DETECTION OF ANALYTES

The present invention relates to an improved method for the detection of analytes on a solid substrate. In particular, the method of the invention is applicable to samples of body fluids such as saliva, serum, whole blood and urine.

The detection of substances in various body fluids by immunoassay methods is well known and is frequently used for a variety of different purposes. Examples include the detection of antibodies in blood, saliva, urine or other body fluid samples as an indication of the presence of pathogens and thus for the diagnosis of various diseases and conditions. Other assays of body fluids include pregnancy tests and tests to determine the amount of alcohol in the blood.

Although such tests can be carried out as bulk liquid assays, it is often easier and more convenient to carry out the test by spotting the sample onto a solid substrate on which a specific binding agent for the analyte is immobilised and then detecting the presence of the specific binding complex. Such tests are popular since they are relatively clean and simple to operate.

However, there are certain disadvantages associated with the carrying out of tests on solid substrates. Firstly, in many assays carried out on a solid substate there is an unacceptable number of false positive results which indicate the presence of an analyte in a sample even if none is present.

The second problem is associated with the use of porous

solid substrates. This type of substrate is, in theory, particularly useful since it enables the bulk of the sample to be removed from the surface of the substrate whilst the analyte remains at the surface bound to the immobilised specific binding molecule. However, in practice there are considerable difficulties because the flow of the sample through the substrate is often very slow so that the assay can sometimes take 20 to 30 minutes. This is a particular disadvantage for tests where the results are required very quickly, for example tests that could otherwise be carried out by a general practitioner during a consultation.

The disadvantages discussed above have been ascribed to the presence in many samples of unwanted particulate matter including cell debris, large proteins, mucopolysaccharides and other macromolecules. Attempts have been made to overcome the problem of such unwanted material in the sample by filtering the sample before attempting to detect the presence of a specific binding complex.

However, surprisingly, this did not appear to be particularly effective and an unacceptable number of false positive results was still obtained and the flow through time of the sample was still unacceptably long even when the sample was filtered twice before adding it to the substrate. It thus seemed that the presence of mucins and particulate material was not the whole cause of the false positive results and long sample flow through time.

After many attempts to solve the problem of false positive results and slow sample flow, however, the

inventors have discovered that when the assay involves the detection of a specific binding complex between an analyte and a specific binding molecule immobilised on a porous solid substrate, the reliability of the test can be significantly improved simply by wiping the surface of the substrate after the sample has been added to it. This was most unexpected because it had been thought that all contaminants had been removed by the filtration steps which had been attempted previously whereas it now seems that this was not the case.

Therefore, in the present invention there is provided a method for detecting the presence of an analyte in a sample of body fluid, the method comprising contacting the sample with a specific binding agent capable of forming a specific binding complex with the analyte wherein the specific binding agent is immobilised on a porous solid substrate; and detecting the presence of specific binding complex; characterised in that before detecting the specific binding complex, the surface of the substrate is wiped to remove unadsorbed contaminants.

The simple step of wiping the substrate before attempting to detect the presence of specific binding complex has a remarkable effect on the success of the assay. The time taken for the sample to flow through the substrate can be reduced from 20 to 30 minutes to as little as 1 to 2 minutes and false positive results can be almost completely eliminated whilst still retaining the sensitivity of the assay to true positive results.

The wiping may be carried out manually or, alternatively, the process may be automated. Generally an absorbent material will be used to wipe the substrate and examples

of suitable materials are cotton, cotton wool and absorbent paper.

The wiping step should be carried out sufficiently vigorously to remove from the surface of the substrate any material which is not bound to a specific binding molecule. In order to make it clear to the practitioner that all of the unwanted material has been removed, a colouring agent may be added to the sample. In cases where a buffer or surfactant solution is added to the sample before transferring the sample to the substrate, the colouring agent may be included in the buffer or surfactant solution but this will not necessarily be the case.

