

## SPECIAL ARTICLE

### The use of saliva for viral diagnosis and screening

#### INTRODUCTION

The strength and diversity of antibody responses to infection are the basis for many of the most rapid and sensitive tests in virology, yet the need to obtain the patient's consent and co-operation, to collect blood from a vein and to separate the serum before the test often deters investigators, particularly if children are involved or if there is added risk, difficulty or cost in doing the venepuncture.

Most body fluids contain antibody; but though researchers have shown that immunoglobulin is present in urine, saliva, breast milk, tears, cerebrospinal fluid, cervical secretions, semen, etc., the concentrations of all classes of immunoglobulin in these fluids are much lower than they are in blood. This has generally been assumed to disqualify these fluids as diagnostic specimens for viral antibody tests in spite of the obvious convenience that some of them, e.g. saliva and urine, might have. However, recent progress in viral serology calls that assumption into question, and this article seeks to explain how saliva can be used as a substitute for serum in investigating clinical and subclinical infection and viral immunity.

#### *Characteristics of salivary antibody*

Salivary antibody comes from two sources: some, mainly of the IgA class, originates in the salivary glands; the remainder, usually referred to as crevicular fluid, transudes from the capillary bed situated beneath the margin between the tooth and the gum (Jenkins, 1978). The composition of the antibody in salivary fluid from the latter source reflects the classes and specificities of immunoglobulin found in plasma. Thus it contains IgG and IgM antibody as well as IgA (Table 1, Lehner, 1982). It is probable that the concentrations of all the plasma-derived immunoglobulins in saliva are raised in subjects with periodontal inflammation, and this condition is common, causes increased plasma transudation (Golub & Kleinberg, 1976) and is typically associated with certain infections, e.g. HIV (Murray, Grieve & Winkler, 1987). Transudation and salivary immunoglobulin concentration is probably diminished in patients who are edentulous, a point currently being investigated. Concentrations of immunoglobulins are thus lower and more variable in saliva than in plasma though, as antibodies, their specificities are very similar. Might it therefore be possible to use saliva as the basis for reliable diagnostic antibody tests?

#### *Antibody capture assays*

The answer to this lies in the technique of 'antibody capture', introduced by Flehmig *et al.* (1979) and Duermeyer, Wieland & Van der Veen (1979), and now extensively used in the diagnosis of virus infection. The essence of this technique is that a particular class of human immunoglobulin (IgM, IgG, IgA) is captured

Table 1. *Immunoglobulin concentrations (mg/100 ml) in plasma, whole saliva and salivary components*

	IgG	IgM	IgA
Plasma	1250	80	220
Whole saliva	1.40	0.20	19.4
Parotid saliva	0.04	0.04	19.4
Creviceular fluid	350	25	110

from the specimen by an antiserum to that immunoglobulin previously fixed to a solid phase such as a polystyrene bead or well. Once the solid phase has been washed, antibodies of that single immunoglobulin class are bound to it free of all other specimen components, including other classes of immunoglobulin. The second part of this procedure is to probe the solid phase for the antibody specificity that is being sought by adding a viral antigen. The third part is to demonstrate antigen binding, for instance by adding a mouse monoclonal antibody to the antigen and an enzyme conjugated anti-mouse immunoglobulin reagent that will generate a colour signal in a substrate.

In an antibody capture assay the occurrence of a signal depends on whether or not the captured immunoglobulin molecules have reacted with the antigen added, and the strength of the signal depends on how many immunoglobulin molecules have bound the antigen. Assay reactivity is thus related not, as in most antibody assays, to the concentration of specific antibody, but to the proportion of the total immunoglobulin of the class being captured that is specific for the antigen. This nice distinction between proportion and concentration of specific antibody is crucial to understanding why capture assays work virtually as well on saliva, which has low immunoglobulin concentrations, as they do on serum, which has high immunoglobulin concentrations. Because saliva is partly made up of plasma transudate, the proportion of specific to total immunoglobulin IgM and IgG is similar in the saliva and serum of each individual and the signals from capture assays on the two sorts of specimen are much the same and almost independent of immunoglobulin concentrations.

#### *The application of antibody capture assays*

All acute virus infections as well as some congenital infections and reactivations of infection are characterized by a specific IgM response detectable in serum, and IgM capture assays are now acknowledged to be the most specific and in many cases the most sensitive diagnostic tests for these. They have become the diagnostic test of choice for hepatitis A and B, rubella, parvovirus B19 and several other virus infections; their use has recently been reviewed by Brown (1986).

It is less well known that capture assays can be used to detect long lasting, IgG class, specific antibodies. These assays have been used for several years to detect antibody to parvovirus B19 and HIV (Cohen, Mortimer & Pereira, 1983; Parry, 1986) and their use to detect anti HAV, anti HBc and anti-rubella virus has also been described (Parry, Perry & Mortimer, 1987). From our experience of using IgG capture assays there has been no sign (as might be the case) that the proportion of IgG that is specific falls below detectable levels in the years following a virus

infection, and therefore IgG capture is probably as suitable as any other assay format as a test for past infection and immunity. IgA capture assays have also been developed (Parry, Perry & Mortimer, 1987), but their usefulness except as a research tool and perhaps as a screening test for EBV related nasopharyngeal carcinoma (Henle & Henle, 1976) is probably very limited.

