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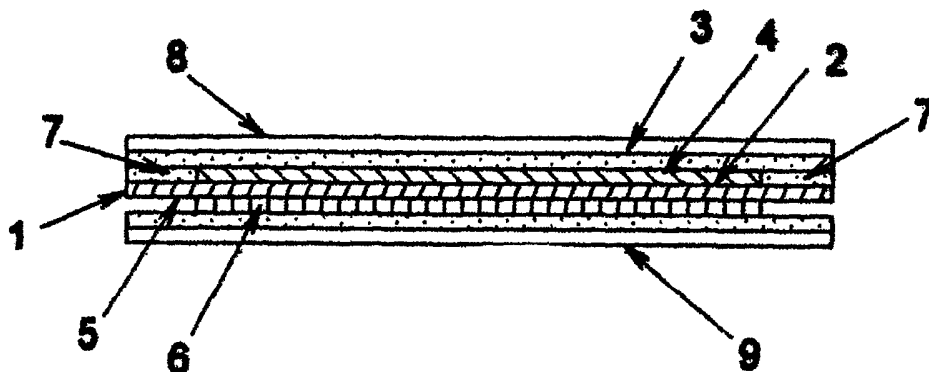
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(54) Title: ANALYTE SAMPLING APPARATUS AND METHOD



(57) Abstract: The present invention provides an apparatus and corresponding method for obtaining environmental samples of specific microbial, viral, toxic or other target analytes from a suspect source such as a meat processing facility, animal carcass and the like, wherein the sampling apparatus, comprising an insoluble substrate, a capture reagent specific for the target analyte immobilised to at least one surface of the insoluble substrate, and an attachment means, is strategically placed on, in, or near the suspect source, and thereafter exposed over a sufficiently extended period of time to sample the target analyte on the suspect source for further analysis.

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## **ANALYTE SAMPLING APPARATUS AND METHOD**

### **FIELD OF THE INVENTION**

The present invention pertains to the field of environmental sampling, and specifically to methods of obtaining analytical samples of target analytes. The target analytes may be of microbial, viral, toxic or other origin.

### **BACKGROUND**

When assessing the safety of an environment, it is necessary to first obtain samples that are truly indicative of the presence of the target analyte. The samples may be subsequently analysed for microbial, viral, toxic or other target analytes. A sample representative of the environment is best collected under standard conditions in relation to time and location. However, there are several factors which complicate such sampling.

A first factor arises when the target analytes are present at low concentration or are not distributed uniformly. There then exists a significant statistical probability that a given analytical sample will not contain the target analyte, leading to falsely negative conclusions regarding the safety of the environment. A typical situation arises in the sampling of food lots for the subsequent detection of microbial analytes. Even though samples may be taken from different units of a lot or from different areas of a lot unit in hopes of improving the probability of effective target analyte sampling, it is impractical and economically prohibitive to take more than a small portion of the lot as analytical samples. A means of increasing the sampling sensitivity would increase the probability of detecting the target analyte.

A second factor arises from the time-dependent dynamics of the contamination process. Such situations occur when levels of contamination fluctuate. For example, excretion of an enteric pathogen may have stopped by the time an animal's faeces is sampled, even though its fur could remain contaminated and may continue to contaminate the carcass at the abattoir. As another example, a small quantity of contaminant such as pesticide or peanut allergen could pass quickly

through a processing point and into a food product. In such cases the contamination could easily be missed by normal quality control sampling procedures. The safety of all such situations could be better controlled if it were possible to sample continuously or over much longer periods than are currently practical.

A third factor arises from the heterogeneous nature of the environmental contaminants, such as when the level of the target analytes is low relative to other environmental contaminants. A typical situation arises in the sampling of meat products or the environment of a meat processing plant. For example, when the sampling method for a pathogen lacks sufficient specificity, tissue cells, fat globules, hair, and the normal microbial flora of the source tissue are unavoidably collected with the sample. As a result, subsequent incubations under enriching conditions are required in order to amplify the target analyte to a detectable level for analysis.

Currently available techniques, therefore, are time-consuming and cumbersome, requiring multiple steps of purification, concentration and detection procedures on the sample to detect the analyte. For example, for many microbial pathogens it is necessary to carry out incubations under various enriching conditions in order to select, purify and amplify the target to a detectable level over contaminants that are unavoidably collected with the sample. Because of these multiple incubations it is quite common for such analyses to require 48 hours or more to complete. A means to begin the selection and purification process during the actual sample-taking period would reduce the subsequent laboratory time needed to detect the target analyte.

Various sampling techniques have been described. In many traditional methods, samples have been collected by rubbing a sterile moist cotton swab or gauze across a suspect surface. While large surface areas may be sampled by this method, the swabbing action displaces analytes already captured in the fabric matrix, resulting in low apparent sensitivity. Furthermore, the method is non-specific and unsuitable for use where the analyte levels fluctuate over time.

U.S. Patent No. 6,203,900B1 describes a method for collecting bacteria, based on a pressure sensitive adhesive sheet which, when pressed adhesive side down against a surface, transfers the micro-organisms which are attached to the test surface. The shortcomings of this method are that

it is non-specific, and does not address the time-dependent dynamics of the contamination process.

US Patent No. 6,207,406 teaches sampling for the presence of a micro-organism using a porous plastic foam strip which is saturated with agar on one surface and has a means of attaching the plastic foam strip to a test surface. While, this device can be used to sample the test surface continuously over extended periods of time, it also lacks the ability to sample for a specific analyte and to generally exclude other analytes.

Devices such as immunoseparation beads, columns, or substance-detection strips such as those used to detect pregnancy hormones, comprising substrates coated with binding antibodies or other affinity agents, have been used for many years in immunological, microbiological, chemical and other analytical procedures, such as ELISA and similar laboratory procedures. The use of these devices requires that analyte-containing fluid be introduced into the device deliberately. Such devices would therefore be difficult to use on, for example, farm and abattoir surfaces.

This background information is provided for the purpose of making known information believed by the applicant to be of possible relevance to the present invention. No admission is necessarily intended, nor should be construed, that any of the preceding information constitutes prior art against the present invention.

### **SUMMARY OF THE INVENTION**

An object of the present invention is to provide an analyte sampling apparatus and method.

In accordance with an aspect of the present invention, there is provided a sampling apparatus capable of being retrievably deployed in environments, said apparatus comprising:

- (a) an insoluble supporting substrate;
- (b) at least one target analyte capture reagent immobilised on a first surface of said insoluble supporting substrate; and

(c) an attachment means.

In accordance with another aspect of the present invention, there is provided a kit comprising:

- (a) the apparatus described herein; and
- (b) instructions for storage, deployment, use, and recovery of the apparatus.