The colouring agent may be an agent such as a stain which is specific for mucins, components of cell debris or other contaminants which remain on the substrate and are a cause of many of the problems of false positives which occur with assays of body fluids. However, it is often simpler to provide as a colouring agent a coloured particulate material such as latex, agarose, polystyrene or another polymer which does not bind to the contaminants but which simply remains on the surface of the substrate because the particles are too large to pass through the pores of the substrate. The particles should thus of course be larger than the pore size of the substrate but must also be smaller than the pore size of any pre-filters which may be used. It has been found appropriate in many cases to use particles of diameter of about 3 μm since, as discussed in more detail below, the filters used in any initial purification steps can be chosen to have a pore size larger than this. The coloured particles will remain on the surface of the

substrate along with the mucins and particulate impurities which appear to be causing the problem of false positives when smaller particles pass through the substrate.

Thus, when the colouring agent is used, it will be a simple matter for a practitioner to wipe the colouring agent from the surface of the substrate and thus to ensure that all surface debris has been removed from the substrate.

The sample may comprise any body fluid, for example, saliva, whole blood, serum or urine but the greatest improvements are seen for saliva and whole blood since, on the whole, these tend to contain greater amounts of cell debris, high molecular weight proteins, mucopolysaccharides and other high molecular weight substances which cannot pass through the pores of the substrate.

The analyte may be any specific binding molecule capable of reacting with the specific binding agent to form a specific binding complex. Examples of specific binding complexes include antibody-antigen complexes and thus the analyte may be either an antibody or an antigen.

In the case where the analyte is an antibody, it may be of any isotype and may be an antibody against any pathogen. Analysis of saliva or whole blood samples is particularly useful in the diagnosis of gut infections caused by pathogens such as *Helicobacter pylori* (formerly known as *Campylobacter pylori*). The presence of *H. pylori* infection is indicated by the presence in saliva of IgG or in the blood and therefore, if the aim of the

test is to detect *H. pylori* infection, the analyte may be IgG specific to *H. pylori* antigen.

The expression "antigen" is used in its broadest sense and includes whole pathogen cells or homogeneous, near homogeneous or heterogeneous extracts from a pathogen, all of which are capable of binding to specific antibody in samples of a body fluid.

When the specific binding agent is an antigen, it may be a protein, polysaccharide or lipid or any combination thereof. Preferred specific binding agents which are antigens include protein, lipopolysaccharide or cell extract of pathogen prepared by, for example, sonication, pressure disintegration, detergent extraction or fractionation.

When the method of the invention is used to detect infection with *H. pylori*, the specific binding agent may be an antigen derived from *H. pylori*. Antigens derived from *H. pylori* suitable for use as specific binding agents in the method of the present invention are disclosed in WO-A-9322682. However, any *H. pylori* derived antigen could be used as a specific binding agent.

As discussed above, the substrate is porous so as to allow the majority of the sample to flow through it whilst retaining the analyte on the surface where it is bound specifically to the specific binding agent. The substrate may be formed from nitrocellulose or other substances, for example polymers such as cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene, but the invention is not limited to these.

The substrate will, however, preferably be a nitrocellulose membrane and may have a pore size of from about 0.5 to 8 μm with from about 1 to 2 μm being preferred.

It is greatly preferred that the solid support is backed by an absorbent wicking material. Absorbent paper will often be the material of choice, generally for reasons of cost, but any absorbent material can be used for this purpose.

The specific binding molecules useful in this invention may be either covalently or non-covalently ("passively") bound to the solid surface. Suitable binding processes are well known in the art and generally consist of cross-linking, covalently binding or physically adsorbing the antigen to the solid support.

The presence of the analyte is diagnosed by means of the present invention by detecting the formation of a complex between the analyte and the specific binding agent. Some form of detecting means is therefore necessary to identify the presence (or, if required, amount) of the specific binding complex.

The detection means may be an antibody, conjugated with a reporter molecule, and which is capable of binding specifically to the specific binding complex.

In the case where an antibody is to be detected, the detection means may comprise a labelled second antibody specific for all antibodies of the isotype of the analyte antibody. In tests for *H. pylori* in humans, the analyte antibody will often be of the IgG isotype and in that

case the second antibody may be anti-human IgG.

