



Handbook on lab methods
for HIV and Syphilis testing
on oral fluid



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Published 2010

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The Capacity Building in HIV/Syphilis Prevalence Estimation using Non-invasive Methods among MSM in Southern and Eastern Europe – SIALON project was funded by the European Commission under the European Commission Public Health Programme 2003-2008. The sole responsibility for the handbook lies with the authors and the European Commission is not responsible for any use that may be made of the information contained therein.

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Introduction

This handbook is one of the deliverables of the EU-funded SIALON project “CAPACITY BUILDING IN HIV/SYPHILIS PREVALENCE ESTIMATION USING NON-INVASIVE METHODS AMONG MSM IN SOUTHERN AND EASTERN EUROPE”. The project strategically addresses the issues of the lack of reliable information on HIV and syphilis prevalence among MSM in Eastern and Southern Europe through the use of non-invasive testing methods as important tools for HIV/STI (Sexually Transmitted Infection) surveillance among hard-to-reach MSM. The overall objective is to obtain reliable and valid information on HIV and syphilis prevalence, risk behaviour, and cultural factors among MSM, using a non-invasive outreach testing method based on oral fluid samples collected in countries of Southern and Eastern Europe (Spain, Greece, Italy, Slovakia, Czech Republic, Slovenia, Romania). The total number of subjects enrolled in the study is 2,688. This handbook is intended as a practical guide for performing HIV and Syphilis tests on oral fluid samples. Its contents were discussed and reviewed by lab technicians from SIALON participating countries during a training workshop held in Verona in February 2010.

Rationale

Forty-five million new HIV infections are expected globally between 2002 and 2010 and MSM feature among the most vulnerable target groups to whom prevention measures should be addressed (see *Communication from the Commission on combating HIV/AIDS for the years 2006-2009*). In many countries, reliable information on HIV and syphilis prevalence is missing or fragmentary, especially among the more vulnerable populations such as MSM. In addition, HIV testing is decreasing in some EU countries. Screening services differ from country to country and the variety of counselling techniques accounts for diversity of access to HIV testing. Furthermore, in some countries screening services are not able to provide low-threshold HIV testing. According to the literature, the current prevalence of HIV infection in gay and bisexual groups is about 10-20% [1]. More specifically, the prevalence of HIV infection is greater in cities than in rural areas, with a positive gradient in big cities. The increased incidence of STIs demonstrates the important association of STIs with HIV. The significant association of syphilis with unprotected active or passive oral-genital sex has recently been highlighted. This tendency has also been confirmed in gay men with a high number of partners [2,3,4].

Despite these findings, few studies have targeted risk groups for HIV / STI such as MSM, using outreach methods collecting behavioural and biological data in line with Second Generation Surveillance System (SGSS) criteria [5,6] and United Nations General Assembly Special Session (UNGASS) indicators [7] . Second generation HIV surveillance relies on data collected from biological surveillance (serosurveillance), behavioural surveillance, and other sources (e.g. HIV/AIDS case surveillance, death registration, STI surveillance, Tuberculosis surveillance..) to describe a

country's HIV epidemic and respond effectively. It aims to improve the integration of data from these sources. It also supports continuous research into new epidemiological tools; improved methods for building estimates and modelling the epidemic; and better ways to use data for advocacy, planning, monitoring, and evaluation [8].

The major advantages of replacing serum with oral fluid (OF) are easy access and non-invasive collection. The collection of oral fluid is, in fact, minimally invasive, safe as virtually devoid of infectious virions and hence is acceptable to the general public. Moreover, it does not require the presence of healthcare workers [9,10] for testing, and has clear compliance advantages over venipuncture in community settings. Therefore, the use of oral fluid as a means for biological testing is of crucial importance in order to gather valid and reliable information about the spread of HIV and other STIs (Syphilis, Hepatitis) among hard-to-reach populations such as MSM. Ethical principles of privacy and confidentiality should be respected and oral fluid samples taken and the test performed on a voluntary and anonymous basis, including a consent form to be signed by the subject giving the sample.

