

TEST DEVICE

5 The present invention relates to a device for detecting the presence of analyte in a sample of a body fluid, in particular a sample other than a serum sample. Such analytes are typically immunoglobulin molecules but may also be other proteins.

10 Immunoglobulin molecules, or fragments thereof, are known to occur in such non-serum body fluids as saliva and urine. In particular, the immunoglobulin molecules of classes IgA, IgG and IgM predominate in saliva. Saliva and urine are secreted body fluids and collection of samples is easily and simply carried out.

15 In the field of medical diagnostics, there are many tests and devices known for detecting analytes in samples of a particular body fluid. These tests include biochemical assays of the amount and/or presence of particular proteins or immunoglobulins; so called 'sandwich assays' for immunoglobulins and the use of electrophoresis to identify individual classes of immunoglobulins.

25 Devices used to detect analytes such as immunoglobulins in saliva include various probes consisting of a stick with an absorbent or adsorbent pad of material at one end. Such probes are disclosed in EP-A-0418739, EP-A-0442231 and US-A-5103838. The probes of the prior art additionally include detection systems capable of identifying antibodies in saliva.

30 Detection systems used in the past on diagnostic probes of this type have employed enzyme-linked assays or particle-agglutination assays to produce visibly

detectable results.

However, diagnostic test devices have suffered from an important disadvantage. All immunodiagnostic tests
5 relying on antibody-antigen binding are subject to the occurrence of false negative results in any one particular sample. In the classic enzyme-linked immunosorbent assay, an antigen which will specifically bind with the antibody of interest is bound to a solid
10 base. A sample containing the antibody of interest is added and forms a specific complex with the bound antigen. Unbound antibody is then washed off and a labelled antibody is added to detect the complexes formed on the surface of the test device. This assay, however,
15 is only reliable when quite large amounts of analyte are present in the sample. Samples of urine and saliva which a clinician might wish to assay generally contain levels of antibody which may be 2 or 3 orders of magnitude lower than the levels of antibody found in serum or plasma
20 samples. Antibody concentration in urine and saliva samples is influenced by several factors, and shows natural variability. The antibody concentration may be lowered when urinary output is increased through ingestion of large volumes of fluid prior to sampling, or
25 when saliva production has been artificially increased through stimulation by citric, or other acids.

This variability can give rise to false-negative results, where the concentration of antibody in the sample is
30 insufficient to give a positive reaction in an enzyme-linked assay or similar test procedure. False negative results can result in the incorrect diagnosis of a medical condition by a clinician. Where the test is for the presence of an antibody to a certain pathogenic

bacteria or virus or for antibodies associated with autoimmune diseases or allergic conditions, a false-negative result due to the use of an inadequate sample can have devastating consequences for the patient and
5 cause the subject unnecessary distress. This is especially so in the case of Human Immunodeficiency Virus (HIV) infection. Recently, it has been reported that the United States Food and Drug Administration (FDA) has decided that all test devices in the immunodiagnostic
10 field must indicate the viability of the sample.

The present invention seeks to solve this problem by providing a device with an inbuilt means for detecting false negative results arising from use of an invalid
15 sample.

In a first aspect of the present invention, there is provided a device for the detection of an analyte present in a body fluid of an animal species, the device
20 comprising a substrate and on the substrate in discrete locations:

- (a) a mobile labelled first specific binding molecule capable of binding to any characteristic molecule of
25 the species being tested to form a binary complex;
- (b) a means for detecting the analyte; and
- (c) an immobilised second specific binding molecule
30 capable of binding to the binary complex or a component of it.

The advantage of this device is contained in the final step of binding the characteristic molecule to a second

immobilised specific binding molecule. This step ensures that a sufficient quantity of characteristic molecule is present to give meaningful results and therefore that particular test can be readily verified. The overall
5 concentration of characteristic molecule in a test sample is easily quantitated and if the level is sufficiently high then the risk of a false negative result is significantly reduced.

10 GB-A-2204398 relates to an assay device which also contains a mobile labelled first specific binding molecule, a means for detecting analyte and an immobilised second specific binding molecule. However,
15 it differs significantly from the device of the present invention because the first specific binding molecule is specific for the analyte rather than for a characteristic molecule of the species being tested as is the case with the present invention. This means that using the device
20 of GB-A-2204398 it is not possible to determine whether the sample is viable and, thus, to eliminate false negatives.

