Cross-sectional study of community serostatus to highlight undiagnosed HIV infections with oral fluid HIV-1/2 rapid test in non-conventional settings

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SUMMARY

The submerged portion of undiagnosed HIV infection in Italy is about 30% of subjects found seropositive. This fact represents one of the most important public health problems hindering the control of infection progression. This means we need to fight unawareness and social stigma and promote easy and friendly access to HIV test. We developed a Prevention Program called "*EASY test Project*", offering a new rapid HIV test on oral fluid, to evaluate the acceptability of an alternative, free and anonymous test available in different settings (on board a "Motor Home" at public events, Points of Care, STDs outpatient prevention units and GP surgeries). From December 2008 to December 2012 we performed 7,865 HIV saliva tests, with 50 new infections found (0.6% of the total) out of 140,000 informed subjects. From the self-reported characteristics of respondents, the population approaching the EAST test project was represented by males (70%) aged between 20 and 50 years, 61% with a medium-high education level, 62% homosexuals (MSM), 88% reported unsafe sexual behaviours, and 48% had never undergone an HIV screening test. In five years of the Prevention Program, 100% of subjects interviewed gave a general favorable consent in approaching rapid and not invasive screening, immediate return of the result, and a timely specialized approach and treatment of HIV positive subjects. Results from our study confirm that the rapid and alternative test may contribute to HIV prevention strategies and to the control of the spread of infection and HIV disease progression by reaching a larger population, particularly when and where regular screening procedures are difficult to obtain or are not preferred.

KEY WORDS: HIV screening test, Late HIV diagnosis, Saliva/oral fluid test, HIV positive submerged people, HIV prevention.

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INTRODUCTION

It is estimated that one-third of HIV-infected persons in the European Union and one in every five in the United States do not know their serological status (Deblonde *et al.*, 2011). Those diagnosed with advanced immune deficiency are known to suffer greater morbidity and mortality (Moreno *et al.*, 2010). Moreover, persons who are unaware of their infection have a transmission rate 3.5 times higher than those who know their serological status (Thompson *et al.*, 2010; Borghi *et al.*, 2008). Thus, the earliest possible diagnosis is one of the most efficient strategies to control the epidemic, and is currently a priority for all infection control plans (National HIV/AIDS Policy, 2010).

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To date in Italy, the submerged portion of undiagnosed HIV infection in Italy is probably about 30% (30-50,000) of seropositive subjects. This fact represents one of the most important public health problems in the field of sexually transmitted diseases (STDs), as is the main deterrent to controlling the progression of the infection to the disease and the spread of the HIV epidemic (D'Amato *et al.*, 2010). Failure or delay in the diagnosis of infection make it difficult to establish a timely and effective treatment and the implementation of all the provisions of appropriate prophylaxis to prevent HIV transmission (Antinori *et al.*, 2010). Timely and effective treatment, and the implementation of the prophylaxis guidelines to prevent the transmission of HIV infection are not possible when the diagnosis of HIV infection is missed or late. Although the current legislation in Italy and the offer of conventional testing by taking blood samples should theoretically be able to guarantee all citizens, the opportunity to obtain a diagnosis of infection free of charge, in reality, individual preference, the procedures and rules for the implementation of testing currently do not solve the problem of "hidden" infection (http://www.salute.gov.it).

For many years, the European Community has advocated that countries should facilitate access to testing, but for various reasons, individual countries can provide only few programs or models of intervention aimed at increasing diagnosis in high risk populations.

In recent years, inadequate information and prevention campaigns in Italy, the medicalization of the tests and the fear of the dreaded diagnosis have contributed to stay away from the test and to raise the proportion of submerged infection (WHO, 2011).

Several studies conducted in the last 15 years have shown that the frequency of late diagnosis of HIV infection in industrialized countries varies between 15% and 43%. According to data provided by the Italian multicentre study *Italian Cohort Näive Antiretrovirals* (I.Co.NA.), of the 968 patients enrolled from 1997 to 2000, 29% received a diagnosis of infection after developing an AIDS-defining illness or condition due to an already advanced immunosuppression (CD4 + lymphocyte count <200 cells/mmc, defined as *Advanced Näives or AIDS Presenters or symptomatic subjects*) (Girardi *et al.*, 2004). There is a strong need to increase awareness, reduce social stigma and facilitate the access to HIV testing. For many years this issue represents the European community goal. It is clear that no single prevention approach alone will be effective and relevant to all populations across Italy. However, strategic actions should be implemented to guide prevention programme planning and development.

