ANALYTICAL METHOD FOR SALIVA

This invention relates to a method of treating saliva. In particular, it relates to a method of treating saliva for use in a diagnostic test for the presence of antigens to *H. pylori*.

Saliva is a complex mixture of secretions originating from various glands within the oral cavity. Among its many constituents are various enzymes, immunoglobulins and mucins, together with cellular debris and a host of bacterial flora. The mucins, in particular, are responsible for the viscoelastic properties of saliva, and act as lubricants with protective and antibacterial properties. The viscoelastic properties of these glycoproteins depend on the protein and carbohydrate composition acquired during synthesis, but are also influenced by the extent of their interaction with other components of saliva. For example, lipids can also contribute significantly to the viscoelastic properties of saliva.

Saliva which contains immunoglobulins can be used for the diagnosis of various diseases. In particular, the detection of IgG specific to antigens produced by pathogens is indicative of infection or recent contact. One example of such an infection is that caused by *H. pylori*. The diagnosis of *H. pylori* infection can be made by microscopy, microbiological culture or urease detection in gastric mucosal biopsies, urea breath test or by the presence of specific antibodies in serum detected by ELISAs. *H. pylori* infects the gastric mucosa and would be expected to elicit an IgA antibody response that could be detected in mucous in gastric secretions.

However, it has been discovered that the predominant *H. pylori*-specific antibody found in mucous secretions is IgG class and not IgA. AU-A-9067676 describes a method for the detection of IgG in mucous secretion specific to *H. pylori* antigen, thereby providing a means of monitoring infection by that microorganism in mammals. The corresponding academic publication is Witt *et al*, *Frontiers in Mucosal Immunology* 1 693-696 (1991).

The presence of IgG antibodies in the saliva of *H. pylori* (*Campylobacter pylori*) positive patients has received some attention in the proceedings of the Annual Meetings of the American Gastroenterological Association. After the disclosure by Czinn *et al* of the presence of such antibodies in the 1989 proceedings, Larsen *et al* concluded in the May 1991 proceedings that salivary IgG levels are a practical, non-invasive marker of therapeutic response during a course of antibiotic therapy. In the April 1992 proceedings, Landes *et al* confirmed earlier observations and observed that measurement of salivary IgG to *Helicobacter pylori* is a simple, non-invasive test for detecting *H. pylori* positive patients, especially in widespread or paediatric populations where other tests are not practical.

WO-A-9322682 discloses a convenient and reliable *in vitro* test for *H. pylori*. This test utilises an antigen preparation that reacts with IgG antibody in a mucous secretion from a mammal being tested.

However, the use of saliva in such diagnostic methods also has its attendant problems. These methods of diagnosis often rely on contacting the saliva sample with antigen bound to a membrane or pad, or other similar

material. Flow-through of the saliva on such a device is therefore an essential element of the test. Also, predictable flow-through times are essential to provide accurate and meaningful results. However, because of the complex nature of saliva, and in particular the presence of particulates and mucins, the flow-through properties of individual saliva samples are subject to both extensive intra and inter subject variability.

Particulates which are present in saliva can bind to the membrane, by simple deposition on the surface and reduce flow times. The mucins have viscoelastic properties and this can result in a gel-like matrix being deposited on the membrane which will reduce liquid flow. The flow-through time of the saliva must be a balance between the need for accurate binding of IgG to antigen, which means that the flow-through time must not be too fast, and the need for the test to be practical, i.e. the flow-through time must not be too slow. The binding of particulates and mucins to the surface of any membrane or pad to which the antigen is bound can lead to greatly reduced flow-through time.

A further problem associated with deposition of mucins and particulates on the membrane is related to signal development. One preferred method of signal development utilises IgG-Colloidal Gold conjugates which can be trapped by the deposits. This can lead to false positive results, and in a worst case, where no conjugate can pass freely, the whole membrane can bind conjugate.

Theoretically, it is possible to remove mucins. However, the reagents and solid-phase media required do not make such an approach commercially acceptable. Thus, an

alternative, more economical way is needed to reduce deposition and hence improve flow-through times.

Currently, saliva can be processed using a system known as Omnisal® in which saliva is collected onto a cotton pad then extracted in a buffer and filtered through a porous plastic filter. However, the liquid so produced can still have poor flow characteristics.

It has now surprisingly been found that by altering the properties, e.g. the viscoelastic properties, of the saliva sample, flow-through times can be improved, leading to a more accurate and sensitive test. This does not require removal of, for instance, the mucins present in the saliva, only an alteration of their properties.

Thus, the present invention provides a method of improving the flow characteristics of saliva, which comprises the step of altering the properties of the saliva sample. In particular, the invention relates to altering the viscoelastic properties of the saliva.

In a preferred embodiment of the invention, the properties of the saliva are altered by passing the saliva through a filtration medium which achieves this effect. Preferred filtration media include charcoal, DEAE-sepharose and diatomaceous earth, e.g. Celite®, with diatomaceous earth being particularly preferred. The filtration medium removes particulates. Mechanical shear, removal of high molecular weight aggregates and lipids may disrupt the polymeric structure of mucins, leading to improved flow characteristics.

