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Swab2know

Manual for the development and implementation of an HIV testing approach using outreach and home sampling strategies and online communication of HIV test results

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KEY MESSAGES

- HIV testing is an integral aspect of HIV prevention. The traditional (medicalized and centralized) approach of HIV testing remains standard for most people.
- Novel HIV testing approaches could increase the uptake of HIV testing, and should therefore be tailored to specific key populations, safeguarding quality throughout each step in the process.
- Introducing a new HIV testing approach covers different phases:
    - Development of the materials;
    - Preparation;
    - Implementation;
    - Evaluation.
- There's no 'one size fits all'-approach in HIV testing. For the project presented in this manual, we chose:
    - Outreach and online collection of samples;
    - Collection of oral fluid samples;
    - A test executed in the laboratory;
    - Delayed communication of HIV test results via a secured website.
- Follow-up and linkage to care should be included in the HIV testing approach.
- Involvement and commitment of communities and stakeholders are prerequisites for a successful HIV testing approach.

INTRODUCTION

In Europe, 29,747 people were newly diagnosed with HIV in 2015. This accounts for 6.3 newly diagnosed individuals per 100,000 inhabitants.(1) The epidemiological situation varies strongly between European regions. Overall, sex between men is the most commonly reported transmission route in Europe, accounting for 42% of newly diagnosed HIV-infections, followed by heterosexual transmission accounting for 32% of new diagnoses. Injecting drug use was less frequently reported as transmission route (4%), and for a considerable proportion of newly diagnosed HIV-positive people (20%), transmission mode was not reported or unknown.(1) New HIV infections via sex between men is increasing in Western and Central Europe, whereas heterosexual transmission is more often observed in Eastern Europe. Transmission via injecting drug use still accounts for one third of new HIV infections in Eastern Europe, and more than half in Russia.(1)

A cure for HIV is probably possible but unrealistic to be available in the coming years. This makes prevention, early diagnosis and treatment, cornerstones of care and follow-up of HIV.

Promoting HIV testing is an integral part of the 90-90-90 Joint United Nations Programme on HIV/AIDS (UNAIDS) plan to end the AIDS epidemic by 2030. In terms of this plan, 90% of all people living with HIV should know their HIV status, 90% should be on treatment, and 90% of these should be virologically suppressed.(2) In Europe, this ambition has not been achieved. The European Centre for Disease Prevention and Control (ECDC) estimates that in Europe, 15% of HIV-positive people are undiagnosed, which is often referred to as a 'hidden epidemic'.(3,4)

HIV counselling and testing is mentioned as a preventive activity since the 1990's.(5) Testing, linkage to care and treatment can be considered as a continuum. It can foster prevention both at the individual and at the population level. People who receive an HIV diagnosis may adapt their behavior towards a less risky sexual lifestyle.(6) Effective testing, linkage to care and treatment of those tested positive will ultimately lead to a diminution of the community viral load in a population,(7) leading to a reduction of new HIV infections.

Many efforts have been invested in increasing the number of individuals from key populations and undiagnosed people to take an HIV-test. This increase of uptake of testing requires tailored approaches to reduce existing barriers on personal, psychosocial or logistical levels.(8) Despite the options, and efforts to reduce barriers, a paradigm switch towards a comprehensive testing approach in HIV testing seems necessary to achieve the first 90% mentioned in the ambitious UNAIDS goals.(2)

The traditional HIV test is offered voluntarily and confidentially by a medically trained health care professional in a health care setting with a strong emphasis on the patient's informed consent.(9) Counseling and test results are provided by trained health care workers during a face-to-face consultation.(10) This approach may remain the standard for most people. For key populations,

alternative HIV testing strategies can be considered. One study found that oral fluid testing is preferred by MSM above giving blood samples.(11) It has also recently been found to be reliable for diagnostic use in groups with an HIV prevalence over 1% (12) like MSM, and several research projects in clinical settings have shown promising results for HIV tests on oral fluid samples.(13,14)

Rapid HIV tests, decentralized HIV testing (i.e., outreach and community-based testing), and self-testing are additional alternatives. Rapid tests are used in a variety of settings, including primary health care settings,(15,16) emergency departments,(17,18) and in dental clinics.(19) Their advantage is that clients receive their results at the time of their visit.(20) A major disadvantage is that in low-HIV-prevalence settings they give a relatively high proportion of false positive results.(14) Outreach testing targeting MSM has been implemented in clubs, bars, and bath houses,(21–23) as well as at large-scale events, such as Gay Pride festivals.(24) Community-based testing among MSM is increasing in recent years.(25,26) In Europe, an increasing number of community-based testing centers (i.e., Checkpoints) have been established.(27,28)

Self-tests for HIV that can be ordered through the Internet are the most recent development in this field.(29) The only US Food and Drug Administration (FDA)-licensed oral fluid-based rapid test is OraQuick ADVANCE Rapid HIV-1/2 Antibody test.(30) The FDA approved the use of this test for home use in July 2012,(31) despite its relatively high risk of false positive results,(32) especially among lower-risk groups.(14) The quality of other test kits that can be ordered online is largely unknown due to their lack of certification. The advantages of self-testing include increased convenience and heightened privacy.(29) The difficulty of ensuring linkage to care in the case of a positive result is a weakness of these tests. In a recent review, supervised and unsupervised self-testing strategies were found to be highly acceptable and preferred, but all studies lacked an evaluation of posttest linkage to counseling and care.(33) Internet-based testing can therefore be an alternative, where posttest linkage is part of the process. The willingness to use Internet-based HIV-testing strategies was high in recently published quantitative and qualitative studies.(34,35)

To address key population's testing needs, we developed the Swab2know project.(36) This project combines two strategies to increase HIV-testing uptake: outreach HIV-test sessions and free online testing. In both strategies, samples are collected using oral fluid collection devices and test results are communicated via secured website.

This nonrandomized, prospective descriptive study aimed at detecting new HIV cases among groups at risk for HIV/sexually transmitted infection (STI) acquisition. The secondary objective was to assess the acceptability and feasibility of an HIV-testing strategy with the use of self-administered oral fluid samples collected through outreach and online activities and Web-based delivery of test results.

GUIDING PRINCIPLES OF THE APPROACH

Involve and engage stakeholders from key population(s)

Involvement and engagement from stakeholders is a crucial element of the success of the HIV testing approach. They should be involved from the very start of the development of the approach, and their involvement does not stop.

Quality standards

Throughout the development of the HIV testing approach, all aspects need to confirm with the existing standards of care. Issues of privacy and confidentiality, qualitative health care, voluntary participation and autonomy should be safeguarded at any time.

DEVELOPMENT PHASE

In the phase pf developing the specific approach, it is important to identify key population(s), partners, including the collaborating laboratory where the HIV tests will be executed.

Identify key population

The approach, with the use of an oral fluid test, is recommended in key populations where the estimated prevalence of HIV is 1% (or higher). In groups where prevalence is lower, the risk of false reactive test results is considered too high to communicate a reliable test result.

In Europe, several key populations meet the required prevalence: men who have sex with men, migrant populations, and sex workers. Locally, potentially other groups may be included, but there should be emphasized that the prevalence should exceed 1%.

Inclusion criteria for participation should be determined: identify as member of a key population, being 18 years of age or older. Additional criteria can be that participants should accept oral fluid sampling, sign informed consent forms (if necessary), provide minimal information (if requested), and understand that the test, if positive, would only be strongly indicative of HIV infection. In the swab2know-project, participants who did not meet these criteria, or were not willing to provide a mobile phone number or email address were excluded from this project and redirected to standard testing facilities.

Identify partners (field workers, venue-owners, etc.)

Organizing outreach activities require close collaboration between project collaborators, venue owners (or people responsible for organizing activities), and volunteers. Discussions should be held on endorsement by venue owners, and on practical aspects of the organization (in which room will the project collaborators be present? During which hours?).

In this phase, involvement of stakeholders is also a prerequisite for a successful project. Who will effectively visit the venues to collect the samples, project collaborators or partnering or community organizations?

Identify collaborating laboratory

A questionnaire was developed to assess the collaborating laboratory's standards of care. The questionnaire is provided as Annex 1.

PREPARATION PHASE

Obtain ethical approval (if required)

In this phase, it is important to check whether ethical approval is required for the implementation of the HIV testing approach.

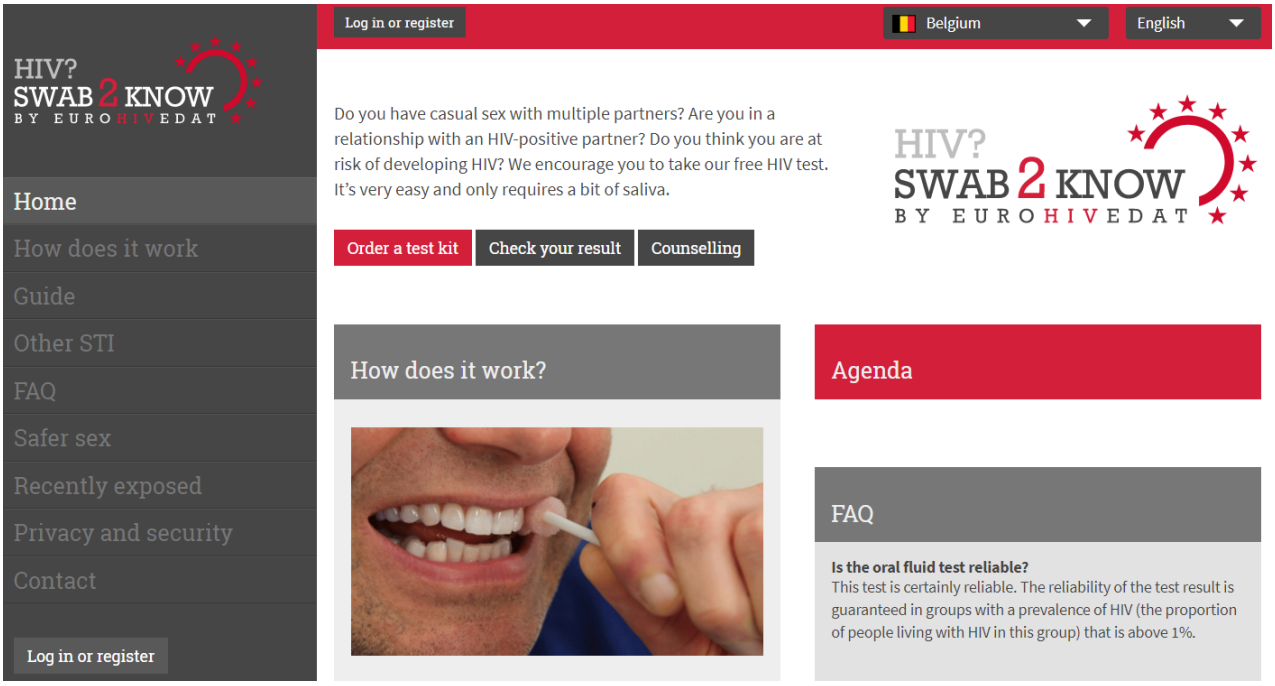
Whether or not ethical approval from an Ethics Committee is required, will depend on the answer to two questions:

- Is the project framed in a 'research' or 'care' program? For research projects, ethical approval is required.
- Does the legal framework in my country allows to implement a project using outreach activities, oral fluid sampling, and delayed communication of HIV test results?

In case ethical approval should be obtained, a protocol to be adapted and submitted can be found as Annex 2.

Develop the website

A website, should be designed for the project. The swab2know project website (www.swab2know.eu) may serve as an example, and can be adapted to local needs and requirements.(36)



The main aim of the website is to provide a platform where visitors can find information, prevention messages, order test kits, and collect their test results. In the development of the website, some elements should be considered:

- Security: the website should be highly secured. In the case of swab2know, it was secured by means of the Secure Sockets Layer protocol, and holds a security certificate provided by Belnet—Belnet is the federal government organization that provides high-bandwidth Internet connection and services to Belgian universities, research centers, and government departments. The certificate confirms the identity of, and encrypts the communication between, the Swab2know Web server and the computer where the information is requested.
- Usability: there should be aimed to develop an easy-to-use, accessible website that is appealing for the targeted population, and is adapted to their needs.
- Transparency

Sampling Procedures

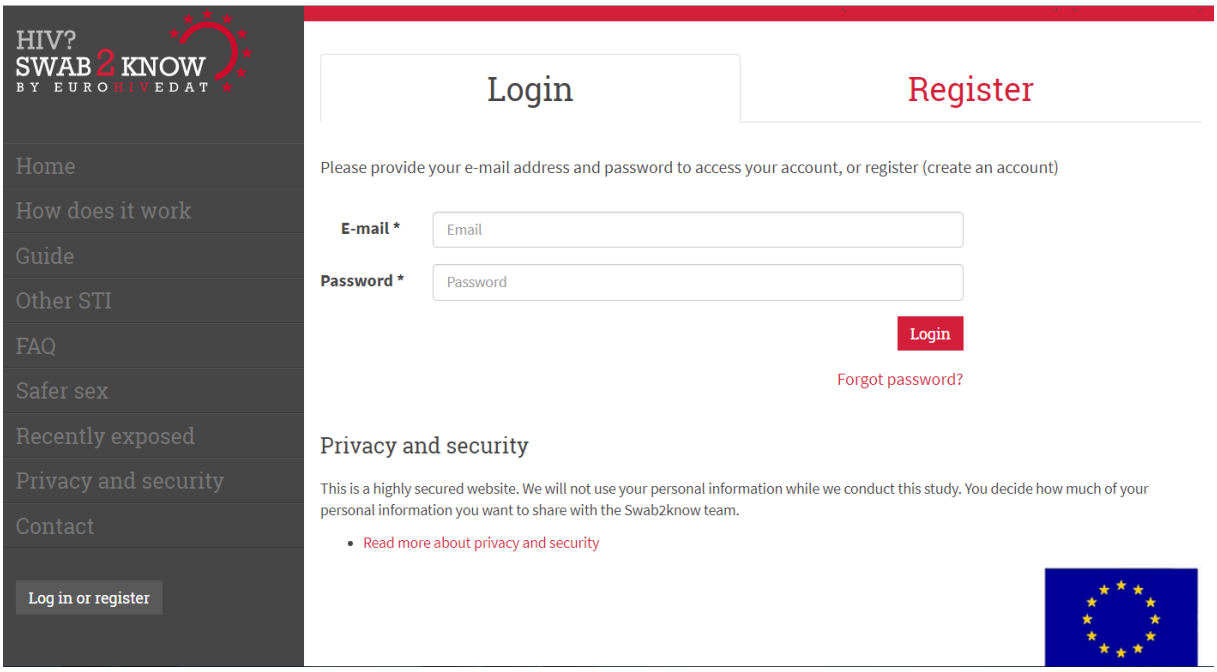
With the organization of outreach activities, practical agreements on sampling during these activities should be made:

- Who will recruit? Sensitizing people attending venues, participating in community events, cruising or involved in transactional sex, etc.
- Who will enroll participants?
- Where will project collaborators be present during the outreach activity?

