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(54) Title: IDENTIFICATION OF PARTICLES AND MACROMOLECULAR SPECIES

(57) Abstract

A method of detection and/or identification of particles, the method comprising the steps of: (a) collecting the particles onto a surface; (b) allowing macromolecular species associated with the particles to diffuse from the particles; (c) immobilising any diffused macromolecular species in close proximity to the particles, wherein an immobilised macromolecular species is sufficiently close to a particle so as to be indicative as being diffused from the particle; (d) analysing the immobilised macromolecular species to determine one or more characteristics of macromolecular species; (e) comparing the determined one or more characteristics of macromolecular species characteristics with a reference base of known characteristics of macromolecular species associated with particles; and (f) using the comparison of the determined one or more macromolecular species characteristics to indirectly detect and/or identify at least one particle type present on the surface associated with the analysed macromolecular species immobilised in close proximity to the particle.

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Identification of particles and macromolecular species

Field of Invention

The present invention relates to methods for identifying particles and macromolecular species of clinical interest which have been collected from air or other fluid streams.

Background of the Invention

There are many situations in which particles in a fluid stream may cause disease, environmental damage, or be of interest for some other reason. In some cases, the fluid stream may be carrying a large number of different particles. In this case it may be difficult to determine which of the particles are causing the effect being studied. A particular example is that asthma is believed to be associated with airborne allergens. There may, however, be many airborne allergens in the air but only one or a few may be causing the asthma.

Asthma is a chronic disease suffered by all age groups in the community. The study of asthma has been hindered by the shortage of suitable methods to identify and measure the characteristics of allergen exposure which is relevant to the disease. These characteristics include:

(1) the identity of the allergen;

(2) the quantity of the allergens; and

(3) the physical characteristics of the particles which are the allergen source.

It may also be of interest to know which particles present in a patient's environment are responsible for the observed symptoms. This knowledge may allow a determination of the source of the allergens and suggest mechanisms for treating the condition.

It has long been conventional to estimate exposure to pollen allergens by collecting pollen particles on sticky surfaces, identifying them by morphological criteria, and individually counting them. Mould exposure has been similarly monitored by biological and/or morphological methods. In each of these cases, the identifiable biological particles are used as a proxy for the quantity of allergens present in the sampled environment. These methods require highly skilled operators and are very labour intensive. The methods also suffer from the problem that the presence of a particular particle does not necessarily indicate that the particle is the source of the allergen of interest.

Immunoassays of allergens from many sources (pollens, mites, fungi, animal dander, etc.) based on aqueous extraction of material collected on filters and traps has the advantages of speed and simplicity, if suitably specific antisera is available. These methods also allow quantification of the exposure to the specific allergens. There is, however, no one-to-one relationship established by such methods between specific allergens and individual particles, as the allergens are extracted from the entire sample of collected particles. Further, because in conventional assays to measure allergens, the immuno-recognition is not performed with the serum from the allergic subject, there is no ability to associate a specific type of particle with sensitisation of the patient in a way commonly associated with an observed disease state.

The known methods of particle detection and analysis do not readily provide information on which aeroallergens are present to which an individual is allergic. Skin-prick testing, Radio allergo sorbent test (RAST) assays and questioning by a physician cannot definitely elucidate which allergens are producing the observed symptoms. In some cases, using such tests may be quite misleading as they cannot determine the time when an individual was exposed to the sensitising allergen nor can they detect sensitisation to allergens not in the test panel.

Not only is it important in a number of clinical situations to identify a specific offending allergen, it is also important in a more general sense to be able to make a cross-sectional study of an exposure situation to understand the composition of different allergens which may be present.

It has been shown in Tovey ER *et al,* Am. Rev. Resp. Dis. 1981; 124:630-635 that individual airborne mite faecal particles can be identified by a ring of immunoprecipitation developed around them following their impaction onto a bed of agarose containing allergen specific antibody. The identification of the particles was made by microscopy. Problems associated with this system are that only very short exposure times of the agarose bed is possible and that only specific polyclonal high-titre (hyper immune) antisera specific for a predetermined antigen can be used. Such high-titre sera is normally only available from an animal, such as a rabbit, that has been repeatedly injected with the allergen or antigen of interest and an adjuvant. The method would not work directly with IgE antibodies in the serum of an

allergic person or with monoclonal antibodies. In neither of these cases is a visible immunoprecipitate formed.

The feasibility of using nitrocellulose membranes to press-blot antigens from individual particles has been described in Schumacker *et al.* J. A1. Clin. Imm. 1988; 82:608-16 and Takahashi Y et al Allergy 1993, vol 48, 94-98. In this method the particles collected and the nitrocellulose allergen blot are separated so that the immunostained antigens cannot be associated with the specific particles from which they originated.

One method for the identification of particles carrying allergenic molecules is described in International Publication No WO 96/07099. In this method, particles are collected and retained on a surface. Macromolecular species are eluted from the particles and are immobilised on nitrocellulose or another macromolecular-binding membrane which is associated with the surface in a manner such that the close proximity of the individual particle and the eluted macromolecules is retained. The immobilised macromolecules near the particles may be specifically stained or labelled and the individual particles thus identified as the origin of the macromolecules.