A "reporter molecule" is a molecule or group which, by its chemical nature, has an analytically identifiable characteristic or provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. Reporter molecules used in this type of assay may be either enzymes, fluorophores or radionuclide containing molecules (ie radioisotopes). In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognised, however, a wide variety of different conjugation techniques exist, which are readily available to those skilled in the art. Commonly used enzymes include horseradish peroxidase, glucose oxidase, β-galactosidase and alkaline phosphatase, among others. The chromophores to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. Chromophores can be soluble or insoluble, depending upon the chosen application. For example, 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium is suitable for use with alkaline phosphatase conjugates; for peroxidase conjugates, 1,2-phenylenediamine-5-aminosalicylic acid, 3,3,5,5-tetramethylbenzidine, tolidine or dianisidine are commonly used. It is also possible to employ fluorophores, which yield a fluorescent product, rather than the chromophores noted above. Examples of fluorophores are fluorescein and rhodamine. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody absorbs the light energy, inducing a state of excitability in the

molecule, followed by emission of the light at a characteristic colour which is usually visually detectable with a light microscope.

However, the present invention is particularly well adapted for use as an 'instant diagnosis' test from which the results will be available in a few minutes and which may be carried out by a general practitioner during a consultation. For this reason, it is greatly preferred that the method of detection is as simple as possible and requires no specialised equipment. Therefore, the reporter molecules preferred in the present invention are colour reagents such as colloidal gold or carbon, polystyrene or latex particles.

When this type of reporter substance is used, it is important that unbound second antibody be removed so that the bound second antibody with the attached colour agent is clearly visible. It is thus particularly suited to the porous membrane backed by an absorbent pad which is used in the present invention since any surplus second antibody will pass through the membrane and be absorbed in the pad leaving only bound second antibody and associated colour agent on the surface of the nitrocellulose membrane.

Although, as discussed above, it does not completely solve the problems of slow sample flow and false positive results, it will often be advantageous to include in the method of the invention one or more initial filtration steps.

Typically, a filter useful in the method will have an effective pore size of from about 1 to 15 μm, preferably

from 3 to 8 μm. This may be achieved using any type of filter but a preferred type is a frit made from a plastics material and having a typical actual pore size of about 5 to 10μm. In this type of frit, the effective pore size is smaller than the actual pore size because the frit is relatively deep and the pores are out of alignment.

A further step which may be included before the filtration step is a primary separation step in which the sample is passed through a coarse filter such as a cotton or cotton wool pad. This step is particularly useful in reducing the viscosity of saliva samples, probably because it removes a large proportion of the mucopolysaccharides which are present in saliva samples but it may also be helpful in assays of other types of body fluid.

A further refinement of the method which assists in the elimination of false positive results is the provision on the substrate of a control reagent which is capable of reacting with the detection reagent. The control reagent will be present in a different location from the specific binding molecule and will be capable of specifically binding the detection agent. Thus, for example, if the detection reagent is anti-human IgG, the control reagent will be human IgG. The presence of the control reagent is a means of monitoring the viability of the method of the invention since if its presence is not detected, then clearly, the detection method is not working correctly.

Before adding the sample to the substrate, it is often preferable to treat it with a solution containing a buffer and/or a surfactant. For whole blood samples, a

lysing buffer may be used. The buffer will usually have a pH of from about 6.8 to 7.8 and preferably about pH 7.2. The buffer will generally contain a surfactant such as the non-ionic polyoxyethylene ether sold under the trade mark TRITON X-100 (Union Carbide Chemicals and Plastics Co, Inc) which may be present in an amount of from 0.05% to 1% and preferably about 0.1% by volume.

The combined use of the wiping step and the buffer has allowed the flow through time of whole blood samples to be reduced from 20 to 30 minutes to 120 to 180 seconds.

When the body fluid is saliva, it will, for preference, be treated with a solution comprising polyoxyethylenesorbitan derivatives of palmitic and/or stearic acids and buffered to pH about 6.8 to 7.8, preferably about pH 7.4.

Suitable surfactants are available under the trade marks TWEEN 40, TWEEN 60, TWEEN 61, TWEEN 65 and TWEEN 80.

It is particularly preferred that the surfactant contains from 40% to 65% stearic acid derivatives and TWEEN 60 which contains about 55% stearic acid derivatives with the balance being palmitic acid derivatives is particularly preferred and provides significantly more reliable results than most other surfactants.

The amount of surfactant present will for preference be chosen so as to maximise the flow of sample through the substrate. The flow is usually maximised when the surfactant is present in an amount of from 0.1% to 1% by volume, typically about 0.5%. This amount of surfactant give the best results for eliminating non-specific

binding without greatly affecting the specific binding and thus the threshold of detection obtainable using the method of the invention.