Project collaborators, volunteers and field workers should be trained in all aspects of the sampling procedures. A manual for the training of field workers is provided in Annex 3.

After being informed and signing the informed consent (IC) form, the process of creating an account and filling in baseline information via a questionnaire starts.

The process for participation starts with creating an account, or logging in if the participant already registered before.



Each account is unique and linked with an email address and phone number. Information provided via the questionnaire is also connected to the account. Oral fluid samples were self-collected by the participants under the supervision of study staff. All samples were identified by a unique sample code, which linked the sample with the personal account, the IC, and baseline data. Samples were kept at room temperature and were brought to the laboratory on the next working day.

Online recruitment happens on the website by occasional visitors who created an account and provide their email address and phone number. The project was advertised by prevention organizations and through articles and announcements in dedicated media, including gay-oriented websites and magazines and a Swab2know Facebook account. Participants provide consent by accepting the terms of the study. A sampling kit identified with a unique sample code is sent to their address, or another address they provide. Participants take the oral fluid sample after having seen a short educational video on the website. Samples are sent to the lab with a prepaid envelope. The participants can also opt to collect their results during a face-to-face consultation.

HIV Test

The accuracy of the HIV ELISA test on oral fluid has been evaluated.(12) Each sample underwent a two-step HIV-test procedure. First, all samples were tested for HIV using Genscreen HIV-1/2 Version 2 by Bio-Rad (Marnes-la-Coquette, France).(37) The results were classified as strong reactive, weak reactive, or non-reactive. In a second step, all nonreactive samples were checked for sample quality using a human IgG quantification test. The quality of the oral fluid samples was measured using the IgG enzyme-linked immunosorbent assay (ELISA) quantification kit (Human IgG ELISA Immunology

Consultants Laboratory, Inc, Portland, OR, USA). If the sample contained more than 3500ng total IgG/mL, the non-reactive result was considered valid and ready to be communicated. All samples containing less than 3500ng total IgG/mL and samples older than 7 days were considered as invalid. Prior to uploading them onto the website, each result of the HIV test performed was technically validated by two persons. Standard operating laboratory procedures for Genscreen™ and IgG testing are provided in Annex 4 and 5.

Another option is using a simple/rapid test on oral fluid. In the laboratory of the Institute of Tropical Medicine in Antwerp, the DPP rapid test (Chembio) was validated, and accuracy of the test was comparable with the HIV ELISA test. Using a simple/rapid test is easier, less labor intensive, and the result is quicker available (one-step procedure, no IgG quantification test is required). The 'Instructions for use' of this DPP rapid test are provided as Annex 6.

In case of a non-reactive test result, the result is decisive negative. There is no need to retest, or confirm the result. In case of a reactive result, it is important to execute state of the art confirmatory HIV testing on a blood sample using validated laboratory protocols. A reactive result is an indication for HIV positive status, but does not have the value of a formal HIV diagnosis. There is consensus to refer to an HIV test using oral fluid as an 'orientation test result'.

Communication of the HIV Test Results

Once the results of the HIV tests are available in the laboratory, they should be uploaded onto the website. Upon uploading, participants receive an email indicating that their result is available. Participants receive one of four standardized messages (for full messages, see Annex 7): (1) a strong reactive test result, strongly indicating HIV infection, to be confirmed by a blood sample, (2) a weak reactive result, indicating a probable false positive result or an early infection, to be confirmed by a blood sample, (3) a nonreactive result, indicating the absence of HIV infection, taking into account a window period of 3 months, and (4) an invalid result, with the suggestion to repeat the oral fluid sample or to take a state-of-the-art HIV test. In the case of a reactive result, a mobile phone number was provided for emergency counseling by a trained paramedic or peer educator.

Follow-up, linkage to care and confirmation test

Participants who did not check their results are contacted by phone or email. All participants with a reactive result were contacted by phone within 24 hours of having picked up their result. The purpose of the call was to offer counseling and to arrange a further confirmation test and guarantee

linkage to care. If confirmation did not take place at the organizing health care center, participants were contacted after the confirmation procedure to collect the confirmation test result.

IMPLEMENTATION PHASE

Organize outreach

During the development phase, contacts with venue owners, managers, organizers of large-scale events and stakeholders are prerequisites for a successful implementation (see also p. 10)

Outreach activities are best announced some weeks in advance via the calendar on the project website, and ideally via social media channels. During the outreach session, additional attention should be drawn to the activity to facilitate participation, and attract participants.

Home sampling

Men and women participate in the home-sampling arm of the project via two ways:

- First participation: via social media channels, advertisement or mouth-to-mouth advertisement, people may find their way to the website directly. If they meet the requirements (see above), they are able to order a sampling kit.
- Repeated testing: participants who are tested in the project receive a reminder email each 3-6 months. Participation is voluntary, but some people accept the offer of repeated testing and order a kit when they are reminded.

Repeated testing

Participants with a nonreactive test result, both through outreach and online participation, were offered the possibility to order a sampling package to be delivered to a Belgian address every 4 to 6 months, allowing frequent and repeated testing. For this purpose, a reminder email was sent 4 to 6 months after participation to the email address linked with the personal account.

EVALUATION PHASE

Effectiveness

Effectiveness is evaluated by the number of participants, the number of reactive results, and newly diagnosed participants.

Linkage to care

An important and integral aspect of an HIV testing project's evaluation is the linkage to care. Intense follow-up of participants with a reactive test result is labor-intensive, but facilitates linkage to care. After linkage, retention in care is equally important. Retention is achieved by offering accessible and qualitative HIV care.

Within the Euro HIV EDAT project, this aspect has been studied and described in work package 6. The manual for optimal linkage to care can be downloaded from the project website (https://eurohivedat.eu/).

Satisfaction and Acceptability

In the delivery message of the test result, participants were asked to fill out an online self-administered survey. In this survey, participants provided information on their impression of the project as a whole (not good, mixed feelings, good) and whether they would participate again in the future (no, not sure, without hesitation).

In work package 7 of the Euro HIV EDAT project, where a toolkit for Checkpoints has been developed, tools to assess satisfaction and acceptability are provided. This tool does also apply for other key populations than men who have sex with men. The toolkit can be downloaded from the project website (https://eurohivedat.eu/).

CONCLUSIONS AND RECOMMENDATIONS

HIV testing is an integral part of HIV prevention, and cannot be seen as a separate entity. It should always be embedded in a preventive approach that is designed for and by a specific population. New technologies facilitate novel HIV testing approaches, and will need to be further developed in order to increase the uptake of HIV testing among key populations. Early adoption of new technologies, and turning them into practice are crucial elements for researchers and health care providers to remain relevant and attractive for users they aim to reach.

Testing as prevention intervention to reduce the number of undiagnosed people living with HIV

Despite a world-wide paradigm shift towards normalization of HIV testing, several barriers remain.(38,39) A way to normalize HIV testing is shifting the testing service delivery towards a more convenient and accessible model for members of key populations. Apart from free and anonymous HIV testing for key populations in community or health care settings, the HIV self-test can be valuable in achieving this goal. The self-test has several benefits for the users, including convenience, privacy, non-invasiveness, and easiness to use. Linkage to care is the main concern about HIV self-testing.(40) Besides linkage to care, concerns about counseling, user error and the prolonged window period of HIV self-tests, compared to clinic-based tests need to be mentioned. Despite these concerns and disadvantages, some men and women use the HIV self-test as an additional tool for prevention.

Another HIV testing approach that offers convenience and accessibility are home sampling, and outreach testing.(21,41) The HIV testing approach described in this manual includes sample collection during outreach activities and a home-sampling approach for members from key populations. The research projects provided evidence for feasibility, and acceptance. Limitations and weaknesses mainly concern workload during outreach activities and follow-up, and false reactive test results when using oral fluid samples.(21,41)

Regarding testing as prevention intervention, additional efforts should be made to test more people unaware of their HIV infection. Innovative testing approaches by adopting the newest technology should guide the development of testing projects. Online activities, convenience and easiness to use will gain importance, and health care workers and community based organizations should follow this evolution. Experiences with our projects show that novelty attracts new users. In line with marketing strategies of other products, an HIV testing approach should be updated when (parts of) the approach are perceived outdated. Constantly adapting the testing approach to the newest technology, both digital and biomedical, is therefore key to a successful project.

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ANNEXES

Annex 1: Questionnaire to assess laboratory's standards of care

Euro HIV EDAT: Laboratory questionnaire

In order to organize a customized training for every collaborating laboratory, we would like to have some feedback and answers on the following questions about your current laboratory situation, skills and knowledge.

Name of the laboratory: ……

Address: ……

Email: ……

Responsible person: ……

Trainee(s) in the Oral fluid test: ……

Is your laboratory familiar with:		Yes	No	Available
1)	A quality system (Good laboratory practices (GLP)) at the minimum	□	□	
2)	Or an accreditation ISO 17025	□	□	
	or ISO 15189	□	□	
	a) use of SOP(Standard operating procedures)	□	□	
	b) EQC (External Quality control)	□	□	
	c) IQC (Internal Quality control)	□	□	
	d) IRC (Internal run control)	□	□	
	e) Temperature monitoring: Room (15-25°C)	□	□	
	Refrigerator (2-8°C)	□	□	
	Freezer (-20°)	□	□	
3)	Performing manual ELISA (Genscreen HIV-1/2 v2 and Total human IgG ELISA (ICL))	□	□	
4)	Other ELISA's	□	□	
	If yes please specify which test:…….			
5)	Is the following equipment available in your laboratory:			
	a) ELISA reader (filters 405,450 and 620-700 nm are needed)	□	□	□
	b) washer (flat bottom 96-well)	□	□	□
	c) incubator (37°C ±1°C)	□	□	□
	d) refrigerator (2-8°C) and freezer (-20°C)	□	□	□
	e) vortex	□	□	□
	f) centrifuge (swabs diameter 16 mm)	□	□	□
	g) calibrated micropipettes (10, 200, 1000 µl)	□	□	□
	h) multichannel (300 µl)	□	□	□
	i) pipetboy	□	□	□
	j) pipettes 5, 10, 25 ml	□	□	□

k)	tubes and recipients	□	□	□
l)	cryotubes 2 ml	□	□	□
m)	deionized or distilled H2O	□	□	□
n)	labels	□	□	□
o)	graduated cylinder (500/1000ml)	□	□	□
p)	timer	□	□	□
q)	dilution rack	□	□	□
r)	plate sealers	□	□	□

6) Maintenance of the equipment:

a)	calibration of the reader	□	□
b)	cross over test of the washer	□	□
c)	rest volume test of the washer	□	□
d)	is the incubator calibrated?	□	□
e)	calibration of pipettes	□	□

7) Statistical data analysis program to create a standards curve by using a four parameter logistics curve (total IgG measurement). For example the program R: is a free software environment for statistical computing and graphics. It compiles and runs on a wide variety of UNIX platforms, Windows and MacOS. □ □

8) A good communication and contact with the reference laboratory where the testing on blood is performed.

General information:

9) What is your national algorithm to test HIV on serum/plasma? Include flowchart.

10) Do you have experience with Simple Rapid Tests? □ □

11) How many specimens/week the laboratory analyses for HIV? …….

Remarks:

……..

Thanks for your collaboration,

Katrien Fransen
Head of the AIDS reference laboratory
Institute of Tropical Medicine

Tine Vermoesen
Laboratory technician

Annex 2: Protocol

Online communication and counseling for oral fluid tests to diagnose HIV infection: EURO HIV EDAT Study

Version 2.3; 15 OCT 2014*

Sponsor:	Institute of Tropical Medicine Nationalestraat 155 B-2000 Antwerpen - Belgium
Coordinating Investigator:	Eric Florence (eflorence@itg.be) Tom Platteau(tplatteau@itg.be)
Investigators/ collaborators	ARL: Katrien Fransen, Tine Vermoesen, Greet Beelaert.

Protocol Number:	
Title:	Online communication and counseling for oral fluid tests to diagnose HIV infection: EURO HIV EDAT Study
Version:	Version 2.3 (15/10/2014)
Previous version:	
Partnering institutions:	Catalan Institute of Oncology (Barcelona, Spain) AIDS Fondet (Kopenhagen, Denmark) AIDS-Hilfe NRW (Cologne, Germany) Checkpoint LX (Lisbon, Portugal) Legebitra (Ljubljana, Slovenia) Romanian Association against AIDS (Bucharest, Romania)
History:	**This study uses the results gathered in the evaluation study on oral fluid tests performed conjointly by the ARC & ARL in 2011.** **This study is an international continuation of the Swab2know-study (submitted 2012, and still ongoing)**

Sponsor:	CHAFEA (Consumers, Health, and Food Executive Agency of the European Commission)
Investigator:	Eric Florence
Department:	Clinical Sciences
Address:	Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium
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Email:	eflorence@itg.be

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Statement of Compliance & Confidentiality

The information contained in this study protocol is privileged and confidential. As such, it may not be disclosed unless specific permission is given in writing by the ITM or when such disclosure is required by federal or other laws or regulations. These restrictions on disclosure will apply equally to all future information supplied which is privileged or confidential.

Once the final protocol has been issued and signed by the Principal Investigator(s) and the authorized signatories, it cannot be informally altered. Protocol amendments have the same legal status and must pass through the mandatory steps of review and approval before being implemented.

By signing this document, the Investigator commits to carry out the study in compliance with the protocol, the applicable ethical guidelines like the Declaration of Helsinki and consistent with international scientific standards as well as all applicable regulatory requirements. The Investigator will also make every reasonable effort to complete the study within the timelines designated.

PRINCIPAL INVESTIGATOR:

Title, Name: Eric Florence, MD, PhD Date:

Signed:

COORDINATING INVESTIGATOR (if applicable):

Title, Name: Tom Platteau, MSc Date:

Signed:

Synopsis

The number of new HIV infections keeps on increasing every year in Europe. We are convinced that regular HIV test among high-risk group in addition to low barrier testing are good strategies to decrease secondary HIV transmission (mostly from people unaware of their status). Unfortunately there are still a lot of barriers for HIV testing. Low-threshold strategies, especially outreach and minimally invasive strategies, could support people from the high risk group to undergo a test. Building on the experiences of Swab2know-project, we aim at evaluating the feasibility of a non-invasive & confidential HIV test strategy among high-risk group of acquiring HIV infection (men who have sex with men, migrant populations, injecting drug users, sex workers) in different European countries (Belgium, Denmark, Germany, Portugal, Romania, Slovenia, Spain).

This is a prospective interventional study where HIV will be tested on oral fluid samples in outreach settings, or through ordering sampling packages online. Oral fluid will be collected on a validated and CE labelled device (Oracol®). A serologic HIV test (Genscreen®) will be executed at the laboratory which will be trained successfully by the AIDS Reference Lab of the ITM. A control, IgG determination, will be performed on all negative samples to assess the quality of the oral fluid sample. The results from the first projects using this method (Swab2know, Together-project, SIALON2) are very promising. We will not perform HIV rapid tests on saliva as with the recent FDA approved oral test for HIV. We think our method is more appropriate for testing groups with increased risk for HIVacquisition, and will lead to a better linkage to care compared to self-testing.