In accordance with a further aspect of the present invention, there is provided a method of sampling the environment for the presence of a target analyte, comprising the steps of:

- (a) applying the apparatus to a predetermined location;
- (b) allowing the apparatus to sample the environment for a sufficient period of time;
- (c) removing the apparatus from the sampling site; and
- (d) rinsing away non-selectively bound materials.

#### **BRIEF DESCRIPTION OF THE FIGURES**

Figure 1 shows a cross-section of one embodiment of the invention;

Figure 2 shows a top view of one embodiment of the invention;

Figure 3 shows a top view of an embodiment of the invention;

Figure 4 shows a top view of an embodiment of the invention;

Figure 5 shows a planar view of an embodiment of the invention;

Figure 6 shows a planar view of an embodiment of the invention; and

Figure 7 shows a side view of an embodiment of the invention.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a sampling apparatus comprising a supporting substrate, one or more capture reagents immobilised to at least one surface of the substrate (providing an affinity surface) and an attachment means that permits the sampling apparatus to be deployed in the environment to be sampled ("the test environment") where it can come into contact with and capture the target analyte for a requisite period of time before being removed for analysis. In accordance with the present invention, the sampling apparatus is capable of retaining the bound target analyte through at least one subsequent purification step, such as a wash step to rinse away non-selectively bound materials. The bound target analyte can then be either released or presented for conventional analytical procedures.

The present invention further provides for a method of sampling an environment for one or more test analytes comprising deploying the sampling apparatus in the environment such that it comes in contact with the target analyte(s) and leaving the apparatus in the deployed position for a suitable period of time to permit sampling. The sampling apparatus can be left unattended and may sample over a period of days. The method of the present invention is thus a qualitative method that increases the apparent volume or proportion of an environment that is sampled and/or the duration of the sampling period allowing for detection of target analytes that are present in low concentrations. The sample apparatus can be easily rinsed or otherwise purified of non-selectively bound materials before the analytical detection of the bound target analyte(s). The method, therefore, additionally provides an initial enrichment step that can decrease the overall analysis time required.

### *Definitions*

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

The term "target analyte," as used herein, refers to a molecule that is the object of attempted capture and whose presence or absence in the environment being sampled is to be determined. Examples of target analytes that can be sampled for using the apparatus and methods of the

invention include, but are not limited to, microbes (such as bacteria, fungi, viruses and parasites), toxins (both synthetic and natural), antibodies, haptens, antigens, tumour antigens, cytokines, chemokines, exotoxins and other proteins, such as bacitracin, shed by bacterial or parasitic organisms, viral DNA or RNA sequences, bacterial or parasitic DNA, mRNA or rRNA sequences, spores, pesticides, hormones, banned or illicit compounds (such as drugs and growth promoting substances), and the like.

The term "captured," as used herein, refers to a state of being removed from a test environment onto an affinity surface.

The term "affinity surface," as used herein, means a surface of a sampling apparatus of the invention that has been coated, directly or indirectly, with an analyte capture reagent.

The term "analyte capture reagent," as used herein, refers to a reagent that binds to and, therefore, captures, the target analyte. Examples of such analyte capture reagents include, but are not limited to, antibodies and antibody fragments that specifically bind the target analyte or an epitope on the target analyte; haptens, epitopes, antigens that specifically bind to an antibody target analyte; ligands, ligand analogues, cofactors and cofactor analogues that bind to a receptor or other protein present in or on the target analyte; substrate analogues and inhibitors that bind to proteins in or on the target analyte; lectins that bind to mono- or polysaccharides or glycoproteins in or on the target analyte; metal ions that bind a target analyte; and oligonucleotides and polynucleotides (including RNA, DNA and mixtures and analogues thereof) that specifically bind other nucleic acid molecules or nucleic acid binding proteins in a target analyte. The analyte capture reagent can thus be a polypeptide, peptide, nucleic acid or other organic molecule or it can be an inorganic molecule. Certain dyes known in the art that bind specific molecules, such as proteins, can also be used as analyte capture reagents. The present invention also contemplates capture reagents that are labelled with a detectable label that facilitates subsequent analysis of the capture reagent-target analyte complex.

The term "binding pair," as used herein, means a pair of moieties whose physiochemical properties are known and can be exploited to allow specific, mutual and reversible binding to, or

interaction with, the other member of the binding pair. Examples of suitable binding pairs for use in the present invention include, but are not restricted to: antigen or hapten with antibody; antibody with anti-antibody; receptor with ligand; enzyme or enzyme fragment with substrate, substrate analogue or ligand; biotin or lectin with avidin or streptavidin; lectin with carbohydrate; digoxin with anti-digoxin; His-tags with  $\text{Ni}^{2+}$  ions; benzamidine with trypsin or other serine proteases; protein A with immunoglobulin; pairs of leucine zipper motifs (see, for example, U.S. Patent No. 5,643,731), bacitracin with undecaphosphopreyll pyrophosphate as well as various homodimers and heterodimers known in the art.

The term "antibody," as used herein, refers to an immunoglobulin, which can be either natural, partially synthetically produced or wholly synthetically produced. Antibody derivatives that maintain specific binding ability are also included in the term, as are proteins having a binding domain that is homologous or largely homologous to an immunoglobulin binding domain. An antibody can be polyclonal or monoclonal.

The term "antibody fragment," as used herein, refers to a derivative of an antibody that is less than full-length and which retains at least a significant portion of the specific binding ability full-length antibody. Examples of antibody fragments include, but are not limited to, Fab, Fab',  $\text{F(ab')}_2$ , scFv, Fv, dsFv, diabody, and Fd fragments. The antibody fragment can be produced by a number of techniques known in the art, for example, the antibody fragment can be enzymatically or chemically produced by fragmentation of an intact antibody, or it may be recombinantly produced from a gene encoding the partial antibody sequence. Alternatively, the antibody fragment can be wholly or partially synthetically produced.

Single-chain Fvs (scFvs) are recombinant antibody fragments consisting of only the variable light chain ( $V_L$ ) and variable heavy chain ( $V_H$ ) covalently connected to one another by a polypeptide linker. Either  $V_L$  or  $V_H$  may be the  $\text{NH}_2$ -terminal domain. The polypeptide linker may be of variable length and composition so long as the two variable domains are bridged without serious steric interference. Typically, the linkers are comprised primarily of stretches of glycine and serine residues with some glutamic acid or lysine residues interspersed for solubility.

“Diabodies” are dimeric scFvs. The components of diabodies typically have shorter peptide linkers than most scFvs and they show a preference for associating as dimers.