Testing policies are undergoing extensive review in many countries and international agencies (http://www.salute.gov.it). All new versions are moving toward ending the "exceptionalism" that previously guided them to facilitate test access. The most interesting tool among the new strategies is the use of rapid and point of care tests in different contexts, including non-clinical settings. These tests are easy to use and provide results within 30 minutes, including counselling (CDC, 2011). The tests available require samples of oral fluid or blood. Although the accuracy may be lower than current reference laboratory serology, the specificities and sensitivities of HIV rapid test kits are high and are comparable to first and second generation conventional EIAs (De la Fuente *et al.*, 2012).

The widespread use of a rapid test on oral fluid, with immediate result, is also considered one of the most important tools to reduce the proportion of people who do not return to pick up the results of the test ("*failure to return*"). In fact, among those who are tested conventionally, a high percentage fail to return to collect their results. In contrast, studies conducted using the rapid test showed that the percentage of failure to return in some cases is even zeroed (Marks *et al.*, 2006). Currently in Europe, rapid HIV tests cannot be sold over the counter for self-testing, but the US Food and Drug Administration (FDA) recently approved the OraQuick In-Home HIV test, based on trials conducted by the manufacturer (FDA, 2012). It should be considered that a device for self-testing not free of charge could be the first limit for an extensive use in Italy.

Some authors have proposed that self-testing can be an innovative component of community-wide HIV prevention strategies by providing testing to persons who, for reasons of stigma or confidentiality, do not wish to reveal their sexual practices; such persons could be tested when they perceive they are at risk of infection, something they may not feel they need again for a long time (Spielberg

et al., 2004; Frith *et al.*, 2007; Vv.Aa., 2002; Merson *et al.*, 1997).
On the other hand, Walensky *et al.* (2006) emphasized the possibility that the availability of self-testing devices would lead to a dangerous stress caused by the immediacy of a possible positive result in individuals carrying out self-testing without training or psychological assistance.
Few studies have addressed this topic, but one did not incorporate interpretation and included only seropositive individuals, and another one was conducted mostly in this group and showed disappointing results (Spielberg *et al.*, 2003). Another study reported very good results, but participants either had seen how they were tested immediately before they performed their own self-test, or performed their own test after a demonstration by a counsellor (Choko *et al.*, 2011).
In 2008, the Infectious Diseases Department of San Raffaele Scientific Institute in Milan developed a Prevention Program called the "*EASY test project*", initially to evaluate the quality characteristics of a saliva test under development, with very interesting results. However, the manufacturer did not continue with the registration in Europe (Parisi *et al.*, 2009).
The same project subsequently continued using a new test, the OraQuick Advance HIV-1/2 (Orasure Technologies Inc.). The OraQuick blood test was first approved in 2002 by the FDA, and in 2004 received approval for use with oral fluid. Today it is globally used in clinics and emergency rooms and outreach community testing schemes (http://www.orasure.com).
To date, the main objectives of our work are no longer to test the reliability of the oral fluid rapid tests (sensitivity and specificity), but to evaluate the acceptability of an alternative, free and anonymous HIV test offer, available in different settings (on board a "Motor Home" at public events, Points of Care, STDs outpatient prevention units and GP surgeries). Furthermore, reaching people with this anonymous and free test offer could reduce or stop this public health problem.
From 2008 to date, the EASY test project has been implemented at various times and locations. The first experience was in 2008 using a mobile unit in Turin, followed by public events and prevention programs in Milan in 2009 (*Convivio, Le piazze della salute*) (http://www.conviviomilano.it/). The "Motor Home" (MH), had four working rooms to offer the general population privacy while waiting and during the three different steps.
In 2010, the project evolved and the rapid oral test became regularly available in three different locations in Milan: on the first Friday of the month in two San Raffaele Point-of-Care sites and one STDs Public Prevention Clinic ("*FreeDay Easy-test*"); and has now been extended to six GP surgeries.
The MH program continues to conduct outreach testing twice a year during the Prevention Program of Milan City ("*Le piazze della Salute 2010*") and on 1st December on International World AIDS Day.
Individuals are counselled by an infectious diseases specialist or by a psychologist and sign an Informed Consent Form before taking the test. In addition, for individuals who do not wish to participate in the study, the test was offered free and anonymously with an identification code for subsequent evaluation of the results.
This report summarizes EASY test data collected from 2008 to date for the individuals who participated in the EASY test study (100% of all tests offered).