Tests to detect HIV antibodies in oral fluid are well documented. Previous studies have shown that the method has high sensitivity (ranging from 96.2% to 100%) and specificity (ranging from 99.6% to 100%) [11]. In 1993 [12] a study in UK showed overall sensitivity of 96.2% and specificity of 100%. In a more recent Slovak study [13], saliva was collected using Omni Sal device and HIV was tested with GACELISA. HIV-reactive samples were re-tested by Test-Pack assay. Both tests were found to be highly specific and sensitive (sensitivity 100% and 100%, specificity 100% and 96 %, respectively). A study on sex workers in Kenya (2001) [14] had a sensitivity of 99% and specificity of 100%, using a modified commercial EIA. A CDC study (2006) [15] on oral fluid using Oral quick had 99.1% sensitivity and 99.6% specificity. A recent study [16] showed that rapid tests may be less sensitive than EIA in detecting early HIV infections. This study also suggest to integrate NAAT into HIV testing programmes that serves populations that undergo frequent testing and that have high rates of HIV acquisition, particularly if rapid antibody testing is employed.

SIALON Validation study of the EIA Genscreen HIV 1/2 version 2 test (BIORAD) for detection of HIV Antibodies in oral fluid

At the time the project started no EIA test for the detection of HIV antibodies in oral fluid had been validated and granted a CE mark, and the formerly available kit, Vironostika HIV-1 kit (Biomérieux), was out of production. It was decided to use an Elisa test for HIV 1-2 and to validate it on oral fluid specifically for our survey. For this reason, a validation protocol was developed according to EU general principles set out in COMMISSION DECISION of 7 May 2002 on common technical specifications for in-vitro diagnostic medical devices as well as WHO/UNAIDS/CDC/USAID Guidelines for the use of HIV testing technologies in surveillance[8]. Based on the same guidelines, we carried out a validation study. The test used in the validation study was GENSCREEN HIV1/2 ver 2

produced by BIO-RAD, CE validated for serum and plasma (HIV-1: 100% sensibility and 99.98% specificity; HIV-2: 100% sensibility).

Paired serum and oral fluid samples were collected from 499 subjects in the 7 Countries participating in the SIALON project. HIV positive subjects (n= 263) were patients with confirmed positivity for HIV attending the health facilities who were invited to take part in the study (regardless of being in HAART therapeutic regimen). Controls (n= 233) were HIV negative individuals recruited among health professionals and volunteers. EIA testing on oral fluid samples for 259 of the 263 HIV positive subjects showed up positive, giving a sensitivity of 98.5% (CI 96.2-99.6) for the EIA on oral fluid. 4 oral fluid samples from these subjects were EIA negative. All 233 controls were found negative for HIV in oral fluid and no false positive was detected (100% specificity; CI 98.4-100). The positive and negative predictive values of the O.F. test according to HIV prevalence are shown in Tab.1 below.

| PREVALENCE | 5% | 15% |
|-------------------|-----------|------------|
| PPV | 100% | 100% |
| NPV | 99.9% | 99.7 |

Tab.1 PPV and NPV according to prevalence

Three of the 4 false negative oral fluid samples showed up positive in Western blotting (see Fig. 1) while 1 oral fluid sample highlighted only one reactive band for HIV 1 (gp160), by using a fivefold amount of regular loading compared to serum testing (100 microlitres instead of 20). This corresponded to a low concentration of functional IgG in the oral fluid and might be explained by IgG degradation, bacterial contamination or erroneous storage.

