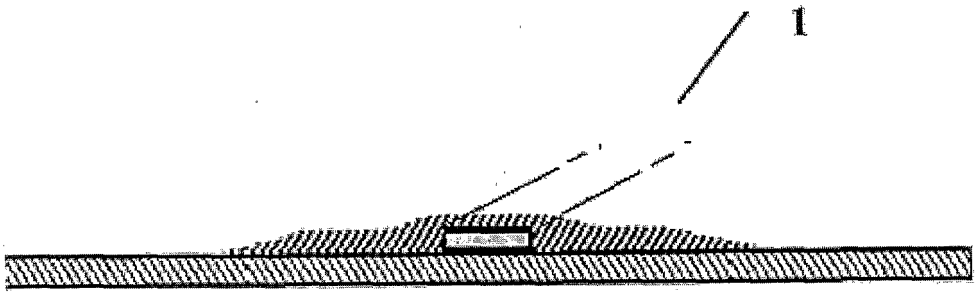


Figure 3

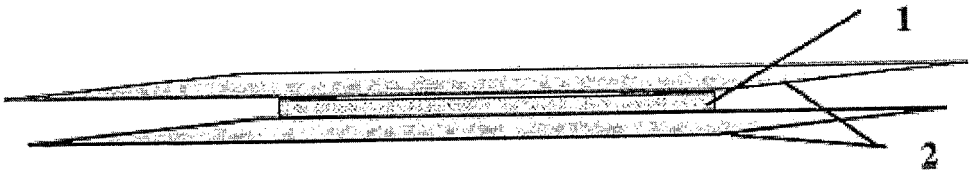


Figure 4

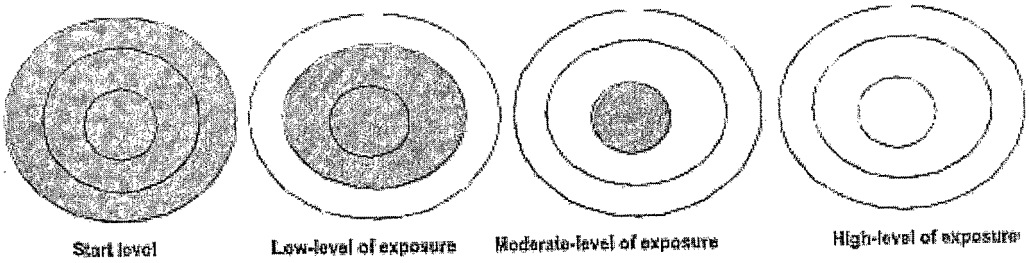


Figure 5

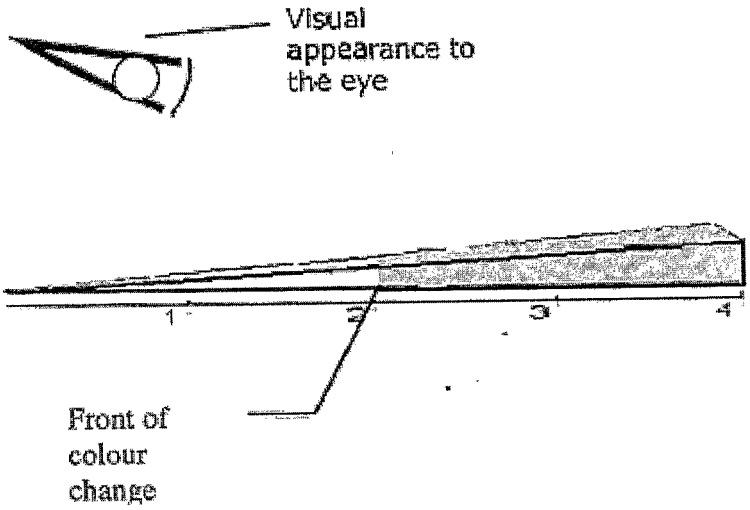


Figure 6

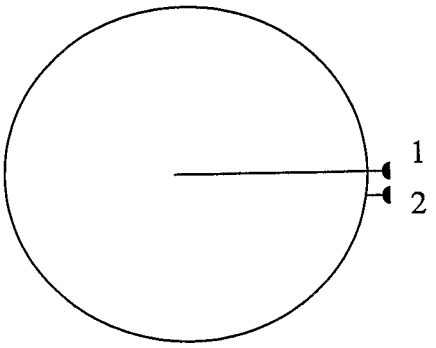