METHODS

This program has been running since 2008 with the aim of promoting HIV early diagnosis using a rapid and non-invasive oral fluid antibody test. This is a cross-sectional community study, implemented with the support of the Infectious Diseases Department of San Raffaele Hospital, in collaboration with Milan Council, Department of Prevention-Reference Centre for HIV and STDs (Local Public Health Unit in Milan), L. Sacco Hospital of Milan, and supported by ANLAIDS-Lombardia association (National Association for the Fight against AIDS).
The study was conducted in Milan between December 2008 and December 2012 on four different occasions:

1. *Convivio* ANLAIDS-Lombardia;
2. Milan Council ("*L'anno della salute*", "*Le piazze della salute*", "*1st December AIDS World Day*");
3. Two Points of Care at San Raffaele Hospital and one HIV-STDs Public Prevention Clinic ("FreeDay Easy");
4. Six GP surgeries.

During each of these appointments, the work team at San Raffaele Hospital, composed of an infectious diseases specialist and two biologists, with one psychologist and many volunteers from the ANLAIDS-Lombardia association, carried out the EASY test project on board the MH, supported by the work team at L. Sacco Hospital in Milan.
On board the MH there were four different spaces to guarantee the privacy of subjects performing the test: a waiting room with STDs prevention information, a pre-test counselling room, a testing room, and a room for giving back results (eventually blood sampling) and post-test counselling. This so-called "Street Lab" is as close as possible to a traditional laboratory, but the HIV test offer was free of charge and anonymous for the study participants.
One of every two subjects approached the Easy test locations just to collect information on HIV prevention and transmission.
A psychologist explained the study objectives and procedures to all participants and each of them signed an informed consent form. Each subject was allocated an identification code number for testing. Subjects who underwent the test were asked to complete an anonymous questionnaire, through which it was possible to collect a series of data on risk behaviours of the population tested. The questionnaire was intended to collect demographic and risk behaviour data, as well as previous HIV testing experience, questions about sex, educational level, nationality, drug use, HIV testing behaviours and use of HIV prevention services.

Recruitment criteria/ Study enrollment
People eligible for enrollment in the project were aged >18 years, unaware of their HIV-1 serological status and able to complete the questionnaire in Italian or in English. Subjects were informed that they could refuse testing at any time. Post-test counselling was provided to all HIV reactive and non-reactive subjects by the Infectious Diseases Department physicians involved in the study.
All those with an HIV reactive test were offered free regular screening (IV generation ELISA followed by Western blot confirmation), and the results and their first specialist infectivology visit were guaranteed within a few days.

Performing the test
The testing step was carried out by a biologist or a practitioner following the manufacturer's instructions.
The first year of the project was carried out using the RAPID TEST HIV® Lateral flow (HealthChem Diagnostics LLC, Pompano Beach, FL, USA). This is a membrane-based solid phase assay that contains antigens capturing the antibodies from a plasma, serum or saliva sample.
Following the discontinuation of the RAPID TEST HIV® Lateral flow, in 2009 our prevention program was performed using the new OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test (OraSure Technologies, Bethlehem, PA, USA).
This test is a single-use qualitative immunoassay to detect antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) and Type 2 (HIV-2) in oral fluid, fingerstick whole blood, venipuncture whole blood and plasma specimens. On the oral fluid the test sensitivity is 99.3%, the specificity is 99.8%, with 95% Confidence Interval (http://www.orasure.com).
It is very important to follow the manufacturer's sampling procedure (saliva collection), because this step can affect the proper performance of the test, resulting in false positive test results. The test is interpreted by a trained biologist or physician. If the HIV oral test is non-reactive, the post-test counselling discusses the prevention of transmission and other safe behaviours to prevent STDs. If the HIV oral test is reactive, a rapid blood test (VIKIA® HIV-1/2, Biomèrieux Diagnostics) is performed immediately, supported by post-test counselling to interview the subject about possible previous risk behaviours and to prepare them for an HIV-positive diagnosis. If both Point of Care rapid tests are reactive, then a blood sample is collected and sent to the serology laboratory of San Raffaele Hospital for standard ELISA and confirmed by Western Blot testing procedures. The results are received in four to five days. At this point, the HIV-positive patient is contacted directly by the infectious diseases specialist to start the process of HIV diagnosis and eventually treatment.