A suitable means for sampling air-born particles is described in WO 96/06657 which is incorporated herein by reference.

The present invention provides an improved means of identifying molecules and/or their associated particles.

Summary of the Invention

In a first aspect, the present invention consists in a method of detection and/or identification of particles, the method comprising the steps of:

a) collecting the particles onto a surface;

b) allowing macromolecular species associated with the particles to diffuse from the particles;

c) immobilising any diffused macromolecular species in close proximity to the particles, wherein an immobilised macromolecular species is sufficiently close to a particle so as to be indicative as being diffused from the particle;

d) analysing the immobilised macromolecular species to determine one or more characteristics of macromolecular species;

e) comparing the determined one or more macromolecular species characteristics with a reference base of known characteristics of macromolecular species associated with particles; and

f) using the comparison of the determined one or more macromolecular species characteristics to indirectly detect and/or identify at least one particle type present on the surface associated with the analysed macromolecular species immobilised in close proximity to the particle.

In a second aspect, the present invention consists in method of detection and/or identification of macromolecular species associated with particles, the method comprising the steps of:

a) collecting the particles onto a surface;

b) allowing macromolecular species associated with the particles to diffuse from the particles;

c) immobilising the diffused macromolecular species in close proximity to the particles, wherein an immobilised macromolecular species is sufficiently close to a particle so as to be indicative as being diffused from the particle;

d) analysing the collected particles to determine one or more characteristics associated with particles;

e) comparing the determined one or more particle characteristics with a reference base of known characteristics of particles from which macromolecular species can to diffuse; and

f) using the comparison of the one or more determined particle characteristics to indirectly detect and/or identify the macromolecular species diffused from the particle and immobilised in close proximity to the particle on the surface.

The advantage of the present invention is that either particles can be indirectly identified from the presence of macromolecular species that have diffused from the particles or that macromolecular species can be indirectly identified from the detection of particles from which the macromolecular species have diffused. Thus, there is no need to directly detect or identify a particle or a macromolecular species to obtain the information required.

In a preferred embodiment of the two aspects of the present invention, the diffused macromolecular species are immobilised non-specifically in close proximity to the particles.

Typically, the diffused macromolecular species are biomolecules including proteins, peptides, antigens, allergens, urine components, toxins, and nucleic acids. The particles may be microorganisms including bacteria, yeasts, fungi, plant materials including pollens, insects including mites and fleas, and waste materials including faeces and urine. The particles may also be of non-biological origin for example dust and diesel particulates which have associated or absorbed biomolecules.

Preferably, in the first step (a) of the methods according to the two aspects of the present invention, the particles are sampled in such a way they are available and appropriately presented for subsequent analysis. The particles can be collected from a fluid stream onto a surface by impaction, filtration onto a membrane, by passive diffusion, or by centrifugation. The surface preferably has the capacity to retain the particles. One preferred method of sampling is to collect the particles by impaction onto a surface provided with adhesive properties. A substantially flat, porous membrane, however, can also be used to filter the particles from a fluid. In both cases, the particles are collected onto, or distributed over, and are presented for analysis on a surface. The preferred method of impaction sampling is to use a device which is either seated at the entrance of, and/or inserted into the first portion of, the nasal cavity. Such a device is described in WO 96/06657 and uses the respiration of the wearer to provide the airflow through the device and such a collection means approximates personal exposure to particles. The impaction collector can consist of a block with a tapered slot through which air is inhaled by the wearer. A sample of particles in an air stream is, on inhalation, deposited on a collection plate which fits into the sampler. Air can also pass around the edges of the collection plate during inhalation. The collection plate can be removed from the device to allow analysis of the particles that have been collected. The device can be sealed into the nose by a combination of adhesion and compression fit of a soft outer seal.

After collection of particles, the macromolecular species associated with the particles are allowed to diffuse from the particles. One suitable method is to cover the collection surface with a thin film of agarose or other aqueous matrix. The macromolecular species are preferably immobilised in close association with the particle. The immobilisation of the macromolecules occurs on a protein binding membrane such as

nitrocellulose, situated just beneath a thin layer of agarose which contains the particles. In a second method, the particles are collected directly onto an adhesive layer which is then overlaid with a macromolecular species-binding membrane (typically a protein-binding membrane). The combined sandwich is then wetted to allow diffusion of the macromolecules and the complete probing and analysis takes place in this combined sandwich. In a third method, the particles are collected onto a macromolecular species-binding membrane by impaction or filtration and the membrane is covered by a layer to retain the particles. The sandwich is wetted to allow the macromolecular species to diffuse and bind to the macromolecular species-binding membrane. Probing and analysis occurs in this combined sandwich. In a fourth method, both the collection and binding of macromolecular species occurs on a layer capable of specifically or nonspecifically binding the macromolecular species of interest. Such a layer could be a form of agarose modified to non-covalently or covalently couple macromolecular species including proteins while in addition possessing properties which allow it to be adhesive and to retain water.