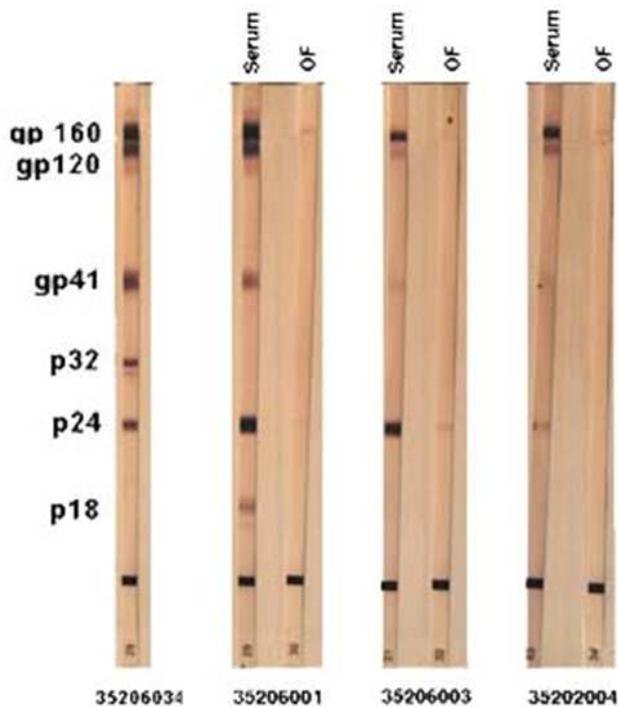


Fig.1 Three positive WB of false negative samples

Tests to detect anti-Treponema Antibodies in oral fluid are more experimental and traditional methods like TPPA or EIA are not sufficiently sensitive for these purposes (as shown in a recent study, not yet published)¹. An innovative method, described in this handbook (Time Resolved Fluorescence Immunoassay, TRFIA [17]) has been shown to be highly sensitive and specific for the detection of anti-Treponemal IgG in oral fluid specimens [18]. According to the study of Baguley et al., 2005, the overall sensitivity and specificity of this oral fluid assay was 95.8% and 86.1% respectively, based on the 5th percentile of the positive results, and 93.7 and 91.1%, respectively, based on a cut-off derived by mixture model analysis. For individuals with primary syphilis the optimum sensitivity of the oral fluid assay was 87.5%, whereas in those with disease classified as secondary and early latent syphilis, the sensitivity was 100 and 94.7%.

Pilot study for detection of treponemal specific IgG in oral fluid samples

In our validation study we challenged the assay on oral fluid samples collected from 49 syphilis seropositive patients (F=4; M=45; median age:44 ys;14 with early - primary, early latent - 35 with late latent syphilis) and 15 seronegative controls. Serum from patients

¹ Lina M. Castro et al. *Evaluation of oral fluid specimens for syphilis testing*, The 2006 National STD Prevention Conference of CDC

was screened using a syphilis enzyme immunoassay (Enzygnost Syphilis, Dade Behring). EIA reactive sera were further confirmed by quantitative TPPA and VDRL assay and by the method for determination of treponemal IgM status. The oral fluid was collected using the Oracol collection device (Malvern Medical Developments, Worcester, UK) and tested for the presence of antibodies against *T. palidum* by time-resolved fluorescence immunoassay (TRFIA) as described by Baguley et al., 2005.

All controls had a reactivity index below the 5 SD cut-off. All samples of 14 patients with early syphilis (primary and early latent) and 27/35 samples of patients with late latent syphilis had reactivity indices above the cut-off, whereas 8 samples of patients with late latent syphilis had reactivity below the cut-off. The test revealed both 100% of sensitivity and specificity and 77% sensitivity and 100% specificity by testing samples of patients with early and late latent syphilis respectively. Testing of all oral samples from patients with positive and negative serology revealed the sensitivity and specificity of the test as 84% and 100%, respectively. See Fig 2. below.

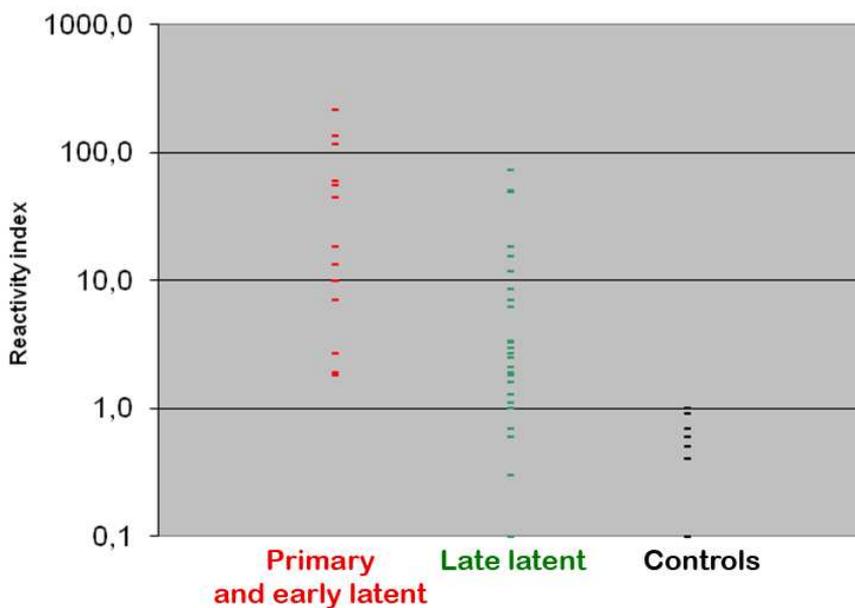


Fig. 2 Reactivity index of oral fluid samples (specimen fluorescence/negative internal fluorescence)

In conclusion, TRFIA on oral fluid for the detection of Anti-Treponemal IgG can be used in syphilis testing with no impact on assay specificity. The test is potentially useful for epidemiological studies when the collection of blood is not possible, but it is not suitable for diagnostic or screening purposes [19].