Ethical committee
The project protocol was approved by the Institutional Review Board of the Ethics Committee of San Raffaele Hospital in Milan. An

informed consent form was prepared explaining the details of the study, the HIV testing procedure and the interventions available for the individual. This information was also orally communicated (pre-test counselling) to the subjects by the personnel performing the test.

Data collection and analysis

HIV test results are entered immediately into a personal electronic file by the biologist responsible. Study data, including the questionnaires, are conveyed to the Department of Infectious Diseases of San Raffaele Hospital, used anonymously under the control of the Head of the project and the Department. Anonymity is guaranteed by assigning to each subject taking the oral test an identification code, of which only the Head of the Department has access for further correspondence.

RESULTS

From December 2008 to December 2012, 140,000 subjects were approached to inform them about HIV infection and other STDs; 7,865 (5.6%) total eligible volunteers underwent HIV tests on saliva and completed the interview in the alternative "street lab" (Table 1). The remaining non-respondents comprised a small number who knew about the alternative test but did not use it reporting fear the results could be less accurate, and others who were unaware of their risk transmission and preferred not to know their serological status.

The total number of subjects tested during the last five years varied and gradually increased due to the different events carried out each year. In the first two years there were few EASY test events, while from 2010 to 2012 the largest number of events was organized (*Convivio*, public events of Milan Council, HIV-STDs Prevention Units and GP surgeries) consequently broadening access to the service.

A high percentage of subjects undergoing monthly HIV saliva tests appears to be represented, on the one hand, by those with a high and altered perception of risk needing to test themselves repeatedly, sometimes obsessively, while not having at-risk sexual behaviours sexual risk. On the other, there are those who are aware and prefer to test themselves to continue their sexual activity at risk but with a clear conscience after having the negative result.

The portrait of the population responding to the EASY test initiative in these five years is quite varied but dominated by Italian men aged between 20 and 50 years, with a medium-high level of education (graduates, managers, 61%), 62% of them homosexuals (MSM), 38% high-risk heterosexuals (HRH) and only one of the total (0.01%) transgender. 52% of subjects had never performed a test for HIV for fear of knowledge or a lack of risk perception, despite claiming to have consistently high-risk sexual behaviours (Table 1).

In the first year of the initiative (2008), 761 saliva tests were performed, 40 of which on African HIV-positive pregnant women considered as a control group at St. Francy's Nsambya Hospital of Kampala in Uganda. Each of 761 tests performed was confirmed immediately by the blood screening test (ELISA), then confirmed by the conventional test (Western Blot). Almost all HIV-positive and negative subjects were confirmed by the reactive and non-reactive saliva rapid tests respectively. Only one reactive saliva test was confirmed by conventional screening test (0.1%, 1/761), four false positives and one false negative results were obtained, probably due to the poor reliability of that oral test used, at that time not FDA approved. The lower prevalence of the test performed (0.1%) is probably related to the test used.

As can be seen from the Table 1, in 2008, 51% of 761 tested subjects were Italian females, with a high level of education (50%), employed, aged between 19 and 40. More than 50% of the total were students, with 80% having had unsafe sex and, in 50% of cases, this represented the first approach to HIV testing.

After the first encouraging numbers of 2008 published by Parisi *et al.* (2009), the EASY test prevention program continued with the same objectives, but using the new rapid saliva test, FDA and Ethical Committee approved, the OraQuick ADVANCE HIV-1/2 Rapid test (OraSure Technologies, Bethlehem, PA, USA).

