

The Diagnosis of HIV Infection in Infants and Children

Alireza Abdollahi^{1,2}, Hana Saffar¹

1. Dept of Pathology, Imam Hospital Complex, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

2. Thrombosis Homeostasis Research Center, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran

KEY WORDS

Diagnosis
HIV infection
Infants
Children

ABSTRACT

It is estimated that the number of HIV infected children globally has increased from 1.6 million in 2001 to 3.3 million in 2012. The number of children below 15 years of age living with HIV has increased worldwide. Published data from recent studies confirmed dramatic survival benefit for infants started anti-retroviral therapy (ART) as early as possible after diagnosis of HI. Early confirmation of HIV diagnosis is required in order to identify infants who need immediate ART. WHO has designed recommendations to improve programs for both early diagnoses of HIV infection and considering ART whenever indicated? It is strongly recommended that HIV virological assays for diagnosis of HIV have sensitivity of at least 95% and ideally greater than 98% and specificity of 98% or more under standardized and validated conditions. Timing of virological testing is also important. Infants infected at or around delivery may take short time to have detectable virus. Therefore, sensitivity of virological tests is lower at birth. In utero HIV infection, HIV DNA or RNA can be detected within 48 h of birth and in infants with peripartum acquisition it needs one to two weeks. Finally it is emphasized that all laboratories performing HIV tests should follow available services provided by WHO or CDC for quality assurance programs. Both clinicians and staffs providing laboratory services need regular communications, well-defined SOPs and nationally validated algorithms for optimal use of laboratory tests. Every country should use assays that have been validated by national reference laboratory.

ARTICLE INFO

Received 23 Jan 2015;
Accepted 30 May 2015;

©Iran J Pathol. All rights reserved.

Corresponding Information: Alireza Abdollahi M.D.: Keshavarz Blvd, Imam Hospitals Complex, Tehran, Iran Email: abdollahi_a@tums.ac.ir
Tel: +98-9121220588

COPYRIGHT © 2016, IRANIAN JOURNAL OF PATHOLOGY. This is an open-access article distributed under the terms of the Creative Commons Attribution-noncommercial 4.0 International License which permits copy and redistribute the material just in noncommercial usages, provided the original work is properly cited.

Introduction

It is estimated that the number of HIV infected children globally has increased from 1.6 million in 2001 to 2.1 million in 2008 and 3.3 million in 2012 in 2008 an estimated 430000 new HIV infections occurred in children whereas in 2012, 260000 children were newly infected with HIV (1-3). It is estimated that the number of children below 15 years of age living with HIV has

increased worldwide (1-3).

Mother to child transmission (MTCT) of HIV is defined as transmission of HIV virus from HIV positive woman to the baby during pregnancy, labor and delivery or breastfeeding without prophylactic treatment (5-6). MTCT of HIV is an important cause of infection. Between 15% and 30% of infants born to HIV-positive women are infected during gestation and delivery while 5-15% is infected through Breastfeeding (3,

5-11). About 90% of new infections in children in 2008 acquired via MTCT by either labor and prepartum period or breastfeeding (12).

Data obtained from previous studies confirmed spectacular survival benefits for infants started anti-retroviral therapy as early as possible after HIV diagnosis (13-18). Early confirmation of HIV diagnosis is required in order to identify infants who need immediate ART (19-22). WHO has designed recommendations to improve programs for both early diagnoses of HIV infection and considering ART whenever indicated.

Diagnostic testing assays

Diagnostic testing procedures are needed to confirm the presence of HIV infection at any age. HIV serological procedures are often used to screen HIV exposure in infants, but they are also used as part of diagnostic tests in children aged over 18 months (23-26). When serological tests are used as screen method for HIV exposure, virological tests are recommended based on one reactive serological test (27). Serological testing identifies HIV antigen/antibody generated following immune response to HIV infection (28).

As we know maternal HIV antibody is transferred passively during pregnancy and decreases with the half-life of 28-30 d in non-breastfed infants (29-31). Most commonly used serological assays are not capable of differentiating between maternal HIV antibody or antibody produced by the infant, so other assays which detect the virus or its components are required to diagnose infection in this period (32-35). The mean/median age at the time of seroconversion ranges between 9-16 months in both developed and developing countries (36) and infants who have positive serological test at 9 months should have virological test to confirm the infection and identify infants who need anti-retroviral therapy (ART)(36).

Antibody to HIV can be measured by variety

of techniques but since all these techniques detect an immune response to the virus, it takes some time to become reactive after infection.

ELISA is a serological test with high specificity and sensitivity that can help detect antibodies to both HIV1 and HIV2 (33, 37).

Selecting serological tests

Multiple factors should be considered in selecting serological tests including the commercial availability, cost of the test, training of staff, volume and type of the specimen required, laboratory quality assurance, specimen collection and processing and need for a cold chain in some circumstances (38).

WHO's description of generic testing algorithm is based on the purpose of HIV testing and prevalence of disease. In a high prevalence situation, the first positive test needs to be confirmed with another test with a different assay and two positive results will lead to HIV seropositivity (1-3, 39, 40).