Figure 7

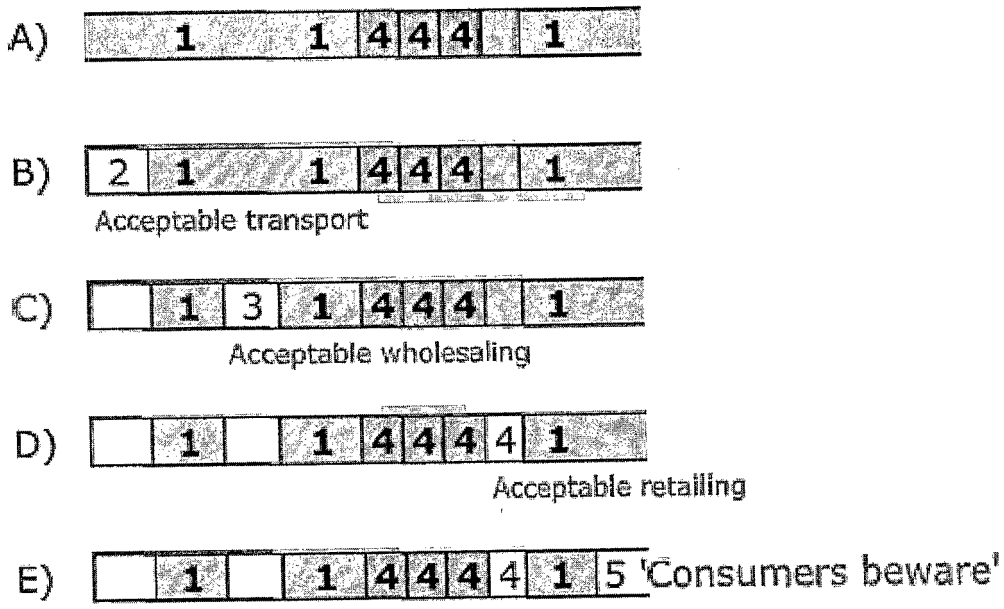

A)
1 1 4 4 4 1
B)
2 1 1 4 4 4 1
Acceptable transport
C)
1 3 1 4 4 4 1
Acceptable wholesaling
D)
1 1 4 4 4 4 1
Acceptable retailing
E)
1 1 4 4 4 4 1 5 'Consumers beware'

