

## TOPICAL REVIEW

**Detection of antibodies in saliva — an effective auxiliary method in surveillance of infectious diseases**

Madar R, Straka S, Baska T

*Institute of Epidemiology, Jessenius Faculty of Medicine, Comenius University, Bratislava, Slovakia. [madar@jfm.uniba.sk](mailto:madar@jfm.uniba.sk)***Abstract**

Saliva is a body fluid containing antibodies of diagnostic significance. Unlike venipuncture, saliva collection (by brushing the teeth and rubbing the gums) is painless, non-invasive, inexpensive, simple and rapid. By using sensitive immunoassays in salivary specimens it is possible to diagnose immunoglobulins against a wide range of infectious diseases e.g. hepatitis A, B and C, measles, mumps, rubella, human immunodeficiency virus, Epstein—Barr virus, parvovirus B 19, human herpesvirus 6 and *Helicobacter pylori* infections. Salivary antibody testing may provide better access to epidemic outbreaks, children, large populations, hard-to-reach risk groups and may thus play a major role in the surveillance and control of infectious diseases. (Tab. 2, Ref. 34.)

**Key words:** saliva, antibody, testing, immunoglobulins, surveillance.

Saliva is a mixture of secretion from the salivary glands and transudate from the capillaries beneath the buccal mucosa, the so-called crevicular fluid, that constantly flows from the crevice between the gum margin and the teeth. It is common knowledge that saliva contains secretory IgA (sIgA), but until recently little attention has been given to the presence of other classes of immunoglobulins in saliva. The contents of the crevicular fluid are similar to plasma, and include significant amounts of antibodies specific to those viruses to which the subject has already made, or is making a humoral response (1—5). Since immunoglobulin concentrations in crevicular fluid are much higher than in salivary gland secretions, the investigator collecting salivary sample must ensure that the crevicular fluid is included in specimen in a sufficient amount. Substantial amounts of crevicular fluid must be represented in the salivary sample, particularly when IgG and IgM are to be detected. To be able to get as big a proportion of crevicular fluid in specimen as possible, it is inadvisable to stimulate salivation before or during sample collection because it may lower the final concentration in saliva. Sputum specimens are not desirable (4, 6, 7).

Several techniques and devices have been designed to achieve collection of salivary samples with a good representation of crevicular fluid. The subject is asked to chew a cylinder of cotton wool (dental roll) so that oral fluid along the tooth-gum margins is taken up. Another device suitable for this purpose is in the form of a toothbrush consisting of a cylinder of expanded

polystyrene foam attached to a handle. The subject is instructed to use it like a toothbrush — brushing the teeth and rubbing the gum until the swab gets wet (about 30—60 seconds). Other devices are similar, for instance OraSure is a small absorbent paddle on the end of a plastic straw that is rubbed along the gums and held between jaw and cheek. After that the wet paddle is placed inside the plastic screw-top container. Subsequent centrifugation and assay (e.g. indirect assays, competitive assays, antigen sandwich assays, class-specific antibody capture assays) performed in laboratory will show whether minimum concentrations of IgG or IgM are present in the specimen (4, 7, 8). Mean immunoglobulin concentrations (mg/L) in selected salivary components are shown in Table 1.

Advantages of saliva specimens include:

- a) greater acceptability: noninvasive and painless,
- b) greater convenience: simple, rapid, inexpensive and self-collected,
- c) less hazardous: to subject as well as to investigator,
- d) better access: in epidemic outbreaks, children, large po-

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Institute of Epidemiology, Jessenius Faculty of Medicine, Comenius University, Bratislava, Slovakia

**Address for correspondence:** R. Madar, MD, PhD, Institute of Epidemiology, JLF UK, Sklabinska 26, SK-037 53 Martin, Slovakia.  
Phone: +421.43.4134715, Fax: +421.43.4134807

**Tab. 1. Mean immunoglobulin concentrations (mg/L) in salivary components and plasma (1).**

Specimen	IgG	IgM	IgA
Plasma	14,730	1,280	2,860
Crevice fluid	3,500	250	1,110
Whole saliva	14.4	2.1	194
Parotid saliva	0.36	0.43	39.5

pulations and in to hard-to-reach groups (e.g. intravenous drug users).