A “Fv” fragment consists of one  $V_H$  and one  $V_L$  domain held together by non-covalent interactions. The term “dsFv” is used herein to refer to a Fv with an engineered intermolecular disulphide bond to stabilise the  $V_H - V_L$  pair.

A “F(ab')<sub>2</sub>” fragment is an antibody fragment essentially equivalent to that obtained from immunoglobulin (typically IgG) by digestion with an enzyme pepsin at pH 4.0-4.5. The fragment may be recombinantly produced.

A “Fab” fragment is an antibody fragment essentially equivalent to that obtained by reduction of the disulphide bridge or bridges joining the two heavy chain pieces in the F(ab')<sub>2</sub> fragment. The Fab' fragment may be recombinantly produced.

A “Fab” fragment is an antibody fragment essentially equivalent to that obtained by digestion of immunoglobulin (typically IgG) with the enzyme papain. The Fab fragment may be recombinantly produced. The heavy chain segment of the Fab fragment is the Fd piece.

The terms “polypeptide” and “peptide,” as used herein, refer to a sequence of amino acid residues linked together by peptide bonds. Typically, a polypeptide or peptide is at least six amino acids long. The polypeptide or peptide can be naturally occurring, recombinant, or synthetic, or a combination of these. The polypeptide or peptide can be a fragment of a naturally occurring protein or polypeptide. The terms polypeptide and peptide also encompass amino acid sequences in which one or more amino acid residues is an artificial chemical analogue of a corresponding naturally occurring amino acid or an “unnatural” amino acid (such as the D-isomer). The polypeptide or peptide may be linear or it may be cyclised, for example, through the formation of a cysteine-cysteine disulphide bridge.

The terms “oligonucleotide” and “polynucleotide,” as used herein, refer to oligomers or polymers of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof. This term

includes oligonucleotides and polynucleotides composed of naturally-occurring nucleobases, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally-occurring portions which function similarly. Such modified or substituted oligonucleotides and polynucleotides can provide desirable properties such as enhanced affinity for a nucleic acid target analyte and/or increased stability in the presence of nucleases. The oligonucleotides and polynucleotides may be linear or they may be cyclic.

The term “immobilised,” as used herein with respect to a reagent indicates that the reagent is attached to a surface with an attractive force stronger than attractive forces that are present in the intended environment of use of the surface which will act on the reagent, for example solvating and turbulent forces. Co-ordinate and covalent bonds are non-limiting examples of attractive forces stronger than typical environmental forces.

The term “non-specific binding,” as used herein, refers to an interaction between a molecule and the capture reagent other than desired binding or interaction between the target analyte and the capture reagent.

The term “self-assembled monolayer,” as used herein, refers to a relatively ordered assembly of molecules spontaneously chemisorbed on a surface, in which the molecules are oriented approximately parallel to each other and roughly perpendicular to the surface. Each of the molecules includes a functional group that adheres to the surface, and a portion that interacts with neighbouring molecules in the monolayer to form the relatively ordered assembly (see, for example, Laibinis, *et al.*, (1989) *Science* 245:845).

The term “self-assembled mixed monolayer,” as used herein, refers to a heterogeneous self-assembled monolayer, *i.e.* one made up of a relatively ordered assembly of at least two different molecules.

## **Sampling Apparatus**

The sampling apparatus of the present invention comprises a supporting substrate, one or more capture reagents immobilised to at least one surface of the substrate and an attachment means. Optionally, the sampling apparatus further comprises a means for permitting the manipulation of the sampling apparatus by the user without contamination of the affinity surface for the purpose of deployment, removal or transport of the apparatus, for example, to a laboratory for subsequent analysis.

### *Supporting Substrate*

In accordance with the present invention, the supporting substrate is a material that can resist moderate stress (*i.e.* is not likely to break, tear or collapse) and is, therefore, distinct from a liquid or a gas. The supporting substrate can be rigid, semi-rigid or flexible.

Numerous materials are suitable for use as a supporting substrate. The supporting substrate may be either organic or inorganic, biological or non-biological, or a combination of these materials. The supporting substrate can be porous or non-porous. For instance, the supporting substrate can comprise cloth, plastic, rubber, latex or a latex gel, cellophane, silicon, silica, silicon nitride, quartz, glass, controlled pore glass, carbon, dextran or a dextran derivative, acrylic resin, tantalum oxide, zeolites, and gallium arsenide. Many metals such as gold, platinum, aluminium, copper, titanium, germanium and their alloys and salts are also options for supporting substrates of the invention. In addition, many ceramics and polymers may also be used as supporting substrates. Synthetic polymers which may be used as substrates include, but are not limited to, polystyrene; poly(tetra)fluoroethylene (PTFE); polyvinylidenedifluoride; polycarbonate; acrylates and acrylic acids such as polymethylmethacrylate and polyhydroxyethylmethacrylate (HEMA); polyvinylethylene; polyethyleneimine; poly(etherether)ketone; polyoxymethylene (POM); polyvinylphenol; polylactides; polymethacrylimide (PMI); polyalkenesulphone (PAS); polypropylethylene, polyethylene; polydimethylsiloxane; polyacrylamide; polyimide, polyvinyl chloride (PVC), polyvinyl chloride vinylidene (PVCDC) and various copolymers.

Biopolymers and derivatives of biopolymers are also suitable. Examples of biopolymers include, but are not limited to, cellulose and cellulose derivatives, fibrinogen, collagen, gelatine, agarose, elastin and alginate. Blends of polymers comprising one or more synthetic polymers and one or more biopolymer are also contemplated as are synthetic polymers cross-linked to biopolymers and synthetic polymers or co-polymers derivatised with pendant reactive moieties that can be used to cross-link a capture reagent.

Selection of the appropriate material(s) for the supporting substrate will be determined in large part by the intended application of the sampling apparatus. For example, for deployment in a fluid, the supporting substrate is constructed of a material insoluble in that particular fluid and for deployment on an irregular surface, the supporting substrate will need to be constructed of a flexible material. The selection of the appropriate supporting substrate for a particular application is within the ordinary skills of a worker in the art.

The geometry of the supporting substrate, *i.e.* size, texture, flexibility, thickness and the like, can be varied. The supporting substrate can be opaque, transparent or translucent. The supporting substrate, therefore, may be in the form of a sheet, a plate, a flexible strip, a tube or other shape and size suited to the intended use. For example, the supporting substrate may have the dimensions of a standard microscope slide or microtitre plate, thereby allowing manipulation of the apparatus by automated devices, for example automated devices capable of processing microscope slides or microtitre plates that are known to a worker skilled in the art. The surfaces of the supporting substrate may be smooth or they may comprise raised regions, etched trenches and the like, or they may comprise combinations of different finishes.