The result of the HIV test on oral fluid will be transmitted as desired through a secured website or during a consultation at the local health care facility or community based test centre. The result of the oral fluid test will be disclosed as indicative of a possible HIV infection. Reactive test results on oral fluid will have to be confirmed on blood with the reference gold standard testing algorithm in use in the country. If negative, participants will be offered the possibility to repeat the test after three to six months. In any case it will be stated that the oral fluid test cannot be substituted for the standard blood test.

1. Introduction

1.1 Background

In Europe, HIV remains an important public health problem. In the EU/EEA-area (European Union Member States, Norway, Iceland, and Liechtenstein and Switzerland), 29.381 new HIV-cases were reported in 2012, an incidence of 5.8 per 100.000 population[1]. The most affected groups are men who have sex with men (MSM; 40.4%), followed by heterosexual transmission (including migrants from endemic regions; 33.8%), and injecting drug users (6.1%). A first step towards reducing HIV incidence is the timely detection of HIV-infection. As considerable thresholds still exist in terms of HIV testing, this study aims at assessing the feasibility of innovative low threshold testing and linkage to care strategies in groups where HIV prevalence is known to be high.

Numerous thresholds are possibly related to a decreased uptake of HIV testing: financial constraints, confidentiality-issues, geographical location of the test facility, fear of blood sampling. [2-4] In recent years, steps have been taken to decrease these thresholds using several strategies: offer free and anonymous HIV-tests, implementing an HIV-rapid tests strategy within the consultation venue, and HIV self-tests. The FDA approved the first oral fluid HIV test already in 2002 but a regain of interest came with the very recent approval of the first rapid HIV test performed on oral fluid. It will be soon marketed as over the counter self-test in North America. Blood sampling remains the gold standard for HIV testing but it represents an obstacle for some people[5]. Furthermore, it has previously been shown HIV that testing using oral fluid samples is preferred by adolescents above donating bloodsamples[2]. Therefore, pilot-research has been conducted on the implementation of oral fluid-sample testing in clinical settings with promising results.[4,6] Based on the evidence from a meta-analysis, point of care oral fluid test are now considered a valid option for HIV screening among populations with a HIV-prevalence above 1%.[7] Although we agree on the beneficial aspects of point of care testing based on oral fluid, we see also some problem with this strategy. Point of care tests may be used as serosorting strategies and give a false feeling of safety for people in early infection and high transmission risk.

In the case of self-testing, linkage to care is not guaranteed.

A screening test strategy based on oral fluid samples has been investigated at ITM. Oral fluid was collected using the Oracol® device. The HIV testing on oral fluid was optimized for three ELISA-tests (Genscreen, Vironostika and Enzygnost). Three hundred samples (90 from HIV positive participants and 210 from HIV negative participants) were assessed. Based on the observed accuracy data for each of the adapted tests, the following testing algorithms were proposed for screening and epidemiological purpose: As the Genscreen test does not require modification of the cut-off for oral fluid and reached 100% (95%CI: 96-100%) sensitivity and a 97.6% (95%CI: 94.5-99.0%) specificity, this test is used as the first and only test for diagnostic purposes. For surveillance purposes using oral fluid Genscreen is used as the first test followed by the Vironostika with a 97.8% (95%CI: 92.3-99.4%) sensitivity and 100% (95%CI: 98.2-100%) specificity and/ or Enzygnost test with a (98.9% (95%CI: 92.3-99.4%) sensitivity and a 99.5% (95%CI: 97.3-99.9%) specificity, if the first test is reactive. Only when the specimen is reactive with both tests the specimen should be considered as HIV infected for surveillance purposes. Quality control tests (IgG determination) were performed on all negative samples according to a previously published procedure[8] but a commercial IgG elisa can also be used

to control the specimen in a more standardized way There was no loss in diagnostic accuracy when the tests were performed at day 0, day 3 or day 7[9].

To facilitate the procedure for a small number of samples, a validation procedure of a delayed simple rapid HIV-test in the laboratory is currently executed in the AIDS reference lab of ITM. The results for this validation study will be available by September – October 2014. On the basis of these results, partners (and the labs they are collaborating with) can opt for the standard ELISA procedure, or the rapid test procedure in the lab. The training for both strategies (if applicable according to the validation results) will be foreseen during the lab training in December 2014.

Counseling is an integral part of the HIV testing process. State of the art communication is a face-to-face discussion with a trained health professional (in our setting mostly a physician) before and after performing HIV test[10].For most people, this approach should remain the standard. But among certain groups, like MSM, HIV is not an exception. They usually know one or more people living with HIV, and most of them take a test on a regular basis. For them, this extensive procedure may withhold them from getting a test . Among these people, one could consider a less invasive approach with delivery of tailored information through innovative communication channels without adopting extensive pre- and post-test counseling.

For this project, we want to use a secured website for communication of test results and information delivery ("online counseling"). This result delivery was pilot-tested before, and evaluated positively.

The project is a continuation of the local, ongoing swab2know-project, financed by the city of Antwerp. The swab2know-methodology will be expanded to different target groups in different European countries.

The project is embedded in the EURO HIV EDAT-project, a project that is supported by the European Commission, through the Consumers, Health and Food Executive Agency (CHAFEA). This project aims to improve the early diagnosis of HIV, and linkage to care for key populations throughout Europe.

The current HIV-testing project is one of the elements to increase HIV-testing uptake among different target groups.

1.2 Rationale

We think that innovative detection methods are needed to improve the diagnosis of HIV among high risk groups. Given our previous experience and the available international recommendations we think that outreach testing, use of oral fluid tests and digital communication may improve the detection and still allow linkage to care. All efforts will be made to decrease the classical thresholds associated with HIV testing by offering cheap if not free, confidential and non-invasive procedures.

2. Study Objectives

2.1. General objective

To assess the acceptability and feasibility of an outreach, and home sampling HIV-testing strategy, as well as web based delivering test results and counselling.

2.2. Specific objectives

1. *To assess the feasibility, and acceptability of outreach, and online strategies to reach different target groups in Europe.*
2. *To assess feasibility, and acceptability of the use of oral fluid samples for HIV-screening among different target populations in European countries.*
3. *To assess the feasibility, and acceptability of online communication of HIV-test results through a specifically designed website.*
4. *To assess uptake of confirmation test, and linkage to care among HIV infected participants newly diagnosed with a non-invasive outreach methodology.*
5. *To study the feasibility and acceptability of a repeated testing strategy among the participants tested negative for HIV.*
6. *To assess the feasibility and acceptability of a novel counseling strategy using an online tool or mobile application ('app').*
7. *To increase the awareness of communities on the importance of HIV-testing.*

3. Study Design

This project is set up as a prospective interventional study.

The project will be carried out in outreach locations for men who have sex with men (MSM), sex workers, and people who inject drugs (PWID), as well as online strategies to reach people from these target groups.

Specific objective 5 will give the opportunity to follow longitudinally a group at high risk of acquiring HIV.

4. Methods

4.1 Study Setting, Population and Sampling Strategy

4.1.1 Study setting

A strong emphasis lies in the role of (local) NGOs and community based organizations (CBO). They will facilitate the outreach activities, process of confirmation, and linkage to care of participants in the project.

MSM

Venues where gay men meet will be selected to take part in the study. These venues will be chosen according to the size of the venue (how many people can we reach?) and the acceptability by the owner.

Migrant populations

The outreach testing intervention will be implemented in venues including cafés, socio-cultural events, integration classes for the newcomers, cybercafés, call centres, and churches. The choice of venues will depend on the size of the venue and the attendance, the acceptability by the organizer of the activity of the venue, and the presence of people from the target group.

Injecting drug users (IDU)

Outreach activities will be organised to reach these groups in countries where IDU are affected with HIV. The local partners will organise sampling sessions, in line with their existing experience and expertise.

Sex workers (SW)

Outreach activities will be organised to reach these groups in countries where SW are affected with HIV. The local partners will organise sampling sessions, in line with their existing experience and expertise.

Other potential participants

- Serodiscordant partners from people living with HIV, who will be recruited at the partner organizations, and local NGOs. A partner can take part in the project using the same procedure as other participants.
- People attending a partnering organization, and who have sexual contacts with multiple partners. The procedure will be similar to the other participants, and strategies.

4.1.2 Sample Size and Power

The main objective is exploratory and assess the feasibility of the methodology and detect new HIV cases in high risk population. We will not assess prevalence or differences between groups. We aim to organize at least 25 outreach test sessions for MSM and migrants each during a period of 24 months. The experience from a pilot study in Antwerp shows that a substantial number of participants can be tested when the session is announced, and prepared sufficiently. When choosing a venue with enough potential participants, a reasonable objective is to reach 20 participants per session.

There could additionally be chosen to elaborate the enrolment strategy to an online platform where people can order a kit, thereby increasing the number of participants in the project.

The objective is to execute 3500 HIV-tests in 2 years in the project. The number of tests will be dependent on the participating partner. Each partner should at least execute 250 tests. .

4.1.3 Inclusion and Exclusion Criteria

In order to be eligible, study participants must meet the following criteria:

» Being at least 18 years old

- Accepting oral fluid sampling
- Signing Informed consent form, where is explicitly stated that participants can be contacted by phone or email.
- Provide minimal information (survey)
- Understand the fact that the test will only be indicative of HIV infection

Potential participants meeting any of the following criteria will not be enrolled in the study:

- Not willing to provide a cell phone and e-mail address
- Pregnant women will be discouraged to participate in the study and will be tested through conventional ways (pregnancy will not be systematically looked at with blood or urine sample).
- Not being a member of 1 of the target groups: in this case, participation will be discouraged because of an increased chance on a false positive test result.

4.2 Procedures

4.2.1 Data Collection

Test sessions

General data on the acceptance of the oral fluid sampling will be collected (how many persons is the test being proposed to and how many accept?).

Secondly, basic data will be collected through the survey filled in by the participants. The survey will take only a few minutes to fill in. If necessary, participants may be assisted by a collaborator of the project, in order to collect all data correctly.

The following information will be gathered:

- Age of the person
- Gender
- Gender of people they have sex with
- Email address and cell phone number (to send them a message when their test result will be available, and in case they don't pick up their results)
- Country where they live
- Country of origin
- Their HIV-status
- Reason for testing
- HIV-testing history
- Having a general practitioner (GP) / Family doctor

- Health insurance status
- Number of sexual partners in the last three months
- Injecting drug use / recreational drug use in the last three months

Additional data on satisfaction with the project method will be collected on the website.

Data will also be collected on outcomes: number of reactive tests, how many participants collect their results, number of participants with a reactive result that had their result confirmed, and linkage to care.

Repeated testing strategy

Participants with a negative result receive a reminder email after 3-4 months to order a new sampling kit directly through the website. This offer will only be implemented upon agreement of each partnering organization. The procedure is identical as the ordering of sampling kits directly through the website.

4.2.2 Subject related Interventions or Procedures

4.2.2.1 Collecting samples

Oral fluid samples can be collected in different ways.

Each participating partner should select the strategies appropriate to reach their most vulnerable participants. Below we describe the different options for sampling strategies.

A. Outreach sessions

Outreach activities can be conceived as formal sampling sessions, but also visits to participants' home, or other extra-mural personal contact with potential participants can result under the outreach-umbrella.

A.1 Test sessions:

Test sessions will be announced at the venues. These announcements will be made through the websites of the study, facebook, and through flyers and posters at the venue.

Additionally, we will promote our outreach activities through contact with partners.

During the sessions, two counselors, and (at least one) outreacher will be present at the venues.

Visitors of the venue will be invited to participate free of charge, and confidential in the project. If clients or attendants of the activities where the session is organized are insufficiently informed about the importance of HIV-testing, there may be chosen for a (collective) pretest counseling. If

showing interest potential participants will be informed about the procedures, benefits, limitations, and their rights when participating in the study. The informed consent form will be used as a guide to provide study related information. After signing the ICF, the participant will collect an oral fluid specimen under supervision of the counselor, and will fill in the survey. An account will be created on the website. The oral fluid sample is collected using the Oracol® device (MMMD, Worcester, UK). Whereas the website is specifically designed for this project, and may be assumed to be the preferred choice of communication of the test result, we will still offer the possibility to receive test result in a face-to-face consultation at a local health or community based facility. If participants choose the latter option, an appointment is given right away. The participant's choice will be respected at all times. The participants will be informed that they may be called during the course of the study. They will finally receive a card with their personal code ('sample code') and a note on how to recollect the result through the website. Care will be taken to keep this procedure as short and simple as possible, as we know that this is critical to ensure participation.

Samples will be kept at room temperature and brought to the local laboratory for lab test on the first working day after the test session.

No incentives will be offered to participants.

A.2 Home visits or extra-mural activities

During these activities, a more personal approach can be adapted. A more 'standard' pre-test counseling may be provided.

The procedure remains the same compared to the test sessions:

1) Information
2) Signing Informed Consent Form
3) Filling in the survey
4) Creating an account on the website
5) Sampling
6) Providing unique sample code.

A.3 Online ordering of sampling packages

We want to make it possible to order the devices directly through the website. The participant needs to register (create an account) on the website, and a test package will be sent to the participant. Using this procedure, participants give up anonymity, because a name, and postal address should be provided. The participant can self-collect the specimen (there's a video available on the website on how to collect the sample), fill in the survey, and send the sample back to the laboratory . Participants will have to confirm their consent (by agreeing to the user terms) when ordering a test kit through the secured website. After the test has been executed in the laboratory, the participant receives a message that the result is available online. Additionally, promotion cards will be available at the venues. It will inform interested people to order a test kit online. It will provide info on how to register, and order this kit. They will have to confirm their consent when ordering a test kit through the secured website. This routine phase will depend on the popularity of the methodology, the uptake of the test during the first phase and the willingness of the owners of the venues, which are all very supportive on the project's objectives, and methodology.

B. Repeated testing

Participants with negative HIV test will have the opportunity to order a free test every three to six months.

If they are interested in receiving a new test, an email will be generated after three to six months to remind them about the possibility to receive a new sampling kit. Using the same account as on the previous test, participants may log on to the website, and will receive instructions on how to order a sampling kit. They will have to agree with the terms of use (which are the same as on the informed consent form) and will fill a short survey (the same as during outreach sessions). Participants can decide to have the test sent at an address of their choice or to pick up a kit at a participating health care facility, or community based centre. A participant may choose another way of communicating the results than for the previous tests. The test kits will consist of a sampling device, a short information note explaining the procedure and a prepaid envelop. All documents will be identified by a unique code.

4.2.2.2 Communication of test results:

As soon as the test results are available, participants will receive an email on the email address or number they provided: "Your test result is available. Please use the following link to login and obtain your result: Insert link. Thank you, Euro HIV EDAT"

Participants may choose at any time to get an appointment with a health care worker, or NGO / peer educator to discuss their test result. They will be identified through their unique code.