In this specification, the term 'binary complex' is used to define the complex formed by the specific binding
25 molecule bound to a particle on the substrate and a characteristic molecule which may include analyte molecules. The analyte may be an antibody.

The term 'mobile', when used in connection with the first
30 specific binding molecule, indicates that the first specific binding molecule is not immobilised on the substrate.

The term 'characteristic molecule' refers to a molecule

which is typically found in the type of sample to be tested and which will therefore be present in detectable concentration if the sample is viable. For example, in saliva samples, it is usual to find detectable quantities of IgG, and IgG would therefore be a suitable characteristic molecule for saliva samples. The first specific binding molecule would, in that case, bind specifically to all antibodies of the IgG isotype.

The device of the present invention is suitable for the detection of an analyte in any body fluid but it is especially useful for overcoming the problems of detecting analytes in saliva and urine samples where the concentrations of analytes tend to vary. It is also useful for other body fluids such as blood.

The analyte to be tested by the device of the present invention may be an antibody to a bacterial, viral, fungal or protozoal pathogen for example *Helicobacter pylori*, the Human Immunodeficiency viruses (HIV 1 and 2), a *Salmonella* spp., a *Streptococcus* species, polio virus, hepatitis A, hepatitis B surface antigen or hepatitis B core antigen, *Candida* spp., *Aspergillus* spp., *Entamoeba* spp. or *Giardia* spp. The analyte may also be an antibody to a tissue or species-specific antibody associated with autoimmune diseases, or allergic conditions.

Alternatively, the analyte may be an antigen, for example a pathogen protein or a hormone, blood group characterising protein or any other protein or glycoprotein which may require detection. Nucleic acids can also be detected using the device of the present invention.

The analyte should also form a portion of the characteristic molecule, or be a subset of characteristic molecules, which bind to the first specific binding molecule to form binary complex.

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Thus, when the analyte is an antibody of a particular isotype, for example IgG, IgM, IgE or IgA, the characteristic molecule will be any antibody of that isotype and the first specific binding molecule will be selected so that it binds to all antibodies of that isotype. Similarly, if the analyte is a glycoprotein, the first specific binding molecule may be a lectin which binds to all glycoproteins present in the sample and labels them and if the analyte is a nucleic acid, a suitable first specific binding molecule would be a labelled low specificity nucleic acid probe.

Because the analyte is a subset of the characteristic molecule, a portion of the labelled binary complex will contain the analyte and this is a particularly advantageous feature of the invention as it eliminates the need for more than one detectable label.

In a preferred embodiment the device provided by this invention is designed for use in diagnosing medical conditions suffered by humans.

As mentioned above, the first specific binding molecule will often be an antibody which will preferably be of the IgG or IgM isotype. Other isotypes of antibody may be similarly so used if required. The first specific binding molecule may be of polyclonal or monoclonal origin, and may be either an intact molecule or a specific binding-site containing fragment derived by

enzymatic or chemical cleavage, or obtained through the application of recombinant DNA technology or peptide synthesis.

5 The first specific binding molecule may be labelled with any convenient label. The label may, in particular, allow the specific binding molecule to be visualised, or otherwise detected, when immobilised. Particles
10 constitute preferred labels for this purpose. Particles coupled to the first specific binding molecule on the test device may be of any known type, for example, latex, colloidal metal, liposomes or polystyrene. Chromophores, fluorophores, and/or other labels may alternatively or additionally be used.

15 The means for detecting the analyte on the test device may suitably comprise one or more discrete areas comprising an immobilised binding molecule specific for the analyte. Because the analyte forms a part of or is
20 a subset of the characteristic binding molecule, it will already be bound to the detectably labelled first specific binding molecule and will therefore be detectable once it has been immobilised in the detection zone.

25 It is particularly useful to provide several analyte detection areas on the device since this allows quantitation of the analyte. Thus, if the analyte is present only in small amounts, it will interact with the
30 immobilised binding molecule in the first detection area, but there will be no surplus analyte which can pass to the further detection areas. If more analyte is present, some surplus may pass to the next detection area and so on so that the amount of analyte present will be

indicated by the number of detection areas in which it is detected.

5 Alternatively, two or more immobilised binding molecules specific for different analytes may be deposited on discrete areas of the test device, permitting the simultaneous detection of the presence of two or more different analytes in the same sample.