Figure 8

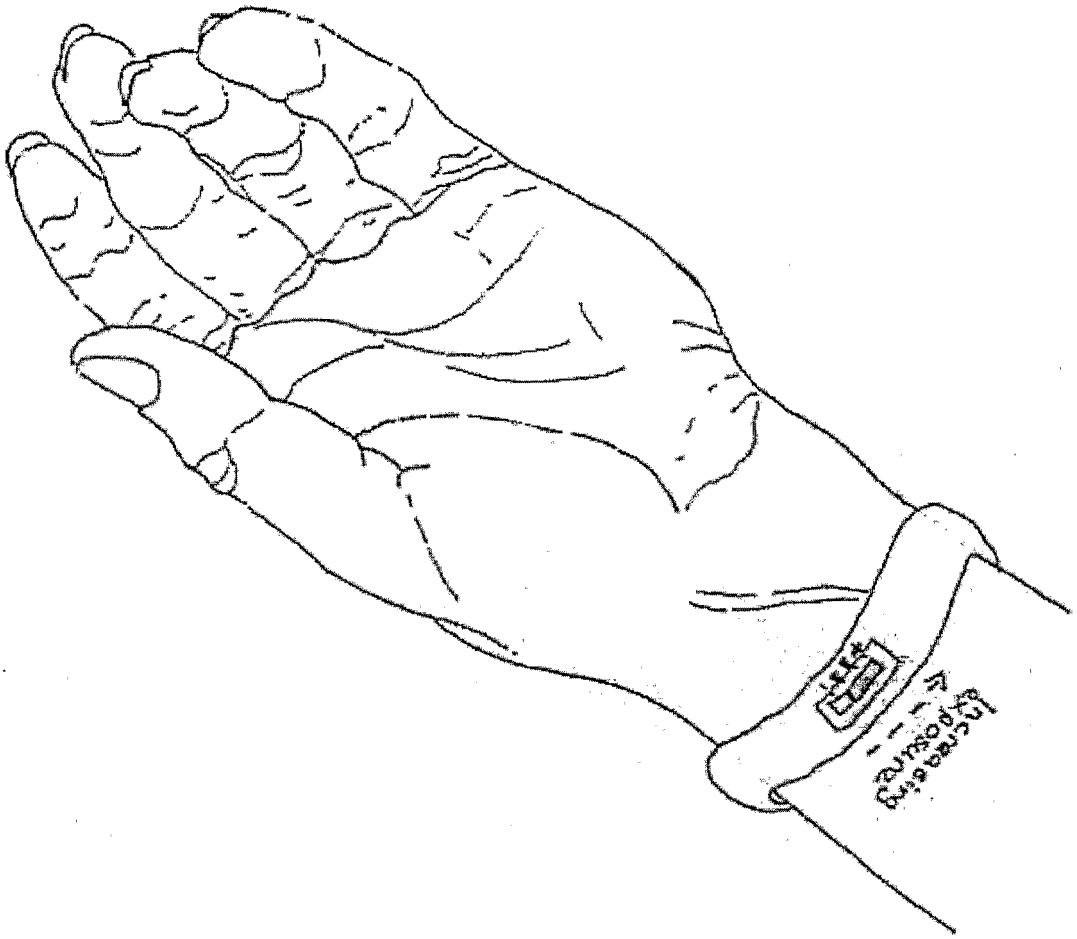


Figure 9a

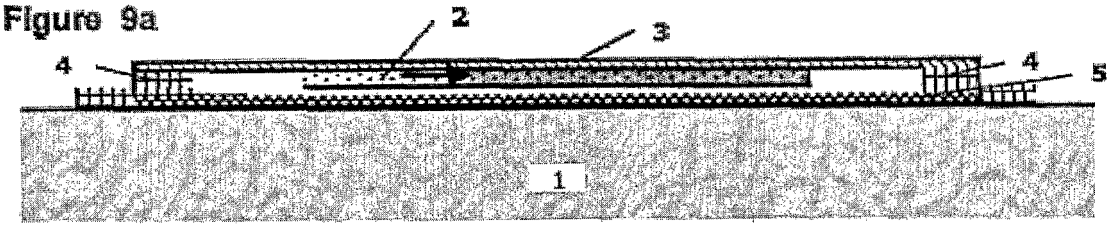