The most advanced application is the salivary diagnosis of measles, mumps and rubella (MMR) antibodies (9). A MMR salivary surveillance program has already been running in United Kingdom successfully for several years. Centres for Communicable Diseases Control (equivalent to State Health Institutes in Slovakia), general practitioners and laboratories supporting the program were collecting saliva specimens of recently diagnosed cases of measles, mumps and rubella (10, 11). Compliance with the program has been excellent and saliva samples from about 50 % of all notified cases have been received. Benefit of the salivary testing was proven on several occasions. For instance, the introduction of a second dose of MMR vaccine in October 1996 to children at pre-school age in Great Britain was accompanied by unsubstantiated reports of the vaccine being associated with Crohn's disease and autism. This has lead some parents to request antibody testing prior to accepting a second dose of MMR vaccine and for this purpose salivary testing was used. Another benefit is that the accurate diagnostic information contributes to maintaining confidence in the vaccination program by demonstrating that the vast majority of suspected cases in vaccinated children are not measles. Laboratory salivary tests confirmed measles in only 11 % of cases diagnosed as measles during the recent increase in the proportion of notified measles cases in British children under 1 year of age (1). On the other hand, notification probably underestimates measles in older age groups. The non-invasive diagnosis of MMR is also important in approaching high-risk groups that tend to ignore or refuse vaccination and other invasive procedures (e.g. minority communities or religious groups in which collecting blood samples could be problematic).

Main public health implications of salivary diagnosis of measles are:

a) Disease surveillance is the cornerstone of program for measles control. The National Salivary Surveillance Programme in the United Kingdom is now well established; by enhancing the laboratory confirmation of measles, mumps and rubella notifications it helped to improve the surveillance system.

b) Surveillance based on clinically diagnosed measles cases is inaccurate — a clinical diagnosis of measles is confirmable by laboratory tests in only about one third of notified cases. The requirement to take blood samples inevitably limits the scale of laboratory investigation — expensive and unnecessary control

**Tab. 2. Antiviral IgG and IgM antibodies detected in saliva (1).**

Infectious agent	IgG	IgM
Human immunodeficiency virus	Yes	No
Hepatitis A virus	Yes	Yes
Hepatitis B virus	Yes	Yes
Hepatitis C virus	Yes	No
Rubella virus	Yes	Yes
Measles virus	Yes	Yes
Mumps virus	Yes	Yes

measures have been instituted on the basis of false positive clinical diagnoses.

c) Of notified cases in subjects vaccinated against measles only 16 % are confirmed as measles

d) There is a 93 % agreement between paired serum and saliva samples; agreement is more likely if an adequate amount of saliva had been collected between 14—42 days of the onset of measles (detection of measles specific IgM in saliva before 2 weeks and after 6 weeks from the onset of the disease is unreliable). This level of sensitivity makes salivary IgM detection a suitable non-invasive method for routine investigation of notified cases.

e) Salivary testing is a suitable non-invasive method for confirming clinical diagnosis of measles and could have an important role in controlling the disease. Detection of measles IgM in saliva is an effective alternative to detection in serum and it could have a major role in measles control program (1).

The World Health Organisation, European Region has accepted some time ago a target to eliminate measles from the region by 2007, and laboratory testing of sporadic cases will be required to achieve this aim. High rates of vaccine coverage have interrupted indigenous transmission of measles, however, in many parts of the world measles is not adequately controlled and therefore there is a constant threat of reintroduction of measles from abroad. The molecular surveillance of measles by genotyping methods used to identify imported strains will become increasingly important as the WHO program for the global eradication of measles moves towards its goal. While most countries will have to rely on the collection of either single or paired sera, in countries using salivary antibody testing e.g. England and Wales, general practitioners will be able to rely on this non-invasive and sufficiently sensitive and specific diagnostic method (1).