1 ORAL FLUID SAMPLES

Oral fluid is a mixture of oral mucosal transudate, saliva and gingival crevicular fluid. The normal concentration of immunoglobulin G (IgG) in oral fluid is 1/10 to 1/1000 lower than in plasma. However, a variety of specially designed devices are commercially available to maximise both collection and IgG concentration and this relies on gum brushing. Gum brushing has been shown to stimulate secretion by crevicular glands thereby enriching oral fluid in IgG. From the different devices available on the market we chose the “Oracol” swab for our study because it combines the cheapest cost with the best performance in IgG recovery [20].

1.1 Specimen collection

Oral fluid collector

The ORACOL kit (Fig. 3) consists of an absorbent foam swab (designed to accommodate up to 1 ml of fluid), a centrifuge tube and a cap. The product is CE marked. This kit has been widely used to collect specimens suitable for the detection of measles, HIV, hepatitis A and B, mumps and rubella antibodies [21,22,23,24].



Fig. 3 ORACOL Oral Fluid Collector

Device for collection

The swab has to be rubbed along the teeth and the gums for approximately 1 minute, rubbing on the gums about 60 times – 30 times on each gum, like a toothbrush. The device proved to be safe and acceptable to individuals of all ages in previous studies and is currently used routinely in the UK for the confirmation of infection of measles, mumps and rubella.



Fig. 4 How to collect oral fluid

1.2 Handling and storage of samples

Sample transport conditions

After collection, oral fluid samples should be maintained between 4°C and 8°C and sent to the laboratory within 72 hours of the collection time, at the latest.

Processing of oral fluid samples

On receipt by the testing laboratory, tubes containing the swab are filled with 0.5 ml of recovering buffer and left to stand at 4°C for 1h. Swabs are then removed from tubes, squeezing and twisting the sponge against the plastic wall, then inverted and placed back inside the tube. Tubes are capped and centrifuged at 2500 rpm for 10 min to remove debris. Oral fluid is then transferred to a sterile microfuge tube (Sarstedt, Numbrecht, Germany) and centrifuged again at 4,000 rpm for a minute. Cleared samples are transferred to a fresh tube, aliquoted and stored at -20° until needed for testing.

RECIPE FOR ORAL FLUID RECOVERING BUFFER

| Stock solution | Volume for 100 ml |
|-----------------------------------|-------------------|
| 10% foetal calf serum | 10 ml |
| 0.2% Tween 20 | 200 microlitres |
| Phosphate buffered saline, pH 7.2 | 90 ml |
| 0.5% gentamicin (50 mg/ml stock) | 500 microlitres |
| 0.2% fungizone (250 ug/ml stock) | 200 microlitres |

1.3 Indicators of specimen quality

ASSAY: HUMAN IgG ELISA QUANTIFICATION KIT (Bethyl Lab.inc) Total IgG measurement

The quality of the oral fluid samples for testing was established by total IgG measurement. The IgG ELISA Quantification Kit is an enzyme immunoassay intended for quantitative determination of human IgG in serum and other biological samples (plasma, saliva, urine, faeces).

The kit is based on the use of purified goat anti-Human IgG antibodies binding the corresponding antibodies in the tested specimens.