The EASY test project was performed during the public Prevention Program of the City of Milan called "*L'anno della salute 2009*" with a MH parked around the City of Milan, and in the first GP surgery in Milan (the pilot study).

By analyzing the population tested for a single year of the initiative, a total of 936 rapid saliva

tests were performed in 2009, and 54,000 booklets were distributed. A total of 12 new HIV infections were detected out of 936 tested (1.2%). The population tested was similar to the previous year: almost an half of them were male (59%), coming from Italy (32%), with a low educational level and a median age of 35 years old. 31% were MSM and 69% HRH, 80% reported unsafe sex but only 35% had been previously tested. During 2010, a total of 1,841 rapid saliva tests were performed. 0.16% (3/1.841) of rapid saliva tests were positive and 60,000 booklets were distributed to the population.

Starting in 2010, the patient demographic data of our project changed: the percentage of males taking the test increased (71%) as did the per-

TABLE 1 - *Self-reported demographic characteristics of eligible respondents among individuals interviewed from December 2008 to December 2012, per year.*

	2008	*2009*	*2010*	*2011*	*2012*	*Total*
N. Informed subjects (Test offer days)	2.000 (15 days)	54.000 (24 days)	60.000 (27 days)	14.000 (26 days)	10.000 (25 days)	140.000 (117 days)
N. Tested subjects (percentage of informed)	761 (38%)	936 (1.74%)	1.841 (3%)	1.931 (13.8%)	2.396 (24%)	7.865 (5.6%)
N. HIV+ saliva test (percentage of tested)	1 (0.1%)	12 (1.2%)	3 (0.1%)	12 (0.6%)	22 (0.9%)	50 (0.63%)
Gender:						
Male	373 (49%)	553 (59%)	1.306 (71%)	1.408 (73%)	1.916 (80%)	5.556 (70%)
Female	388 (51%)	383 (41%)	534 (29%)	523 (27%)	480 (20%)	2.308 (30%)
Transgender (percentage of tested)	0	0	1 (0.05%)	0	0	1 (0.01)
Education:						
High level school (percentage of tested)	380 (50%)	281 (30%)	921 (50%)	1.545 (80%)	1.677 (70%)	4.804 (61%)
Employment:						
Workers (percentage of tested)	304 (40%)	113 (12%)	1.141 (62%)	1.641 (85%)	2.108 (88%)	5.307 (67%)
Median age (Range)	30 (19-40)	35 (20-50)	35 (20-50)	35 (20-50)	35 (20-50)	34
Race:						
Italian (percentage of tested)	396 (52%)	300 (32%)	737 (40%)	580 (30%)	1.437 (60%)	3.450 (44%)
HIV Risk status:						
MSM*	300 (40%)	293 (31%)	1.287 (70%)	1.267 (65%)	1.724 (72%)	4.871 (62%)
HRH**	461 (60%)	643 (69%)	553 (30%)	664 (35%)	672 (28%)	2.993 (38%)
Transgender (percentage of tested)	0	0	1 (0.05%)	0%	0%	1 (0.01)
Unsafe sex (percentage o tested)	609 (80%)	750 (80%)	1.694 (92%)	1.738 (90%)	2.156 (90%)	6.947 (88%)
Previous tested (percentage of tested)	380 (50%)	328 (35%)	552 (30%)	1.216 (63%)	1.557 (65%)	4.033 (52%)

*Male who have Sex with Male; **High-Risk Heterosexual.

centage of MSM (70%), with 50% having a medium-high level of education. We also tested a member of the transgender community (0.05%, 1/1.841), who resulted HIV-negative. 92% of subjects reported at-risk sexual behaviours and only 30% had had a previous HIV test (Table 1).
During 2011, 1,931 total rapid oral tests were performed (14,000 information packages were distributed), and 12 (0.6%) reactive tests were found and confirmed positive by the blood test. A new and fixed appointment took place each first Friday of the month from February to December called *"FreeDay EASY test 2011"*: two San Raffaele Points of Care, located in two different points of the City, and one HIV-STDs Prevention Unit were dedicated to prevention, counselling and screening for HIV infection. Most people who underwent the test were male (73%), 65% of whom were MSM, with high-risk behaviours (90% of unsafe sex), workers with a high level of education (85%), aged between 20 and 50 years.
Also during this last year 2012, the San Raffaele initiative of *FreeDay EASY test* was started in February and finished in December. A total of 2,396 saliva tests were performed in 2012 and 22 (0.9%) new HIV infections diagnosed. 65% of those who participated in the EASY test project had had a previous test for HIV, and half of them