Salivary antibody testing can also bring benefit in some other viral infections e.g. human immunodeficiency virus infection (7, 12—18), hepatitis A, B and C (2, 7, 19—26) (Tab. 2). In the developing world, the ability to perform salivary screening for infections like human immunodeficiency virus (HIV) reduces the risk of blood-borne infections arising from multiple use of disposable needles for venipuncture. In addition to that, salivary screening would help to reduce the percentage of HIV negative persons tested at testing centres allowing more time and effort to be spent on those truly infected with HIV virus. Salivary antio-

dy tests for immunoglobulins have recently proved to be effective in diagnosing the Epstein—Barr virus (EBV) infection as well (27). The quantity of total IgG in salivary samples depended on the collection device used. Some of the specimens with total IgG concentration less than 2.0 mg/l gave false positive reactions. EBV antibodies detection in salivary samples will enable easier access to larger population groups, including children, and thus will permit wider epidemiological studies of EBV infection that are currently not possible because of limitations imposed by need of blood samples.

Salivary antibody tests are also being developed for parvovirus B19 and human herpesvirus 6 (HHV 6), which are known to be responsible for some of the incorrectly diagnosed cases of measles (1, 28—30). However, salivary antibodies against non-viral infectious agents have been studied as well. It was proved that the enzyme-linked immunosorbent assay for detection of specific salivary IgA antibodies to *Giardia lamblia* can be used for the detection of the mucosal immune response to the parasite, and may serve as a screening tool in monitoring the exposure of various populations to *Giardia lamblia* (31). The ELISA antibody assay can be applied for detection of salivary IgG antibodies against an important cause of peptic ulcer disease and chronic gastritis — *Helicobacter pylori* (32, 33). However, despite some interesting results the assays used had limited diagnostic value and ought to be improved.

On the basis of several research studies and existing public health practices in the United Kingdom we can conclude that saliva is a body fluid containing antibodies of diagnostic significance, which can be collected readily and cheaply, with minimal hazards. Compared to venipuncture, its collection is much more convenient. Thanks to sensitive immunoassays, it is possible to diagnose a wide range of viral antibodies in salivary specimens. Since the salivary antibody testing can be performed in the doctor's office, a clinic or even at a home; in some countries it is recommended and used for rapid screening of several infectious diseases (34). If the screening test is positive, the person is referred to a health care provider for counselling and additional diagnostic blood tests. Because the test of saliva can be performed outside a formal hospital setting, its future is in superseding serological tests for some infectious diseases allowing serological testing on a broader front e.g. in children, hard-to-reach risk groups and in epidemiological studies (1, 10, 24, 26, 34).

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## SHORT COMMUNICATION

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# HLA system and autoantibodies in primary Sjögren's syndrome

Panchovska M, Sheytanov Y, Martinova F

*Clinic of Rheumatology, Medical University, Sofia, Bulgaria. mar\_pan@abv.bg*

### Abstract

The primary Sjögren's syndrome is an autoimmune exocrinopathy in which is established a correlation between the production of antibodies and certain HLA determinants.

A significant number of antigens from A, B, C, DR and DQ loci was investigated in 50 patients with primary Sjögren's syndrome with positive autoantibodies against dsDNA, ssDNA, Ro (SS-A), La (SS-B), RNP antigens, as well as with higher titer of anticardiolipin antibodies from IgM and IgG classes. The HLA antigens were investigated by a microlymphocytotoxic test and a prolonged test, while the autoantibodies by an indirect immunofluorescence, counter electrophoresis and ELISA.

A significant correlation was established between HLA-A1 and HLA-DR3 and the anti-Ro, anti-La, anti-DNA and anti-RNP antibodies, as well as between the higher titer of JgM and JgG anticardiolipins and the HLA-A1, B8 and DR3 antigens. No association was found between the specific for the disease anti-Ro and anti-La autoantibodies and certain genetic markers.