In still further method, a film of agarose which has been modified to enable it to rapidly covalently or non-covalently couple macromolecular species non-specifically can be used to cover the collection surface. A method to perform this is to include components of a macromolecular species-binding material, such as small fibres or particles (~1 um) of nitrocellulose, into the agarose layer. A layer of agarose may be used to permanently cover the particles after they have been collected onto the surface.

The proximal association between particle and bound macromolecular species is preferably such that it allows the macromolecular species derived from a particle to be available for analysis.

The characteristics of the particles suitable to be analysed for the methods according to the present invention include, but not limited to, morphology like size, shape, density, refractive index, colour, texture, roughness and the possession of particular surface or interior features. It will be appreciated, however, that many of the suitable characteristics may be measured by automated means and the results recorded electronically. In this case, the comparison between the measured characteristic(s) and the reference base may be made in an automated manner. Alternatively, the

comparison may be made manually by an operator by comparing the observed characteristics with the reference base. Either way, the particle may be identified and its presence in the sample recorded.

The characteristics of the macromolecular species that may be analysed include antigenicity, immunoreactivity, chemical, labelling, enzymatic properties, presence of specific gene sequences, and physical. The characteristics may be measured directly or indirectly by using specific probes that react with or to a macromolecular species of interest. The reaction of the probe with the macromolecular species may then be measured.

There are a variety of ways the labelling of the macromolecules can be analysed. One particularly suitable way is the linking the immunolabelling of macromolecules to image analysis and feature extraction leading to the identification of pollens and fungi. For some purposes the main information outcome would be the number of particles associated with specific staining of their bound macromolecules, in other cases the number, intensity or staining and its relationship to particle size would be of interest.

Detectable probes can be provided directly to the side of the surface to which the particles are collected. Alternatively, the detectable probes can be provided at the opposite side of the surface to which the particles are immobilised and transfer of the probes occurs substantially perpendicular through to the side of the surface to which the particles are immobilised. Furthermore, detectable probes can provided by diffusion and/or capillary flow substantially parallel to the surface to which the particles are immobilised.

It will be appreciated that one or more particle types or macromolecular species may be detected and identified in a given sample by measuring for the particles or macromolecular species of interest.

Data sets from both macromolecular species and from image analysis can be analysed to provide information of the particle's morphological features. Furthermore, analysis of specific labelling of macromolecular species associated with given particles may also provide quantitative information about the macromolecular species. The characteristics from analysis of the macromolecular species and the image analysis of the particle can be used interactively and in any order with information in a separate reference base containing digitised information about known particles of

interest and their associated macromolecular species. This database will have previously been assembled and will enable the identification of the particle and/or the macromolecular species associated therewith to a reasonable degree of probability.

Although the principle method used to obtain the images would be using a microscope, and probably a video camera. It has become apparent to the present inventors that one simple way to perform collection of information is to directly use a 35 mm slide scanner. This is a very cheap method to provide crude digital information about the numbers and intensity of staining. If required, such scanning can be combined with visualisation of the particle through a microscope and a more manual, interactive, algorithmic process using a database of information.

While the present invention has particular application to the identification of allergenic macromolecular species and/or their associated particles, the methods can be used for other purposes. For example, the methods could be used to characterise particles in exhaled air to assist in diagnosis of a disease state. The presence of microorganisms, endotoxins, and industrial or agricultural materials (such as dusts) could also be analysed using the present invention. The method may also be used to identify cells or microorganisms exporting, producing or expressing specific macromolecules.

The analysis is adapted to provide information about immunological, physical, enzymic, gene sequence or chemical properties of the macromolecular species which forms a set of characterising information which can be used for identification or measurement of the macromolecular species.

In an automated system, for example, a set of one or more images of morphological features of the particles can be collected. These features can include observable physical characteristics (such as dimension), more abstract shape concepts (such as roundness), analytical parameters (such as expressions of surface or subsurface regularity) or the possession of characteristic physical features.

Data sets from the analysing and from image analysis can be used, in conjunction with an existing reference base of known characteristics of particles, to predict the identity of particles collected by the sampling.

Analysis of the macromolecular species can be performed by one or more methods and image analysis of each analysis can be performed and the results recorded electronically. The different analyses can be performed in any order as required.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

In order that the present invention may be more clearly understood, preferred forms will be described with reference to the following drawings.

Brief Description of the Drawings

Figure 1 is a simplified cross sectional view of one embodiment of a particle collection device suitable for use with the present invention;

Figure 2 is a depiction of the symbols used in Figures 3-7;

Figure 3 is a cross-sectional view of one means of binding macromolecular species in association with particles according to the present invention;

Figure 4 is a cross-sectional view of a second means of binding macromolecular species in association with particles according to the present invention;

Figure 5 is a cross-sectional view of a third means of binding macromolecular species in association with particles according to the present invention;

Figure 6 is a cross-sectional view of a fourth means of binding macromolecular species in association with particles according to the present invention; and

Figure 7 is a cross sectional view of a fifth means of binding the macromolecular species in association with particles according to the present invention.

Modes of Carrying Out the Invention

The invention relates to the measurement and identification of particles sampled from a fluid medium by a process involving several integrated stages.

