

Rapid test to detect HIV antibodies in pediatric patients

José Guillermo Vázquez-Rosales

Servicio de Infectología, Hospital de Pediatría, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, México, D.F., México

Abstract

During the past years, rapid tests to detect human immunodeficiency virus (HIV) antibodies have been introduced and include new formats and different types of samples. Rapid assays that can be used with whole blood or oral fluid specimens have now been developed and make point-of-care HIV testing feasible. They are suitable for application in untested pregnant women during labor or delivery and prevent vertical transmission. Acceptance of rapid test among young persons that begins with sexual activity is superior to the traditional algorithm for HIV diagnosis, because of the following: 1) immediate results, 2) lack of invasive procedures and 3) accessibility in areas outside of hospital facilities. New clinical research studies, responsibility and education by users and social organization are needed in order to provide greater access to rapid testing, prevention and medical care for persons living with HIV in Mexico.

Key words: laboratory techniques and procedures, rapid test for HIV antibodies, HIV infection, pediatrics.

In Mexico, infection due to the human immunodeficiency virus (HIV) type 1 (HIV-1) has affected part of the

pediatric population. It is calculated that there have been ~2972 cases reported from the start of the pandemic and, at present, the infection in those <15 years of age occupies 2.4% of the total cases.¹ In a large number of cases, transmission was through blood transfusions, and many of the children affected had a history of hemophilia or of having had transfusions for surgical procedures.² However, at present and due to control of hemoderivatives in blood banks, in >90% of the cases of HIV-1 infection in children <15 years of age, transmission is via mother/child, which could reach up to 40% in the exposed fetus.³

Since 1994 it has been known that this manner of transmission could be prevented through antiretroviral therapy in the mother and in the fetus.⁴ Gradually over the years, the percentage of vertical transmission of HIV-1 has decreased due to the different antiretroviral schemes used during pregnancy. A decrease has taken place from the beginning of monotherapy with azidotimidine (AZT) to 9%, up to the highly active antiretroviral therapy (HAART) with elective cesarean, reducing this percentage to <2%. Other alternatives for urgent antiretroviral prophylaxis for women in labor and who are known to be seropositive for HIV-1 antibodies but do not have established treatment have reduced transmission to 9-12%.⁵

However, all these benefits for the fetus remain without effect if there is no maternal diagnosis during pregnancy or at least during labor. Determination of serum antibodies against HIV-1 using highly sensitive

Correspondence to:

*Dr. José Guillermo Vázquez Rosales
Servicio de Infectología, Hospital de Pediatría
Centro Médico Nacional Siglo XXI
Instituto Mexicano del Seguro Social
México, D.F., México*

E-mail: vazguill@aol.com

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conventional testing such as immunoenzymatic assay (IEA) and its confirmation through more specific assays such as Western blot (Wb) is sufficient to support the diagnosis in the mother and to initiate some prophylactic measures.⁶

Even though in some countries the frequency of HIV-1 infection in women of childbearing age is <0.01%, it is ideal that all pregnant women have a voluntary determination of antibodies against this virus at least once during pregnancy. For those who do not have this opportunity, the implementation of rapid testing during labor, or even at birth, would allow immediate access to any antiretroviral prophylaxis and appropriate obstetric management. The U.S. Centers for Disease Control and Prevention (CDC) reported that ~40% of mothers who had infected children were unaware of their infection prior to the birth.⁷

In this study we review some characteristics of rapid tests and their application in pediatrics, attempting to answer the following questions: What is a rapid test for antibodies against HIV? What are the situations where these tests are useful in the pediatric population?

Rapid test for the detection of antibodies against HIV
Although the determination of antibodies against this virus has been performed as a diagnostic test shortly after the first isolation of HIV,⁸ the EIA method has been improved in successive generations.

Its high sensitivity is maintained because we are dealing with a screening test, but its reactivity should be corroborated using tests of greater specificity such as the Wb. The performance of both techniques requires an already established laboratory infrastructure and trained personnel. It also requires <24 h for its performance and the report generally takes 1-2 weeks.⁹ For these reasons, more than 10 years ago rapid tests emerged for determination of antibodies against HIV-1 and HIV-2 (Genetic Systems Genie and Abbot Testpack), which consisted of solid-phase immunoassays comprised of synthetic peptides reporting a sensitivity of 97% and a specificity >99% in populations with a high probability of infection.¹⁰⁻¹²

However, when analyzed in field studies with populations showing less prevalence such as those who attend hospitals, a not entirely satisfactory specificity was observed. In a study performed in a New York