Figure 9b

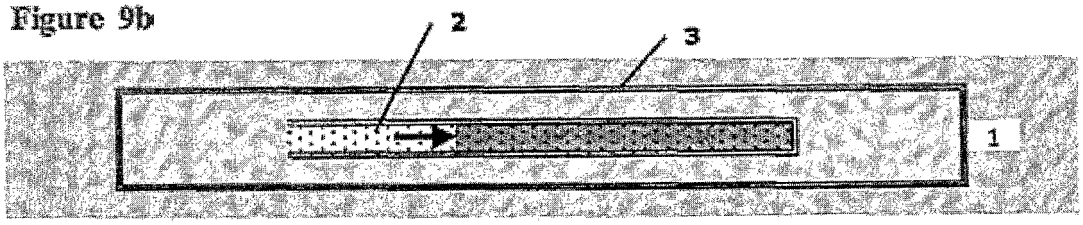


Figure 10 a

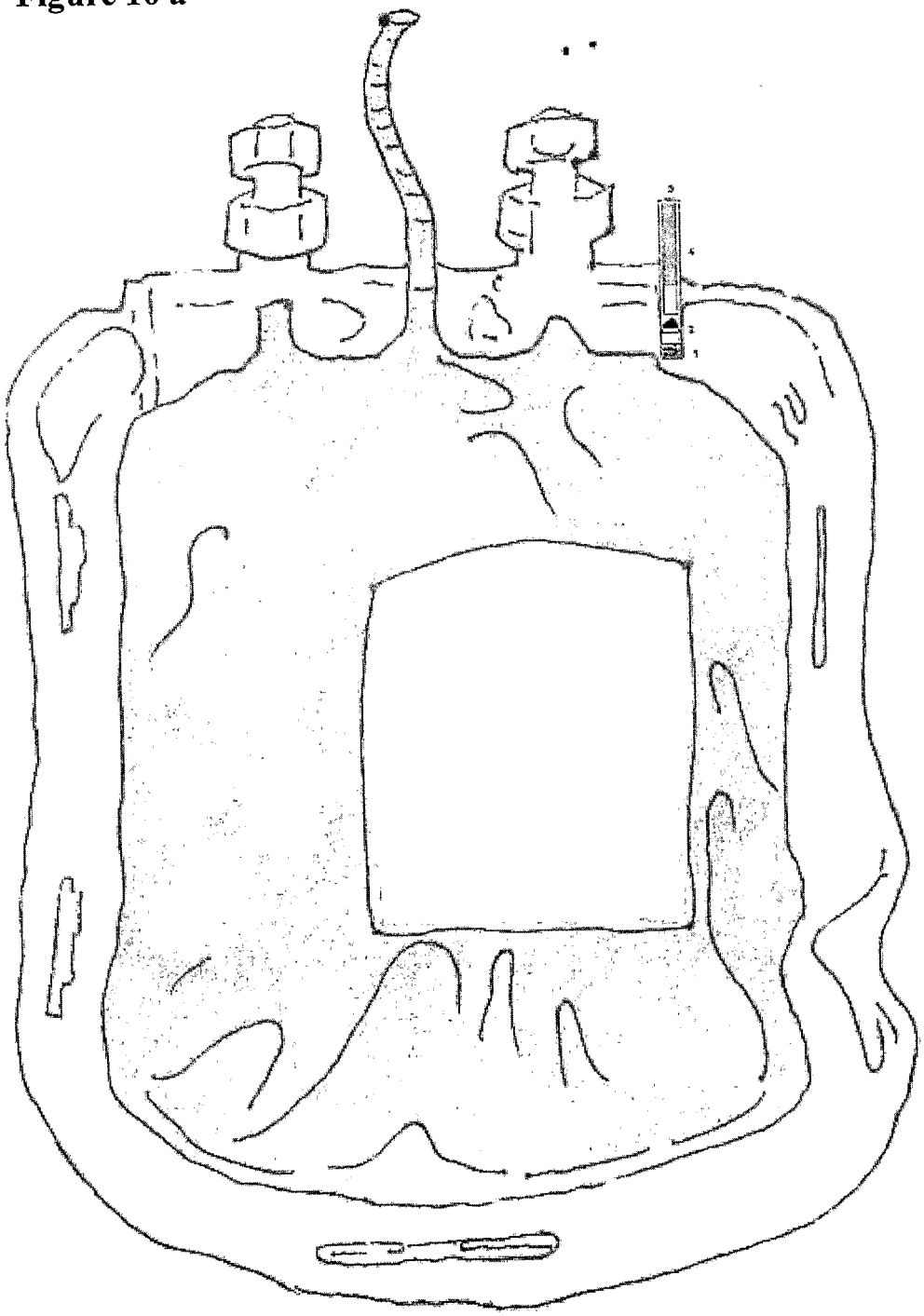