TABLE 2 - *Self-reported demographic characteristics of HIV-positive saliva test subjects per year, from December 2008 to December 2012.*

	2008	*2009*	*2010*	*2011*	*2012*	*Total*
N. HIV+ saliva test	1	12	3	12	22	50
Gender:						
Male	1 (100%)	12 (100%)	3 (100%)	12 (100%)	22 (100%)	50 (100%)
Female	0	0	0	0	0	0
Transgender	0	0	0	0	0	0
(percentage of tested)						
Education:						
High level school	0	5	2	4	19	30
(percentage of tested)		(42%)	(67%)	(34%)	(86%)	(60%)
Employment:						
Workers	0	5	2	10	19	36
(percentage of tested)		(42%)	(67%)	(84%)	(86%)	(72%)
Median age (Range)	25	30 (20-40)	30 (20-40)	37.5 (25-50)	37.5 (25-50)	32 median age
Race:						
Italian	0	6	1	10	19	36
(percentage of tested)		(50%)	(34%)	(84%)	(86%)	(72%)
HIV Risk status:						
MSM*	1 (100%)	7 (58%)	3 (100%)	10 (84%)	22 (100%)	43 (86%)
HRH**	0	5 (42%)	0	2 (16%)	0	7 (14%)
Transgender	0	0	0	0	0	0
(percentage of tested)						
Unsafe sex	1	10	3	10	22	46
(percentage o tested)	(100%)	(84%)	(100%)	(84%)	(100%)	(92%)
Previous tested	0	2	0	4	10	16
(percentage of tested)		(16%)		(34%)	(46%)	(32%)

*Male who have Sex with Male. **High-Risk Heterosexual.

chose our *FreeDay* as the usual date to undergo screening after having unsafe sexual behaviours. 80% of total tested subjects were male with a high level of education, in employment and aged between 20 and 50. 72% of the subjects, in this last year, comprised MSM who reported consistently unprotected sex (90%) (Table 1).

Regarding the 50 (0.6% of the total) subjects who

TABLE 3 - *Self-reported demographic characteristics of eligibles respondents among individuals interviewed from December 2008 to December 2012, per different location.*

	Convivio ANLAIDS (2008-2012)	*Public events of Milan City and 1° December AIDS World Day (2008-2012)*	*San Raffaele Point of care and HIV-STDs Prevention ambulatory (2010-2012)*	*General Practitioner ambulatories (2010-2012)*	*Total*
N. Subjects informed on STD prevention	34.000	45.000	52.000	9.000	140.000
N. Tested subjects (percentage of informed)	1.582 (4.6%)	2.276 (5%)	3.738 (7%)	269 (2.9%)	7.865 (5.6%)
N. HIV+saliva test (percentage of tested)	11 (0.7%)	12 (0.5%)	22 (0.6%)	5 (1.8%)	50 (0.63%)
Gender:					
Male	900 (57%)	1.700 (75%)	2.806 (75%)	150 (56%)	5.556 (70%)
Female	682 (43%)	576 (25)%	932 (25%)	118 (44%)	2.308 (30%)
Transgender (percentage of tested)	0	0	0	1 (0.3%)	1 (0.01)
Education:					
High level school (percentage of tested)	600 (38%)	1.204 (53%)	2.800 (75%)	200 (74%)	4.804 (61%)
Employment:					
Workers (percentage of tested)	767 (48%)	1.300 (57%)	2.980 (80%)	260 (62%)	5.307 (67%)
Median age (Range)	25 (20-30)	30 (20-40)	35 (20-45)	46 (30-62)	34 median age
Race:					
Italian (percentage of tested)	560 (35%)	1.138 (50%)	1.552 (42%)	200 (74%)	3.450 (44%)
HIV Risk status:					
MSM*	900 (57%)	1.266 (56%)	2.700 (72%)	5 (1.8%)	4.871 (62%)
HRH**	682 (43%)	1.010 (45%)	1.038 (28%)	263 (97.9%)	2.993 (38%)
Transgender (percentage of tested)	0	0	0	1 (0.3%)	1 (0.01)
Unsafe sex (percentage o tested)	947 (60%)	2.260 (99%)	3.660 (98%)	80 (30%)	6.947 (88%)
Previous tested (percentage of tested)	1.000 (63%)	933 (41%)	2.000 (53%)	100 (37%)	4.033 (52%)

*Male who have Sex with Male. **High-Risk Heterosexual.

were HIV oral test reactive (50/7.865) in five years, all of them were confirmed by rapid blood test and by the ELISA and Western Blot conventional test. Almost all (86%) HIV-positive subjects were homosexual men (MSM), aged between 20-50, coming from Italy (72%), with a medium-high education level (60%), and with unsafe sexual practices (92%). 32% of them were previously tested for HIV screening (Table 2). All 50 patients were asymptomatic and without a history of HIV-related symptoms or pathology. In particular, none had signs or symptoms of primary HIV infection or serological conversion. They were immediately directed to our clinical department for all medical needs related to HIV infection and almost all are still in treatment programmes at our HIV Centre.

Evaluating and looking at the EASY test data from another point of view, that is the distribution in the different initiatives (Table 3), the events approached by the largest number of people were the FreeDay EASY test, carried out each first Friday of the month in two San Raffaele Points of Care and one HIV-STDs Prevention Unit, now a fixed prevention appointment for many people coming from Milan and the surrounding area. In fact, during the FreeDay Easy test, a total of 3,738 HIV rapid oral test were performed from 2009 to date, and 22 new HIV infections were detected, keeping the average of 0.6% of new HIV infections diagnosed. The other event approaching many subjects was the Public event of Milan City and the *1st December AIDS World Day*, during which 2,276 tests were performed and 12 (0.5%) new HIV infections were diagnosed.

The average population tested during the four different initiatives was represented by Italian men (70%), in employment with a high education level (61%), 62% of whom were MSM practising unsafe sex (Table 3). The percentage of subjects previously tested was on the rise over the past two years, mainly thanks to the *FreeDay Easy* appointment, that ensures an anonymous, fast and easy screening, much appreciated by the population.

DISCUSSION

The development of rapid tests for the diagnosis of HIV infection is not only to facilitate access to testing, overcoming the "barrier" of fear over invasive conventional tests and to reduce HIV transmission, but also and especially to reduce the individual risk of disease progression and social costs associated with the diagnosis of "late presenters" (Lee *et al.*, 2007).

Overall, awareness and consciousness of alternative HIV test methods was limited across all the interviews, particularly in the first four years of the project. Previous studies on acceptability and preference for alternative HIV tests, as well as misconceptions about the accuracy of the tests as reported in our analysis suggest that an appropriate information and education about alternative HIV tests has the potential to increase testing among at-risk and not at-risk individuals (FDA, 2012).

Some studies have shown that alternative HIV tests are accepted with a 99% of satisfaction level and may be preferred when people are educated about these tests (Gauthier *et al.*, 2012; Spielberg *et al.*, 2003). Studies looking at adolescents' preferences for HIV tests have shown that non-invasive HIV antibody tests (such as oral mucosal transudate) and tests with rapid results were preferred. Similar studies have been done to look at the HIV testing preferences of adults. Most participants in these studies preferred oral fluid rapid testing, followed by rapid testing using blood. Throughout all of the studies, the least preferred method was standard blood testing (Branson *et al.*, 2006; http://www.bhiva.org).

The literature on the costs and the cost-effectiveness of HIV counselling and testing demonstrates that the costs of new HIV diagnoses vary according to the strategies used to recruit people for testing (e.g., outreach, partner notification, and social networks), testing technologies (e.g., rapid or conventional HIV testing), and costs included in the analysis (e.g., variable vs. fixed costs).