If the result of the HIV test is not reactive, participants will receive the following message (see Annex 1 for full message): "Your saliva has not reacted to the test. We can conclude with high reliability that you are not infected with HIV. Please note that an HIV test does not give any information about the risk you may have had during the last three months." Additionally, information on how to stay HIV-negative (including referral for sexual counseling if required), window phase, e.g. that risks from the past three months are not covered in the test, and the importance of being tested for other STI, is provided. They will be offered the possibility to receive a new test later (see c. follow-up).

If the result of the HIV test is strongly reactive, participants will receive the following message (see Annex 1 for full message) : "Your saliva has reacted to the test performed to detect HIV. This result means that there is a risk that you carry an HIV infection. However, in order to be able to give a definitive diagnose, it is necessary to perform a blood test to confirm this result. Please note that a saliva test is not yet officially recognized to pose a diagnosis of HIV infection." They will be given practical information on the modalities of a confirmation test at a GP, local health care facility, or specialized HIV treatment center. Participants will receive basic information on HIV, treatment possibilities, importance of HIV follow-up. Each participant will be contacted for counseling once he has picked up this result.

If the test result is weakly reactive, a more nuanced message will be given (see Annex 1 for full message): "Your saliva has weakly reacted to the test performed to detect HIV. This result means that there is an indication that you carry an HIV infection. However, previous research results have learnt that a weak reaction could also indicate that the test has detected antibodies for another viral

infection. Therefore, the risk that you are infected with HIV is very small. It remains absolutely necessary to take a confirmation test on a blood sample too obtain certainty on your HIV-status."

" Each participant will be contacted for counseling once he/she has picked up his/her result.

If a result is inconclusive due to an invalid sample, participants will receive the following message: "Due to an invalid sample, we cannot provide you with a reliable result. This is most likely because your sample didn't contain enough oral fluid for a reliable analysis. We advise you to take a new test using a blood sample to know your HIV-status." Participants will be referred to a local health care facility, participating community based centre, or a general practitioner for a (rapid) HIV-test on blood.

Participants receiving their result through the website will be provided with an emergency phone number for use during business hours. Calls will be answered by the study coordinator, or counselor. In case of urgency (e.g. psychological distress) they will be seen at a participating health care facility, or community based centre, at latest on the next business day.

For the purpose of the study, an online counseling tool will be developed. This tool will offer the possibility to get in touch with the counselor, and will combine sound and video (participant and counselor can hear and see each other). This tool needs also to be mobile phone compatible ('app').

In case participants don't pick up their result through the website, they will be contacted 2 times within two weeks by phone or email by the study-team. In case we are unable to reach them, we assume that they choose not to get their result.

When participants receive an e-mail saying that their test result is available, we monitor whether at least the participants with a reactive test checked their result.

When they pick up their result, we contact participants 1-2 days later to ask them whether they would like to talk about this result, and offer them the possibility to visit the a local health care facility, participating community based centre, for confirmation of the test on a blood sample, or offer them to make an appointment at another centre (of GP) for a confirmation test.

When people don't pick up their result, they are actively encouraged (by e-mail and/or phone) to check the result. This service is provided for all participants who don't pick up their test result, but is more actively followed-up in case of a reactive result.

All participants will be asked to provide feedback on the project. Data will be collected either via the website (when participants log-in to receive their results) or using a paper-pencil questionnaire when collecting the results at the local health care center. Following questions will be asked (see annex 2):

- Satisfaction with the project;
- Satisfaction with the mode of communicating the test result;
- Preference on donating oral fluid vs. blood samples;
- Satisfaction with self-collection of oral fluid samples (if appropriate);
- Motivation to use this method, even if it is no longer free of charge;
- Comments or suggestions.

4.2.2.3 Overview of partners, sampling collection strategies, target groups, and number of envisaged tests

Partner	Number of tests	Target group(s)	Sampling strategy
CEEISCAT (ES)	1000	MSM	Outreach
AIDS Fondet (DK)	250	MSM	Outreach
AIDS Hilfe (DE)	250	MSM	Home sampling
GAT (PT)	400	FSW	Outreach
Legebitra (SL)	350	MSM	Outreach
ARAS (RO)	250	PWID	Outreach
ITM (BE)	1000	MSM	Outreach
			Home sampling

Role of CBVCT in the project

In the Euro HIV EDAT project, several partners are CBVCT centers. The role of these centers is crucial in the project. The role of CBVCT (partners as well as CBVCT collaborating with our partners) can be described on various levels in the project:

- Organization or collaboration in outreach activities as 'outreacher' or 'study collaborator'
- Follow-up (contacting participants with reactive result)
- Counselling (online, or face-to-face) for participants with reactive, and non-reactive test results. CBVCT have a vast experience in counselling of people who take an HIV-test. The project should acknowledge this experience, and involve CBVCT as much as possible.
- Confirmation test for participants with reactive and non-reactive test results. CBVCT have a vast experience in the confirmation procedure of people with an reactive HIV-test. The project should acknowledge this experience, and involve CBVCT as much as possible
- Linkage to care for participants with reactive, and non-reactive test results. CBVCT have a vast experience in the linkage to care of people with an reactive HIV-test. The project should acknowledge this experience, and involve CBVCT as much as possible

4.2.3 Website

The website (Insert link) is specifically designed for this project and will be embedded in the EURO HIV EDAT website.

The website will consist of an open (public), freely available part with information on the project, reliability of the oral fluid test, window-phase of the test result, preventive HIV-messages, medical information on HIV, and information on PEP and STI. A second part (restricted) part will be highly secured using HTTPs protocol (as used for credit card payments and internet banking). The access will be restricted to participants (who are registered to the website), they will be asked to create a personal profile at their first visit. Minimal information for this profile is a cell phone number, and an

email address. This part of the website will serve to communicate the test results and information related to the test results, as well as ordering the test kits. After logging in , the participants will only be able to see their own test results (they have to fill in the unique code that is linked to their account). Samples will be randomly coded (to prevent that people try to search for the test result of the person that underwent sampling during the same session). There are several advantages of using a website over a cell phone message to communicate results. First the participants decide when to pick up their results. Second more information can be made available on a website ("online pre- and posttest counseling").

Personal data will be collected. Of each participant, email address and phone number are mandatory (for follow-up purposes). For all participants, a sample code (unique identifier), as well as test result, and information from the surveys is gathered. For partners offering repeated testing, additional personal information (full name, postal address) might be collected (in case participants choose the package to be sent to their home address). Data collection, and storage will be executes according to data protection laws from all participating countries.

The website will be developed by a third party under supervision of the ITM webmaster and IT department. The chosen partner is All Directions (http://www.alldirections.be/), a longstanding trusted partner for development of IT applications within ITM. They already realized the website of ITM and several databank applications.

The website of the study will be hosted on the ITM servers, these servers are also protected.

Within the website, an online counseling tool will be developed. This tool should combine both video and sound in order to facilitate online counseling.

Process

- When visitors open the website, a language will be selected based on browser settings.
- Users will be able to change the language using a drop down menu.
- Users will only be requested to select a country when ordering a sample kit. (Sample kits and the corresponding code will always be traceable to a country.)
- Some users will order a sample kit in a different country than the one they originate in. For identification purposes, only the test can be assigned to a particular country, because a person might have himself tested while staying abroad. Users will show up in the contact information database of a particular country once a sample has been assigned to a user account (see management tool, below).

Public website

- The website will be hosted and managed at ITM.
- The website will be multilingual.
- The website will contain the following pages:
    - A homepage, containing:
        - Internal links to pages with more information about the Swab2Know-project.
        - The calendars, listing all test sessions of all partner countries. A filter allows the user to see only the scheduled sessions of his or her country.
        - A list of external links (includes filter to show only local results).
    - A registration page

    - A login page
    - Account section:
        - Update account
        - Request sampling kit + questionnaire
        - Log off page
        - Satisfaction questionnaire
    - Forgot password-page
    - Contact information page, containing all partners
    - Manual page
    - Page with more information on privacy/security settings
    - What is EURO HIV EDAT?
    - A page explaining testing on location + calendar
- All pages will by default show the English version of the page. If content is available in another language, the page will be displayed in the chosen language.
- The content of these pages will be managed centrally, at ITM.

Database

- All data will be stored in a central database.

Management tool

- Every country will have its own local management tool.
- The local management tool allows administrators to:
    - Manage contact information (user accounts will only be shown for a particular country when sample kits have been assigned to a particular user)
    - Generate sample codes (code specific to each country)
    - Import sample results
    - Generate reports on sample results
    - Generate questionnaire reports
    - Manage test kit requests
    - Manage automated e-mail messages
    - Add and remove session dates (calendar) to be shown on the public website (homepage)
    - Add and remove links on the public website (homepage)
- The central management tool has access to all data and content of the webpages in all languages.

4.2.4 Laboratory Procedures

Within seven days of collecting the sample, the collaborating laboratory will perform the Genscreen ™ HIV-1/2 Version 2 test (Bio-Rad) according to the package insert using oral fluid specimens.

In case of a non-reactive Genscreen test, a commercial total IgG test 'Human IgG Elisa Kit (Immunology consultants Laboratory) will be performed. If the IgG test is positive (sufficient IgG present in the sample) the sample will be considered valid for analysis and the result of the HIV test will be reported as negative. A negative test is considered as strongly indicative for the absence of

HIV infection. If the IgG test is negative (not enough IgG detected in the sample) the sample will be considered as non-valid for analysis and the result of the HIV test will be considered as inconclusive.

If a result is inconclusive due to an invalid sample, participants will be referred to a participating health care, community based centre, or a general practitioner, for a test on blood sample.

If the HIV test is reactive the result will be reported as positive. Distinction will be made between strongly, and weakly reactive test results (see also 4.2.2). A strongly reactive result is considered as strongly indicative for HIV infection, the participant will therefore be considered as eligible for further confirmation test on a blood sample. A weakly reactive result is most likely a false reactive result, but this result should be confirmed on a blood sample. All samples outside of the seven days window period will be excluded for analysis, they will be reported as "non-suitable for analysis, please provide new sample".

For samples collected on site the date on the survey will be registered to assure the timeframe between sample collection and HIV-test in the lab (maximum 1 week)

For samples sent by post, participants are asked to fill in the date on the sample device to ensure the correct date.

4.3 Data Analysis

4.3.1 Quantitative Data Analysis

Data will be analyzed by the work package leader, and stored at an ITM-server.

Personal data will be collected for contact purposes (follow-up of the participants), not for data analysis. Participants will be informed that their personal data will be stored on a server, using state-of-the-art security procedures, and certified in the most reliable way.

Descriptive analysis will be performed on all variables available. In uni- and multivariate analysis, associated factors with the HIV test result will be calculated.

The proportion of participants who take a test on a regular basis will be calculated from the data extracted from the second phase of the project.

A yearly descriptive report will be produced by the work package leader (ITM). Each partner can use the country data for local reports (upon agreement by the work package leader).

All statistical analysis will be performed using SPSS software. A significance level of 5% will be used.

5. Ethical Issues

5.1. Use of oral fluid specimens

The use of oral fluid specimens to detect HIV infection is generally less reliable than the gold standard technique performed on plasma samples because of lower amount of IgG in oral fluid. Nevertheless, thetest performed on oral fluid specimen is deemed valuable for individual diagnosis in a high prevalence setting, defined in the literature as a prevalence above one percent[11].

Our method using a routine ELISA technique has not yet been used in previous research. Our method seems valuable to detect and exclude HIV infection. Tests have shown that oral fluid samples remained stable without loss of diagnostic accuracy up to seven days after sampling. We will build a quality control on all negative samples (measure of the total IgG). If the IgG is insufficient(< 3500 ng/ml) the result will be communicated as inconclusive and the participant will be asked to give a new specimen.

We will clearly state in the informed consent (and the message people receive) that the result of the test is only indicative.

A positive test is only strongly indicative of HIV infection and it should be confirmed on a blood sample taken at a health care facility. We are nonetheless aware that false positive results may lead to unneeded anxiety among the participants. We will give the possibility to all participants tested positive to call a participating health care, or community based centre,. Clear instructions will be given to participants on the website to be timely referred for confirmation test. Several options will be given: their GP or another GP in the neighborhood, a participating health care facility, or community based centre. Facilities where rapid test on blood sample are routinely offered will be detailed. Participants collecting their results at a participating health care facility, or community based centre, will be proposed an HIV rapid test.

A negative test is only indicative of the absence of HIV infection. Our preliminary results showed this method is highly reliable to exclude established HIV infection. The diagnostic accuracy is nevertheless not guaranteed in acute and recent HIV infection (3 months). Previous experience shows that some are picked up, showing a weakly reactive result. This will be clearly stated for the participants. Participants collecting their results through the website will be asked to repeat testing regularly or to go for a blood test in case of persisting risk or suggestive clinical conditions.

5.2. HIV test results communicated by a secured website

The use of a website for the communication of test results offers several advantages: people pick up their result at a moment when it suits them, information can be delivered, and people can receive an emergency phone number.

We assume that there is a need for 'easier' testing, without the state-of-the-art counselling procedures, certainly among MSM. The need is shown in a pilot project in Antwerp, using a similar methodology.

Although the knowledge is not yet broadly distributed, there is a real interest in self-testing especially among internet using MSM[11]. Even in most European countries where they are not officially authorized, self-test kits can be ordered very easily online from numerous suppliers. The quality of those kits is mostly unknown and no information is provided to put the result in perspective. Linkage to care is not guaranteed.

The tested strategy allows test quality, delivery of medical information on HIV, and on risks of HIV-transmission and urgent referral to specialized treatment centres when necessary. Moreover, we will facilitate linkage to care for all positive results by actively contacting all participants with a reactive result.

An online counselling tool will be developed to facilitate easy counselling for participants in the project.

5.3. Potential risks

With self-sampling, there's a risk that samples are not correctly collected. The risk is low given the fact the procedure is very simple, taken under supervision (during outreach sessions) and clearly explained in the documentation. We will build a quality control on all negative samples as detailed above. An inconclusive result may lead to disappointment among the participants.

There is a possibility that our technique leads to some false positive results. We will try to overcome these potential problems through clear messages on the website and by the physician, and by offering (re)test strategies. If needed the participants will have the possibility to join someone from the study team for further explanation or psychological support. A timely referral will be organized for very anxious patients.

There is always a potential risk that people receive their test result without proper counseling when using a (secured) website for communicating test results. Messages on the website may be refined according the feedback received during the course of the study.

5.4. Potential benefits

Participants will benefit from the opportunity to perform a very low-threshold, and reliable HIV-test, which is free of charge and confidential. Participants do not have to attend a consultation. Linkage to care is an integral aspect of the study.

5.5. Informed consent procedure

During the first phase (assisted sampling), people will be informed about the project, and asked for a written informed consent at the venue (pen-paper).

Later on the participants may receive test packages without intervention of study staff. Informed consent will consist of signing the 'Terms of use' on the website.

For the longitudinal part of the study, participants will also receive a test package at regular time interval. Informed consent will be collected on the website when collecting a negative result. Participants will have to confirm their consent on the website after each negative result to receive a new test package later.