In the first stage, particles are collected, usually by a particle-collection device or apparatus. One embodiment of such a device is generally depicted

as 20 in Fig. 1. The collection device 20 comprises an inlet passage 22 for the airstream which carries the particles which are impacted onto the sample collection plate 23. The collection device 20 is supported in the nose by a soft flexible seal 24 to the nasal wall. Other sampling devices which fit partially or wholly within or beneath the nostrils can be envisaged.

In the second stage, if required, is to allow the macromolecular species associated with the particles to diffuse away from the particle. The particles may be optionally solubilised to assist in the diffusion process.

In the third stage, following collection of the particles and diffusion, the macromolecular species are immobilised, bound or trapped in sufficiently close proximity to the particle. This stage is normally referred to as 'presentation'. Subsequent labelling of the macromolecular species can provide the association with the particle of origin. The macromolecular species may include proteins, glycoproteins, carbohydrates, nucleic acids, lipids, and complexes or combinations thereof. This 'presentation' of the macromolecular species, ie. the binding of macromolecular species in association with the particles, can occur in a number of ways; five of which are shown by way of illustration in Figs. 3-7. Fig. 2 depicts the general symbols used to depict the particle and macromolecular species in Figs. 3-7.

In Fig. 3, the macromolecular species 7 from the particle 6 are directly bound in a matrix 8 which has the capacity to covalently or non-covalently bind macromolecules. The matrix ideally has the additional properties of porosity, optical clarity and stability. It can also have adhesive properties to retain particles. If not, an additional layer 5 can be used to ensure particles are not lost on subsequent probing. One method of providing the matrix 8 is to use agarose modified in such a way to include groups to provide the non-covalent and/or covalent binding.

In Fig. 4, the macromolecular species 7 from the particle 6 are bound to a matrix, film or membrane 8 which has the capacity to covalently and/or non-covalently couple the macromolecular species 7. The surface of the matrix may be coated with or contain, one or more materials 9 to provide adhesive properties and enhance the retention of surface moisture. An additional overlay 5 may or may not be required to retain particles during analysis and additional under-layer 10 may be required to provide a ballast of moisture. The type of matrix would include nitrocellulose, PVDF, nylon or

similar macromolecular species-binding membranes or materials used for protein blotting and Southern blotting.

In Fig. 5, the particles 6 are collected on an adhesive surface 11 bound to a clear film 12 and later overlaid with a matrix 8 which is capable of binding the macromolecular species 7. The nature of this matrix would include those described in either Fig. 3 or Fig. 4.

In Fig. 6, the particles 6 are collected by suction on a porous membrane 13 which may be coated with one or more materials 11 to increase particle adhesion. The type of porous membrane 13 would include track-edged polycarbonate membranes and teflon membranes. The adhesive material does not block the membrane pores and does not cause the macromolecules to be extracted. The porous membrane 13 is later overlaid with a matrix 8 which will bind the macromolecular species 7 when they have been solubilised. The nature of the matrix may be similar to either those in Fig. 3 or Fig. 4.

In Fig. 7, the particles 6 are collected onto a membrane 8 which is both porous and capable of binding the macromolecular species 7. After collection, the membrane 8 is overlaid with an adhesive film (11, 12) to retain the particles 6 and the eluted macromolecular species 7 are bound to the membrane 8.

The rate of release of individual macromolecular species from the particle will vary and may occur over short (seconds) to extended (days) periods of time depending on circumstances and the nature of the particular source. Once released, diffusion (second stage) into and binding to (third stage) the macromolecule-binding matrix is envisaged to occur rapidly (seconds) to retain the required proximal relationship between source particle and bound macromolecular species.

The fourth stage of the present invention is analysing which may take a variety of forms, depending upon the application. Analysing of macromolecular species involves specific interaction with one or more different characterising molecules with the occurrence of such interaction enabling a characteristic of a macromolecular species to be identified. The analysing step is usually directed toward specific parts of the macromolecular species and involves the occurrence of specific and stable binding between the macromolecular species and a probe of some type. Examples include antigenic epitopes on macromolecular species and specific

antibody probes, carbohydrate groups on macromolecular species and specific lectin probes, and between oligonucleotide regions of the macromolecular species and complementary oligonucleotide probes.

The interaction of the probes can be detected in a number of ways. Where essentially irreversible physical coupling of the macromolecular species and probe occurs, the probing molecule may be prelabelled in such a manner that its presence can be detected, for example it may carry a fluorescent or radioactive label. Another alternative is to label the probe associated with an enzyme which, on reaction with a suitable substrate, will produce a coloured reaction product or other detectable product or signal including the transfer of electrons. Probing can involve secondary or further probe binding or reaction steps to increase sensitivity or specificity.

Macromolecular species can also be characterised by their activity (detection of enzymic or other biological activity with specific substrates) or chemical properties (binding of specific dyes).

The reliability of the information obtained by probing is usually dependent on the characterisation of the probing system, particularly the specificity and sensitivity of a particular probe. When using information from probing to identify the particle from which the macromolecular species are derived, the usefulness of the information may also require a knowledge of alternative macromolecular species which could carry the region bound by the probe.