City hospital, comparing a rapid test against a non-rapid conventional assay, a sensitivity of 100% with a specificity of 99.1% and with a positive predictive value (PPV) of 86% was found.¹³ This demonstrated a high number of false positive cases indicated by the rapid test. Other studies have provided similar results, perhaps with a slight improvement in the number of false positives, which motivated the need to recommend a confirmatory test after a reactive result from the rapid test.¹⁴ With a positive vision of these tests, it should be pointed out that the time of result availability was reduced to 40 or 60 min, which allowed an increase of 27% in patients who knew their serological status, as well as the possibility of establishing early counseling with regard to HIV infection.¹⁵

Formerly, the rapid and conventional tests utilize serum, plasma or whole blood, which implied a potential risk of accidents with sharp materials. Beginning in 1995, various publications reported the determination of antibodies against various HIV components in saliva. This allowed distinguishing a high concentration of antibodies against HIV-1 reverse transcriptase in asymptomatic carriers from its absence in healthy individuals, with a reported sensitivity and specificity of 100%.¹⁶ The determination of antibodies against diverse viral proteins using commercial EIA equipment (ELISA) allowed establishing 98-100% sensitivity among crevicular fluid, submandibular and parotid saliva and mixed samples. However, obtaining the saliva mix with a flat absorption device facilitated the operation and a greater sensitivity was obtained.¹⁷ In newborns of mothers with HIV infection, the determination of antibodies in saliva obtained by means of a sponge was 100% concordant with the determination performed in serum, raising the mother's confidence for the study in children because it was noninvasive.¹⁸ In recent years, the search for antibodies in saliva by rapid testing was implemented, with an initial report of a similar specificity to blood tests but with lower sensitivity.¹⁹ At present there are three main techniques used in rapid tests: a) particle agglutination, in which HIV antigen-coated latex particles bind to antibodies of the sample, b) membrane immunoconcentration in which the HIV antigen is applied to a solid and porous base that allows flow of the sample and the concentration of antibodies on the antigen, requiring several steps including addition of signal reagent, c) online immunochromatography that is performed in a single step and includes the signal reagent and the HIV antigen into nitrocellulose strip.

The specimen is applied to an absorbent pad and the antibodies migrate through the strip incorporating the signal reagent and binding to the antigen.²⁰

In the U.S. there are currently four tests for the rapid HIV antibody approved by the Food and Drug Administration (FDA): OraQuick Advance Rapid HIV-1/2 Antibody Test (OraSure Technologies, Inc.); Reveal G2 Rapid HIV-1 Antibody Test (MedMira), Uni-Gold Recombigen HIV Test (Trinity BioTech) and MultiSpot HIV-1/HIV-2 rapid test (Bio-Rad Laboratories).

Like conventional EIA, all are screening tests that require confirmation if they are reactive, their interpretation is visual and do not require special instruments, and they may even take place at the point of medical care.²¹

Initially, the OraQuick Rapid HIV-1 test was approved for use with plasma or whole blood collected digitally or by venipuncture. However, beginning in 2004, OraQuick Advance version can be used for detecting antibodies to HIV-1 and 2 in plasma, whole blood and oral cavity fluid. The device consists of a nitrocellulose strip on which transverse bands of synthetic peptides gp41 of HIV-1 and gp36 of HIV-2 envelope have been applied. Another transverse band of goat antibodies against human IgG serves as a control.

Saliva obtained by passing a tongue blade over the gums or blood or plasma to test is applied to the developer vial where it is placed at the side of the nitrocellulose strip. If there are specific antibodies in the sample, these bind to the peptides and a red line is formed. The control band also becomes red on uniting the nonspecific antibodies present. The result should be read between 20 and 40 min after the test device is inserted in the developer vial: a reactive test will have two color bands, and a negative one will have only a colored control band. If neither of the two bands stain, the test should be repeated.

The sensitivity of the test is similar if performed using oral fluids, blood or plasma (99.3-99.6%), but specificity is lower if oral fluid is used (99.8%) with a PPV of 90%. This has implications for populations with low HIV prevalence because the amount of false positives can be expected to be up to two to six times higher in saliva testing compared with whole blood.²²

In locations where obtaining blood samples is difficult, the use of OraQuick in oral fluid can help in identifying

persons with HIV infection; however, due to its slightly lower sensitivity, when conditions allow for obtaining blood samples, this method is preferred.²³

The Uni-Gold Recombigen test determines HIV-1 antibodies in whole blood, serum or plasma using a nitrocellulose membrane covered by antigens from an immunodominant region of the HIV-1 envelope, with a control region that indicates the appropriate functioning of the test. The test should be read 10-12 min after application of the sample and has a sensitivity of 100% and specificity between 99.7 and 99.8%.²¹