Other agents may be present in the solution in order to minimise the non-specific binding of mucins and particulate material in the test sample to the test reagents. Such agents include inorganic salts such as sodium chloride and proteins such as bovine serum albumin (BSA).

Sodium chloride may be present in a concentration of from about 0.1 to 0.2 M. It is greatly preferred that the upper concentration limit of 0.2 M is not exceeded since this would tend to discourage specific binding. Typically, the concentration of sodium chloride present in the solution is about 0.125 M.

BSA, if present will typically be included in an amount of about 0.05% to 0.5% by weight, preferably of 0.1%.

Phosphate is a particularly preferred buffer for the treatment of both blood samples and saliva samples but other examples of buffers which could be used to ensure that the pH of the solution is within the preferred range are familiar to those skilled in the art.

Any water soluble salt such as a sodium, potassium or ammonium salt may be used for the preparation of the buffer solution although sodium salts often give the best results. It has been found that effective buffering is obtained using a 0.001 - 0.05 M, preferably about 0.02 M, solution of sodium phosphate.

As briefly mentioned above, a colouring agent for visualising the contaminants which are to be wiped from the surface of the substrate may be included in the buffer or surfactant solution. When it is included in a buffer or surfactant solution, a particulate colouring agent may be present in an amount of from about 0.005% to 0.05% and preferably from about 0.01% to 0.02% by weight. If a stain is used, however, the concentration may be somewhat higher than this.

The buffer and/or surfactant solution comprising a colouring agent is new and itself forms a part of the invention.

Therefore, in a second aspect of the invention there is provided a buffer solution for the treatment of a sample of body fluid to be assayed and comprising a surfactant and a colouring agent.

As discussed above, the colouring agent may be specific for some contaminant which it is desirable to remove but it will, for preference comprise a coloured particulate material such as latex, agarose, polystyrene or another polymer. The particles should of course be larger than the pore size of the substrate but must also be smaller than the pore size of any pre-filters which may be used. It has been found appropriate in many cases to use particles of diameter of about 3 μm.

The buffer solution may be included in a kit for carrying out the method of the invention, which kit itself forms a further aspect of the invention.

In this aspect of the invention there is provided a kit

comprising:

i. a buffer solution comprising a surfactant and a colouring agent;

ii. a specific binding agent immobilised on a porous substrate and capable of forming a specific binding complex with the analyte; and

iii. a detection reagent for detecting the presence of specific binding complex.

The present invention is particularly useful for the detection of antibodies against *H. pylori* which may be present in the saliva of *H. pylori* infected patients. As discussed above, *H. pylori* is unusual in that infection gives rise to antibodies of the IgG isotype present in the saliva.

Therefore in a fourth aspect of the invention there is provided a kit for the detection of IgG specific for *H. pylori*, the kit comprising:

i. a solution comprising a surfactant and a colouring agent;

ii. an antigen derived from *H. pylori* immobilised on a porous substrate; and

iii. a solution of a labelled antibody capable of binding specifically to human IgG.

When the kit is for the assay of whole blood or saliva samples, the preferred components of the solutions are those which are described for the method of the first

aspect of the invention. The preferred substrates and detection reagents are also as described for the first aspect of the invention.

The kit may also contain a collection device appropriate to the body fluid to be assayed. A coarse filter such as a cotton or cotton wool pad for preliminary filtration of the sample may also be included and may optionally form a part of a saliva collection device. Furthermore, the kit may include a filter for removing particulate material from the sample. As discussed above in relation to the method of the first aspect, the filter may have an effective pore size of from about 1 to 15 μm, preferably from 3 to 8 μm. This may be achieved using any type of filter but a preferred type is a frit made from a plastics material and having a typical actual pore size of about 5 to 10μm.

The invention will now be described in detail with reference to the following examples.

EXAMPLE 1

Preparation of Buffered Surfactant Solution for Saliva Sample

A buffered surfactant solution was prepared from the following ingredients:

0.02M sodium phosphate solution	100 mL
sodium chloride	0.73 g
Bovine serum albumin	0.1 g
TWEEN 60™	0.5 g
dark blue latex particles (3.0 μm diameter)	0.1 g

by mixing at room temperature. The dark blue latex

particles are available from Polymer Laboratories, UK.