There are many examples of the analytical use of sets of previously organised information to effect an identification. These are generally based on identified morphological or macromolecular species features. Morphological information can often be determined visually (eg possession of serrated or non-serrated edge of a leaf). Sometimes such information sets are arranged in hieratical form in tables and presented as flow diagrams to combine sets of different information groups to effect an identification. Not all information sets need to be intuitively predictable and it is possible to combine or analyse sets of information in different ways such that the outcome is a better or more useful predictor of identity than the unanalysed characteristics. An example would be the digitisation of features extracted from sets of images.

The application of sets of extracted features to identify pollen grains, fungal spores or other aerobiological material differs from the usual approach

applied by professionals trained in these skills. Typically, such analysis is effected by visual analysis and determining whether the particle possesses a small group of features which may only be visible under certain conditions of focussing, high magnification, staining or can only be assumed to be present from the regularity of other observable features. Example are the apertures and furrows present on some pollens with the number and appearance of these being fundamental to an analysis by conventional means. For the identification of particles that an individual has an allergic response to following exposure, the preferred method is to probe the macromolecular species, which include the allergens, with serum from the subject so the subject's IgE binds to the allergic macromolecular species. This binding can subsequently be detected with labelled commercial anti-IgE molecules and the relevant particles identified.

Other probing systems may be employed. These could be used to quantify specific macromolecular species associated with the particles, such as allergenic proteins. In this case probing would be performed with labelled specific monoclonal or polyclonal antibodies. Probes could be used which enable the particle to be specifically identified or to be differentiated from other similar particles.

The format of the assay influences the probing and analytical systems used. In the presentation systems depicted in Figs. 3-7, in some cases fluorescent labels for probes would be used, in other cases coloured substrates would be used. Where Nitrocellulose or other opaque membranes are used, they may be rendered translucent in mounting solutions of similar refractive index.

Analysing is followed by the fifth stage that can be termed 'detection'. Detection can be performed at a range of levels of sophistication and human interaction. These would range from a basic level where most tasks were performed by a human operator to a more complex level where many of the tasks such as movement of the microscope stage, focusing and image acquisition were performed and controlled by microprocessors. The functional steps remain similar.

A preferred outline of the analytical stages would proceed as follows. The field of total particles are initially viewed under a microscope to determine which particles have specific labelling surrounding the particle. The coordinates of these particles is determined and images collected. Either

individual images can be handled separately for analysis, or images of particles could be categorised as groups, each with common characteristics. Individual images of the particles are subjected to a range of feature extraction techniques to obtain digitised data on features such as length, maximal and minimal diameters, depth, roundness, perimeter, regularity, perimeter roughness, etc. Additional features such as fractal patterns of interior structural elements may also be extracted and compiled. These together form a morphological data set or fingerprint, characteristic of the particle.

Quantitative information can also be obtained about the probed macromolecular species associated with the particle, for example, the concentration of macromolecular species per particle, the size distribution of allergen carrying particles and the quantity of allergen associated with different sized particles.

In another option, information can be obtained from image analysis of non-labelled particles which maybe of clinical significance - for example the number of inhaled particles less than 10 or 2.5 microns in diameter (PM_{10}, $PM_{2.5}$) or small needle-shaped particles which may be asbestos fibres.

In the preferred option, the information from the morphological data set of the IgE-probed particles would be compared with a database of information of known allergenic particles. This database could have a number of forms and structures. In the preferred option, it contains not only the digital data on a range of features, but also obtains information and illustrations on the appearance and geographic distribution of the allergen sources.

This allows predictions as to the likely identity of the particles to be made. To discriminate between options, confirm identity or quantify allergens, a second round of probing with specific non-IgE antibody probes can be conducted. These would use a second labelling system to enable them to be distinguished from IgE probing.

For some applications, only non-IgE probing would be performed.

There are numerous ways in which the four stages could be used and combined to form applications. In the preferred scenario, subjects requiring such a diagnosis, on advice of a medical practitioner (such as general practitioner (GP)), would purchase the sampling units from an outlet, such as a pharmacist and by following contained instructions collect the samples

from situations associated with symptoms. These samplers, along with a serum sample are returned via the GP to a pathology laboratory where the probing and analysis would occur. Results, including graphics, would be communicated to the patient by the GP.

Many other optional scenarios are possible for these technologies such as the analysis of longitudinal samples collected with time based collectors, such as Burkard Spore Traps. This could be used to quantify specific environmental allergens, examine the nature of allergenic particles and to identify sources of unknown allergens in a regulatory or public health setting. The use is not confined to allergenic particles and many other biological particles - presence from microorganisms, products from microorganisms (eg endotoxins), indoor air quality, water quality, and industrial or agricultural materials (dusts etc) could also be analysed.

Although identification of particles functioning as sources of macromolecules is one application of the method according to the present invention, another application is the assessment of disease-risk posed by the environment where sampling was conducted. For example, if more than 100 particles which were detected following the detection of their macromolecular species and these were collected during 10 minutes of dust raising activity by a subject, it might be concluded that the particular subject was in a high-risk environment.

EXAMPLES

Air samples were collected for 1 hour by inertial impaction during normal breathing in houses using intra-nasal samplers (IRM Technologies, Sydney, Australia). The characteristics of such samplers have previously been described in *Sercombe JK, Pavlicek PK, Xavier ML, Lea SA, Graham JAH. (Abstract 337) Assessment of nasal and pocket air samplers. J Allergy Clin Immunol 1998;101:S80.* Inhaled particles were collected within the nasal sampler onto a transparent adhesive tape (for example: ARcare 7759 ®, Glenrock, Pa., USA) which was overlayed with the protein-binding PVDF membrane (0.45 μm pore size, Du Pont Polyscreen ®) after sampling was completed. The membrane/adhesive sandwich was wetted briefly with 80% methanol and then incubated in borate buffer overnight to allow allergens from the particles to bind to the membranes. Vacant binding sites on the membranes were blocked in 5% skin milk powder, rinsed and the membranes immunostained with an allergen-specific antibody system. As an example,

the monoclonal 10A6 (Indoor Biotechnologies, University of Virginia) which is specific for the cockroach allergen Bla g 1, was diluted to 2 ug antibody/ml and incubated for 2 hours. This was followed by incubation with an anti-mouse antibody conjugated to alkaline phosphatase enzyme (Sigma, St. Louis, USA) for 2 hours. An insoluble immunostain colour was developed using BCIP/NBT substrate (Sigma, St. Louis, USA). Particles containing Bla g 1 are identified by the presence of a halo of stain around the particle.

An example of an alternative way of combining the air sampling with the adhesive membrane was to collect the particles by filtering the air through the dry PVDF membrane (1 μm pore size, Millipore, MA, USA). A suitable air filtering system is Airchek Model 224-52 (Eighty Four, PA, USA) air sampling pump operating with a air flow of 2 L/min with an IOM sampling head (SKC Limited, Blandford, Dorset) to hold the membrane. After sampling, the PVDF membrane was overlayed with the transparent adhesive tape as described above. Again this forms a permanent sandwich of the adhesive tape and the protein binding membrane which was immunostained as previously described. Particles containing allergen were identified by the presence of a halo of stain around the particle.

Another method of performing the sampling and immunostaining was to impact the airborne particles directly onto a protein-binding membrane - for example using the previously described nasal sampler. In this case, prior to sampling, the membrane was prepared by coating (by particle deposition) on the collection side with a thin film (~10- 30 μm thick) of 0.5% agarose containing 2% CMC (carboxy methyl cellulose) in phosphate buffered saline and the membrane has been coated on the reverse side with a ~0.5 mm thick layer of 0.5% agarose containing 20% sorbitol in phosphate buffer saline. The layer on the collection side was to maintain adhesive properties and the layer on the reverse side was to assist in maintaining moisture in the membrane. After collection of particles, the membranes were stored at 4°C for 12 hours to allow allergens to elute from the particles and to bind to membrane in close proximity to the particle. The collection surface of the membranes were then re-coated with a second layer of agarose to retain the particles in their original position. Membranes were blocked in 5% skim milk in PBS for 1 hour and then incubated with a biotinylated monoclonal antibody which was specific for cat allergen Fel d 1 (3E4C4, Indoor Biotechnologies Inc.) for 3 hours, washed 3 times in PBS/Tween, followed by

incubation with Extravidin-alkaline phosphatase conjugate (Sigma Aldrich Chemical Co., Sydney, Australia) for 2 hours, followed by 3 washes and development of colour with BCIP/NBT (Sigma Aldrich Chemical Co., Sydney, Australia). Particles containing Fel d 1 were identified by the presence of a halo of stain around the particle.

Not all allergenic particles require the same elution times and conditions in order to elute macromolecules. For example, for the fungus *Alternaria alternata* elution times of 12 hours may be used and elution may be enhanced by the addition of the detergent sodium dodecyl sulphate (SDS) or Coca's solution.

The image analysis of features on the immunostained membranes was performed using a Pulnix TM1001, progressive scan, monochrome video camera (Pulnix, Australia) mounted on an Olympus BH2 microscope, an XPG1000 frame grabber (Dipix Pty. Ltd., Vancouver, Canada) and the "WiT" commercial image analysis software (Logical Vision, Coreco, USA), running on a Pentium PC.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

CLAIMS:

1. A method of detection and/or identification of particles, the method comprising the steps of:

a) collecting the particles onto a surface;

b) allowing macromolecular species associated with the particles to diffuse from the particles;

c) immobilising any diffused macromolecular species in close proximity to the particles, wherein an immobilised macromolecular species is sufficiently close to a particle so as to be indicative as being diffused from the particle;

d) analysing the immobilised macromolecular species to determine one or more characteristics of macromolecular species;

e) comparing the determined one or more macromolecular species characteristics with a reference base of known characteristics of macromolecular species associated with particles; and

f) using the comparison of the determined one or more macromolecular species characteristics to indirectly detect and/or identify at least one particle type present on the surface associated with the analysed macromolecular species immobilised in close proximity to the particle.