The Reveal G2 HIV-1 and Multispot HIV-1/HIV2 tests can only analyze serum or plasma, which make their application in the field difficult. Both trials have a sensitivity of 99.8-100% and specificity of 99.1-99.91% but require various steps for their performance, which lends greater complexity than the previously mentioned tests.²¹

Other rapid tests are available worldwide, and the majority demonstrate adequate sensitivity and specificity: Determine HIV-1/2 (Abbott Labs), Genie II HIV-1/2 (Bio-Rad Labs), etc.²⁴ Their characteristics are described on the Internet (www.medadvocates.org/diagnostics/rapidhiv).

Rapid tests in pregnant women

Pregnant women living with HIV are among the groups of people in whom a rapid diagnosis is essential for establishing prophylactic measures for prevention of infection to the fetus. The prevalence of infection among pregnant women may be different if they are tested during pregnancy, at the time of labor or during delivery. In a study conducted in Tijuana, Mexico using a rapid test (Determine HIV1/2), the prevalence was 0.33% in pregnancy and 1.2% during delivery. Risk factors that were associated with infection were drug use and having no prenatal care.²⁵

The speed of obtaining the results, as well as counseling when results are given, is important in both developed and developing countries because it motivates the person to seek help and treatment. Factors associated with the acceptance of the test are being young, black or Hispanic, gestational age <32 weeks and having had no prenatal care.²⁶ The time frame from taking of the sample to the time the patient is given the results is significantly different if rapid tests over conventional

tests are used. With the rapid tests the time frame is between 60 and 90 min and with the conventional tests it is 1 to 2 days.^{26,27} However, having the results in a timely manner is not always associated with acceptance of beginning antiretroviral therapy as shown in a study in Kenya in 1282 mothers who had rapid tests or conventional tests performed randomly. Of the 162 mothers who were determined to be infected in both groups, only 24 accepted antiretroviral prophylaxis.²⁸ The sensitivity and specificity of a rapid test performed in pregnant women are generally adequate;^{19,25} however, there persist a certain number of false positives, which varies according to the prevalence of HIV infection in the population, the test and type of sample utilized. Based on clinical trials for clinical license, the CDC has published the best use of OraQuick in whole blood throughout populations with different prevalences, maintaining a PPV of 100%.⁷ Determination with saliva utilizing the same test demonstrated a sensitivity similar to that with whole blood but with a different specificity.²⁹ Based on the MIRIAD study (rapid intervention in the mother and child at birth), which included 7381 women in labor with a search for antibodies in the blood by OraQuick and where they reported a sensitivity of 100%, specificity of 99.9% and PPV 90%,²⁶ the CDC recommends performing a rapid test routinely during labor or delivery as well as informing the patient that such a procedure is routinely performed if the serological status is unknown, unless the patient does not consent.

Due to the possibility that some mothers/children may receive antiretroviral prophylaxis unnecessarily, algorithm studies for alternative confirmation have been performed based on the combination of screening tests that have produced comparable results to the standard algorithm of EIA-Wb.^{11,19,23,30,31} Therefore, the WHO has recommended the use of different test combinations for strategies of study, including rapid tests in place of EIA-Wb.⁹

In a cost-effectiveness analysis where the diagnosis of HIV infection was introduced by means of rapid tests and the consequent implementation of monotherapy with AZT in pregnant women in order to prevent vertical transmission, it was found that rapid testing may prevent infection of 183 newborns, as well as achieving a savings of almost \$58,000 due to case prevention.³²

Rapid tests in children

Currently, rapid tests are indicated at the extremes of pediatric life. When the serological status for HIV is unknown in the mother before the beginning of labor and if it is not possible to perform a rapid test during labor, the CDC recommends performing a routine rapid test immediately after labor with the purpose that antiretroviral prophylaxis be provided to the child exposed to HIV. When the pharmacological prophylaxis is started during labor (labor or birth) or in the neonatal period, rates of transmission of 9 to 13% could be reached, according to clinical and observational data. This represents a reduction of 50% in the rate of transmission without any intervention.³³