Given the fact that the pre-counselling phase occurs partially in group in the migrant group, there is a risk of peer-pressure for participating in the study. Visitors may feel forced to participate in the study under the influence of the group. We have planned a short personal talk between the potential participants and the counsellor before participation in the study. The counsellor will make all effort to mention in the information talk that the participation is fully voluntary and that the will to participate in the study is completely independent of the potential HIV status (e.g.: it is not because you do not participate that you are automatically HIV positive and vice versa).

5.6. Which bodies will review and approve the research

This study will be submitted for formal review and approval to the local Ethics Committee of each participating partner. The study needs also to be submitted for local EC for each of the participating sites. No participants will be enrolled or subject related activities performed before written approval from these bodies is obtained.

The study will be carried out according to the principles stated in the Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects) as amended in 2008, all applicable regulations and according to established international scientific standards.

5.7. Storage of the samples

Left overs will be frozen at -20°C at the laboratory until the end of the study. Additional tests (such as confirming positive samples with INNOLIA or retesting samples with other test methods) may be performed during this period. No genetic tests will be performed on these samples.

6. Monitoring And Quality Control

The local center must allow the ITM's monitor or a monitor designated by the ITM access to any study-related files or source document present at the local center, or collaborating laboratory.

7. Insurance

This is an non-interventional study, not a clinical trial. This type of study is covered by an insurance. (For ITM, this is covered by the umbrella insurance).

8. Timelines

The study starts on the first of January 2015, and ends at the end of December 2016.

The protocol will be sent out to all participating partners by half October, 2014. The protocol should be submitted to local Ethics Committee (EC), to obtain ethical clearance by December 2014. During this period, contacts with owners and organizations of activities will be arranged. The website and study databank will be designed.

Outreach sessions will be organized between January 2015 and December 2016, only when ethical approval by the local EC is obtained.

From January 2017 – July 2017, data will be analyzed, and reports will be written; results will be presented at the final project meeting in September 2017.

9. Data Management And Archiving

9.1 Data Management

Data will be stored in a secured databank linked with the website. Test results will be entered in the database by the data clerks . All participants will be identified through their unique code. The database will not be accessible remote or from the website. Survey filled by participants will be entered in the database by a sub-investigator. The data will be content protected en will only be accessible to the study coordinator and designees.

Data will receive the highest possible degree of protection. All requirements of data-protection will be followed, and the responsible organizations and services will be requested for their approval.

Each partner should reach their local degree of data-protection.

9.2 Archiving

The Principal Investigator is responsible for ensuring a secure and appropriate location for storage of the Investigator's File and any other study related documentation present at site, as well as for ensuring that only site staff that is competent and delegated to work for the study has got access to the files.

After study completion, all the relevant study documentation will be retained in accordance with the local legislation and should be retained for a minimum period of 20 years after completion of the study. The Investigator's File should at all times remain available for internal audits and/or inspections of regulatory authorities, also after completion of the project.

After the project has been finalized, files will be stored on an ITM-server, available for researchers, but inaccessible for outsiders.

10. Financial Aspects

Details of funding bodies

This project is funded by the European Commission (European Agency for health and consumers).

Budget including direct and indirect costs

Total project budget (of which 60% is reimbursed): € 1.179.927

Total budget ITM (of which 60% is reimbursed): € 100.041

11. Dissemination of Results

A final project meeting will be organized to disseminate the project's results in September 2017.

Results will also be disseminated through at least one article in an international, peer-reviewed journal, and by attending national and international conferences, where the results will be submitted and presented.

12. Limitations Of The Study And Future Research

12.1. Generalizability of the results

Whereas we will offer the test to clients or participants to events (convenience sample), the population will probably not be representative of the general HIV population in the participating countries. Therefore, results from this study will only be applicable to high risk population with high HIV prevalence (>1%).

12.2. Impact on new infections

We do not yet know the number of persons who will be interested to participate in the study, although we think there is interest for non-invasive sampling and home-testing. We cannot foresee the real impact of this project on the number of new HIV infections in high risk population.

12.3. Practical concerns

Due to the use of oral fluid samples, we are only able to test HIV. Ideally, other STI would also have to be included in the strategy.

12.4. Sensitize communities of the importance of HIV-testing

Whereas this is an objective in the study, this will be very hard to assess. This is also due to the selection bias (only the convinced will take a test). Those who are not (yet) convinced of the importance, will test later or not.

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14. List Of Abbreviations

ARC	AIDS Reference Centre
ARL	AIDS Reference Laboratory
GP	General Practitioner
(I)EC	(Independent) Ethics Committee
IRB	Institutional Review Board
ITM	Institute of Tropical Medicine
MSM	Men who have Sex with Men
PEP	Post-Exposure Prophylaxis
PI	Principal Investigator
STD/I	Sexually Transmitted Disease/Infection
VCT	Voluntary Counseling and Testing
95% CI	95% Confidence Interval

15. Annexes

Annex 3: Manual for field workers

Swab2know-manual for field workers and project collaborators

Field workers are the people who collect the data in the field, and are therefore responsible for approaching respondents, for administering the questionnaire, for collecting oral fluid and for implementing prevention activities. They are chosen at country level by local partner's coordinator.

Field workers should be:

- good communicators, able to explain the scope and aim of the research, in particular if respondents request further information;
- inspire trust, able to persuade and motivate respondents, overcoming any diffidence;
- reliable, able to carry out their functions as specified;
- able to manage any critical situations that might arise, both in terms of their own safety and the behavior of respondents;
- blend into the various settings, look and feel comfortable where data is collected.

Profile of the data-collector; the following characteristics can be used as a checklist for potential data collectors during intake:

Knowledge

- Knowledge of the project: procedures, materials, etc.
- Knowledge of the transmission of HIV and STI's.
- The physical, mental and social aspects and the effects of alcohol and (other) drugs and the relation of these aspects on risk increasing sex concerning HIV and STI transmission.
- Knowledge of the local MSM/migrant/SW scene. Know where to find what activities, type of visitors and knowing how to get there.
- Familiar with the local health map and wellbeing services.

Skills

- Create an atmosphere without prejudices where the responded feels save and respected services.
- Create a professional atmosphere where there are no taboos in speaking about sexuality.
- Good communication skills: Communication with all kinds of people will occur. For example, different kind of staff member and visitors of different socio-economic status, rural vs. city background, all kind of personalities, different subcultures.

- Creative in finding solutions when things don't happen as planned.

Attitude

- Respectful: no judgments of lifestyle of clients.
- Open minded: being able to work in completely different venues, from a cozy atmosphere to venues where explicit sexual actions can be seen.
- Flexible: Irregular hours should not be a problem, as well as adapt to the local situation.
- Firm where needed: being able to communicate with clients which have used alcohol or other drugs. Decide if somebody can participate or not.
- Discrete: Information given by and seen from visitors and staff concerning life style and sexual behavior will not be shared with people outside the projectteam.
- Team player: being able to working in a team, the ability to back up your colleague and having trust in each other is a must.
- To bear in mind: During the introduction, the field workers should show respect, interest and appreciation for their respondent's participation. Previous experiences have shown that this attitude will increase participation rates.

A. Outreach activities

A.1 PRIOR TO THE SESSION

A.1.1 Appointments with venue owners and S2K-collaborators

Sampling in commercial locations involves arranging set times so in this case **an agreement with the owner is required,** so he/she should be contacted. When meeting with venue owners, the field workers should emphasize individual and community benefits of the study to the community, and that sampling activities will be conducted in ways to minimize burden on venue management and patrons.

In particular, various aspects should be agreed upon to prevent misunderstandings later which might involve data collectors in having to renegotiate their presence each time.

These include:

- An exact timetable;
- If and how customers are to be approached (where, what they are asked to do, etc.);
- Positioning in the location (at the entrance, casually inside, at a dedicated table, etc.) and logistics (spacious enough, wifi/4G connection, etc.);

In crowded locations or when the respondent is surrounded by others, a private area should be found for the respondent to fill in the questionnaire, in agreement with the

owner/manager. Moreover, a compromise needs to be found between being in a position which enables the approach to respondents and not creating confusion and misconceptions about the role of the data collectors.

List of demands interview area

- Enough light to write
- Not too much light to give an idea of privacy (which influences the honesty of answering).
- Sound isolation towards a level where a conversation in normal voice is possible.
- Possibility to separate the respondent from other visitors.

Check whether they want or need or wish anything from you: flyers, posters, condoms.

Give owners a phone call around a week, and the day before your visit, as a reminder and to check their presence.

Volunteers (if applicable): sufficient volunteers must be provided. To attract participants, to give some information, to distribute condoms. Also remind them a few days before by email.

The **laboratory**: inform the lab about your session. They can organize their work to receive the swabs.

A.1.2 Preparation of the materials

A.1.2.1 Creation of sample codes

A sample codes is a unique code which is linked to one sample. A label with the sample code should be stuck on each Informed consent form, questionnaire, sample and business card for participants (they need this code to access their result). It identifies all documents and the sample, and links it to a participant's profile.

During the course of the project, 1 participant may get tested several times (using the same e-mail address and phone number), but the sample code will differ for each test.

Each sample code list is country specific, and consists of the date (yymmdd), time (hhmm), and an index number (three digits). By creating a sample list, you will generate a set of codes with a fixed date and time, followed by a series of numbers. So you can generate 999 samples per minute (which is absolutely sufficient!), which will be assigned to one outreach session.

For example: you plan a session in a sauna, and create 34 sample codes for this session. If you visit another venue one week

later, you will need to create new sample codes, because the sample code list is assigned to the session in the sauna.

We usually create a multiple of 34 samples. This is not an arbitrary choice, but our label-page contains 34 rows.

Dependent of the outreach session, you can choose how many codes you'll create. After the session, the codes aren't usable anymore, because they are designated to the session. Each set of codes is linked to one outreach session, to avoid mistakes. (So don't prepare too many codes for 1 session). De-labeling is not always possible (Informed consent forms, business cards), which means you have to throw away some cards and Informed consent forms.

HOW TO CREATE A SAMPLE LIST?

Enter to the administrator link: https://admin.swab2know.eu. Ask Tom Platteau (TPlatteau@itg.be) or Lieselot Ooms(looms@itg.be) for a login and a password.

Go to the tabpage "Samples". Enter the number of samples you want to generate and click on "Generate new samples". Enter a multiple of 34 (there are 34 sample codes for 1 hard-copy page of labels), and generate the sample codes. An excel-file will be generated. You should now save this file.

If you choose to use the same label-pages as we do, we'll provide you with an example in which you can copy-paste the sample codes in order to be printed on the labels. Use any printer to print these codes on a hard-copy page.

A.1.2.2 Creation of QR-codes:

A QR-code is a unique code which refers to a webpage that is designated to 1 sample code. In other words: one QR is linked to one sample code.

This code is only useful for participation during outreach activities. This QR-code facilitates the process of participation as it simplifies the procedure.

HOW TO CREATE QR-CODES

The list of QR-codes is automatically generated with the sample list (see above). You can find the list of QR-codes as the third icon in the 'sample list' tab page in the admin module. When you click on this icon, a new window will show the list of QR-codes with the linked sample codes.

This list can easily be printed on a label-page.

A.1.2.3 Creation of laboratory list

To link the sample codes, test results, and email addresses, an Excel-file should be created with .xls extension (Excel 2003 version). This file (annex XX) contains following 5 columns: (1) sample codes ,

(2) result of the HIV test, (3) email address, (4) if participant wants to receive result through the website, or in a consultation, and (5) if the result has been picked up if participant chose to

receive it during a consultation.

A template of this Excel-sheet will be sent out by ITM to all collaborating partners. It's absolutely crucial to include sample code, test result, and email address in this document, as this document will be used to upload the result (and the 3 parts of information need to be checked by at least two different people). This Excel sheet should be sent to the lab, where it will be completed with the test result.

TO SUMMARIZE, PRIOR TO A SESSION, THESE DOCUMENTS NEED TO BE PRINTED:

- Labels with the samples codes. we usually print 5 labels for each sample code (1 for ICF, 1 for business card, 1 for sample, 1 to keep for the lab, 1 reserve)
- Labels with QR Codes to be stuck on the informed consent form (ICF).
- Excel file (can also be sent by e-mail, as this is still without test results at this point) for the laboratory.

A.1.2.3 Materials to prepare for a session

Packet:

We prepare individual packets for each sample/ participant during outreach sessions. Each packet contains pre-labeled (use your printed labels (see above) material for each participating client:

- **Informed consent form (ICF)** with QR-code and sample code .

- **Questionnaire**: in case you don't work with a tablet/iPad, you will need paper/pencil questionnaires (if you do work with a tablet/iPad, the questionnaire will directly be filled in on the tablet/iPad) which needs to be labeled as well. Use the extra label for this purpose. ***Beware that participant fills in his/her e-mail address on this questionnaire, as it is the only place where e-mail address and sample code are linked***. If you don't do this, we will have a list of sample codes, and a list of participant e-mail addresses, but no link between them; you will not be able to communicate anyone's test result… After the session you will create an account for the participant and will fill in the questionnaire online with the information collected on paper.)

- **Business card** with the sample code stocked on one side of the card and the link to access to the project website to collect the results of the test on the other side. Ask the participant to keep this
carefully. This code is necessary to retrieve the result, in combination with his email address and password.

- **Swab** for the saliva, also coded with the sample code.

Put these pre-labeled materials in a little bag or envelope; this is what we call 1 'packet'. Don't prepare too much packets. When you don't use all your packets, you cannot re-use your (pre- labeled) ICF, business cards, and questionnaires. You will just have to throw them away to avoid confusion in the future, as each sample code is linked to 1 session..

A.1.3 Materials for the session

Take in a **backpack:**

- The individual **packets**
- **extra material**: sample codes (that are created, but not pre-labeled), swabs, questionnaires, ICF just in case you may need them.
- **condoms and lubricant to distribute in the venue.**
- **Flyers with information of the activities of your organization, and the swab2know project (including project website).**
- **Ipad/tablet with internet access**(fully charged battery, and install a QR code reader on each device; we use 'QR Code Reader' by 'Scan, Inc.')
- **Banner, if you have it**
- **t-shirts** (preferably with the logo of S2k), **, if you have it**

A.2 DURING THE SESSION

A.2.1 In the venue

A.2.1.1 Outreacher

Approach clients about offer to get tested. Distribute condoms if possible.

Provide information on procedure, and answer questions.

It will take personality and persuasion to get potential subjects to stop and agree to be screened for eligibility. Having lists of common reasons and retorts for nonparticipation is recommended. It is helpful to practice these responses in advance of sampling events (e.g., role play with staff).

In approaching participants, recruiters should quickly identify themselves and their organization and succinctly describe their purpose.

To avoid misunderstandings of various kinds it is important to say that the test is a screening test and in the case that there is a reactive result a confirmation with a blood test will be required.

A.2.1.2 Data collection:

In general, the following conditions should be guaranteed for data collection and sampling:

- Safety of and lack of physical risk for field workers
- Clarity and non-ambiguity of the role of field workers in the specific setting
- Logistic feasibility for field workers
- Privacy for participants

These guarantees differ for the various settings. The most evident distinction to be made is between outdoor cruising and commercial settings (bars, discos, saunas, etc.).