2. A method of detection and/or identification of macromolecular species associated with particles, the method comprising the steps of:

a) collecting the particles onto a surface;

b) allowing macromolecular species associated with the particles to diffuse from the particles;

c) immobilising the diffused macromolecular species in close proximity to the particles, wherein an immobilised macromolecular species is sufficiently close to a particle so as to be indicative as being diffused from the particle;

d) analysing the collected particles to determine one or more characteristics associated with particles;

e) comparing the determined one or more particle characteristics with a reference base of known characteristics of particles from which macromolecular species can to diffuse; and

f) using the comparison of the one or more determined particle characteristics to indirectly detect and/or identify the macromolecular

species diffused from the particle and immobilised in close proximity to the particle on the surface.

3. The method according to claim 1 or 2 wherein the particles are collected from a fluid stream onto a surface by impaction, filtration, passive diffusion, or centrifugation.

4. The method according to claim 3 wherein the surface has the capacity to retain the particles.

5. The method according to claim 4 wherein the particles are collected by impaction onto a surface provided with adhesive properties.

6. The method according to claim 4 wherein the particles are collected by filtration on a substantially flat, porous membrane forming the surface.

7. The method according to claim 1 or 2 wherein the particles are collected on a surface formed of a thin film of agarose or other aqueous matrix layered on a macromolecular species-binding material.

8. The method according to claim 7 wherein the macromolecular species-binding material is selected from the group consisting of nitrocellulose, PVDF, and Nylon.

9. The method according to claim 1 or 2 wherein the particles are collected directly onto an adhesive surface which is then overlaid with a macromolecular species-binding material to form a sandwich.

10. The method according to claim 1 or 2 wherein the particles are collected by impaction or filtration onto a surface formed of a macromolecular species-binding membrane and the membrane is then covered by a layer to retain the collected particles.

11. The method according to claim 1 or 2 wherein the particles are collected and the diffused macromolecular species are immobilised on a surface capable of specifically or nonspecifically binding a macromolecular species.

12. The method according to claim 11 wherein the surface is formed of agarose modified to non-covalently or covalently couple macromolecular species and having properties which allow it to be adhesive and retain water.

13. The method according to claim 1 or 2 wherein the diffused macromolecular species are immobilised non-specifically in close proximity to the particles.

14. The method according to claim 1 wherein the characteristics of the macromolecular species analysed include antigenicity, immunoreactivity,

chemical, labelling, enzymatic properties, possession of genetic material, and physical.

15. The method according to claim 1 wherein the characteristics of the macromolecular species are analysed directly or indirectly by using detectable probes that react with or to a macromolecular species of interest.

16. The method according to claim 15 wherein the detectable probes are provided at the opposite side of the surface to which the particles are immobilised and transfer of the probes occurs substantially perpendicular through to the side of the surface to which the particles are immobilised.

17. The method according to claim 15 wherein the detectable probes are provided by diffusion and/or capillary flow substantially parallel to the surface to which the particles are immobilised.

18. The method according to claim 2 wherein the characteristics of the particles analysed include morphology, colour, texture, roughness and possession of surface or interior features.

19. The method according to claim 18 wherein the morphology comprises size, shape, density, or refractive index.

20. The method according to claim 2 wherein digitised images of the particles are analysed to provide information on the particle's morphological features.

21. The method according to any one of claims 1 to 20 wherein the reference base of known characteristics associated with particles and macromolecular species is comprised of digitised information or images of known particles and characteristics of associated macromolecular species.

22. The method according to claim 21 wherein the images are obtained using microscopy, video photography or digital image scanning.

23. The method according to claim 22 wherein the digital image scanning is obtained using a 35 mm slide scanner.

24. The method according to any one of claims 1 to 23 wherein the particles and associated macromolecular species cause allergies in mammals, cause diseases in mammals, are microorganisms, cells, toxins, or industrial and agricultural materials.