10 When the analyte is one of the antibodies discussed above, the immobilised binding molecule in the detection area will generally be an immobilised pathogen protein, carbohydrate or other fragment, or a human tissue-derived protein, carbohydrate or other fragment, or a non-human
15 species-derived protein, carbohydrate or other fragment. The immobilised binding molecule may also be an anti-idiotypic antibody.

20 When the analyte is a protein, the immobilised binding molecule may be a monoclonal or polyclonal antibody which binds specifically to the protein or some other binding molecule specific for the particular protein to be detected. For nucleic acids, a suitable immobilised binding molecule would be a high specificity nucleic acid
25 probe.

Binary complexes which contain a characteristic molecule other than the analyte will not be immobilised or otherwise removed at location (b), where the analyte is
30 detected and will pass to location (c), which functions as a verification station. A second specific binding molecule immobilises the complexes, which are then detectable by means of the label. The second specific binding molecule may specifically bind any part of the

complex. It is preferred for the second specific binding molecule to bind the characteristic molecule; so, for example, if the characteristic molecule is a human antibody isotype, the second specific binding molecule may again be anti-human antibody such as anti-human IgG. Other suitable binding molecules are as discussed above in relation to the first specific binding molecule and will again be chosen depending on the analyte and the characteristic molecule.

The device may further comprise a sample receiving portion of adsorbent material continuous with or separate from the substrate. This flexibility of construction permits maximum utility of operation depending on how the sample is to be applied.

The substrate material of the test device may be composed of paper or membrane. The substrate material may also optionally be a carbohydrate and may preferably be a nitrocellulose material.

It is preferred that the device be constructed so that body fluid (or components of it) can flow to locations (a), (b) or (c) successively, for example by capillary action. Other means of delivering body fluid to the locations are within the scope of the invention.

In a preferred aspect of the invention, as mentioned above, sample is wicked along the membrane or paper substrate by capillary action. No additional solvent is required although particular sample diluents may increase the speed at which a result is obtained, or enhance the signal obtained with a positive test result.

In a second aspect of the present invention there is provided a method for the detection of analytes present in the body fluid of an animal, the method comprising:

- 5 (i) adsorbing a sample onto an adsorbent substrate;
- (ii) bringing the sample into contact with a
10 mobile labelled specific binding molecule
 such that characteristic molecules of the
 tested species present in the sample bind
 to the specific binding molecule to form
 a binary complex;
- 15 (iii) bringing the binary complex comprising in
 contact with an immobilised analyte-
 specific binding molecule such that binary
 complex comprising analyte binds to the
 analyte-specific binding molecule to give
20 a detectable reaction product; and
- (iv) bringing binary complex not comprising
 analyte into contact with an immobilised
 specific binding molecule which reacts
25 with the binary complex to form a visibly
 detectable product.

The detectable products in steps (iii) and (iv) are preferably visibly detectable, as they are when the label
30 referred to in step (ii) is a particle.

Preferred features of the second aspect of the invention are as for the first aspect, *mutatis mutandis*.

The invention will now be further described by way of example with reference to the following drawing in which:

5 **FIGURE 1** shows a perspective view of a device in accordance with the invention.

Referring now to Figure 1, a sampling device 10 comprises a substrate 12 formed from an adsorbent material, typically nitrocellulose paper, and, attached to the substrate, a sample receiving portion 14. On the
10 substrate is positioned a first area 16 in which is contained an anti-human IgG antibody coupled to a particle. The particles may be of any known type, for example latex, colloidal metal, liposomes or polystyrene.
15 Sample detection areas 18, 20 contain antibodies specific for the analyte immobilised on the nitrocellulose substrate 12. A particularly preferred analyte which this device may be used to detect is antibody to *H. pylori* and, therefore, in that case, the detection areas
20 18, 20 would contain an immobilised antigen specific for *H. pylori* antibody.

The final area on the device is the verification area 22 which contains immobilised anti-human IgG. This may be
25 the same as the anti-human IgG which is bound to the particles but this is not necessarily so.