Figure 10 b

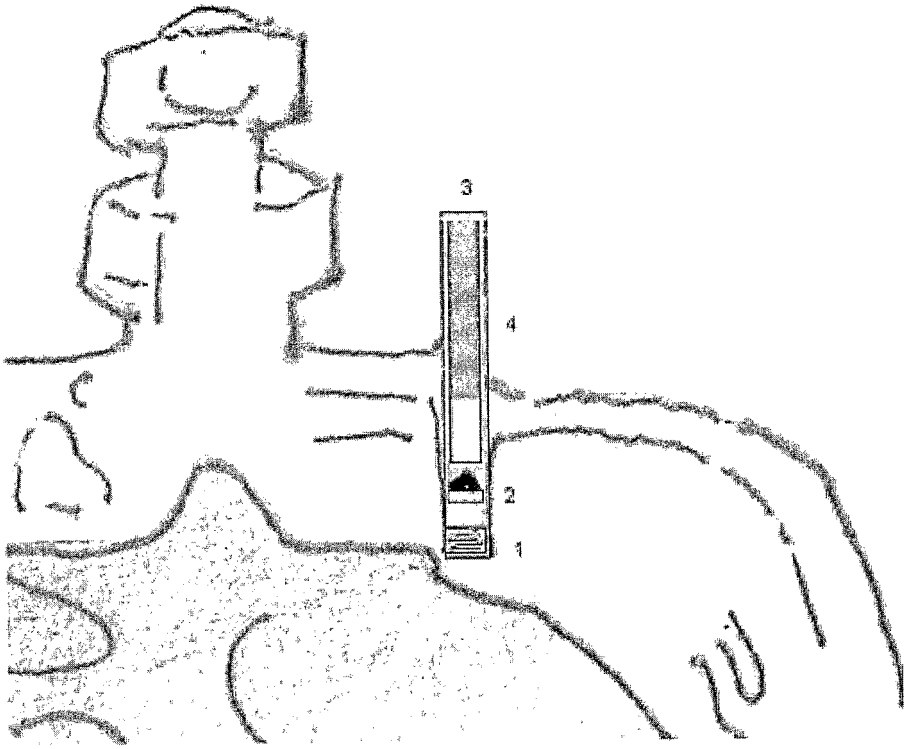
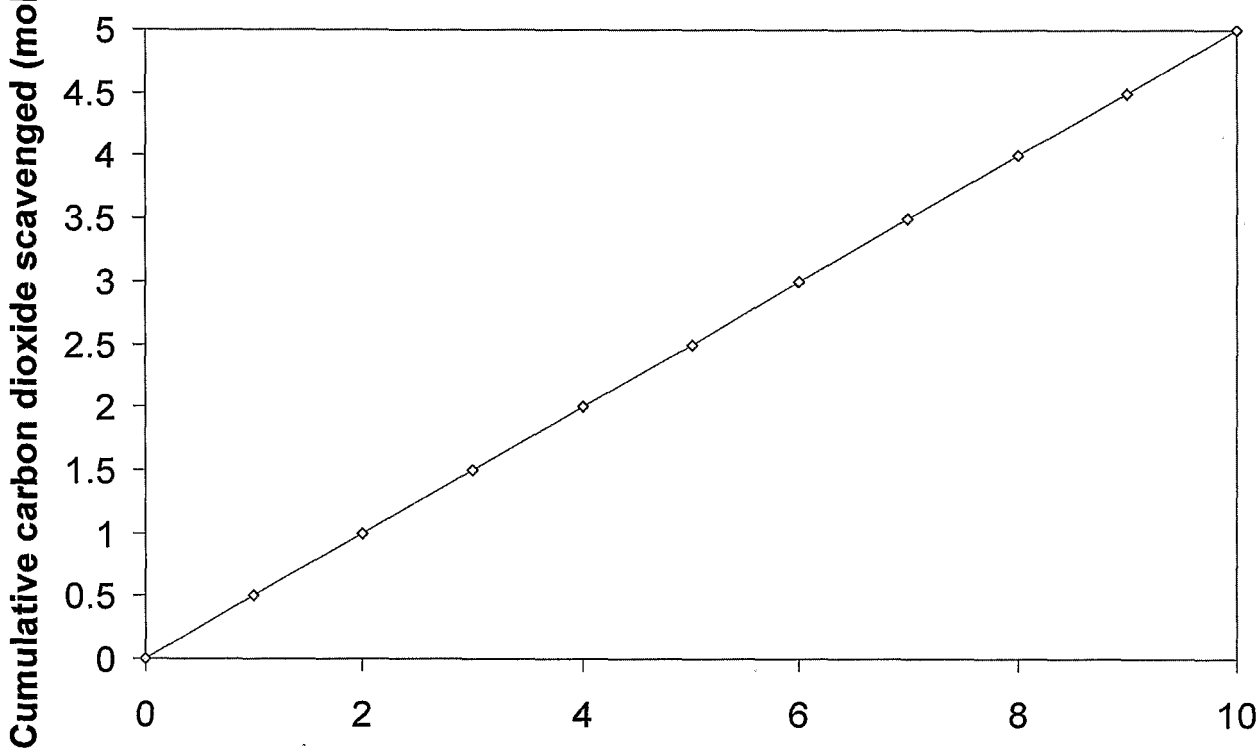