figure 1

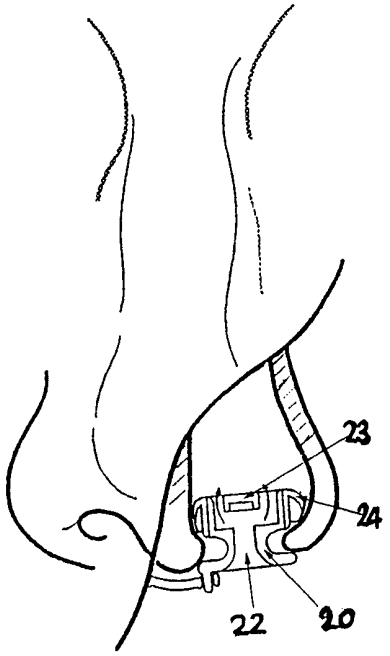


figure 2

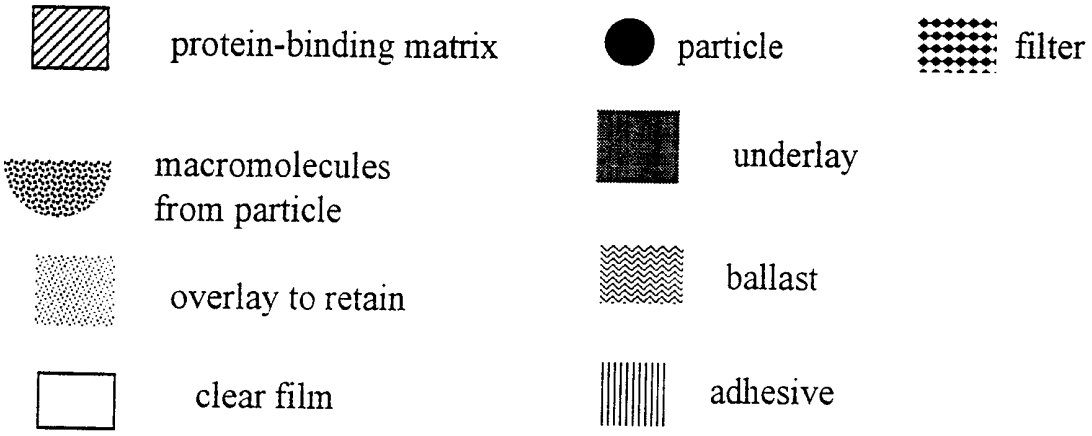


figure 3

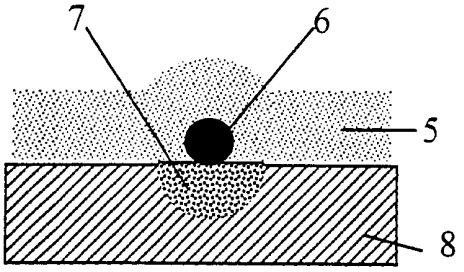


figure 4

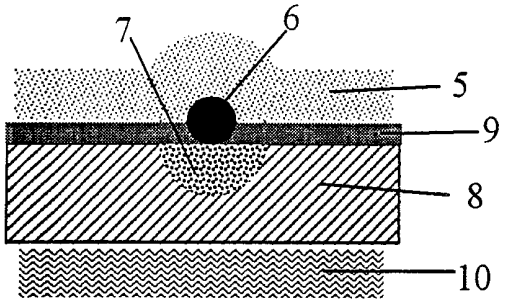


figure 5

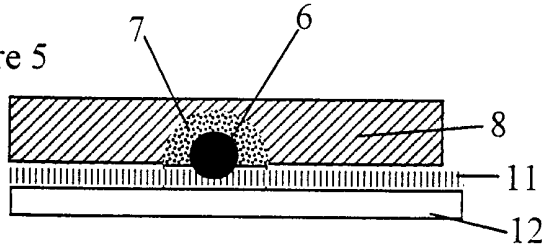


figure 6

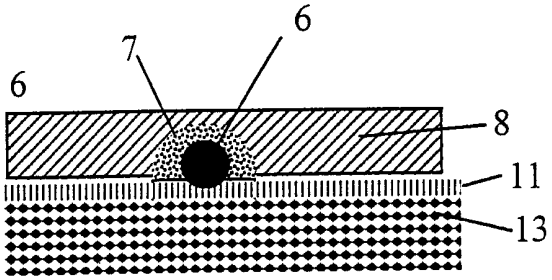
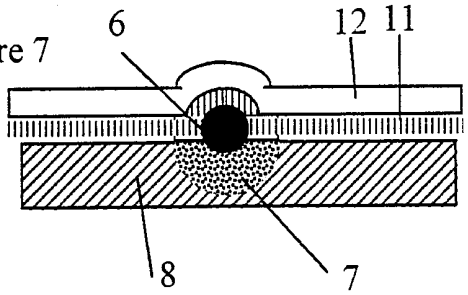


figure 7



INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/00017

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: G01N 33/483, 33/53, 33/559

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)
FILE WPAT: G01N

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)
FILE WPAT: KEYWORDS: ALLERGEN, PARTICLE, PARTICULATE, DATABASE, DIFFUSE,
FILE MEDLINE: KEYWORDS AS ABOVE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AU 33348/95 (687618) B (UNIVERSITY OF SYDNEY) 7 March 1996 Whole article	1-24
Y	US 5633140 A (KERSTIN WEX) 27 May 1997 Abstract, claims	1, 3-17, 21-24
Y	AU 65109/90 A (MILSTEIN) 18 April 1991 Abstract, claims	1-17, 21-24

[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
28 January 1999	-3 FEB 1999
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929	Authorized officer **ROSS OSBORNE** Telephone No.: (02) 6283 2404

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/00017

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Electrophoresis, Vol. 18 No. 15, December 18 1997,p 2803-2810 Posch A et al. "Latex allergen database." Abstract	1-24
Y	Journal of Allergy and Clinical Immunology Vol. 81 No. 3 March 1988 p 611-614 Tovey E.R. et al. "An on-line computer data bank of monoclonal antibodies that recognise allergens - Invitation for entries." Abstract	1-24
A	US 4173415 A (WYATT) 6 November 1979 Whole article	1-24
A	US 4884886 A (SALZMAN) 5 December 1989 Whole article	1-24

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU 99/00017

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					
AU	33348/95	WO	9607099	EP	777858		
US	5633140	CA	1088772	DE	2737488	FR	2362387
		GB	1583511	IT	1085232	JP	53026199
		JP	62006141	SE	7706982		
US	4884886	EP	190628	EP	190628	JP	61193049
AU	65109/90	WO	9105260				

END OF ANNEX