- **Outdoor cruising: logistics feasibility and safety**

Cruising settings (parks, beaches, etc.) involve **usually outdoor locations which are hard to control** by field workers and in general anyone else: it is impossible to control who is coming and going, and often areas are hidden from view (particularly at night). This increases the degree of risk. Hence it is important to make sure there is a safe exit route in the event of critical situations and establish procedures beforehand, the method of approach and working method, as well as the positioning of field workers in the setting, should be such as to ensure that the situation does not slip out of control. Field workers must always be in pairs, or, better still, two pairs for all outdoor cruising settings.

Cruising settings also involve **logistics problems**, particularly if they are areas frequented at night or on foot: is the lighting good enough to fill in the informed consent and the questionnaire? This and other aspects of the location need to be investigated and proper logistics solutions found.

- **Commercial locations: privacy**

The following should have been discussed and agreed upon during the preparation phase of the project; it's quite complicated to start negotiating when arriving at the venue. Ideally, a separate room with enough light, providing some privacy, should be sufficient.

A compromise needs to be found between being in a position which enables the approach to respondents and not creating confusion and misconceptions about the role of the field workers.

List of demands interview area:

- Enough light to write
- Not too much light to give an idea of privacy (which influences the honesty of answering).
- Sound isolation towards a level where a conversation in normal voice is possible.
- Possibility to separate the respondent from other visitors.

A.2.2 Specimen collection

- Give the respondent the sterile sampling device and tell him he should open it so he can see for himself that it was sealed and container sterile material.
- After opening the package, the respondent should take out the sampling swab.
- The swab should be rubbed back and forth over the upper and lower gums, left and right, for 1 full minute: make sure the respondent does this properly. Specifically, the swab should be primarily rubbed against the gums and not the teeth in order to obtain enough oral fluid to analyze in the lab.
- The participant should place the swab back in the tube (the sponge first), and close the sampling tube.
- When the participant hands back the sampling tube the data collector hands over the card required to pick up the test result. It is important to remind the respondent to keep the Card with sample code on him; it is the only means of picking up the test result: only the sample code identifies the person providing the sample.

Full procedure of participation:

- **Check** the sample codes are pasted on every item in the package (ICF, swab, business card, questionnaire when necessary).
- **Read** the IC, together with the participant and **sign** it (both). The participant can sign it anonymously, making a mark.
- Register the participant by reading/scanning the QR-Code pasted on the ICF with the tablet or the smart phone of the participant. Follow the procedure. An account is created during the procedure; if this procedure doesn't work, fill in the form paper-pencil and explain how to proceed later to register the participant. The field worker should ask the email address and write in the questionnaire? Should create a password and send it to the participant?
- **Fill in the questionnaire** online.
- Self-collection of the sample: the participant **rubs the swab on his/ her gums for 1 minute**, like brushing their teeth.
- Inform the participant how **to get their result**.
- give the participant the **card with the personal code and the link to the project website.**
- put **everything back in the bag and check** if the whole procedure is completed.

To summarize: How can participants register on the website?

- During an outreach session, when the QR-code is scanned, registration will be done during the process. The sample code will be automatically linked with the account.
- When there's no possibility to register on site (for instance, when no internet connection is available, or field workers prefer not to use valuable materials during outreach activities, delayed registration through the homepage should be considered. Go to the homepage (www.swab2know.eu), go to 'Login or register' and click tab page 'Register'. The field worker should discuss with the participant who will create the account (safer / more reliable option
  = field worker; more participant-empowering option = participant).
- During the process of online ordering of a sampling kit, participants should create an account (when they first participate) or login (when they have already been registered).

It's important to emphasize that using the latter two options, sample codes are not automatically linked to the account. This link will be established by uploading the test results onto the website (see below). This implies that participants, when they try to check their result before it has been uploaded, receive the message that the sample code has not been linked to an account, which might give them the idea that something went wrong during the process. It's important to inform them sufficiently.

A.3 AFTER THE SESSION

1. **Laboratory**: bring the samples as soon as possible to the lab. The time between the sampling and processing may be maximum 7 days. If it's not possible to bring them directly to the lab, store them at room temperature.
2. Participants didn't register on site. Create an account (use e-mail address, and phone number they filled in on the paper/pencil questionnaire).
3. Create the excel-sheet, or complement it if you created it beforehand. The samples that were used during the session should be in the list, as well as the e-mail addresses.

After the tests are executed in the lab, the reference person from the lab will fill in the test results in the Excel-sheet. Once this document is ready, the results can be uploaded.

To upload the results:

Go to https://admin.swab2know.eu, choose "Import sample results".

Before uploading the results, beware that 'Testmode' is ticked. This testmode will execute everything as 'for real', but the results will not be uploaded yet, and the e-mails to participants (informing that result is available) will not be sent. All the other steps in the procedure will be executed. This means that if there's a mistake (wrong e-mail address, result cannot be delivered, etc.) you will be informed about that. If all is OK, you can 'de-tick' the testmode, and re-upload the results.

This is done by browsing (button on the right hand side of the screen) to the correct Excel-file (each session requires a new excel-sheet). All participants will automatically receive an e-mail, stating that the result is available. They have to visit the website (www.swab2know.eu), choose 'check your result'. Then, during the process of accessing their result, they will need to provide their e-mail address, password, and sample code.

A.4 FOLLOW UP OF THE PARTICIPANTS

A few days after uploading the results, you may check who has picked up his/her result.
This is done in the administrator module, under the tabpage "Samples". Click on the first icon, the one located below "Samples":

An excel-file which all sample codes and email addresses listed will be generated. The excel file contains the following information:

- Sample ID: not important, is an automatically generated number from the database system
- SampleCode: the sample code that you created
- Result: test result
- ResultDate: date on which the test result was uploaded onto the website
- ResultConsultedDate: date on which participant picked up his/her test result. This is in particular important to check whether participants picked up their result.
- PickedUpInHelpcenter / NotPickedUpInHelpcenter: the colums that you filled in on the Excel-sheet. This can be adapted when you add information in the database.
- MailResultAvailableSent: whether or not (TRUE/FALSE) e-mail informing participant that result is available is sent.

You will be able to see if a participant has checked his or her result (True) or not (false).

A.4.1 Follow up non-reactive samples:

The participants with a non-reactive sample who haven't pick up their result will be contacted by email or phone at least once to remind them that the result is ready. In Belgium, Lieselot Ooms is responsible for the follow-up, and contacts participants. We prefer to call them, but this decision can be adapted locally. Participants could be contacted by email by the partners in each participating country.

A.4.2 Follow up weak reactive sample: when the saliva gives a weak reactive result, it's necessary to repeat the test on a blood sample. Maybe it's a recent infection, most likely it's a false reactive result.
Therefore, it is important to contact participants with a weak reactive result by email or preferably by phone. While waiting for the blood test with clear result, telephone counseling is needed to provide more information and to reassure.

A.4.3 Follow up reactive sample:

It's necessary to repeat the test using a blood to confirm the test result, and to come to a diagnose with HIV.
If the participant doesn't collect his result, he or she must be contacted several times by email or by phone. The number of times can be adapted locally.

There's always the responsibility of the partners to motivate participants to pick up their result; but participants also have (1) a responsibility, and (2) the right to withdraw from the project at any time during the process (including the moment of picking up their test result). The ITM (Belgium) always contacts participants, even if they have picked up their results, to counsel them, and to invite them to attend the organization headquarters for confirmation test, or to refer them to a local health care facility where they can access a confirmation test. If the participant prefers to take a confirmation test elsewhere, he or she will be followed-up about their confirmation test result.

B. Online test

B.1 PREPARATION:

B.1.1 Sample codes:

Generate enough sample codes (340 for example), it will save you a lot of

time. HOW?

Enter to the administrator link: https://admin.swab2know.eu.

Go to the tabpage “Samples”. Enter the number of samples you want to generate and click on “Generate new samples”. Enter a multiple of 34 (there are 34 sample codes for 1 hard-copy page of labels), and generate the sample codes. An excel-file will be generated. You should now save this file.

Whenever you create new codes, paste them in an excel-file (Annex XX, this is not the same as the ‘result Excel sheet’ described above). All codes are to be linked to the correct addresses (email and postal address).

For example:

Sample code	e-mail address	Postal address	Date kit sent	Date kit received
A1505172923154				
A1505172923155				
A1505172923156				
A1505172923157				

Beware that the second sample is in red, indicating that it hasn’t arrived yet.

B.1.2 Preparing sampling packets:

An individual packet for each participant will be prepared. Each packet will contain the following items:

- Completely white envelope without any logo (in order to guarantee discretion) (230 X 310 mm)
- swab with 2 labels:
    - 1 label with sample code
    - 1 label where participants will write their email address and date of sampling (Annex XX)
- Business card with sample code and the link of the project website (Annex XX)
- Information leaflet with the instructions to collect the sample, send it to the lab and how to get the results.
- condom and lubricant
- printed and prepaid envelope (240 X 160 mm), so the participant send back his or her sample, free of charge. Laboratory mail address and name of lab-reference person

should be addressed.

B.2 SENDING SAMPLING PACKETS:

When people order a sampling kit directly through the website, the order will be saved in the admin tool. Under the tab 'Testkits' you find a list of participants who ordered a kit.

You find 6 columns on this page:

1. a packet-code, automatically generated by the 'system'
2. name of the person who ordered the kit
3. Delivery on address / picks up kit elsewhere: during the process of ordering a kit, participants may choose to have it sent to their home, or pick it up at a health care facility/NGO headquarter (in case they don't want to provide their postal address, for instance). Their choice how to receive the kit can be found here.
4. Date and time when they ordered the kit
5. If ticked, the packet is sent to them
6. If ticked (you should do it yourself), packet has arrived back at the lab

Once you have sent a packet to a participant, you tick the 'packet sent' box. In the Excel-list of ordered kits (see above), complete information

Sample code	e-mail address	Postal address	Date kit sent	Date kit received
A1505172923154	tests2k@gmail.com	Chimaysteenweg 22, 2650 Edegem	20/10/2015	
A1505172923155	tplato@itg.be	Louis Tobacklei 4, 9000 Gent	20/10/2015	
A1505172923156	liesooms@itg.be	Kerkstraat 14A, 9140 Temse	20/10/2015	
A1505172923157	kmaikels@gmail.com	Kerkplein 46, 8840 Westrozebeke	20/10/2015	

Samples arrive at the laboratory:

Create an Excel-sheet for uploading test results. This file is the same as above. The lab person will add test results in the excel file (cfr. above)

Adapt the excel file for ordered kits, as shown below:

Sample code	e-mail address	Postal address	Date kit sent	Date kit received
A1505172923154	tests2k@gmail.com	Chimaysteenweg 22, 2650 Edegem	20/10/2015	27/10/2015
A1505172923155	tplato@itg.be	Louis Tobacklei 4, 9000 Gent	20/10/2015	
A1505172923156	liesooms@itg.be	Kerkstraat 14A, 9140 Temse	20/10/2015	27/10/2015
A1505172923157	kmaikels@gmail.com	Kerkplein 46, 8840 Westrozebeke	20/10/2015	27/10/2015

Note that as a supplementary check, we write samples sent (not arrived) in red, arrived in black.)

B.3 FOLLOW UP

Cfr. testsessions.

Annex 4: Standard operating procedures for the use of Genscreen™

Standard Operating Procedure

Title: Screening for HIV on oral fluid: Genscreen™ HIV-1/2 v2

Subtitle: Genscreen HIV ½ v2 (BioRad - ref nr 72279 - 5 plates)

Table of Contents

1. General Information

1.1 Aim and application

- The acquired immunodeficiency syndrome is a virus induced infectious disease characterized by strongly depressed immunity. The Genscreen™HIV1/2 Version 2 allows simultaneous detection of anti HIV-1 and anti HIV 2 antibodies in blood.
- Screening on oral fluid specimen is not a gold standard for HIV antibody testing because of a lower presence of antibodies in oral fluid and the absence of antigen. On the other hand oral fluid HIV reactive samples should be confirmed on a blood specimen tested by the national HIV algorithm. Oral fluid testing can be a useful tool for research purposes as an orientation test for

HIV outreach testing and for epidemiological studies. Therefore screening tests using oral fluid specimen are considered as indicator tests for a possible HIV infection.

- This test is used to detect HIV antibodies on oral fluid using ELISA.

1.2 Principle

- Genscreen™HIV1/2 Version 2 is an enzyme immunoassay based on the principle of the two step sandwich technique for the detection of the various antibodies associated with HIV-1 and/or HIV-2 virus in human serum, plasma or oral fluid. The test is based upon the use of a solid phase coated with purified antigens (gp160 and p25) recombinant proteins of HIV-1 and a peptide mimicking the immunodominant epitope of the HIV-2 envelope protein) and of an antigens-peroxidase conjugate (peptides mimicking the immunodominant epitopes of the HIV-1 and HIV-2 envelope glycoproteins , and nucleocapsid recombinant protein).
- The assay procedure includes the following reactional steps:
  - The oral fluid samples and controls to be assayed are pipetted into the microplate wells. HIV-1 and/or HIV-2 antibodies, if any, bind to the antigens immobilized on the solid phase.
    Sample deposition is validated through a colour change, from purple to blue.
  - Peroxidase labelled purified HIV-1 and HIV-2 antigens are added. They bind in turn to the IgG, IgM or IgA captured on the solid phase, from the patient samples.
  - The presence of the enzyme immobilized on the complexes is shown by incubation in the presence of the substrate, after the unbound conjugate fraction has been removed.
  - The reaction is stopped and the absorbances are read using a spectrophotometer at 450/620-720 nm. The absorbance measured on a sample allows the presence of absence of HIV-1 and/or HIV-2 antibodies to be determined.
    In order to validate the specimen, a total IgG ELISA will be performed (see SOP Human IgG determination).

1.3 Apparatus, equipment and materials required provided in kit

- R1 Microplate
- R2 Concentrated Washing solution (20X)
- R3 Negative Control
- R4 Cut-off Control
- R5 Positive Control
- R6 Sample Diluent
- R7a Conjugate
- R7b Conjugate diluent
- R8 Peroxidase Substrate Buffer
- R9 Chromogen
- R10 Stopping solution

1.4 Apparatus, equipment and materials required not provided in kit

Phosphate buffered saline (PBS) (1X) + Tween 20:

Prepare this bufferfirst and then use it for the preparation of the recovery buffer (RB).
Add 10 tablets Oxoid (PBS) to 1l H_2O_{demi} + 2000 µl Tween 20 (=0.2%)

Shelf life: 3 months at 2-8°C.