#### *Affinity Surface*

All or part of a first surface of the supporting substrate forms an affinity surface comprising at least one analyte capture reagent that is capable of binding to its target analyte when immersed in liquid or attached to a test surface, or otherwise deployed in the test environment. In accordance with the present invention, the capture reagent is immobilised either directly or indirectly on the affinity surface. The immobilisation of the capture reagent may be reversible or irreversible.

Methods of immobilising reagents onto a variety of support surfaces are known in the art and include simple absorption or adsorption, ultra-violet cross-linking and covalent attachment (either directly or through a linker). When a capture reagent is adsorbed or absorbed onto a surface, the use of a porous substrate provides a higher surface area allowing for optimised absorption and presentation of the reagent. Cellulose nitrate and nitrocellulose esters have been shown to absorbatively bind a number of substances, in particular polynucleotides and polypeptides, so strongly that they do not require a chemical bond for immobilisation. Fluorocarbon polymers have also been used as supports to which biomolecules have been attached by adsorption (see, for example, U.S. Patent No. 3,843,443).

Numerous methods have been developed for the covalent attachment of various molecules onto solid supports (see, generally, Affinity Techniques. Enzyme Purification: Part B. *Methods in Enzymology*, (1974) Vol. 34, ed. W. B. Jakoby, M. Wilchek, Acad. Press, N.Y.; Immobilized Biochemicals and Affinity Chromatography, *Advances in Experimental Medicine and Biology*, (1974) Vol. 42, ed. R. Dunlap, Plenum Press, N.Y.). The supporting substrate can be treated or derivatised to contain an appropriate reactive moiety or, in some cases, may be obtained commercially already containing the reactive moiety. The capture reagent(s) can then be reacted with the reactive moiety, either directly or through an appropriate linker, in order to attach the capture reagent to the supporting substrate. Examples of reactive groups include, but are not limited to, amine, hydroxyl, carboxyl, aldehyde, hydrazine, epoxide, bromoacetyl, maleimide, silyl and thiol groups, which can be introduced using standard surface chemistry techniques known to those of skill in art.

The capture reagent can, therefore, be conjugated to one member of a binding pair that is reversibly attached to the other member of the binding pair affixed to the affinity surface. In this instance, the capture reagent complex can be removed from the affinity surface by disrupting the binding pair interaction once the capture reagent has captured the analyte and formed a capture reagent-analyte complex, and optionally, once the non-specific contaminants have been removed from the affinity surface. Alternatively, the capture reagent can itself be one member of a binding pair reversibly attached to the other member of the binding pair which is affixed to the affinity

surface. The capture reagent can thus be removed from the affinity surface by disrupting the binding pair interaction.

The present invention also contemplates the immobilisation of the capture reagent on the affinity surface by means of a binding compound. The binding compound may be a polymer matrix to which the capture reagent is attached, or in which the capture reagent is embedded.

An affinity surface of the present invention may optionally further comprise a coating between the supporting substrate and the capture reagent. The term "coating" means a layer that is either naturally or synthetically formed on or applied to at least the first surface of the supporting substrate. For instance, exposure of substrates such as silicon to air results in the formation of a silicon oxide coating on the surface. In other instances, the coating is not derived from the substrate and may be placed upon the surface of the substrate via mechanical, physical, electrical, or chemical means. The present invention contemplates coatings of various thickness. In one embodiment, the coating has a thickness less than that of the supporting substrate.

The coating may either be formed on the supporting substrate or applied to the supporting substrate. The supporting substrate can be modified with a coating by using thin-film technology based, for instance, on physical deposition, vapour deposition, or thermal processing. Alternatively, plasma exposure can be used to directly activate or alter the supporting substrate and create a coating. For instance, plasma etch procedures can be used to oxidise a polymeric surface (for example, polystyrene or polyethylene to expose polar functionalities such as hydroxyls, carboxylic acids, aldehydes and the like) which then acts as a coating.

The coating can alternatively comprise a composition or matrix that contains a reactive group as described above that can react with the capture reagent or a suitable linker. Compositions or matrices comprising silicon, silicon oxide, titania, tantalum oxide, silicon nitride, silicon hydride, indium tin oxide, magnesium oxide, alumina, glass, hydroxylated surfaces, and various polymers may be suitable. The coating can also be a metal film. Examples of metal films include, for example, those based on aluminium, chromium, titanium, tantalum, nickel, stainless steel, zinc, lead, iron, copper, magnesium, manganese, cadmium, tungsten, cobalt, and alloys or oxides

thereof. The metal film can be a noble metal film, such as a film of gold, platinum, silver, or copper or an alloy thereof. Electron-beam evaporation may be used to provide a thin coating of gold on the surface of the supporting substrate. Metal films are typically between about 50 nm to about 500 nm in thickness, for example, from about 1 nm to about 1  $\mu$ m in thickness.

The coating may be a self-assembled monolayer (SAM). SAMs are also known in the art and are generally defined as a type of molecule that can bind or interact spontaneously or otherwise with a metal, metal oxide, glass, quartz or modified polymer surface in order to form a chemisorbed monolayer. A self-assembled monolayer should be the thickness of a single molecule (*i.e.*, it is ideally no thicker than the length of the longest molecule included therein). Each of the molecules making up a self-assembled monolayer thus includes a reactive group that adheres to the conductive base layer and may also include a second reactive moiety that can be used to immobilise the capture reagent onto the supporting substrate.

Immobilised artificial membranes (IAMs) may also be used as a coating (see, for example, U.S. Patent Nos. 4,931,498 and 4,927,879). IAMs mimic cell membrane environments and may be used to bind molecules that preferentially associate with cell membranes (see, for example, Pidgeon *et al.* (1990) *Enzyme Microb. Technol.* 12:149).

Deposition or formation of the coating (if present) on the supporting substrate is typically performed prior to the deposition of the capture reagent thereon. Alternatively, the capture reagent may be bound to the coating material prior to deposition of the coating. Several different types of coating may be combined. The coating may cover the whole first surface of the supporting substrate or only parts of it. In one embodiment of the invention, the coating covers the supporting substrate only at the site of the affinity surface. Techniques useful for the formation of a coating on the surface of the supporting substrate are well known to those of ordinary skill in the art. For instance, the coating on the supporting substrate may be fabricated by photolithography (for example, U.S. Patent No. 5,143,854 describes a process of synthesising a polymer directly onto a solid support), micromolding (PCT Publication WO 96/29629), wet chemical or dry etching, or a combination of these techniques.