MATERIALS and PROCEDURES

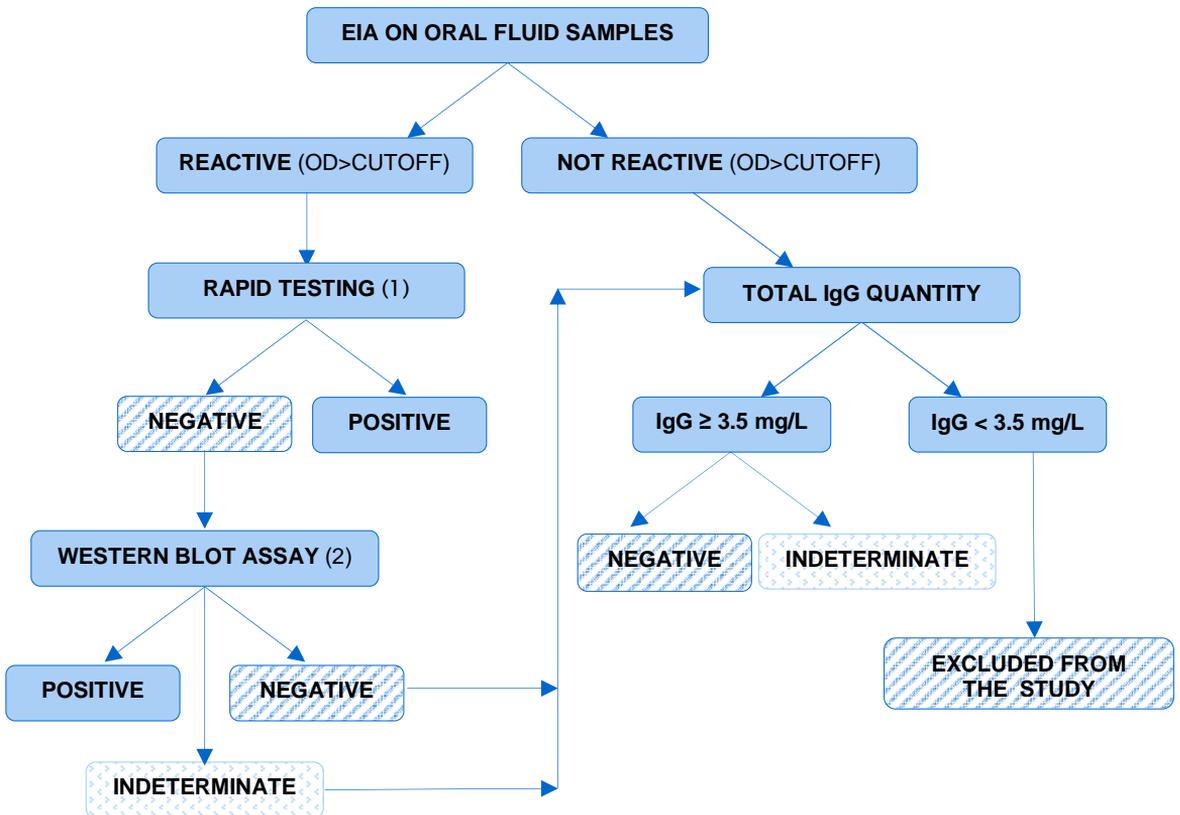
The assay procedure includes the following steps:

- Microplate loading with 100 μ L of oral fluid diluted 1:250 and standard dilutions of human IgG to be analyzed in duplicate.
- Coating microtitre well strips with capture anti-human IgG antibody.
- Microplate incubation at room temperature for 1 hour to allow for IgG binding to the capture antibody.
- Washing out of unbound sample material.
- Addition of horseradish peroxidase (HRP).
- Anti-human IgG conjugate.
- Microplate incubation at room temperature.
- Washing out with wash solution.
- Incubation with a tetramethylbenzidine (TMB) substrate revealing the presence of the bound conjugate by a blue reaction product.
- Addition of sulphuric acid to stop the reaction.
- Detection of the reaction by reading the absorbance with a microtitre plate reader at 450 nm of wave of length. Given mean absorbance readings of each set of oral fluid samples, IgG concentration values are calculated by creating a standard curve.

Samples containing a concentration of IgG \geq 3.5 mg/L are considered suitable for further testing.

2 DETECTION OF ANTI HIV1/2 ANTIBODIES

Algorithm for oral fluid testing



(1) we used rapid Immunochromatography to determine HIV 1/2 (Unipath Ltd, Bedford UK) CE marked for serum.

(2) we used HIV 1/2 BLOT 2.2 (MP Biomedical, Singapore, Cina) CE marked for serum.

Negative samples with IgG concentration < 3.5 mg/L should be considered invalid and excluded from the study. IgG measurement is not mandatory on oral fluid samples that tested positive as positive samples must be included in any case.

ASSAY: GENSCREEN® HIV1/2 version 2

The assay is based on the use of a solid phase, coated with purified recombinant antigens (HIV1 recombinant proteins gp 160 and p25 proteins of the HIV1 virus and a peptide corresponding to an immuno-dominant epitope of the glycoprotein of the HIV2 virus envelope glycoprotein) and a recombinant antigen-peroxydase compound (peptides corresponding to HIV1/2 immuno-dominant epitopes of the envelope glycoproteins and nucleocapsidic protein of the HIV1 and HIV2 virus envelopes and nucleocapsidic protein in the recombinant form).

MATERIALS and PROCEDURES

Testing proceeds through the following reaction steps (according to the manufacturer's instruction):

- Microplate loading with 75 l of oral fluid together with the various samples and control sera to be assayed.
- Microplate incubation at room temperature for 30 min. During this time the anti-HIV1 and/or anti-HIV2 antibodies potentially present in the samples bind to the antigens fixed to the solid phase. The sample deposit is shown by a change of colour, from purple to blue (SDP=Sample Deposition Proof).
- Washing out of unbound antibodies.
- Addition of peroxydase-conjugated, purified HIV1 and HIV2 antigens.
- Incubation at room temperature for 30 min and then washing out of the unbound conjugate fraction.
- Incubation with a substratum revealing the presence of the enzyme-conjugated antigens
- Detection of the reaction by reading absorbance (OD values) with a spectrophotometer at 620-700nm wavelength (OD values).
- Cut-off values are established based on the mean absorbance of three cut-off positive and negative controls according to the manufacturer's instructions, to discriminate positive from negative samples.

3 DETECTION OF ANTI-TREPONEMA ANTIBODIES

ASSAY: Time Resolved Fluorescence Immuno Assay (TRFIA) for detection of Anti-Treponema IgG.

The TRFIA is a very sensitive recent immunoassay which uses a time-resolved mode of measurement, i.e. it measures a label with long lifetime emission and excludes the background signal, which often consists of rapidly decaying fluorescence and light scattering. The DELFIA (Perkin Elmer) is a competitive system where the labelled and unlabelled antibodies being sought compete for antigens immobilized on a solid matrix.

MATERIALS

- ETI-Treponema plus (DiaSorin S.p.A., Italy): the plates are coated with a mixture of the 15Kd, 17 Kd, and 47 Kd recombinant antigens of *Treponema pallidum*.
- DELFIA reagents (Perkin Elmer Life Sciences, Cambridge, UK): enhancement solution, assay buffer, wash concentrate buffer, Europium-labelled anti human IgG antibodies diluted 1:500 in DELFIA assay buffer).
- Mini-orbital shaker.
- Plate washer (Perkin Elmer).
- Fluorometer (Wallac 1420 Multilabel counter Victor2– Perkin Elmer).



PROCEDURES

Testing involves the following steps according to the manufacturer's instructions: Treponemal antigen-coated microtitre plates are loaded with 100 µl oral fluid specimens. In a typical assay the loading scheme is as follows:

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | E | S | S | S | S | S | S | S | S | S | S |
| A | E | S | S | S | S | S | S | S | S | S | S |
| B | E | S | S | S | S | S | S | S | S | S | S |
| B | E | S | S | S | S | S | S | S | S | S | S |
| C | E | S | S | S | S | S | S | S | S | S | S |
| C | F | S | S | S | S | S | S | S | S | S | F |
| D | F | S | S | S | S | S | S | S | S | S | F |
| D | F | S | S | S | S | S | S | S | S | S | F |

A, blank; **B**, high positive internal control; **C**, low positive internal control; **D**, negative control¹; **E**, positive oral fluid sample²; **F**, negative oral fluid sample pool; **S**, oral fluid sample.

High positive and low positive internal control are from the kit (serum); oral fluid positive controls are from a patient with known serological positivity (reactivity index above the cut-off); oral fluid negative controls are prepared by pooling oral fluid samples collected from 15 healthy donors with negative syphilis serological assay.

The plates are shaken at 100 rpm on a Stuart Mini-Orbital shaker for 2h at room temperature and then washed eight times with DELFIA wash buffer by using DELFIA plate washer (or equivalent).

Europium-labelled anti-human IgG conjugate diluted 1:500 in DELFIA assay buffer is added, at 100 l per well, to all wells of the plates, which are then shaken at 100 rpm for 2h at room temperature.

After washing eight times with DELFIA wash buffer, 200 l of DELFIA enhancement solution is added to all wells.

The plates are put in a dark box on the shaker and incubated for 10 min and are read using a Wallac 1420 multilabel counter Victor2 fluorometer.

A reactivity index is calculated for each specimen by dividing the specimen fluorescence by the cut-off. Cut-off values, above which a specimen was deemed to be positive, are calculated as the mean OD (Optical density) value of the six negative oral fluid pool samples, +3 standard deviations (SD).

The Europium counts of negative samples should be between 45000 and 55000.

N.B.: the instruments used for all the procedures described may be replaced by others with the same technical performance.

¹ was not considered as it is a serum control (not oral fluid).

² a positive oral fluid sample was used occasionally as a control.

4 GUIDANCE FOR THE INTERPRETATION OF ORAL FLUID TEST RESULTS

4.1 HIV EIA Testing

HIV prevalence can be estimated using this oral fluid test following the algorithm presented above. The sensitivity (98.5%) does not allow the use of the test on oral fluid for diagnostic purposes (the revised recommendations of UNAIDS and WHO state that HIV tests used should have a sensitivity of at least 99% and a specificity of 98%) [25] but it is useful for epidemiological surveillance, given that it is not expensive (about 3 Euros/sample) and that the level of sensitivity allows for precise prevalence estimation (the revised recommendations of UNAIDS and WHO state that HIV antibody tests used for surveillance need to have a specificity of at least 95% and recommend EIAs for areas with good laboratory infrastructure and trained staff and for hospitals that perform 100 or more tests per day, as EIAs are very efficient for batched testing and easily implemented in laboratories at the national or regional level - UNAIDS/WHO 1998 [26].

If subjects recruited during the survey want to know their HIV status (after a positive or negative oral fluid result), a confirmation test based on a blood sample, in line with the traditional guidelines for HIV testing, should be performed. If respondents do not want to be tested again they can be given a test result clearly stating that the result is only designed as epidemiological information and that its validity must be confirmed by another diagnostic blood test.

4.2 TRFIA Testing For Anti-Treponema Antibodies

At the moment, TRFIA is the only oral fluid test able to detect Anti-Treponema antibodies in oral fluid and it could be useful to estimate the IgG seroprevalence of syphilis in high risk populations. Nevertheless further validation studies, recruiting a higher number of subjects (with different stages of syphilis) should be carried out in order to better interpret the results, as for the moment it is not possible to distinguish active syphilis from former/treated infection.

Bibliography

- 1 Hamers FF, Downs AM. *The changing face of the HIV epidemic in western Europe: what are the implications for public health policies?*. Lancet. 2004;364,83-94.
- 2 Bellis MA, Hale G, Bennett A, Chaudry M, Kilfoyle M. *Changes in substance and sexual behaviour amongst young people visiting an international night-liferesort.Ibiza uncovered*. International Journal of Drug Policy. 2000;(11):235-44.
- 3 Sadiq ST, *Factors associated with gonorrhoea in men aware of being positive for HIV infection: case-control study*. BMJ. Oct1998;317:1052-1053.
- 4 Truong HM, Kellogg T, Klausner JD, Katz MH, Dilley J, Knapper K, Chen S, Prabhu R, Grant RM, Louie B, McFarland W. *Increases in sexually transmitted infections and sexual risk behaviour without a concurrent increase in HIV incidence among men who have sex with men in San Francisco: a suggestion of HIV serosorting?*. Sex Transm Infect. Dec2006;82(6):461-6.
- 5 Joint United Nations Programme on HIV/AIDS (UNAIDS) and World Health Organization (WHO). *Initiating second generation HIV surveillance systems: practical guidelines*. Available from: [/www.who.int/hiv/pub/surveillance/guidelines/en/index.html](http://www.who.int/hiv/pub/surveillance/guidelines/en/index.html)
- 6 Joint United Nations Programme on HIV/AIDS (UNAIDS) and World Health Organization (WHO). *The pre-surveillance assessment. Guidelines for planning serosurveillance of HIV, prevalence of sexually transmitted infections and behavioural components of second generation surveillance of HIV*. Available from: <http://www.who.int/hiv/pub/surveillance/sti/en/index.html>
7. Joint United Nations Programme on HIV/AIDS (UNAIDS) and World Health Organization (WHO). *Monitoring the Declaration of Commitment on HIV/AIDS: guidelines on construction of core indicators: 2010 reporting*. Geneva: UNAIDS 2009. Available from: <http://www.unaids.org>
8. UNAIDS/WHO Working Group on Global HIV/AIDS/STI: *Guidelines for Using HIV Testing Technologies in surveillance*, World Health Organization and Joint United Nations Programme on HIV/AIDS, 2001
- 9 Nokes DJ, Enqueselassie F, Vyse A, Nigatu W, Cutts FT, Brown DWG. *An evaluation of oral-fluid collection devices for the determination of rubella antibody status in a rural Ethiopian community*. Transactions of the Royal Society of Tropical Medicine and Hygiene. Volume92,Issue6,679-685.
- 10 Vyse AJ, Cohen BJ, Ramsay ME. *A comparison of oral fluid collection devices for use in the surveillance of virus diseases in children*. Public Health 2001;115:201-7.
- 11 Hodinka RL, Nagashunmugam T, Malamud D, *Minireview. Detection of Human Immunodeficiency Virus Antibodies in Oral Fluids*. Clinical And Diagnostic Laboratory Immunology, 1998July;Vol.5,No.4.419-426.