Mucins present are not removed by the method, however,

their polymeric structure may be altered, thus improving flow characteristics.

In a second embodiment of the invention, the properties of the saliva sample can be manipulated by the addition of detergents and adjusting the ionic strength and/or pH of the saliva sample. This can be achieved by passing the saliva sample into a suitable buffer solution. Of course, if the properties of the saliva are adjusted in this way, it is also usual to include a separate filtration step to remove particulate matter.

Thus, in a particularly preferred embodiment of the invention, the saliva sample is not only passed through a filtration medium which alters the properties of the saliva, but also has its pH and/or ionic strength adjusted and detergent added by means of passing into a suitable buffer solution.

The method of the present invention finds particular application in rapid diagnostic tests for specific antibodies found in saliva. Therefore, in a further aspect, the present invention provides a method for the detection of an antibody in a sample of saliva which comprises the step of altering the properties, e.g. the viscoelastic properties, of the saliva to improve flow characteristics. As described herein, this can be achieved by means of filtration and/or buffer manipulation.

Preferably the test is for the detection of *H. pylori* specific antibody, usually IgG.

Such diagnostic methods are usually carried out using a

diagnostic kit comprising a membrane or pad to which the antigen is bound. In yet a further aspect, therefore, the present invention provides a kit for the detection of an antibody in a sample of saliva which comprises a means for altering the properties, e.g. the viscoelastic properties, of the saliva sample, those means being provided in the form of a filtration material and/or a buffer solution, either or both of which have the ability to alter the flow properties of the saliva sample before application to the test area.

The invention will now be described with reference to the following example, which should not be construed as in any way limiting the invention.

The example refers to the figures in which:

Figure 1: shows saliva flow times with or without a Celite® filtration step;

Figure 2: shows IgG Gold conjugate flow times with or without a Celite® filtration step.

EXAMPLE

Saliva samples were collected from a number of individuals using the Omnisal® (Saliva Diagnostic Systems, Inc., 11719 NE 95th Street, Vancouver, WA 98682, USA) saliva collection and processing system. However, the saliva was extracted into the following buffer: 20 mM Phosphate pH 7.0-8.0 containing 0.5% v/v Tween 60, 125 mM NaCl, 10 mM Sodium azide and 0.1% w/w BSA.

The saliva was either extracted into the buffer by

filtering through the Omnisal® filter or through Celite®. 1 ml of the resulting solutions were then placed onto a Helisal® rapid test card, which contains membrane spotted with antigen, and the flow through times recorded. After saliva, 1 ml of IgG-gold conjugate was added and the flow through time also recorded. The results are shown below.

RESULTS

Sample	Saliva flow time		IgG-Gold Conjugate flow time	
	No Celite filtration (sec)	With Celite filtration (sec)	No Celite filtration (sec)	With Celite filtration (sec)
1	120	98	166	213
2	132	102	196	215
3	168	72	284	164
4	100	89	195	182
5	160	79	275	160
6	120	81	285	239
7	167	79	373	171
8	104	69	245	190
9	82	70	182	158
10	119	97	203	181
11	95	89	162	198
12	112	94	245	190
13	163	83	325	162

DISCUSSION

Figures 1 and 2 are a representation of the above data graphically. The following points are clear:

i) flow times of both the saliva samples themselves and the IgG-Gold conjugates are reduced as a result of the processing steps;

ii) in addition, the flow times in both cases show much

less variation between samples.

Samples processed as described above also show a significant reduction in background colour due to trapping of IgG-Gold, and the number of false positives is greatly reduced.

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CLAIMS

1. A method of improving the flow characteristics of saliva, which comprises the step of altering the properties of the saliva.

2. A method as claimed in claim 1 wherein the viscoelastic properties of the saliva are altered.

3. A method as claimed in claim 1 or claim 2 wherein the properties of the saliva are altered by passing the saliva through a filtration medium.

4. A method as claimed in claim 3 wherein the filtration medium is charcoal, DEAE-sepharose or diatomaceous earth.

5. A method as claimed in claim 4 wherein the filtration medium is Celite®.

6. A method of improving the flow characteristics of saliva which comprises the step of manipulating the pH and/or ionic strength of the saliva and adding detergent.

7. A method as claimed in claim 6 wherein the saliva sample is also subjected to a filtration step as defined in any one of claims 3 to 5.

8. A method for the detection of an antibody in a sample of saliva which comprises the step of altering the properties of the saliva to improve its flow characteristics.

9. A method as claimed in claim 8 modified by any one

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or more of the features of claims 2 to 7.

10. A method as claimed in claim 9 wherein the antibody is *H. pylori* specific antibody.

11. A method as claimed in claim 10 wherein the *H. pylori* specific antibody is IgG.

12. A kit for the detection of an antibody in a sample of saliva which comprises a means for altering the properties of the saliva to improve its flow characteristics.

13. A kit as claimed in claim 12 wherein the means comprises a filtration medium.

14. A kit as claimed in claim 13 wherein the filtration medium is diatomaceous earth.

15. A kit as claimed in claim 14 wherein the diatomaceous earth is Celite®.

16. A kit as claimed in any one of claims 12 to 15 wherein the kit further comprises a buffer and/or detergent.