The coating can be an organic thinfilm used to immobilise the capture reagents to the affinity surface. An "organic thinfilm" is a thin layer of organic molecules which has been applied to a supporting substrate (see, for example, U.S. Patent Nos. 6,329,209; 6,475,808 and 6,365,418). Typically, an organic thinfilm is less than about 20 nm thick. Optionally, an organic thinfilm may be less than about 10 nm thick. An organic thinfilm may be disordered or ordered, may be heterogeneous or homogeneous, and may be a monolayer or a bilayer. Optionally, the organic thinfilm may comprise a combination of more than one form of organic thinfilm. For instance, an organic thinfilm may comprise a lipid bilayer on top of a self-assembled monolayer. A hydrogel may also compose an organic thinfilm. The organic thinfilm will typically have functionalities exposed on its surface which serve to enhance the surface conditions of a supporting substrate or the coating on a supporting substrate in any of a number of ways. For instance, exposed functionalities of the organic thinfilm are typically useful in the binding or covalent immobilisation of the capture reagents to the affinity surface. Alternatively, the organic thinfilm may bear functional groups (such as polyethylene glycol (PEG)) which reduce the non-specific binding of molecules to the affinity surface. Other exposed functionalities serve to tether the thinfilm to the surface of the supporting substrate. Particular functionalities of the organic thinfilm may also be designed to enable certain detection techniques to be used with the affinity surface. Alternatively, the organic thinfilm may serve the purpose of preventing inactivation of the capture agent from occurring upon contact with the surface of a supporting substrate. Optionally, the coating may contain humectant moieties such as glycerol or other substances that serve to protect the captured analyte from desiccation.

A variety of different organic thinfilms are suitable for use in the present invention. For instance, a hydrogel composed of a material such as dextran can serve as a suitable organic thinfilm on the supporting substrate, or the organic thinfilm can be a monolayer of polyarginine or polylysine adsorbed on a negatively charged supporting substrate, or a lipid bilayer or monolayer. Another option is a disordered monolayer of tethered polymer chains. The organic thinfilm can also be a self-assembled monolayer. For example, the organic thinfilm can be a self-assembled monolayer which comprises molecules of the formula X-R-Y, wherein R is a spacer, X is a functional group

that binds R to the supporting substrate, and Y is a functional group for binding capture reagents to the monolayer.

Methods for the formation of organic thinfilms include, but are not limited to, *in situ* growth from the surface of the supporting substrate, deposition by physisorption, spin-coating, chemisorption, self-assembly, or plasma-initiated polymerization from gas phase.

The present invention contemplates affinity surfaces to which a plurality of capture reagents are attached which provide for enhanced detection of a target analyte. For example, when the target analyte is a microbe, the affinity surface may comprise capture reagents that bind to different surface proteins of the microbe, or when the target analyte is a protein, the affinity surface may comprise antibody or antibody fragment capture reagents that bind different epitopes present within the same protein.

#### *Affinity Surface Protection Means*

The affinity surface of the present invention can further comprise an affinity surface protection means to protect the affinity surface from contamination and dehydration before and during the deployment of the apparatus. For example, the affinity surface protection means can comprise a removable covering overtop the affinity surface, wherein the affinity surface protection means can be removed during the deployment of the apparatus. The affinity surface protection means may be reversibly removable, whereby the affinity surface protection means may be replaced on the apparatus to cover the affinity surface after sampling. In this way, the affinity surface is protected from contamination after sampling, while the apparatus is being manipulated prior to any subsequent shipping and analysis. The affinity surface protection means can alternatively comprise a soluble matrix which dissolves upon contact with the fluid to be sampled, thereby exposing the affinity surface beneath.

The present invention also contemplates an affinity surface protection means that is composed of a porous material that permits access of a target analyte to the affinity surface but restricts access of debris and other undesirable larger moieties. Such an affinity surface protection means would remain in place during deployment of the sampling apparatus.

*Attachment Means*

The apparatus further comprises an attachment means for attaching the apparatus to the site to be sampled. Examples of attachment means include mechanical means such as a clip, clasp, magnet, tack or a combination thereof for reversibly mounting the apparatus to the site to be sampled. The mechanical attachment means may be connected directly to the sampling apparatus, or may be connected to the sampling apparatus by means of a string, ribbon, or chain, allowing the apparatus to be suspended from the attachment point by a distance determined by the length of the string, ribbon, or chain.

Alternatively, the attachment means comprises an adhesive surface, wherein an adhesive is coated on a second surface of the support substrate. Optionally, the adhesive surface further comprises an adhesive surface protection means to protect the adhesive surface from desiccation, degradation, or unwanted adhesion before and during the deployment of the apparatus. In one embodiment, the adhesive surface protection means comprises a removable covering overlying the adhesive surface, wherein the adhesive surface protection means can be removed during the deployment of the apparatus. Optionally, the adhesive surface protection means may be reversibly removable, whereby the adhesive surface protection means may be replaced on the apparatus to cover the adhesive surface after sampling, while the apparatus is being manipulated prior to any subsequent shipping and analysis.

The attachment means can be associated with one supporting substrate or it may be associated with a plurality of supporting substrates. The apparatus of the present invention, therefore, can comprise two or more supporting substrates, each having a different capture reagent attached thereto. The different capture reagents may be designed to capture target analytes from the same source, for example, different parts of a bacteria or virus, or they may be designed to capture target analytes from different sources, for example, two or more different viruses.

The present invention also contemplates an attachment means that comprises a frame with an adhesive backing or an associated mechanical attachment means. The frame is dimensioned to

hold at least one supporting substrate. The frame may be designed such that the supporting substrate(s) can be removed and replaced with a new supporting substrate (s).

### *Manipulation Means*

The apparatus can optionally further comprise a manipulation means whereby the apparatus may be manipulated by the user and by which the apparatus may be positioned on the surface to be sampled prior to the deployment of the apparatus, and removed from the surface after sampling for transport to a laboratory for subsequent analysis, without contamination of the affinity surface by the user. In one embodiment of the invention, the manipulation means comprises a plurality of removable tabs flanking the affinity surface and extending therefrom, but which are devoid of capture reagent.

The manipulation means can be adapted for use with a device such as a robotic arm or automated slide or plate reader. For example, the manipulation means may comprise areas or sectors on the supporting substrate that are devoid of affinity reagent, and may further comprise a textured surface on the areas or sectors of the supporting substrate, allowing facilitated gripping of the sampling apparatus during automated manipulation.