EXAMPLE 2

Preparation of *H. pylori* derived antigen

An antigen derived from *H. pylori* was prepared according to the method set out in Example 1 of WO-A-9322682. In summary, a crude sonicate of *H. pylori* was prepared and fractionated. A 440 kDa protein was removed leaving a mixture containing 265 and 340 kDa proteins.

EXAMPLE 3

Preparation of Test Device

Between 1 and 5 μL of a 0.05% (w/w) solution of the antigen of Example 2 was spotted onto a substrate to form a test area. The substrate was a 1.2 μm SARTORIUS™ nitrocellulose membrane supported upon a backing layer of Schleicher & Schuell chromatography paper No 3469 (available from Anderman & Co, Kinston upon Thames, UK) which acts as a wicking material.

From 1 to 5 μL of a 0.005% (w/w) solution of purified normal human IgG (Sigma Chemical Company Ltd, Poole, Dorset, UK) was spotted onto the substrate in a control area distinct from the test area.

EXAMPLE 4

Preparation of Disclosing Agent

A disclosing agent was prepared by diluting colloidal gold conjugated to goat anti-human IgG (heavy and light chains) (Biocell Research Laboratories, Cardiff, UK) in phosphate buffered saline (PBS) containing 0.05% by

volume of the surfactant available under the trade mark TWEEN 20 and 0.1% by weight BSA to an absorbance at 520 nm, 1 cm path length of 0.5 optical density units.

EXAMPLE 5

Assay of Saliva Sample

Saliva (1mL) was collected using the collection device available under the trade mark OMNISAL (Saliva Diagnostic Systems, Vancouver, Washington, USA) in which the sample is collected in a pad which also acts as a coarse filter. The collection device containing the sample was then transferred to a tube containing 1.0 mL of the solution of Example 1. The collected saliva was filtered using a Porex Ultrafine serum separator having an approximate exclusion of 5 μm and was then added to the test device prepared in Example 3.

After the diluted saliva sample had flowed through the nitrocellulose membrane into the chromatography paper backing layer, the blue latex particles formed a layer on the surface of the substrate. This layer was removed by wiping firmly but gently with cotton wool until no blue colour remained.

0.5 mL of the disclosing agent of Example 4 was then added to the test device. The disclosing agent was allowed to drain through the nitrocellulose membrane and then the test was read.

A single pink spot in the control area indicates a viable but negative test result whereas a test which results in a spot in the test area and a spot in the control area indicates a positive result.

The test was capable of detecting levels of anti-*H. pylori* IgG of as low as 0.8 units (on a scale of from 0 to 10) and did not give false positive results.

EXAMPLE 6

Preparation of Buffered Surfactant Solution for Blood Sample

A buffered surfactant solution was prepared from the following ingredients:

0.02M sodium phosphate solution	100 mL
TRITON X-100	0.1 mL
dark blue latex particles (3.0 μm diameter)	0.1 g

by mixing at room temperature. The dark blue latex particles are available from Polymer Laboratories, UK.

EXAMPLE 7

Assay of Blood Sample

The assay was carried out according to the method of Example 5 except that the blood sample was collected using a UNISTIK 2™ collection device and a VOLAC™ heparinised glass tube. The blood sample was treated with the solution of Example 6 rather than the solution of Example 1.

Again, a single pink spot in the control area indicates a viable but negative test result whereas a test which results in a spot in the test area and a spot in the control area indicates a positive result.

The test was capable of detecting levels of anti-*H. pylori* IgG of as low as 0.8 units (on a scale of from 0

to 10) and did not give false positive results.



CLAIMS

1. A method for detecting the presence of an analyte in a sample of body fluid, the method comprising contacting the sample with a specific binding agent capable of forming a specific binding complex with the analyte wherein the specific binding agent is immobilised on a porous solid substrate; and detecting the presence of specific binding complex; characterised in that before detecting the specific binding complex, the surface of the substrate is wiped to remove unadsorbed contaminants.

2. A method as claimed in claim 1, wherein the wiping step is carried out manually.

3. A method as claimed in claim 1, wherein the wiping step is automated.

4. A method as claimed in any one of claims 1 to 3, wherein a colouring agent which will remain on the surface of the substrate is added to the sample before the wiping step.