The achieved results confirm the hypothesis that the autoantibodies' production in primary Sjögren's syndrome is to a certain extent genetically determined. (*Tab. I, Ref. 5.*)

**Key words:** primary Sjögren's syndrome, HLA, autoantibodies.

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Clinic of Rheumatology, Medical University, Sofia, and Department of Haemotransfusiology and Immunogenetics, Institute of Emergent Medical Care, Sofia, Bulgaria

M. Panchovska, MD, PO Box 136, 4023 Plovdiv, Bulgaria.

**Tab.1. Correlation between autoantibodies and HLA antigens in primary Sjögren's syndrome.**

HLA antigens	n	A1	B8	B18	DR3	DR4
Antibodies to DNA, Ro, La	36	16 44,44%	12 33,33%	9 25%	17 47,22%	11 30,55%
Antibodies to Ro/La	18	7 38,89%	4 22,22%	5 27,78%	8 44,44%	7 38,89%
Antibodies to dsDNA/ssDNA	16	8 50%	5 31,25%	3 18,75%	9 56,25%	3 18,75%
aCL-IgM	13	4 30,76%	3 23,08%	2 15,38%	5 38,46%	2 15,38%
aCL-IgM+IgG	20	10 50%	7 35%	3 15%	10 50%	2 10%

Primary Sjögren's syndrome (pSS) is a chronic inflammatory autoimmune exocrinopathy, affecting predominantly the lacrimal and the salivary glands. In the patients' serum are detected a significant number of organ specific and organ non specific autoantibodies (1, 2). In part of the antibodies: anti Ro (SS-A), anti La (SS-B), anti RNP, anticardiolipines (aCL) and anti-DNA is established an association with certain HLA antigens. This association is particularly typical, referring to the disease pathognomic autoantibodies as anti-Ro (SS-A) and anti-La (SS-B) (3).

The purpose of the present study is to be found an association between the autoantibodies and the genetic determinants of the patients with pSS in the Bulgarian population.

#### Material and methods

We investigated 50 patients between 21 and 74 years of age with pSS according to the European Criteria for diagnosis (4). The patients were firmly positive for anti Ro, anti La, anti RNP and anti DNA antibodies and showed a high titer of aCL antibodies of IgM and/or IgG type. The antibodies against the abovementioned antigens were investigated using a counter electrophoresis and ELISA.

There were investigated 36 antigens from locus A, 67 from locus B, 8 from locus C, 53 from locus DR and 9 antigens from locus DQ. The HLA-A, B and C antigens were investigated by a standart lymphocytotoxic test, while HLA DR and DQ antigens by a prolonged test.

For statistical data processing antigenic frequency and chi<sup>2</sup>-test.

#### Results

The achieved results referring to the genetic determination of the autoantibodies are presented on Table 1.

A statistically significant association between the firmly positive anti Ro, anti La, anti DNA, RNP autoantibodies', aCL IgM and IgG type and HLA-A1, HLA-DR3 antigens was established ( $p < 0.05$ ).

Anyway we did not determine any independent significant association between the disease's pathognomic anti Ro and anti La autoantibodies with certain genetic markers HLA-A1, B8 and DR3 ( $p > 0.05$ ).

#### Discussion

Primary Sjögren's syndrome is an autoimmune exocrinopathy in which a genetic predisposition is established. It is also proven a genetic background for the production of some autoantibodies (1, 3).

Our study revealed a statistically significant high frequency of presenting HLA-A1 and DR3 antigens in the patients with firmly positive anti Ro, anti La, anti RNP and anti DNA antibodies. The established association between the disease's specific anti Ro and anti La antibodies and HLA-A1, B8 and DR3 determinants was not statistically significant.

Other authors find a significant genetic predisposition, referring to the production of anti Ro and anti La antibodies and the following HLA determinants: B8, DR3 (1, 2), DR5, DQ2/DQ6 heterozygotes (3).

We established a significant association between HLA-A1, B8 and DR3 antigens and the increase of aCL-IgG and IgM type. A similar association, concerning B8 antigen is reported in other investigations (5).

The production of autoantibodies in patients with pSS is to a certain extent genetically determined.

This production is also influenced by other factors, such as: infections (viral), gender, stress, the administered therapy (1, 3). These complex reasons might explain the immunogenetic differences among the patients with primary Sjögren's syndrome from different populations.

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