### *Exemplary Embodiments of the Sampling Apparatus*

Referring to Figure 1 which schematically shows a section through one embodiment of the invention, and Figure 2 which shows a top view of the embodiment, a flexible plastic film supporting substrate **1** comprises an affinity surface **2**. Capture reagent **3** specific to a target analyte for which the embodiment will be used coats affinity surface **2**, and is optionally bound to affinity surface **2** by a member of a binding pair **4** to ensure the durability of its attachment. Capture reagent **3** and the member of the binding pair **4** may be coated as an admixture onto affinity surface **2** in the same layer, or may each comprise a separate layer. Supporting substrate **1** further comprises an attachment surface **5**. Adhesive **6** of a type and composition suited to the eventual use, for example, permitting removable attachment of the invention to a carcass, coats attachment surface **5**. Optionally, removable tabs **7** permit the manipulation of the present invention by the user for the purpose of deployment, removal or transport of the apparatus to a

laboratory for subsequent analysis without contamination of affinity surface **2** by the user's fingers. Optionally, an affinity surface protection means **8** comprising a removable layer protects affinity surface **2** before deployment of the apparatus, and an attachment surface protection means **9** comprising a removable layer protects attachment surface **5** before attachment.

Referring to Figures 3 and 4 which show schematic plans of other embodiments, the overall shape and size of the supporting substrate **1** and the shapes and sizes of the various layers **3**, **4**, **6**, **7**, **8** and **9** may be any shape and size suited to the efficient deployment of the invention. Typically for attachment to a carcass the invention may be rectangular, circular or more or less oval, or have projecting areas for the tabs **7** and have an area of 10 to 100 cm<sup>2</sup>, but other shapes and sizes are not excluded.

Referring to Figure 5 which shows a schematic projection of another embodiment, a porous cloth **10** is coated with capture reagent **3**. The cloth **10** may be kept spread open by an optional frame **11** having a weight suited to keeping the apparatus immersed in a fluid, and optionally may have attachment means such as string **12** and clip **13** or other attachment means permitting it to be suspended in a fluid tank or conduit where it can bind target analyte from a fluid supply.

Referring to Figure 6 which shows a schematic projection of another embodiment, an open-ended tubular supporting substrate **14** is coated internally with capture reagent **3**, and is attachable by string **12** and clip **13** or other attachment means, permitting it to be immersed in a fluid where it can bind target analyte.

Referring to Figure 7 which shows a side view of another embodiment having a spike **15** or other means of retaining the affinity surface **2** of the invention above the surface of the ground.

### **Sampling Method**

The present invention further provides for a method of sampling a test environment for one or more target analytes. The sampling method of the present invention comprises the steps of:

- (a) applying the apparatus to a test environment;

- (b) allowing the apparatus to sample the test environment for a suitable period of time;
- (c) removing the apparatus from the sampling site; and optionally
- (d) rinsing away non-selectively bound materials.

To employ the sampling method, a suitable form of the sampling apparatus is first selected. For example, if the test environment is a carcass in an abattoir, the selected sampling apparatus could be in the form of a flexible film comprising an adhesive surface.

The location within a test environment for deploying a sampling apparatus is typically one in which the apparatus will receive maximum exposure to the target analyte. Appropriate locations can be readily determined by a worker skilled in the relevant art. For example, when the test environment is an animal carcass, the apparatus is preferably applied at a drip point or pool point on the surface, where it will be exposed to fluid running down or pooling on the carcass during subsequent abattoir procedures. The term “drip point” as used herein refers to a physical point on a surface such as a food processing surface or an animal carcass suspended in an abattoir to which fluid such as carcass rinse run-off or carcass-sourced fluid (*i.e.* blood, urine, and other biological fluid) is drawn by means of gravitational attraction, and from which the fluid drips. Generally, the drip point is a low point of the surface. The term “pool point” as used herein refers to a physical point on the surface to which the fluid is drawn by means of gravitational attraction and at which the fluid pools. Generally, the pool point is a relative low point of the surface located on a depression of the surface.

If the test environment is a liquid medium such as a water supply, the apparatus may comprise an attachment means such as a string and clip, and either a coated cloth held open by a weighted frame, or an open-ended tube comprising an internal affinity surface. The user will remove any protective wrapping, and immerse the apparatus in the water supply or allow the water supply to flow through the tubular apparatus, fastening it for easy retrieval by means of its attachment means.

During the sampling time a proportion of any target analyte in the fluid that contacts the apparatus will bind to its affinity surface. After the apparatus has been allowed to sample for a suitable period of time, the apparatus is removed from the sampling site and transferred to an appropriate analysis facility. Non-selectively bound materials such as non-target microorganisms, skin cells, fat globules, hair, and other substances can be removed by rinsing prior to, or after, removal of the apparatus from the test environment.

The period of time that the apparatus will be allowed to sample is typically dependent on the environment sampled and the type of analyte targeted. A suitable sampling time is typically a period of time that permits sampling of the target analyte and can be readily determined by a worker skilled in the relevant art. For example, the sampling apparatus can be allowed to sample over a period of minutes to a period of days.

For analysis of the target analyte that is reversibly bound to the supporting substrate, the target analyte can be released from the supporting substrate by carrying out an appropriate releasing or decoupling step after the washing step. Once the target analyte has been released, or the analyte-capture reagent has been decoupled, the recovered analyte is subjected to analysis. As will be appreciated by a worker skilled in the art, the method of analysis of the bound target analyte will be dependent on the particular target analyte being sampled and may comprise one or more standard analytical techniques such as gas chromatography, high-performance liquid chromatography, mass spectrometry, fluorescence spectroscopy and the like. Alternatively, when the target analyte is a microbe, the affinity surface of the apparatus can be contacted with the surface of a Petri dish of diagnostic agar, thereby transferring the microbial analyte to the diagnostic agar surface, or the bound analyte can be removed using a cell scraper to scrape the analyte into a small volume of diluent and this diluent is incubated on Petri dishes of suitable agar, followed by biochemical tests on suspect colonies.

## Uses

The sampling apparatus of the present invention has a broad applicability in a number of environments. Examples include, but are not limited to, cattle pens, cow's tails, fruits, vegetables

trucks, crates, conveyors, grinders, mixers, processing shop floors, processor's overalls, chill cabinets, kitchen counters, cutting boards, refrigerators, food processing plants, hospitals, nursing homes, water facilities, runoffs, airports, ventilation systems, crop growing surfaces and other areas of concern.

### **Kits**

The present invention further provides for kits for sampling a test environment. A kit according to the present invention comprises one or more sampling apparatus and instructions for use.