Figure 11



Relationship between carbon dioxide scavenged from blood vessels transcutaneously into a monitoring skin patch and the migration of a reaction front

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2007/000956

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. *G01N 21/75* (2006.01) *G01N 33/48* (2006.01) ***A61B 5/00*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DWPI: A61B-005/IC, G01D/IC, G01N/IC & Keywords (Substrate, Carrier, Diffuse, Porous, Reagent, Analyte, Scavenge, React, Indicator, Scale, Exposure, Visual, Well Being, Health, Monitor) and similar terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6986869 B2 (TUOHY et al.) 17 January 2006 See whole document.	
A	US 6267724 B1 (TAYLOR) 31 July 2001 See abstract and figures 1 to 4.	
A	WO 2002/095401 A2 (BERLIN) 28 November 2002 See whole document.	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 September 2007	19 SEP 2007
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer **ROGER SMALL** AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No : (02) 6283 7998

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2007/000956

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5443080 A (D'ANGELO et al.) 22 August 1995 See whole document.	
A	WO 2005/084534 A1 (LIFE PATCH INTERNATIONAL, INC.) 15 September 2005 See whole document.	
A	US 6479015 B1 (LONG et al.) 12 November 2002 See abstract and figures 1 to 6	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2007/000956

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: **21**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

The numerous possible combinations of features which may be obtained from the invention residing in "any alternative combination of features indicated in the specification" render the scope of this claim indeterminate.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
US	6986869	AU	36754/97	AU	45307/97	AU	48126/96
		AU	60859/96	AU	61241/90	AU	83618/91
		BR	9603273	BR	9705636	CA	2024064
		CA	2050677	CA	2172681	CA	2182545
		CA	2214292	CA	2226069	CN	1143763
		CN	1186956	CN	1202620	CZ	9602284
		CZ	9704161	EP	0415679	EP	0475692
		EP	0735369	EP	0759555	EP	0826777
		EP	0852336	GR	90100628	HK	1009180
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		US	2002106710	US	2002106711	ZA	9606614
US	6267724						
WO	2002095401	US	6585646	US	2002115921	US	2003199743
US	5443080	AU	21242/95	BR	9507431	CA	2185555
		EP	0754005	US	5462064	WO	1995024859
WO	2005084534	AU	2004317007	BR	PI0414067	CA	2537796
		CN	1874720	EP	1670356	KR	2006012310
		US	2005106713				
US	6479015	AU	28928/99	GB	2350190	WO	1999045140

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX