DETECTION OF ANALYTES

The present invention relates to a method for the detection of analytes, in particular specific binding molecules such as antibodies or antigens in saliva samples. The invention is especially concerned with the detection of analytes in fresh saliva samples which have not been stored or frozen.

The detection of antibodies in saliva is a convenient method for the diagnosis of various diseases and conditions, in particular gut infections. Gut infections in mammals, and in particular humans, stimulate an immune response in mucous secretions, such as saliva, through activation of the common mucosal immune system. This response often initially parallels an antibody response in serum although is generally characterised by the presence of IgA antibodies. However, the immune response in secretion, including saliva, rapidly diminishes following elimination of the antigen (eg bacteria or virus) from the body. Accordingly, the presence of antibody in saliva reflects current, ie contemporary, infection. In the case of a microbial infection, for example, antibodies in saliva, hereinafter referred to as secretious antibodies, reflect the current status of colonisation of the microbe, such as in the gut, and thus is a useful monitor of contemporary infection. Serum antibody, on the other hand, persists for some time after the microbe is eliminated from the body. A positive serum antibody test, therefore, reflects both past and present exposure to antigen which is less helpful to the clinician. A positive secretious antibody test indicates present or contemporary infection by the microbe.

The provision of saliva samples is greatly preferred by patients to the provision of samples of other body fluids, particularly serum. Further, in obtaining a sample of saliva, there is a very low risk of infection of either the patient or the clinician since it is not necessary to use a needle as is the case with serum samples.

Methods of diagnosis from saliva samples are known and, in particular, AU-A-9067676 is directed to the detection of IgG specific to *Helicobacter pylori* antigen in mucous secretions such as saliva and thereby provides a means of monitoring current, ie contemporary, infection by that microorganism in mammals. The corresponding academic publication is Witt *et al*, *Frontiers in Mucosal Immunology* 1 693-696 (1991).

WO-A-9322682 relates to a saliva-based test for *H. pylori* in which IgG antibodies to *H. pylori* are detected.

One disadvantage with currently available saliva-based tests is that they may, in certain circumstances, be unreliable; in particular, the number of false positives obtained in the test can be unacceptably high. In experiments which were carried out in an attempt to develop immunoassays for salivary antibodies to *H. pylori*, 78 subjects were asked to pool saliva flow into a clean sterile container. When the samples were tested for the presence of antibodies to *H. pylori*, it was found that the results did not correlate well with the results of serum tests because of a significant number of false positive results.

A further disadvantage which saliva based tests have in

common with most other types of diagnostic test is that in order to obtain the results, it is necessary to send the sample to a laboratory in order for it to be analyzed. This may take several days and in part destroys the advantage of being able to obtain an indication of present infection by a microbe. In addition, the patient may forget to collect the results of the test, particularly if any symptoms have disappeared in the interval between submitting the sample and receiving the results of the test.

A test kit for the detection of an analyte in a sample of saliva which would provide reliable results in a few minutes would therefore be of great value as the test could be carried out during a consultation with a general medical practitioner. This would have the added advantage of ensuring that the patient collects the results and is prescribed treatment if necessary. However, the problems of false positive results which exist with any saliva based test are greatly multiplied when saliva samples are analyzed within a few minutes of collection, so much so that any results from assays carried out on fresh saliva samples have, in the past, been almost completely meaningless.

It is believed that this lack of reliability, particularly in fresh saliva samples, may arise because of the presence in the sample of agents which bind non-specifically to the test reagent and thus give the false positive results. It is, of course, possible to add to the sample agents which reduce non-specific binding but this has also proved problematic as many such agents also reduce specific binding to such an extent that true positive results are eliminated along with the false

positives. The present invention makes it possible to overcome this problem and to eliminate false positive results whilst retaining the true positive ones.

In a first aspect of the present invention there is provided a method for detecting the presence of an analyte in a sample of saliva, the method comprising contacting the saliva sample with a specific binding agent capable of forming a specific binding complex with the analyte and detecting the presence of specific binding complex; characterised in that the saliva sample is initially contacted with a solution comprising polyoxyethylenesorbitan derivatives of palmitic and/or stearic acids.

The advantage of the method of the present invention is that the results of the test can be obtained quickly and reliably by a non-invasive method and, in addition, it overcomes the problems which arise when fresh saliva is used and enables reliable results to be obtained from "on the spot" tests. In the context of the present invention the term "fresh saliva" refers to saliva which has been stored for not longer than about thirty minutes, preferably for not longer than ten minutes and often for a shorter length of time than that.

It is essential to choose the surfactant extremely carefully and, indeed, one of the surprising features of the present invention is that from the vast range of surfactants available, the only ones which enabled us to obtain reliable results were those defined above. 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, when a solid substrate is used as is discussed below, 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.

The best results are obtained when the solution is buffered to a pH of from about 6.8 to 7.8 but a solution buffered to pH 7.4 has been found to be optimal. Any buffer may be used provided that it results in the pH of the solution being maintained in the preferred range. Phosphate is a particularly preferred buffer for use in the present invention 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.

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%.

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 samples is particularly useful in the diagnosis of gut infections caused by pathogens such as *Helicobacter pylori* (formerly known as *Campylobacter pylori*). As discussed above, *H. pylori* infection is indicated by the presence in saliva of IgG 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 saliva.

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.

The specific binding agent will for convenience and preference be bound to a solid support. Suitable solid supports include a nitrocellulose membrane, glass or polymer solid supports. The most commonly used polymers for this purpose are cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene, but the invention is not limited to these. The solid supports may be in the form of strips, tubes, beads, discs or microplates, or any other surface suitable for conducting

an immunoassay.

A particularly useful solid support may comprise a nitrocellulose membrane backed by an absorbent pad so that, on adding the sample to the solid support, the analyte will be immobilised by the specific binding agent on the top surface of the nitrocellulose membrane whilst the remainder of the sample passes through the membrane and is absorbed on the pad. This ensures that any unwanted material is removed from the area in which the specific binding complex is detected.

The nitrocellulose membrane may have a pore size of from about 0.5 to 8 μm with from about 1 to 2 μm being preferred.

The pad may be formed from any absorbent material but absorbent paper will often be the material of choice, generally because of considerations of cost.

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. This can be achieved by the provision of the specific binding agent immobilised on a nitrocellulose membrane backed with an absorbent pad as described above. 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.

Further investigations into the problems of obtaining reliable results from saliva based assays suggested that one possible cause of the false positive results was the presence of mucins and particulate material in the saliva sample which bind non-specifically to the test reagents thus producing false positive results. Therefore, it is advantageous to include in the method of the present invention the step of filtering the sample before attempting to detect the presence of a specific binding complex.

Typically, the filter 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.

The removal from the saliva sample of particulate material helps to ensure that a substrate to which the specific binding agent is bound does not become contaminated. This is particularly important when the substrate is a membrane since particulate material may block the pores of the membrane preventing unbound sample from passing through the membrane and being removed from the test surface and thus increasing the flow through time for the sample.

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 reduces the viscosity of the

saliva sample, possibly by the removal of high molecular weight mucopolysaccharides. A sample of reduced viscosity is helpful when the specific binding molecule is immobilised on a membrane substrate since a viscous saliva sample may pass through the pores of the membrane only with difficulty and thus the test takes much longer when this step is not present. In addition, unless this step is included, a residue is often left on the test surface which may interfere with either the specific binding reaction or with the detection step.

Even these preliminary filtration steps do not appear to be completely effective in removing mucins and particulate material from saliva and the flow through time is slow and a number of false positive results are often obtained even when the sample has been pre-filtered.

However, it has now been shown that it is possible to reduce the number of false positive results obtained in the test of the present invention simply by wiping the surface of the substrate after the sample has been adsorbed onto it. This was most unexpected because it had been thought that all contaminants had been removed by the filtration step which had been attempted previously whereas it now seems that this was not the case.

Therefore, in cases when the specific binding agent is adsorbed on a substrate, it is preferred that the method of the invention includes the step of wiping the substrate after the sample has been added to it and before attempting to detect the specific binding complex. The wiping step both dramatically decreases the flow

through time of the sample and reduces the occurence of false 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 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 that all of the unwanted material has been removed, a colouring agent may be added to the sample on the substrate. For convenience, the colouring agent may be included in the surfactant solution but this will not necessarily be the case. The surfactant solution may contain from about 0.005% to 0.05% (w/w) of a particulate colouring agent and preferably from about 0.01% to 0.02% but other types of colouring agents may be present in greater amounts.

The colouring agent may be an agent which is specific for the mucins and other contaminants which remain on the substrate and are a cause of many of the problems of false positives which occur with assays of saliva. However, it is often simpler to provide as a colouring agent 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 since, as discussed above, 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.

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.

Thus the present invention provides a simple and effective method for assaying saliva samples even when the saliva samples are fresh.

The surfactant solution is an important part of the invention since it has been found that only the surfactants discussed above in relation to the method are

at all effective in preventing false positive results in the assay.

Therefore, in a second aspect of the invention there is provided a surfactant solution for use in the method of the invention, the solution comprising from 0.1% to 1% by volume polyoxyethylenesorbitan derivatives of palmitic and/or stearic acids; a buffer capable of maintaining the pH of the solution at a level of from 6.8 to 7.8; and optionally, a water soluble salt, a protein such as BSA and a particulate colouring agent.

The preferred buffers and concentrations of salt, BSA and colouring agent are as discussed above in relation to the first aspect of the invention.

The method may be carried out using a kit which itself forms a further aspect of the invention.

In this aspect of the invention there is provided a kit comprising:

i. a solution comprising polyoxyethylenesorbitan derivatives of palmitic and stearic acids;

ii. a specific binding agent immobilised on a 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 polyoxyethylenesorbitan derivatives of palmitic and stearic acids;

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

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

The preferred components of the solution, and preferred substrates and detection reagents are those which are described for the method of the first aspect of the invention.

The kit may also contain a saliva collection device. 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 the 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. 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 colouring agent capable of remaining on the surface of the substrate may also be included if the method of the invention is to include the wiping step mentioned above. The colouring agent may be an agent which is capable of staining the mucins and particulate impurities but will preferably consist of coloured particles of latex, agarose, polystyrene or some other polymer in which the particle size is chosen so that the particles will not be removed in any preliminary filtration steps which are carried out but will be too large to pass through the pores of the substrate.

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

EXAMPLE 1

Preparation of Surfactant Solution

A 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.



CLAIMS

1. A method for detecting the presence of an analyte in a sample of saliva, the method comprising contacting the saliva sample with a specific binding agent capable of forming a specific binding complex with the analyte and detecting the presence of specific binding complex; characterised in that the saliva sample is initially contacted with a solution comprising polyoxyethylenesorbitan derivatives of palmitic and/or stearic acids.

2. A method as claimed in claim 1 wherein the sample of saliva comprises fresh saliva.

3. A method as claimed in claim 1 or claim 2, wherein the surfactant is available under the trade mark TWEEN 40, TWEEN 60, TWEEN 61, TWEEN 65 or TWEEN 80.

4. A method as claimed in any one of claims 1 to 3, wherein the surfactant is present in an amount of from 0.1% to 1% by volume.

5. A method as claimed in any one of claims 1 to 4, wherein the surfactant solution is buffered to a pH of from about 6.8 to 7.8.

6. A method as claimed in any one of claims 1 to 5, wherein the analyte is an antibody.

7. A method as claimed in claim 6, wherein the analyte is an antibody specific for an *H. pylori* antigen.

8. A method as claimed in claim 7, wherein the antibody



is of the IgG isotype.

9. A method as claimed in any one of claims 1 to 8, wherein the specific binding molecule is an antigen.

10. A method as claimed in claim 9, wherein the antigen is derived from *H. pylori*.

11. A method as claimed in any one of claims 1 to 10, wherein the specific binding molecule is immobilised on a solid support for example a nitrocellulose membrane or a glass or polymer solid support.

12. A method as claimed in claim 11, wherein the solid support comprises a nitrocellulose membrane backed by an absorbent pad.

13. A method as claimed in any one of claims 1 to 12, 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.

14. A method as claimed in claim 13, 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.

15. A method as claimed in claim 13 or claim 14, 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 or polystyrene



particles.

16. A method as claimed in any one of claims 1 to 15, further including the step of filtering the sample before attempting to detect the presence of a specific binding complex.

17. A method as claimed in claim 16, wherein the filter has an effective pore size of from about 3 to 8 μm.

18. A method as claimed in any one of claims 1 to 17, further including an initial coarse filtration step.

19. A method as claimed in any one of claims 11 to 18, further including the step of wiping the substrate after the sample has been added to it and before attempting to detect the specific binding complex.

20. A method as claimed in claim 19 wherein a colouring agent which will remain on the surface of the substrate is added to the sample before the wiping step.

21. A method as claimed in claim 20, 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.

22. A method as claimed in any one of claims 11 to 21, wherein there is immobilised on the substrate a control reagent capable of being detected by the same means as the specific binding complex.

23. A method as claimed in claim 22, wherein the control

reagent is human IgG.

24. A surfactant solution suitable for use in a method as claimed in any one of claims 1 to 23 and comprising from 0.1% to 1% by volume polyoxyethylenesorbitan derivatives of palmitic and/or stearic acids; a buffer capable of maintaining the pH of the solution at a level of from 6.8 to 7.8; and optionally, a water soluble salt, a protein such as BSA and a particulate colouring agent.

25. A kit comprising:

i. a surfactant solution comprising polyoxyethylenesorbitan derivatives of palmitic and stearic acids;

ii. a specific binding agent immobilised on a 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.

26. A kit for the detection of IgG specific for *H. pylori*, the kit comprising:

i. a surfactant solution comprising polyoxyethylenesorbitan derivatives of palmitic and stearic acids;

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

iii. a solution of a labelled antibody capable of



binding specifically to human IgG.

27. A kit as claimed in claim 25 or claim 26, wherein the surfactant solution is a solution as claimed in claim 24.

28. A kit as claimed in any one of claims 25 to 27, wherein the surfactant solution further comprises a colouring agent, for example coloured particles, capable of remaining on the surface of the substrate.

29. A kit as claimed in any one of claims 25 to 28, further including one or more of the following:

a saliva collection device;

a coarse filter such as a cotton or cotton wool pad which may form a part of the saliva collection device; and

a filter for removing particulate material from the sample.