Optionally, the kit further comprises a washing solution for post-sampling washing of the apparatus to remove non-specific contaminants while retaining the target analyte bound thereto, or a releasing solution for releasing the target analyte from the capture reagent, or a decoupling solution for decoupling the capture reagent and target analyte-capture reagent complex from the other member of the binding pair.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

**THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:**

1. A sampling apparatus capable of being retrievably deployed in environments, said apparatus comprising:
  - (a) an insoluble supporting substrate;
  - (b) at least one target analyte capture reagent immobilised on a first surface of said insoluble supporting substrate; and
  - (c) an attachment means.
2. The apparatus of claim 1 wherein the supporting substrate is selected from a group comprising a plastic film, a cellophane film, a metal sheet, a rubber sheet, a cloth, and a sponge.
3. The apparatus of claim 1 wherein the supporting substrate is tubular.
4. The apparatus of claim 1 wherein the supporting substrate is maintained in an open configuration by a frame, and is immersible in fluid by means of a weighted frame.
5. The apparatus of claim 1 wherein the capture reagent is selected from the group comprising an antibody, a hapten, an epitope, an antigen, a ligand, a cofactor, a substrate analogue, a lectin, and a polynucleotide.
6. The apparatus of claim 1 further comprising a coating between said supporting substrate and said capture reagent.
7. The apparatus of claim 1 wherein the attachment means comprises an adhesive on a second surface of said insoluble supporting substrate.
8. The apparatus of claim 7 wherein the adhesive is protected by a removable protective layer.

9. The apparatus of claim 1 wherein the supporting substrate comprises manipulation means permitting the apparatus to be handled without contaminating the capture reagent immobilised on the first surface.
10. A kit comprising:
  - (a) the apparatus of any one of claims 1 to 9; and
  - (b) instructions for storage, deployment, use, and recovery of the apparatus.
11. The kit of claim 10 further comprising a washing solution and a solution selected from the group comprising a releasing solution and a decoupling solution.
12. A method of sampling the environment for the presence of a target analyte, comprising the steps of:
  - (a) applying the apparatus to a predetermined location;
  - (b) allowing the apparatus to sample the environment for a sufficient period of time;
  - (c) removing the apparatus from the sampling site; and
  - (d) rinsing away non-selectively bound materials.