5. A method as claimed in claim 4, wherein the colouring agent comprises coloured particles of diameter smaller than the pore size of any filters used in filtration steps but larger than the pore size of the substrate.

6. A method as claimed in any one of claims 1 to 5, wherein the body fluid comprises saliva, whole blood, serum or urine.

7. A method as claimed in any one of claims 1 to 6, wherein the analyte is an antibody.



8. A method as claimed in claim 7, wherein the analyte is an antibody specific for an *H. pylori* antigen.

9. A method as claimed in claim 8, wherein the antibody is of the IgG isotype.

10. A method as claimed in any one of claims 1 to 9, wherein the specific binding molecule is an antigen.

11. A method as claimed in claim 10, wherein the antigen is derived from *H. pylori*.

12. A method as claimed in any one of claims 1 to 11, wherein the substrate is formed from nitrocellulose, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

13. A method as claimed in any one of claims 1 to 12, wherein the substrate has a pore size of from about 0.5 to 8 μm, preferably from about 1 to 2 μm.

14. A method as claimed in any one of claims 1 to 13, wherein the solid support is backed by an absorbent wicking material.

15. A method as claimed in any one of claims 1 to 14, wherein the specific binding complex is detected using an antibody, conjugated with a reporter molecule, the antibody being capable of binding specifically to the specific binding complex.

16. A method as claimed in claim 15, wherein the analyte is an *H. pylori* specific antibody of the IgG isotype and the specific binding complex is detected using an



antibody capable of binding specifically to human IgG.

17. A method as claimed in claim 15 or claim 16, wherein the reporter molecule is an enzyme such as horseradish peroxidase, glucose oxidase, β-galactosidase or alkaline phosphatase, a fluorophore such as fluorescein or rhodamine, a radionuclide containing molecule or a colour reagent such as colloidal gold or polystyrene particles.

18. A method as claimed in any one of claims 1 to 17, further including the step of filtering the sample before attempting to detect the presence of a specific binding complex.

19. A method as claimed in claim 18, wherein the filter has an effective pore size of from about 3 to 8 μm.

20. A method as claimed in any one of claims 1 to 19, further including an initial coarse filtration step.

21. A method as claimed in any one of claims 1 to 20 for the assay of whole blood samples, the method comprising the step of treating the sample with a buffered surfactant solution comprising a surfactant such as the non-ionic polyoxyethylene ether sold under the trade mark TRITON X-100 in an amount of from 0.05% to 1% by volume and buffered to a pH of about 6.8 to 7.8 before adding the sample to the substrate.

22. A method as claimed in any one of claims 1 to 21 for the assay of saliva samples, the method comprising the step of treating the sample with a buffered surfactant solution comprising polyoxyethylenesorbitan derivatives of palmitic and/or stearic acids and buffered to pH about



6.8 to 7.8 before adding the sample to the substrate.

23. A method as claimed in any one of claims 1 to 22, wherein there is immobilised on the substrate a control reagent capable of being detected by the same means as the specific binding complex.

24. A method as claimed in claim 23, wherein the control reagent is human IgG.

25. A buffer solution for the treatment of a sample of body fluid to be assayed and comprising a surfactant and a colouring agent which will remain on the surface of a substrate during an assay.

26. A buffer solution as claimed in claim 25, wherein the colouring agent comprises coloured particles of diameter smaller than the pore size of any filters used in filtration steps but larger than the pore size of the substrate.

27. A solution as claimed in claim 25 or claim 26, wherein the particles comprise latex, agarose, polystyrene or another polymer.

28. A kit comprising:

i. a buffer solution as claimed in any one of claims 25 to 27;

ii. a specific binding agent immobilised on a porous substrate and capable of forming a specific binding complex with the analyte; and

iii. a detection reagent for detecting the presence



of specific binding complex.

29. A kit comprising:

i. a buffer solution as claimed in any one of claims 25 to 27;

ii. an antigen derived from *H. pylori* immobilised on a porous substrate; and

iii. a solution of a labelled antibody capable of binding specifically to human IgG.

30. A kit as claimed in claim 28 or claim 29, further including one or more of the following:
a body fluid collection device;
a coarse filter such as a cotton or cotton wool pad which may form a part of the collection device; and
a filter for removing particulate material from the sample.