In Mexico, the age group with the greatest rate of HIV infection is from 15 to 44 years of age and, of these patients, 31.7% are between 20 and 29 years of age.¹ Because of the delay in developing symptoms of HIV infection, it is probable that it was contracted during adolescence. During this stage, young people exhibit a sense of invulnerability. Sexuality is in the process of change, often dependent on the experience of other adolescents. Some factors associated with high-risk behavior such as lack of parental support, adverse life circumstances and addiction to any type of substance have been detected.³⁴ The adolescent also may not accept help from people other than their peers, which makes orientation and providing diagnostic methods difficult.³⁵ A study performed in secondary school students in Tanzania reported a prevalence of HIV infection of 5.5% in young people from an urban environment vs. 1% from a rural environment. The poll was carried out utilizing a rapid test on saliva (OraQuick Advance), which received high acceptance from the participants.³⁶ One of the strategies of prevention proposed at the international level is to develop diagnostic modules outside of hospitals, managed by young people and facilitated by availability of easy-to-perform rapid tests.³⁵ However, although some rapid tests may be self-administered and 90% of the instructions are understood by the users, in a Singapore study up to 56% of the participants had invalid results due to the incorrect performance of the test.³⁷ The most accepted rapid tests by adolescents are the ones performed on saliva because they are quick and noninvasive. However, digital puncture tests also have a high acceptability because of their rapidity.³⁸

In Mexico, the availability of rapid tests is limited to civil or governmental organizations, which makes their access difficult to other segments of society. In order

for these types of assays to be acquired in a more open manner in our country and contribute to the control of this pandemic, health education and implementing

responsibility for users, as well as clinical studies and improvement of social organizations, are necessary.

References

- 1.SS/DGE. Registro nacional de casos de SIDA. Datos al 14 de Noviembre 2008. Centro Nacional para la Prevención y el Control del VIH/SIDA. Available at: <http://www.censida.salud.gob.mx>
- 2.Vázquez-Rosales G, Solórzano-Santos F, Álvarez-Muñoz MT, Sánchez-Huerta G, Miranda-Novales G. Infección por VIH-1 en pacientes pediátricos en México. *Rev Invest Clin* 2004;56:153-168.
- 3.SS/DGE. Registro nominal de seropositivos al VIH. Datos al 14 de Noviembre 2008. Centro Nacional para la Prevención y el Control del VIH/SIDA. Available at: <http://www.censida.salud.gob.mx>
- 4.Connor EM, Sperling RS, Gelber R. Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. *N Engl J Med* 1994;331:1173-1180.
- 5.Public Health Service Task Force. Recommendations for use of antiretroviral drugs in pregnant HIV-1 infected women for maternal health and interventions to reduce perinatal HIV-1 transmission in the United States. November 2007. Available at: <http://AIDSinfo.nih.gov>
- 6.Lampe MA. Human immunodeficiency virus-1 and preconception care. *Maternal Child Health J* 2006;10:S193-195.
- 7.Centers for Diseases Control and Prevention. Rapid HIV-1 antibody testing during labor and delivery for women of unknown HIV status. January 30, 2004. Available at: <http://cdc.gov>
- 8.Barre-Sinoussi F, Cherman JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 1983;220:868-871.
- 9.Branson BM. Point-of-care rapid test for HIV antibodies. *J Lab Med* 2003;27:288-295.
- 10.Kline RL, Dada A, Blattner W. Diagnosis and differentiation of HIV-1 and HIV-2 infection by two rapid assays in Nigeria. *J Acquir Immune Defic Syndr* 1994;7:623-626.
- 11.Brattegaard K, Kouadio J, Adom ML. Rapid and simple screening and supplemental testing for HIV-1 and HIV-2 infections in West Africa. *AIDS* 1993;7:883-886.
- 12.Malone JD, Smith ES, Sheffield J. Comparative evaluation of six rapid serological tests for HIV-1 antibody. *J Acquir Immune Defic Syndr* 1993;6:115-119.
- 13.Irwin K, Olivo N, Schable CA. Performance characteristics of a rapid HIV antibody assay in a hospital with a high prevalence of HIV infection. *Ann Intern Med* 1996;125:471-475.
- 14.Kassler WJ, Alwano-Edyegu MG, Marum E. Rapid HIV testing with same-day results: a field trial in Uganda. *Intern J STD AIDS* 1998;9:134-138.
- 15.Kassler WJ, Dillon BA, Haley C. On-site, rapid HIV testing with same-day results and counseling. *AIDS* 1997;11:1045-1051.
- 16.Ishikawa S, Hashida S, Hashinaka K. Diagnosis of HIV-1 infection with whole saliva by detection of antibody IgG to HIV-I with ultrasensitive enzyme immunoassay using recombinant reverse transcriptase as antigen. *J Acquir Immune Defic Syndr Hum Retrovir* 1995;10:41-47.
- 17.Lamey PJ, Nolan A, Follett EAC. Anti-HIV antibody in saliva: an assessment of the role of the components of saliva, testing methodologies and collection systems. *J Oral Pathol Med* 1996;25:104-107.
- 18.Tess BH, Granato C, Parry JV. Salivary testing for human immunodeficiency virus type 1 infection in children born to infected mothers in Sao Paulo, Brazil. *Pediatr Infect Dis J* 1996;15:787-790.
- 19.Bhore AV, Sastry J, Patke D. Sensitivity and specificity of rapid HIV testing of pregnant women in India. *Int J STD AIDS* 2003;14:37-41.
- 20.Branson BM. Rapid test for HIV antibody. *AIDS Rev* 2000;2:76-83.
- 21.Greenwald JL, Burstein G, Pincus J, Branson B. A rapid review of rapid HIV antibody tests. *Curr Infect Dis Rep* 2006;8:125-131.
- 22.Delaney KP, Branson BM, Uniyal A. Performance of an oral fluid rapid HIV-1/2 test: experience from four CDC studies. *AIDS* 2006;20:1655-1660.
- 23.Pai NP, Tulsy JP, Cohan D. Rapid point-of-care HIV testing in pregnant women: a systematic