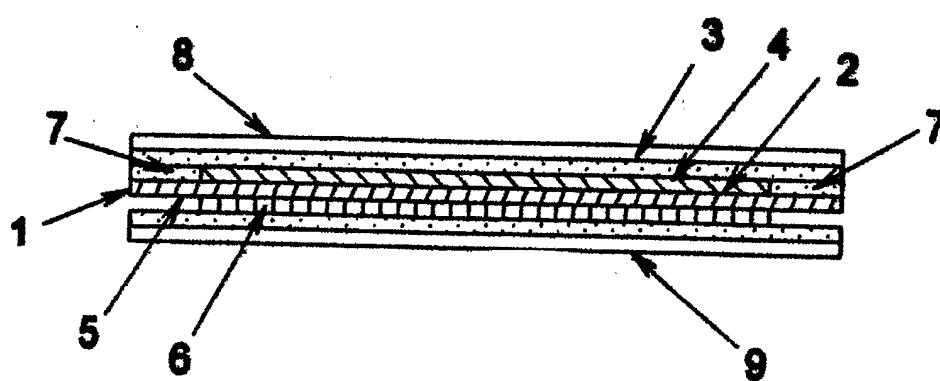


Figure 1

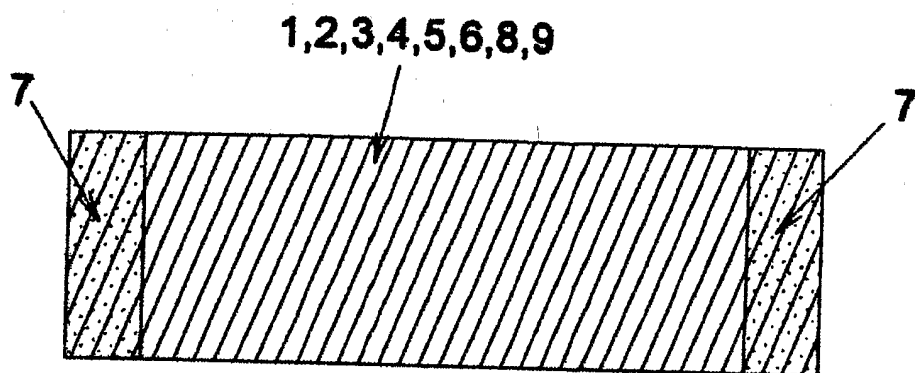


Figure 2

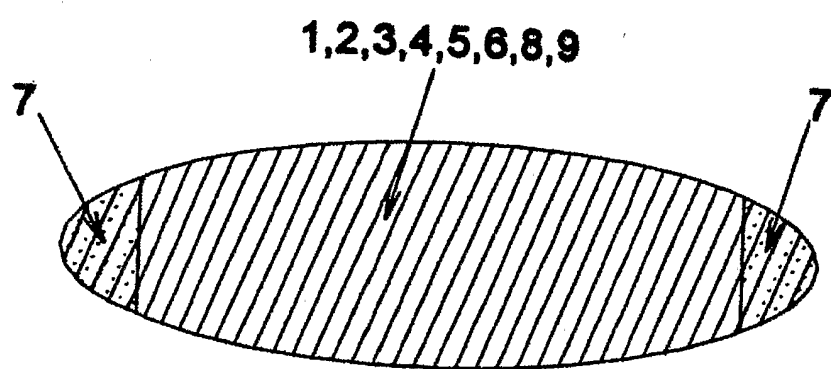


Figure 3

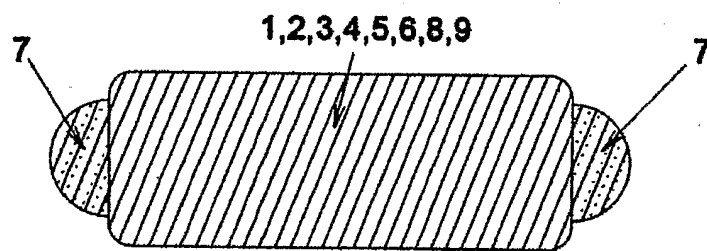


Figure 4

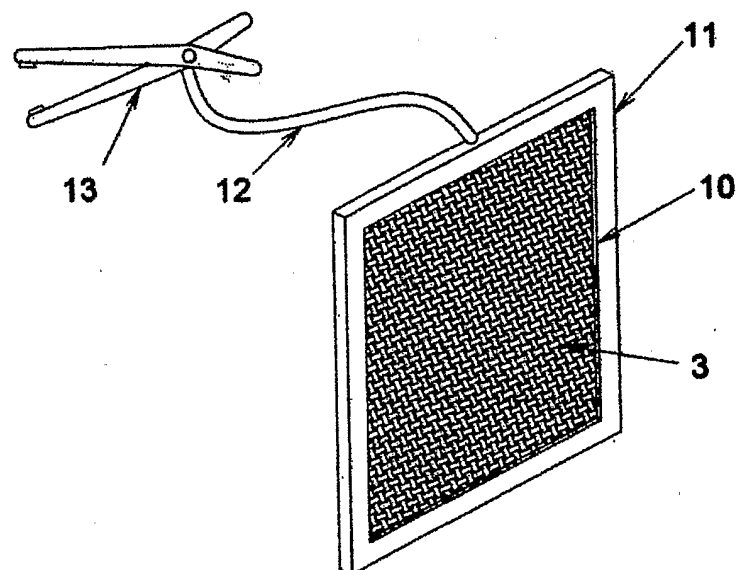


Figure 5

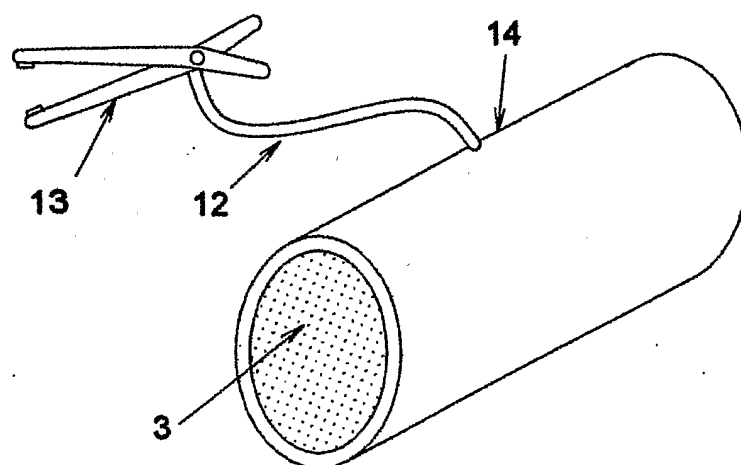


Figure 6

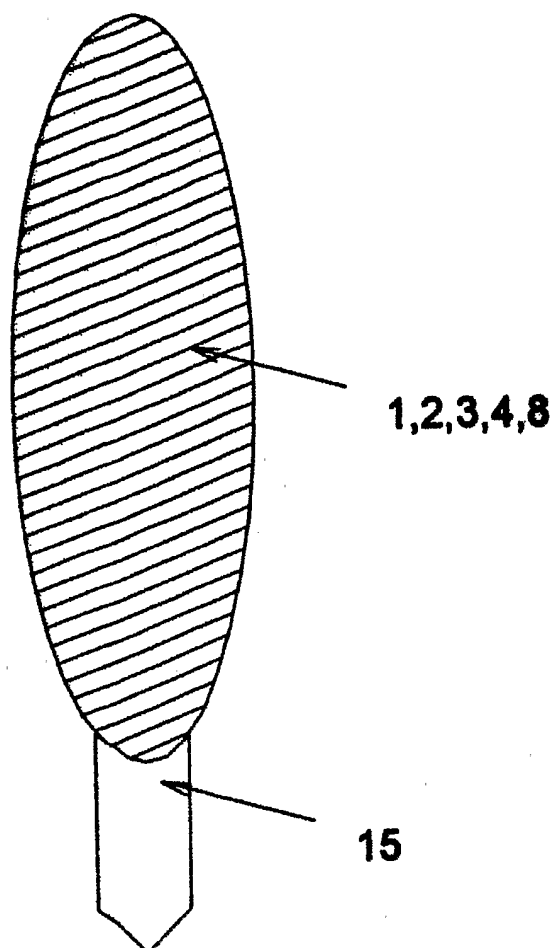


Figure 7 .

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/CA2004/000768

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 G01N33/543 C12M1/26 C12Q1/24 G01N33/52

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)

IPC 7 G01N C12M C12Q

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 practical, search terms used)

EPO-Internal, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                  | Relevant to claim No |
|------------|-------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| P,X        | WO 03/060479 A (SERITELLA KEITH A ; WICKSTEAD JAMES C (US); SLOWEY PAUL D (US); SALVO) 24 July 2003 (2003-07-24) the whole document | 1,5,6,9,12           |
| X          | WO 02/50541 A (NANOSPHERE LTD ; OLDFIELD CHRISTOPHER (GB)) 27 June 2002 (2002-06-27) the whole document                             | 1,5,9,12             |
| Y          |                                                                                                                                     | 2-4,6-8,10,11        |
| Y          | US 5 641 642 A (PEYTON BRENT M ET AL) 24 June 1997 (1997-06-24) the whole document                                                  | 2-4                  |
|            | -/--                                                                                                                                |                      |



Further documents are listed in the continuation of box C



Patent family members are listed in 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 document 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

15 November 2004

Date of mailing of the international search report

22/11/2004

Name and mailing address of the ISA

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Authorized officer

Diez Schlereth, D

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA2004/000768

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                                 | Relevant to claim No |
|------------|----------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| Y          | WO 00/61802 A (LINGARD LABEL COMPANY LIMITED ; PASTORAL AGRIC RES INST NZ LTD (NZ); S) 19 October 2000 (2000-10-19)<br>the whole document<br>----- | 6-8,10,<br>11        |
| A          | US 6 207 406 B1 (WILKINS JUDD R)<br>27 March 2001 (2001-03-27)<br>cited in the application<br>the whole document<br>-----                          | 1-12                 |
| A          | EP 0 816 513 A (NITTO DENKO CORP)<br>7 January 1998 (1998-01-07)<br>cited in the application<br>the whole document<br>-----                        | 1-12                 |

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/CA2004/000768

### Box 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.  
The scope of claim 12 embraces embodiments directed to a method for taking samples of living human/animal bodies by using a chirurgic step.
2. ☐ Claims Nos.:  
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
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 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 an additional fee, this Authority did not invite payment of any additional fee.
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.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA2004/000768

| Patent document<br>cited in search report |    | Publication<br>date | Patent family<br>member(s)                                                                                             | Publication<br>date                                                                            |
|-------------------------------------------|----|---------------------|------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|
| WO 03060479                               | A  | 24-07-2003          | US 2003205097 A1<br>CA 2473514 A1<br>EP 1474672 A2<br>WO 03060479 A2<br>US 2004014203 A1                               | 06-11-2003<br>24-07-2003<br>10-11-2004<br>24-07-2003<br>22-01-2004                             |
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| US 6207406                                | B1 | 27-03-2001          | NONE                                                                                                                   |                                                                                                |
| EP 0816513                                | A  | 07-01-1998          | JP 10070975 A<br>JP 10070976 A<br>DE 69718245 D1<br>DE 69718245 T2<br>EP 0816513 A1<br>US 6203900 B1                   | 17-03-1998<br>17-03-1998<br>13-02-2003<br>14-08-2003<br>07-01-1998<br>20-03-2001               |