Some studies specified the costs of identifying new cases of HIV infection by recruitment strategy (http://www.has-sante.fr). For example, Katz and colleagues studied a peer-referral approach for HIV counselling and rapid testing among MSM in an STDs clinic in King Country, Washington. They found that the cost per new HIV diagnosis ranged from $5,600 to $12,000 (adjusted to 2005 US dollars value) when the HIV serological prevalence rates were 4.4% and 1.3%, respectively. The costs of clinical management of

late diagnoses have doubled (Katz *et al.*, 2012).
We successfully conducted this rapid HIV testing and counselling program with the goal of spreading the use of saliva test anonymously and free of charge. We aimed to facilitate access to testing in alternative settings, to establish if the "submerged" population would access salivary rapid testing versus the conventional settings.
In the present study, the non-respondents commonly cited concern for accuracy as the reason for not using rapid tests. The standard blood test and home collection kit use different collection methods and specimens and all have been shown to have sensitivity and specificity greater than 99%. The rapid oral tests, which use a different testing method, have been shown to have equally high specificity, and a lower value of sensitivity, not representing an obstacle, due to the priority of the saliva test to identify more or less recent new HIV infections (Pavie *et al.*, 2010).
Therefore, the concern that alternative tests may be less accurate is not supported by experience with the test and is likely due to lack of knowledge on test performance. This suggests a need for increased education among high-risk populations about the accuracy of these tests (FDA, 2012).
A common reason reported by respondents from all events for complaining about the low uptake of the oral test was that it was not offered to them in conventional settings. Providers might not offer alternative tests if "standard" testing is accepted by a client. A provider survey to ascertain the levels of knowledge and situations in which alternative testing may or may not be offered by physicians and HIV testing centres would help to determine specific educational needs.
Our analysis has some limitations because is a broad approach to offer an easier test in different settings with a standardized procedure, but it is not a selection of a target population. Although a formative research process was conducted to select locations representative of communities at risk for HIV infection, sites were not randomly selected, and therefore our findings may not be generalizable to all individuals at risk for HIV infection in the areas where the survey was conducted. In addition, our response rate of 5.6% (7,865 tested/140,000 informed) of interviewed individuals may introduce some bias if those refusing interviews were different from those accepting interviews. We did not collect demographic information from those who declined to be interviewed and tested, and thus could not characterize this potential bias.
The results from this analysis suggest that the promotion of alternative HIV test screening has not yet been fully developed as a strategy to increase levels of HIV testing among people at risk for HIV infection. Increasing awareness of these alternative tests among individuals at risk and providers may be an appropriate strategy to increase the numbers of people who know their serological status. However, our analysis does not make clear the extent to which availability of alternative HIV tests would increase testing among those high-risk individuals previously untested for HIV. This question, which relates most directly to CDC's strategic goal of increasing awareness of serological status among people living with HIV infection, may be answered by questions in future interview studies on the willingness or intention to test with alternative HIV tests among individuals untested in the past.
The recent introduction of rapid oral HIV antibody testing has completely changed the HIV diagnosis approach by facilitating the possibility of testing millions of people worldwide. The availability of affordable, point-of-service HIV testing is especially important, not only in low-income, high-HIV-burden countries which lack the financial and technological resources to perform more sophisticated laboratory-based assays, but also in developed countries were the HIV prevalence, morbidity and mortality continue because of lack of awareness.
Our short experience in Uganda seems confirm the above suggestion: the high acceptability of the local population during our preliminary screening in a small village emphasized the high potential of the rapid saliva test in low-income countries, especially in places where there is awareness but a lack of resources (Parisi *et al.*, 2009).
CDC launched the Advancing HIV Prevention Initiative with the objective to implement new models for diagnosing HIV infections outside medical settings, also to reduce the number of people who do not return for their test results. The possibility of rapid and more accessible testing methods gives the option of immediate counselling and referral to medical treatment as well as partner notification (ECDC, 2011). Public

health agencies should consider strategies to make rapid tests with well-documented performance characteristics available in epidemiological and clinical settings.

For these reasons, we hope the oral-based rapid HIV tests could become the gold standard to facilitate HIV screening access and become the standard of care and the basis for the national HIV testing algorithm in many countries with HIV epidemics to increase the proportion of persons aware of their HIV serological status.

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