Recovery buffer (transport medium):

- Phosphate Buffered Saline (pH 7.2) + 0.2% Tween 20	90 ml
- 10% Foetal Calf Serum	10 ml
- 0.5% Gentamicin (50mg/ml stock)	500 µl
- 0.2% Fungizone (250µg/ml stock)	200 µl
	100 ml

Shelf life 1 month after adding Tween 20 at 2-8°C

Apparatus and equipment:

- ELISA reader (filters 405,450 and 620-700 nm)
- washer (flat bottom 96-well)
- incubator (37°C +1°C)
- refrigerator (2-8°C) and freezer (-20°C)
- vortex
- centrifuge (swabs diameter 16 mm)
- calibrated micropipettes (10, 200, 1000 µl)
- multichannel (25,300 µl)
- pipetboy
- pipettes 5, 10, 25 ml
- tubes and recipients for buffers
- cryotubes (2 ml)
- deionized or distilled H2O
- labels for specimens
- graduated cylinder (500/1000ml)
- timer
- dilution rack
- plate sealer

1.5 Sample for analysis

- Oral fluid (OF) is collected by using an ORACOL swab. By rubbing the sponge for 1 minute gently along the gums (lower or/and upper). OF specimens can be stored up to 7 days at room temperature. OF can be tested immediately or can be frozen at -20°C until testing.

2. Responsibilities

- Only declared competent Medical laboratory technologist can perform the test, execute the data entry, review the entered results and compare with the raw data.
- Validation of the results is done by the laboratory manager or supervisor.

3. Definitions and abbreviations

ELISA	Enzyme linked immunoadsorbent assay
CO	Cut-Off
HIV	Human Immunodeficiency Virus
IRC	Internal run control
MLT	Medical Laboratory Technologist
OD	Optical density
OF	Oral fluid
RB	Recovery buffer
PBS	Phosphate buffered saline
RT	Room temperature
TMB	Tetramethylbenzidine

4. Method

4.1 Sample preparation

- Add 1 ml Recovery buffer (transport medium) to the Oracol swab.
- Agitate the swab, use bench vortex for 20 seconds to ensure foaming of transport medium.
- Remove the swab from the tube using a twisting motion to extract as much liquid as possible from the swabs.
- Invert swab and replace it in the tube so that the pink foam is now at the top of the tube. Replace the cap.
- Centrifuge at 2000 rpm (~ 805 g) for 5 minutes.
- Discard the swab.
- Extracted oral fluid can now be recovered from the tube, using a pipet (or Pasteur pipet).
- Store at -20°C prior to testing.

4.2 Kit Storage Conditions

- All un-opened kit materials are stable at 2-8°C until the expiration date mentioned on the package except specific instruction:
  - R1 (microplate): After the vacuum-sealed bag has been opened, the microwell strips stored at +2-8°C in the carefully resealed bag can be used for 1 month
  - R2 (concentrated washing solution): The diluted washing solution can be stored at +2-30°C during 2 weeks. The concentrated washing solution can be stored at +2-30°C.
  - R7a +R7b (conjugate + conjugate diluent): The reagents stored at +2-8°C can be used for 4 weeks after the vials have been opened and reconstituted.
  - R8 + R9 (substrate + substrate buffer): After reconstitution, the reagent stored in the dark can be used for 6 hours at RT (18-30°C).
- Do not use kit components beyond the expiration date.

4.3 Testing Procedures

- At all steps, apply Good Laboratory Practice and follow the instructions of the leaflet that will be summarized in this SOP.
- Bring all reagents and specimens to RT before use. To avoid condensation, allow micro-well strips to reach RT before opening the foil packet. If less than a full plate is to be used, return unused strips to the foil packet with desiccant and reseal completely. Store unused strips at 2-8° C.
- Prepare identification plan or worksheet. Prepare washing solution starting from the concentrated washing solution (20X) and carrier with strips.
- Apply 25µl diluent in each well.
- Apply 75µl of the specimens to the appropriate wells.
- Apply 75µl of negative control (A1), cut off control (B1 – C1 – D1) and positive control (E1).
- Homogenize by a minimum of 3 aspirations by pipette.
- Cover plate with a sealer.
- Incubate at 37°C (± 1°C) for 30 minutes (± 5 minutes).
- Remove the sealer, empty all wells by aspiration and wash a minimum of 3 times (0.370 ml washing solution and soak time of 30 seconds). Repeat this procedure 2 times (3 washes). The residual volume must be lower than 10 µl
- Tap the plate upside down to remove any residual liquid on absorbent paper.
- Dispense 100µl conjugate into all wells.
  Remark: Conjugate must be shaken gently before use.
- Cover plate with a sealer.
- Incubate at RT (18-30°C) for 30 minutes (± 5 minutes).
- Remove the sealer, empty all wells by aspiration and wash a minimum of 5 times as described above.
- Tap the plate to remove any residual liquid on absorbent paper.
- Dispense 80µl substrate (freshly prepared before use) into all wells.
- Incubate in the dark at RT (18-30°C) for 30 minutes (± 5 minutes) (do not cover the strips).
  Remark: turn on reader.
- Add 100 µl stopping solution into each well. Positive specimens will become yellow.
- Carefully wipe the bottom of the plate.
- Read the optical density at 450/620-700nm at least 4 minutes and within 30 minutes after adding stopping solution.

5. Quality Control

- Each plate run must include a negative control serum R3, a cut-off control serum R4 a positive control serum R5 and an internal run control (2):
  1) IRC swab: prepare a dilution of 1/100 of an HIV reactive specimen in HIV negative OF (derived from a seronegative person). Make aliquots of 200µl and store at -20°C. Before you start to remove OF from the swab, add an aliquot IRC to a clean swab and vortex for 20 sec. Process this swab the same as the specimen swabs. The IRC will be tested in the same way as the other specimens.

2) IRC OF: if possible prepare aliquots of an HIV reactive OF specimen in a dilution 3-5 times the CO of Genscreen. The OF specimen will be diluted in RB. Make aliquots of 100µl and store at -20°C.

- IRC's will be added to the plate at the end of the group of specimens.

6. Technical validation and calculation of cut-off value

- The assay can only be considered valid if:
  - The SOP has been followed correctly.
  - The absorbance of the negative control serum should be less than 70% of the cut-off value:

    ODR3 < 0.7 X C.O.

  - The mean absorbance of cut-off control serum should be greater than 0.80: $\overline{ODR4}$ >0.80
    The ratio ODR5/ODR4 should be greater than or equal to 1.3:

    ODR5/ODR4 ≥1.3

  - The cut-off value is calculated by the mean absorbance of the cut-off control serum ODR4 divided by 10:

    CO = $\overline{ODR4}$/10

  - IRC values (IRC swab and IRC OF) must be reactive. If the IRC value is non reactive, you need to evaluate the procedure of the test and the results first for a possible cause, in order to validate the results.

7. Recording and interpretation of results

- Samples with absorbance values less than the cut-off value are considered to be negative by the Genscreen™ HIV-1/2 version 2 test.
- All Genscreen negative OF test results should be validated for the sample quality through the Human IgG test. (see SOP Human IgG determination)
- Samples with absorbance values equal to or greater than the cut-off value are initially considered to be positive (reactive) by the Genscreen™ HIV-1/2 version 2 test and could be an indication of an HIV infection. Further confirmation on a blood specimen is needed.

8. Clinical validation/release of results

- Not applicable

9. Reporting

- All data are filled in the worksheet
- The results from the datasheet and specimen data will be transcribed into an Excel file in conformity to the procedure MICRO_STD_ PRO_203 'Het invoeren van resultaten (studies en projecten)'

10. Storage of samples

- After analyzing, the samples are stored at -20°C as long as needed for the study.

11. Training

- The trainee follows the complete procedures with an experienced person and then makes an analysis on a blind panel.

12. References

- Leaflet Genscreen™ HIV1/2 Version2: Screening kit for the detection of antibodies to HIV-1 and HIV-2 in serum/plasma by enzyme immunoassay.

13. Method validation and literature

- Katrien Fransen et al. Using Conventional HIV tests on oral fluid. Journal of Virological Methods 194 (2013) 46-51.
- Veronique Hutse et al. Oral fluid as a medium for the detection of hepatitis B surface antigen. Journal of Medical Virology 77 (2005) 53-56

14. Safety and environment

- All manipulations are carried out in a restricted laboratory level 2 in accordance with the Belgium law and in conformity with the quality standards of the HIV/STI reference laboratory and the ITM.
- Some reagent contain sodium azide as a preservative. Sodium azide may react with laboratory plumbing to form copper of lead azides. Such azides are explosive. To prevent azide build-up, flush the pipes with a large quantity of water if solutions containing azides are disposed of in the sink after inactivation.
- The stop reagent contains sulphuric acid. Do not allow to contact skin or eyes. If exposed, flush with copious amounts of water.

15. Attachments and forms for completion

- Worksheet: MICRO_ARL_ F_0001

16. Revision

- New SOP

17. Approval and distribution

Approval and distribution	
Initiated by:	Tine Vermoesen

Approved by:	Katrien Fransen
Manual distribution:	Present in the serology laboratory

Annex 5: Standard operating procedures for human IgG determination

Standard Operating Procedure

Title: Human IgG determination

Subtitle: Human IgG ELISA (Immunoperoxidase Assay for Determination of Total IgG in Human Sera (ICL – ref nr E-80G – 1 plate)

Table of contents

1. General Information

1.1 Aim and application

- The total Human IgG test kit is a highly sensitive two site enzyme linked immunoassay (ELISA) for measuring IgG in Human biological samples.

1.2 Principle

- The ELISA is based a double antibody sandwich principle. In this way the IgG present in samples reacts with the anti-IgG antibodies which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, anti-IgG antibodies conjugated with horseradish peroxidase, are added. These enzyme-labeled antibodies from complexes with the previously bound IgG. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine. The quantity of bound enzyme varies directly with the concentration of IgG in the sample tested; thus the absorbance, at 450 nm, is a measure of the concentration of IgG in the test sample. The quantity of IgG in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

1.3 Apparatus, equipment and materials required provided in kit

- Diluent concentrate
- Wash solution concentrate
- Enzyme-antibody conjugate
- Chromogen-substrate solution
- Stop solution
- Anti-human IgG Elisa micro plate
- Human IgG calibrator

1.4 Apparatus, equipment and materials required not provided in kit

- Distilled water
- Elisa plate strip washer
- 1L graduate cylinder
- 10 µl, 100 µl and 1000 µl pipettors with disposable tips
- Multichanel pipette 100 µl
- 1 ml & 5 ml pipette or dispenser
- Timer
- Vortex Mixer
- Elisa plate spectrophotometer with wavelength 450nm
- Fluid trays
- 70% ethanol for decontamination
- Sealers
- Gloves
- Disposable paper towels

1.5 Sample for analysis

- Serum, plasma or Oral fluid specimen of humans can be used.

- OF is collected by using an ORACOL swab. By rubbing the sponge for 1 minute gently along the gums (lower and/or upper). OF specimens can be stored up to 7 days at room temperature. OF can be tested immediately or can be froze at -20°C until testing.

2. Responsibilities

- Only declared competent medical laboratory technologist van perform the test, execute the data entry, review the entered results and compare with the raw data.
- Validation of the results is done by the laboratory responsible or head of the laboratory.

3. Definitions and abbreviations

ELISA	Enzyme linked immunoadsorbent assay
CO	Cut-Off
HIV	Human Immunodeficiency Virus
IRC	Internal run control
MLT	Medical Laboratory Technologist
OD	Optical density
OF	Oral fluid
RB	Recovery buffer
Ab	Antibody
IgG	Immunoglobulin Gamma
PBS	Phosphate buffered saline
RT	Room temperature
TMB	Tetramethylbenzidine
RB	Recovery Buffer (or transport medium)

4. Method

4.1 Sample preparation (see also SOP Genscreen HIV ½ v2)

- Add 1 ml recovery buffer (transport medium) to the Oracol swab.
- Agitate the swab, use bench vortex for 20 seconds to ensure foaming of transport medium.
- Remove the swab from the tube using a twisting motion to extract as much liquid as possible form the swabs.
- Invert swab and replace it in the tube so that the pink foam is now at the top of the tube.
- Replace the cap.
- Centrifuge at 2000 rpm (800g) for 5 minutes.
- Discard the swab.
- Extracted oral fluid can now be recovered from the tube, using a pipet (or Pasteur pipet).
- Store at -20°C prior to testing.

4.2 Kit storage conditions

- All un-opened kit materials are stable at 2-8°C until the expiration date mentioned on the package except specific instruction:
  - Prepared sample diluent: stable for one week at 2-8°C
  - Prepared wash solution: stable for one week at 2-8°C
  - Do not use kit components beyond the expiration date.

4.3 Testing procedures

- At all steps, apply Good Laboratory Practice and follow the instructions of the leaflet that will be summarized in this SOP (see below).
- Prepare 1x diluent solution: for 1 plate: 20ml 5x diluent + 80ml deionized or distilled H2O (stable for 1 week, store at 2-8°C).
- Prepare wash solution: for 1 plate: 40ml 20x wash solution + 760ml deionized or distilled H2O (stable for 1 week, store at 2-8°C).
- Dilution of the samples: the OF samples need to be diluted 1/250: 3µl OF + 747µl 1x diluent (mix well).
- Prepare Human IgG standards (freshly prepared before use): check leaflet for current concentration of the Human IgG calibrator (different from lot to lot) and follow 2 fold dilutions steps as described in the leaflet.

Standard	ng/ml	Volume added to 1x diluent	Volume of 1x diluent
A	See leaflet	See leaflet	See leaflet
7	500	See leaflet	See leaflet
6	250	300µl stand 1	300µl
5	125	300µl stand 2	300µl
4	62.5	300µl stand 3	300µl
3	31.25	300µl stand 4	300µl
2	15.6	300µl stand 5	300µl
1	7.8	300µl stand 6	300µl
0 (blanco)	0		300µl

- Prepare identification plan.
- Pipette 100µl of standard 0-7 in duplicate according to identification plan.
- Pipette 100µl of specimen according to identification plan.
- Cover plate with a sealer.
- Incubate at RT for 60 min (± 2 minutes).
- Remove the sealer, empty all wells by aspiration and wash 4 times.
- Tap the plate upside-down to remove any residual liquid on absorbent paper.

- Freshly prepare before use. For 1 strip: 10µl enzyme-antibody conjugate to 990µl 1x diluent and mix gently, avoid foaming (stable for 1h when stored in the dark).
- Pipette 100µl of the diluted conjugate to each well.
- Incubate at RT for 20 min (± 2 minutes) in the dark.
- Empty all wells by aspiration and wash 4 times.
- Tap the plate upside-down to remove any residual liquid on absorbent paper.
- Pipette 100µl of TMB substrate solution (ready to use) to each well.
- Incubate in the dark for precisely 10 min.
- Add 100 µl stop solution to each well.
- Read the optical density at 450nm. (read as soon as possible but stable up to 2h after adding stop solution).

5. Quality Control

- Each plate must include standard series in duplo, a positive OF (IRC) and a positive OF internal run control.
    1. Standard series: see above
    2. IRC OF: prepare a dilution of 1/100 of an HIV reactive plasma/serum specimen in HIV negative OF (derived from a sero-negative person). Make aliquots of 200µl and store at -20°C. Before preparing the OF specimens for testing, add an aliquot IRC to a clean swab and vortex for 20 sec. Process this swab the same as the specimen swabs. The IRC will be tested in the same way as the other specimens.
    3. IRC OF: if possible prepare aliquots of an HIV reactive OF specimen in a dilution 3-5 times the CO of Genscreen. The OF specimen will be diluted in RB. Make aliquots of 100µl and store at -20°C.