- 12 Hunt AJ, Connell J, Christofinis G, Parry JV, Weatherburn P, Hickson FC, Coxon AP, Davies PM, McManus TJ, Sutherland S. *The testing of saliva samples for HIV-1 antibodies: reliability in a non-clinic setting*. Genitourin Med. 1993 Feb;69(1):29-30.
- 13 Staneková D, Jíreová K, Habeková M, Wimmerová S, Gramblichová I. *HIV infection and sexual behaviour among homosexuals and bisexual men in Bratislava*. Cent Eur J Publ Health 8. 2000;No.3,172–175.
- 14 Chohan BH, Lavreys L, Mandaliya KN, Kreiss JK, Bwayo JJ, Ndinya-Achola JO, Martin HL Jr. *Validation of a modified commercial enzyme-linked immunoassay for detection of human immunodeficiency virus type 1 immunoglobulin G antibodies in saliva*. Clin Diagn Lab Immunol. 2001 Mar;8(2):346-8
- 15 Delaney KP, Branson BM, Uniyal A, Kerndt PR, Keenan PA, Jafa K, Gardner AD, Jamieson DJ, Bulterys M. *Performance of an oral fluid rapid HIV-1/2 test: experience from four CDC studies* AIDS. 2006 Aug;20(12):1655.
- 16 Joanne D, Stekler et al. *HIV Testing in a High-Incidence Population: Is Antibody Testing Alone Good Enough?* HIV/AIDS. 2009;49 (1 August); 444-453
- 17 Baguley SDK, Horner PJ, Maple PAC, Stephenson I. *An oral fluid test for syphilis*. International journal of STD & AIDS. Vol16, Number 4, 2005 April;299-301.
- 18 Maple PAC, Simms I, Kafatos G, Solomou M, Fenton K. *Application of a noninvasive oral fluid test for detection of treponemal IgG in a predominantly HIV-infected population*. Eur J Clin Microbiol Infect Dis. 2006;25:743–749.
- 19 Lambert NL, Fisher M, Imrie J, Watson R, Mercer CH, Parry JV, Phillips A, Iversen A, Perry N, Dean GL. *Community based syphilis screening: feasibility, acceptability and effectiveness in case finding*. Sex. Transm. Inf. 2005;81:213-316.
- 20 Vyse AJ, Cohen BJ, Ramsay ME. op. cit.
- 21 Marianne Morris A, Bernard Cohen, Nick Andrews, David Brown. *Stability of total and rubella-specific IgG in oral fluid samples: the effect of time and temperature*. Journal of Immunological Methods. 2002;(266):111–116.
- 22 O'Connell T, Thornton L, O'Flanagan D, Staines A, Connell J, Dooley S, McCormack G. *Oral fluid collection by post for viral antibody testing*. Int J Epidemiol. 2001 Apr;30(2):298-301.
- 23 Hutse V, Verhaegen E, De Cock L, Quoilin S, Vandenberghe H, Horsmans Y, Michielsens P, Van Damme P, Van Vlierberghe H, Claeys F, Vranckx R, Van Oyen H. *Oral fluid as a medium for the detection of hepatitis B surface antigen*. J Med Virol. 2005 Sep;77(1):53-6.
- 24 Quoilin S, Hutse V, Vandenberghe H, Claeys F, Verhaegen E, De Cock L, Van Loock F, Top G, Van Damme P, Vranckx R, Van Oyen H. *A population-based prevalence study of hepatitis A, B and C virus using oral fluid in Flanders, Belgium* Eur J Epidemiol. 2007;22(3):195-202.
- 25 HIV/AIDS programme Strengthening health services to fight HIV/AIDS. *Guidance on provider-initiated HIV testing and counselling in health facilities*. World Health Organization 2007 http://whqlibdoc.who.int/publications/2007/9789241595568_eng.pdf

26 UNAIDS/WHO Working Group on Global HIV/AIDS/STI: *Surveillance Guidelines for Using HIV Testing Technologies in surveillance* op.cit.