In the case of IgM capture assays commercial kits, some of them incorporating monoclonal antibodies and other refinements, are available. This means that certain capture assays are readily accessible to diagnostic laboratories. We have recently been investigating the application of these and other antibody capture assays to saliva specimens for the diagnosis of recent (i.e. IgM positive) and past or persisting (i.e. IgG positive) infection, and our early findings using specific IgG tests have been reported elsewhere (Parry, Perry & Mortimer, 1987). Our preliminary findings with specific IgM tests can be summarized as follows: for hepatitis A, IgM anti HAV was present in the saliva of all of 15 serologically confirmed acute infections; for hepatitis B, IgM anti HB<sub>e</sub> was present in the saliva of 5 out of 6 confirmed cases of acute hepatitis B; for measles, specific IgM was found in the saliva of 67 out of 69 clinically diagnosed cases: and for patients with rubelliform rashes, IgM anti rubella was present in 16 out of 46 cases. For the last group, the good discrimination between the 16 positive results (T:N values 7.0–57.9, median 21.0) and the 30 negative results (T:N values 0.6–1.6, median 1.2) suggests that most of the negative cases were not due to rubella. However, this study, done in schoolchildren, lacked serological control so that it is not certain whether all true cases of rubella were diagnosed by the salivary IgM assay. An analogous assay for mumps antibody in saliva has been set up (Brown DWG, unpublished) and detects specific IgM during parotitis and for up to 2 months afterwards. It is hoped that these last three assays will contribute to the successful use of MMR vaccine by helping to recognize residual clinical cases of measles, mumps and rubella and allowing intensive immunization of possibly susceptible contacts.

Saliva specimens are easily collected, either by asking the subject to dribble into a pot, or by wiping the margin between gum and tooth with a swab from which the saliva can then be eluted, or by asking the subject to chew a small cotton wool cylinder, e.g. a dental roll. The last is part of a convenient device for saliva collection (*Salivette*™). All these collection methods yield specimens which require no further treatment before their use in a capture assay, typically at a dilution in the range 1 in 2 to 1 in 20. A useful feature of salivary capture assays is that precise specimen dilution is not important: all that has to be achieved is saturation of the immunoglobulin capture sites. For the same reason the three methods of collecting saliva are unlikely to vary much in the results they yield: as long as enough crevicular fluid is collected and eluted from the specimen the capture sites on the solid phase will be saturated. In comparative tests for IgM anti measles on 23 patients with clinical measles positive results were, with a single exception, obtained on specimens collected by dribbling into a pot, by wiping the gum margin with a swab and by salivette. Reactions from specimens collected by swab or into a pot were mostly slightly stronger than reactions from salivette specimens, but the greatest single variable was probably the care with which the specimens were collected. Saliva specimens can be stored at 4 °C for several days

and at 20 °C for several years without deterioration, and they do not seem to be affected by repeated freezing and thawing.

We anticipate that variations of the standard capture assay will be developed that minimize the steps in the test procedure, with simultaneous addition of some reagents and the use of simpler end points than enzyme conjugates and radiolabels. As with all new assays, salivary antibody capture assays have to be compared with existing serum assays, a process which, in view of the many specific IgM and IgG assays that may be involved and the refinements still being introduced, will take several years. Standard materials of agreed unitage need to be prepared and substantial volumes of human saliva may be required for control purposes. It is difficult to collect saliva in large amounts, and it may in the end be necessary to substitute diluted serum. Once the process of development and evaluation is complete, however, salivary tests for viral antibodies should offer obvious advantages. It is likely that many patients, and their doctors, will prefer saliva collection to venepuncture when laboratory diagnosis is needed. Most patients can collect these saliva specimens themselves and future technical developments may permit self-diagnosis by the application of saliva to a solid phase made available in kit form. This innovation may not be welcomed by all, but it is highly predictable.

Another application of saliva testing is in epidemiology. The necessity for venepuncture often hinders sero-epidemiological studies, especially in areas where there are cultural barriers to collecting blood and in groups (e.g. children, injectable drug users) on whom venepuncture is ethically unacceptable or practically difficult. Salivary testing allows large numbers of individuals to be investigated with minimum fuss. It has recently been used in three such situations: to investigate two school outbreaks in which children were exposed to hepatitis A; to test for anti HIV the clients of a needle exchange programme for drug misusers; and to screen the female staff of a large retail company for rubella antibody. In the first case salivary testing confirmed all of the cases of jaundice that occurred as hepatitis A and revealed several sub-clinical infections. It also demonstrated a high level of susceptibility in family and school contacts, justifying the use of human normal immunoglobulin to control the two outbreaks. Results from the other two investigations are as yet incomplete.

This brief review is intended to encourage virologist colleagues to broaden their application of antibody capture assays to the testing of saliva specimens. It may demand changes in attitude to accept that saliva can be an adequate substitute for serum in a wide variety of virological tests; but it seems to us that serum testing has become a strait-jacket for virological diagnosis from which there is now a chance to break out. The opportunity should be taken.

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