- IRC's will be added to the plate at the end of the set of specimens.

6. Technical validation

- The assay can only be considered valid if:
    - The SOP has been followed correctly
    - To assess the quality of the standard curve, the back-fitted standard concentration values must be in an acceptable range of +/- 15% of the nominal standard concentration values. If not, you need to repeat the IgG test.

      BC = (NC +/- 15%)

      Also the back-fitted curve must fit the known values of the standards visually well, if there is a big deviation between fitted curve and known values (see curve) you need to repeat the IgG test.
    - IRC values (IRC swab and IRC OF) must be reactive. If the IRC value is non reactive, you need to evaluate the procedure of the test and the results first for a possible cause, in order to validate the results.

7. Recording and interpretation of results

- Subtract the average background value from the test values for each specimen.
- Construct a standard curve: the appropriate curve fit is a four-parameter logistics curve.
- Calculate concentration IgG for each specimen, correct for the dilution factor (250) to obtain the IgG concentration of the original specimen.
  Remark: useful tool for standard curves: R is a free software environment for statistical computing and graphics. It compiles and runs on a wide variety of UNIX platforms, Windows and MacOS.
- ≥3500 ng/ml total IgG is considered as sufficient IgG for acceptance of an Genscreen negative test result.
- If the specimen contains <3500 ng/ml total IgG, the specimen is repeated in duplo (= 2 tubes with a 1/250 dilution of the specimen, 1 tube for 1 well) in a next run. Calculate the IgG concentration for each dilution (= 2 values in total), if the IgG concentration remains < 3500 ng/ml, the specimen is invalid for HIV testing. If the IgG concentration is > 3500 ng/ml, you consider the specimen valid for HIV testing.

8. Clinical validation /release of results

- Not applicable

9. Reporting

- All data are filled in the worksheet
- The results from the datasheet and specimen data will be transcribed into an Excel file in conformity to the procedure MICRO_STD_PRO_203 'Het invoeren van resultaten (studies en projecten)'.

10. Storage of samples

- OF can be stored in the refrigerator up to 7 days, after that it must be stored at -20°C.

11. Training

- The trainee follows the complete procedures with an experienced person and then makes an analysis on a blind panel.

12. References

- Leaflet Human IgG ELISA (Immunology Consultants Laboratory, Inc, Portland, OR USA)
- Article R program

13. Method validation and literature

- Not applicable

14. Safety and environment

- All manipulations are carried out in a restricted laboratory level 2 in accordance with the Belgian law and in conformity with the quality standards of the HIV/STI reference laboratory and the ITM.
- Some reagent contain sodium azide as a preservative. Sodium azide may react with laboratory plumbing to form copper of lead azides. Such azides are explosive. To prevent azide build-up, flush the pipes with a large quantity of water if solutions containing azides are disposed of in the sink after inactivation. The stop reagent contains sulphuric acid. Do not allow to contact skin or eyes. If exposed, flush with copious amounts of water.

15. Attachments and forms for completion

- Worksheet: MICRO_ARL_F_0001

16. Revision

- New SOP

17. Approval and distribution

Approval and distribution	
Initiated by:	Tine Vermoesen
Approved by:	Katrien Fransen
Manual distribution:	Present in the serology laboratory

Annex 6: Instructions for use of DPP rapid test

DPP® HIV 1/2 Assay — **Quick Reference Instructions**

CHEMBIO DIAGNOSTIC SYSTEMS, INC.

ORAL FLUID Test Procedure

For *IN VITRO* diagnostic use

CLIA Complexity WAIVED for oral fluid, fingerstick whole blood, and venous whole blood.

- Before performing testing, all operators MUST read and become familiar with Universal Precautions for prevention of transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and other blood borne pathogens in Health-Care Settings.
- These instructions are only a Reference Guide for use in CLIA waived settings. Read the complete procedure, including the QC procedure, before performing the test.
- **Laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test. Any modification by the user(s) to the manufacturer's test procedures will result in the test no longer meeting the requirements for waived classification.**

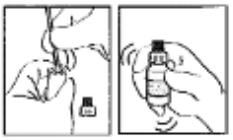
External Quality Control:

A Chembio DPP® HIV 1/2 Rapid Test Control Pack is available separately for use with the Chembio DPP® HIV 1/2 Assay. The HIV Controls are used to verify the operator's ability to properly perform the test and interpret the results. Run the controls as described in the Test Procedure section for a whole blood sample and follow the Interpretation of Results section below. We recommend running controls: • with each new operator • with each new device lot • with each new shipment of devices • if improper device storage is suspected and • at periodic intervals. It is the responsibility of each facility using the Chembio DPP® HIV 1/2 Assay to establish an adequate quality assurance program to ensure the performance of the device in their environment and conditions of use.

IMPORTANT

Follow the oral fluid sample collection instructions carefully, as not doing so may produce incorrect results.

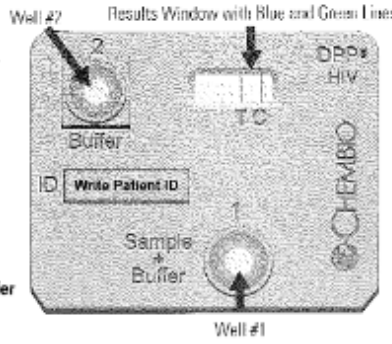
- Slowly swab upper & lower gums in a circular motion 4 TIMES for at least 30 SECONDS.
- IMPORTANT: Collection time of 30 seconds is needed for accurate results.
- DO NOT SWAB roof of mouth, inside of cheek or tongue.
- Insert Oral Fluid Swab into DPP® SampleTainer® bottle and snap at break notch
- Replace black/white cap and SHAKE the DPP® SampleTainer® bottle for at least 10 SECONDS



BEFORE YOU BEGIN

- Gather the materials you will need.
- Cover your work space with a clean, disposable absorbent workplace cover.
- Put on disposable gloves.
- Let the test reach room temperature (between 18-30°C or 64-86°F) before opening the pouch.
- Remove the DPP® HIV 1/2 test device from pouch and become familiar with it.

DPP® HIV 1/2 Test Device



The DPP® HIV 1/2 Assay kit contains:

- 20 DPP® HIV 1/2 Individually Pouched Test Devices
- 20 Disposable Sample Loops (10µL)
- 20 Oral Fluid Swabs
- 20 DPP® SampleTainer® Bottles (1mL, Black and White Cap)
- 1 DPP® Running Buffer Bottle (6mL, Green Cap)

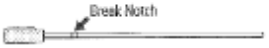
DPP® Running Buffer Bottle

Materials required but not provided:

- Clock, watch or other timing device
- Sterile gauze
- Disposable gloves
- Antiseptic wipes
- Biohazard disposal containers
- Sterile Safety Lancet (for fingerstick)
- Collection devices for venous blood

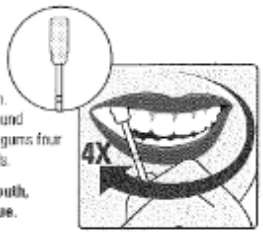
DPP® SampleTainer® Bottle

- Before collecting the sample, **write the sample ID on the DPP® SampleTainer® bottle** (with the black and white cap).
- Unscrew the WHITE CAP keeping the BLACK CAP in place.



Oral Fluid Swab

1 Obtain Sample



- Insert Oral Fluid Swab into mouth above teeth and against outer gum as shown.
- Gently swab completely around both upper and lower outer gums four times for at least 30 seconds.

DO NOT SWAB: roof of mouth, inside of cheek, or tongue.

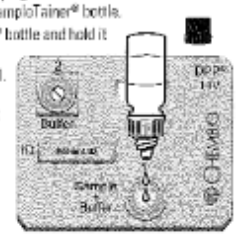
2 Add Sample to DPP® SampleTainer® Bottle

- Insert Oral Fluid Swab into DPP® SampleTainer® bottle with swab resting on bottom of bottle.
- Snap shaft at break notch to release tip of swab into bottle as shown.
- Replace black/white cap assembly onto bottle and shake bottle for at least 10 seconds.

3 Add Sample to Test Device Well #1

- Unscrew the **BLACK CAP** keeping the **WHITE CAP** with the dropper tip on the DPP® SampleTainer® bottle.
- Invert the DPP® SampleTainer® bottle and hold it VERTICALLY (not at an angle) over SAMPLE-BUFFER Well #1.
- Slowly add 2 FULL drops of sample into SAMPLE-BUFFER Well #1

Important: Adding less than 2 drops may produce invalid results.



Wait 5 minutes. After 5 minutes, the blue and green lines should disappear from the Results Window. If they do not disappear, discard test device and repeat the procedure with a new DPP® test device.

4 Add Running Buffer to Test Device Well #2

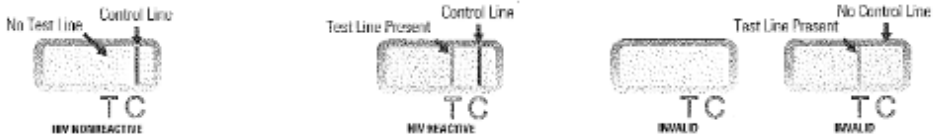
- **Five minutes** after adding the sample, add 4 drops of Running Buffer (green cap) into BUFFER Well #2. Hold bottle VERTICALLY.

Important: Adding less than 4 drops or more than 8 drops of Running Buffer may produce invalid results.

- Read the test result between 25 and 40 minutes after adding Running Buffer to BUFFER Well #2. If a test is nonreactive, you MUST wait the full 40 minutes to report a nonreactive result.

Interpretation of Results: ORAL FLUID

ADEQUATE LIGHTING REQUIRED



NONREACTIVE RESULT

One pink/purple line in the CONTROL (C) area, with no line in the TEST area, is a Nonreactive result. A nonreactive result at **40 minutes** means that HIV antibodies were not detected.

The test result is NEGATIVE for HIV antibodies. However, **this does not exclude possible infection with HIV.**

REACTIVE RESULT

A pink/purple line in the CONTROL (C) area, with a line in the TEST area, is a Reactive result. If ANY VISIBLE LINE appears in the TEST area and in the CONTROL area, no matter how faint, the result is Reactive. A reactive result **between 25 and 40 minutes** means that HIV antibodies were detected.

A REACTIVE test result means that HIV antibodies were detected in the specimen. The test result is Preliminary POSITIVE for HIV antibodies.

INVALID RESULTS

If no control line appears, results are invalid whether or not a test line is present.

An INVALID test result means there was a problem running the test. The problem could be due to the specimen, the test device or the procedure.

An INVALID test result cannot be interpreted. An INVALID test should be repeated with a new device.

Contact Chembio Diagnostic Systems if you are unable to get a valid test result upon repeat testing.

Annex 7: Full messages that participants receive

SWAB 2 KNOW

Doe de test swab2know.be

Strong reactive sample

Your saliva has reacted to the test performed to detect HIV.

This result means that there is a risk that you carry an HIV infection. However, in order to be able to give a definitive diagnose, it is necessary to perform a blood test to confirm this result. Please note that a saliva test is not yet officially recognized to pose a diagnosis of HIV infection.

What can you do?

You can contact a physician (either your GP or www.helpcenteritg.be), where a blood sample will be taken to confirm or exclude this HIV test result. We advise you to adopt safe sex practices in the meantime. We will contact you in the future to get a feedback on this result.

You can make an appointment at a specialized HIV treatment center: https://www.swab2know.be/en/arc.aspx.

If you want you can contact us by phone during business hours at 03 247 64 33 (Tom Platteau, study coordinator) or 0474 10 20 79. If you do not receive an answer at this number, you can also call the physician on duty at the HIV clinic of the Institute of Tropical Medicine at 03 247 64 65. These persons will answers your urgent questions and will refer you to an urgent medical consultation if needed.

For more information about HIV and AIDS, please visit http://www.soaaids.nl/english/stihivaids

Finally, it would help us enormously if you took the time to complete a small questionnaire: https://www.swab2know.be/en/account/satisfaction-questionnaire.aspx.

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Helpcenter voor seksuele gezondheid

SWAB 2 KNOW

Doe de test swab2know.be

Weak reactive sample

Your saliva has weakly reacted to the test performed to detect HIV.

This result means that there is a possibility that you carry an HIV infection. However, previous research results have learnt that a weak reaction could also indicate that the test has detected antibodies for another viral infection. Therefore, the risk that you are infected with HIV is very small.

THEREFORE, IT IS ABSOLUTELY NECESSARY TO TAKE A CONFIRMATION TEST ON A BLOOD SAMPLE TO OBTAIN CERTAINTY ON YOUR HIV-STATUS.

You can contact a physician (either your GP or www.helpcenteritg.be), where a blood sample will be taken to confirm or exclude this HIV test result. We advise you to adopt safe sex practices in the meantime. We will contact you in the future to get a feedback on this result.

If you want you can contact us by phone during business hours at 0474 102079 (cell phone swab2know), or at 03/247 64 33 (Tom Platteau, study coordinator).

If you do not receive an answer at this number, you can also call the physician on duty at the HIV clinic of the Institute of Tropical Medicine at 03/2476465. They will answers your urgent questions and will refer you to an urgent medical consultation if needed.

For more information about HIV and AIDS,
please visit http://www.soaaids.nl/english/stihivaids

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SWAB 2 KNOW

Doe de test swab2know.be

Non-reactive sample

Your saliva has not reacted to the test. We can conclude with high reliability that you are not infected with HIV. Please note that an HIV test does not give any information about the risk you may have had during the last three months. Furthermore an oral fluid test is not officially recognized to pose a diagnose of HIV infection.

If you ran a sexual risk during the last three months it is better to have a combined HIV test performed on your blood (antibody and antigen determination). This give better (but not absolute) results for recent risks. After having had unsafe sexual contacts, it can be important to test for sexually transmitted infections. To discuss the necessity of such tests, you can contact your GP, a HIV specialized treatment center or Helpcenter. (www.helpcenteritg.be)

When you have casual sex with different partners, it is recommended to have an HIV test every 3-6 months. We 'd like to contact you by email or text message in 3-6 months so you can order a test kit for a new oral fluid sample. If you don't want to receive this email, please let us know by changing the settings of your account.

If we send a test to you, you can collect the sample yourself and send it to ITM's laboratory using the envelope provided. The procedure for obtaining your result will be the same.

We would like to ask you some additional questions on this project.
Please follow the link to the questionnaire:
https://www.swab2know.be/en/account/satisfaction-questionnaire.aspx

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voor seksuele gezondheid

SWAB 2 KNOW

Doe de test swab2know.be

Invalid sample

Due to an invalid sample, we cannot provide you with a reliable result. This is most likely because your sample didn't contain enough oral fluid for a reliable analysis. We advise you to take a new test to know your HIV-status.

What can you do?

You can contact a physician (either your GP or Helpcenter), to have a blood sample taken. We advise to adopt safe sex practices in the meantime.

You can make an appointment at a specialized HIV treatment centre:
https://www.swab2know.be/en/arc.aspx

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