In use, a sample is placed on the sample receiving portion 14 and, because of the adsorbent nature of the
30 substrate 12, the sample wicks along the substrate until it reaches the first area 16. At this point, any human IgG present in the sample binds to the anti-IgG coupled to the particles to form a binary complex. The binary complex continues to wick along the substrate 12 until it

encounters the first sample detection area 18. In this area, binary complex containing analyte binds to the immobilised antibody to form a discrete visible clustering on the sample detection area 18. Unbound
5 binary complex continues to pass along the substrate until it reaches the second sample detection area 20 where any remaining analyte-containing binary complex will be immobilised to form a second discrete visible clustering. Finally, remaining binary complex passes
10 along the substrate until it reaches the verification area 22, at which point it will bind to the anti-IgG in this area and will form a further discrete visible clustering. This visible signal in the verification area is an indication that the initial sample contained a
15 sufficient amount of human IgG for the sample to be viable and any positive result to be relied upon.

Clearly, therefore, the device of the present invention is simple to use and provides a quick and easy method of
20 verifying that the sample is a viable one.

CLAIMS

1. A device for the detection of an analyte present in a body fluid of an animal species, the device comprising a substrate and on the substrate in discrete locations:
- 5
- (a) a mobile labelled first specific binding molecule capable of binding to any characteristic molecule of the species being tested to form a binary complex;
- 10
- (b) a means for detecting the analyte; and
- (c) an immobilised second specific binding molecule capable of binding to the binary complex or a component of it.
- 15
2. A device as claimed in claim 1, wherein the body fluid is saliva, urine blood.
- 20
3. A device as claimed in claim 1 or claim 2, wherein the analyte is an antibody to a bacterial, viral, fungal or protozoal pathogen for example *Helicobacter pylori*, the Human Immunodeficiency viruses (HIV 1 and 2), a *Salmonella spp.*, a *Streptococcus* species, polio virus, hepatitis A, hepatitis B surface antigen or hepatitis B core antigen, *Candida spp.*, *Aspergillus spp.*, *Entamoeba spp.* or *Giardia spp.*, an antibody to a tissue or species-specific antibody associated with autoimmune diseases, or allergic conditions, an antigen, for example a pathogen protein or a hormone, blood group characterising protein or any other protein or glycoprotein which may require detection or a nucleic acid.
- 25
- 30
4. A device as claimed in any one of claims 1 to 3,





wherein the characteristic molecule is an antibody, a protein or a nucleic acid.

5 5. A device as claimed in claim 4, wherein the antibody is of the IgG, IgM, IgE or IgA isotype.

10 6. A device as claimed in any preceding claim, wherein the first and/or the second specific binding molecule is an antibody, a lectin or a nucleic acid probe.

7. A device as claimed in any preceding claim, wherein the species is human.

15 8. A device as claimed in any preceding claim, wherein the first specific binding molecule is labelled with a particulate label, for example latex, colloidal metal, liposomes or polystyrene particles.

20 9. A device as claimed in any preceding claim, wherein the means for detecting analyte comprises one or more discrete areas comprising an immobilised binding molecule specific for the analyte.

25 10. A device as claimed in claim 9, wherein the immobilised binding molecule in the detection area is an immobilised pathogen protein, carbohydrate or other fragment, or a human tissue-derived protein, carbohydrate or other fragment, or a non-human species-derived protein, carbohydrate or other fragment, a monoclonal or
30 polyclonal antibody or a high specificity nucleic acid probe.

11. A device as claimed in any preceding claim further comprising a sample receiving portion of adsorbent





material continuous with or separate from the substrate.

12. A device as claimed in any preceding claim wherein the substrate is of paper or membrane material.

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13. A device as claimed in claim 12, wherein the paper or membrane substrate is a carbohydrate material.

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14. A device as claimed in claim 12, wherein the substrate is nitrocellulose.

15. A method for the detection of analytes present in the body fluid of an animal, the method comprising:

15

(i) adsorbing a sample onto an adsorbent substrate;

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(ii) bringing the sample into contact with a labelled specific binding molecule such that characteristic molecules of the tested species present in the sample bind to the specific binding molecule to form a binary complex;

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(iii) bringing the binary complex comprising in contact with an immobilised analyte-specific binding molecule such that binary complex comprising analyte binds to the analyte-specific binding molecule to give a detectable reaction product; and

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(iv) bringing binary complex not comprising analyte into contact with an immobilised specific binding molecule which reacts with



the binary complex to form a visibly detectable product.

16. A method as claimed in claim 15, wherein in step
5 (iv) the immobilised specific binding molecule reacts with the characteristic molecule of the binary complex.

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