- review and meta-analysis. *Trop Med Int Health* 2007;12:162-173.
24. Rouet F, Ekouevi DK, Inwoley A. Field evaluation of a rapid human immunodeficiency virus (HIV) serial serologic testing algorithm for diagnosis and differentiation of HIV type 1 (HIV-1), HIV-2, and dual HIV-1-HIV-2 infections in West African pregnant women. *J Clin Microbiol* 2004;42:4147-4153.
25. Viani RM, Araneta MR, Ruiz-Calderon J. Perinatal HIV counseling and rapid testing in Tijuana, Baja California, Mexico: seroprevalence and correlates of HIV infection. *J Acquir Immune Defic Syndr* 2006;41:87-92.
26. Bulterys M, Jamieson D, O'Sullivan M, Cohen M, Maupin R, Nesheim S, et al. Rapid HIV-1 testing during labor. *JAMA* 2004;292:219-223.
27. Viani RM, Hubbard P, Ruiz-Calderon J. Performance of rapid HIV testing using Determine (R) HIV-1/2 for the diagnosis of HIV infection during pregnancy in Tijuana, Baja California, Mexico. *Int J STD AIDS* 2007;18:101-104.
28. Malonza IM, Richardson BA, Kreiss JK. The effect of rapid HIV-1 testing on uptake of perinatal HIV-1 interventions: a randomized clinical trial. *AIDS* 2003;17:113-118.
29. Delaney K, Branson B, Uniyal A, Kerndt P, Keenan P, Jafa K, et al. Performance of an oral fluid rapid HIV-1-2 test: experience from four CDC studies. *AIDS* 2006;20:1655-1660.
30. Nogueira SA, Lambert JS, Albuquerque AL. Assessment of a rapid HIV test strategy during labor: a pilot study from Rio de Janeiro, Brazil. *J Hum Virol* 2001;4:278-282.
31. Bruzzone B, Bisio F, Ventura A. HIV serological screening in a population of pregnant women in the Republic of Congo: suitability of different assays. *Trop Med Int Health* 2008;13:900-903.
32. Stringer JS, Rouse DJ. Rapid testing and zidovudine treatment to prevent vertical transmission of human immunodeficiency virus in unregistered parturients: a cost-effectiveness analysis. *Obstet Gynecol* 1999;94:34-40.
33. CDC's mother-infant rapid intervention at delivery (MIRIAD). Available at: <http://www.aidsinfo.nih.gov/guidelines>
34. Walter HJ, Vaughan RD, Cohall AT. Psychosocial influences on acquired immunodeficiency syndrome-risk behaviors among high school students. *Pediatrics* 1991;88:846-851.
35. Centers for Diseases Control and Prevention. HIV/AIDS among youth. CDC HIV/AIDS fact sheet. June 2006: 1-5.
36. Holm-Hansen C, Nyombi B, Nyindo M. Saliva-based HIV testing among secondary school students in Tanzania using the OraQuick (R) Rapid HIV-1/2 antibody assay. *Ann NY Acad Sci* 2007;1098:461-466.
37. Lee VJ, Tan SC, Earnest A. User acceptability and feasibility of self-testing with HIV rapid tests. *J Acquir Immune Defic Syndr* 2007;45:449-453.
38. Peralta L, Constantine N, Griffin D. Evaluation of youth preferences for rapid and innovative human immunodeficiency virus antibody tests. *Arch Pediatr Adolesc Med* 2001;155:838-843.