If the second test is negative, the specimen needs a new testing by a third assay and in case the result is still non-reactive, the individual is seronegative (1-3). If the result of the third test is reactive, the individual should be referred for further testing in three weeks. In infants in relation to breastfeeding, the test can be interpreted after cessation of breastfeeding for 6 months. Therefore, in infant who is still breastfeeding, negative HIV test result cannot exclude HIV infection. In a low prevalence setting, again the first reactive test should be confirmed with a second assay (1-3). If the result of second test is non-reactive, the individual is HIV seronegative and if both tests are reactive, then a third different assay must be performed on the same specimen to confirm seropositivity and if the result of third test is non-reactive, the individual should be referred for further testing in three weeks (32).

WHO/UNAIDS define a generalized HIV epidemic as prevalence of $\geq 1\%$ in antenatal

clinic and for the purpose of selecting HIV testing strategies, thresholds of $\geq 5\%$ in target population are suggested. The assays chosen are required to have or exceed sensitivity $\geq 99\%$ and specificity $\geq 98\%$ (3, 41-45).

Combination assays allow detection of P24, HIV antigen and HIV antibody and so have shortened window period (46). These rapid tests are available these days with diagnostic performance compatible to traditional EIA methods (sensitivity $\geq 99\%$, specificity $\geq 98\%$) and should be validated by national reference laboratory. They are quick, easy, cost-effective, require no equipment, there is lesser chance of error and the results are usually ready after 10-30 min (46-49).

The western blotting detect HIV antigen on a strip of nitrocellulose and the results can be interpreted as positive, negative and indeterminate (neither + nor - usually beginning of seroconversion at the time of testing or cross reactivity) (1-3, 50). In this situation, test should be repeated 3 weeks later. Because western blot is sophisticated equipment and needs expertise in interpretation, today with improvement in performance characteristics of serological and virological tests it is no longer essential as a confirmatory test in HIV infection (50).

Virological testing detects presence of viral nucleic acid (i.e. viral RNA or viral DNA) or viral products such as P24 antigen. Most of these assays amplify genetic material of HIV and can be either qualitative or quantitative. PCR based techniques are the most widely used assays even in resource-limited settings (51-53).

In general, it is recommended to use HIV DNA on whole blood or dried blood spot (DBS), HIV RNA on plasma or DBS and ultrasensitive (US) P24 antigen on plasma or DBS. 500-1000 μl of plasma or serum is required for virological tests whereas DBS requires 25-100 μl whole blood. There is reasonable agreement between viral load results from plasma and DBS (47-48), however in plasma viral load (PVL) ≤ 1000 copies/ml

the DBS can represent higher results and lower limit of detection for DBS is 3000 copies/ml but this situation is only of concern when total suppression of HIV viral load is sought and for diagnosis in children who generally have higher viral loads than adults this will be problematic (53, 54).

It is strongly recommended that HIV Virological assays for diagnosis of HIV have sensitivity of at least 95% and ideally greater than 98% and specificity of 98% or more under standardized and validated conditions (55-57). HIV Virological tests should be used to diagnose HIV infection in infants and children below 18 month of age and all HIV-exposed infants should have virological testing at 4-6 weeks of age or at the earliest opportunity thereafter. It is strongly recommended that in infants with initial positive virological test, (ART) should be started without any delay and at the same time a second specimen should be collected for confirmatory test and it is not acceptable to delay ART while waiting for result of confirmatory test (58-59).

HIV DNA could be recognized in the blood mononuclear cell and lymphoid tissue of HIV positive children who have received anti-retroviral drugs (41, 60- 67). RNA detection in plasma seems is sensitive during the first couple of weeks of life (61, 68- 70). Despite the association of administration of maternal ART with reduced replication of HIV, studies have in general shown no loss of sensitivity (41, 42, 44, 69, 71). Some of the newer molecular assays detect both DNA and RNA in whole blood. Increased sensitivity and reduced window period are the main advantages (48, 51, 66).

P24 assays measure HIV core P24 in whole blood, serum or plasma. When antibody to HIV becomes detectable, P24 antigen is no longer demonstrable. Available data suggest that US P24 is better to be used up to 18 months of age and the sensitivity may decline with increasing age. Since detection of P24 antigen depends on viral replication and ART inhibits this, there

are some concerns that US P24 detection assays are not suitable when the mother or infant is on ART. HIV RNA and HIV DNA testing are recommended in these circumstances (54, 61).

Recently technical improvements have enabled the newer immune complex-dissociated US P24 test that can be performed as well as HIV DNA PCR in diagnosis of HIV in infants and can be done in laboratories that can perform EIA testing. These tests are less complex and less costly and can be performed on whole blood, plasma or DBS with no need for nucleic acid extraction (32, 66).

General factors that should be considered in virological testings are commercial availability, equipment required for performance and handling, costs, number of specimens, specimen storage and transport and ongoing laboratory quality control (64). The timing of virological testing is also important. Infants infected during delivery may take short time to have detectable virus. Therefore, the sensitivity of virological tests is lower at birth (3).

In utero HIV infection, HIV DNA or RNA can be detected within 48 h of birth but in infants with peripartum acquisition it needs one to two weeks. Generally, by six weeks of age almost all infants can be identified by virological tests (1-3). Disease progression is rapid in young infants, so the turned around time from specimen collection to giving results should not be more than 4 weeks. If HIV infection is considered as underlying cause of symptoms and signs in sick infants and virological tests are not available, using serological testing accompanied by clinical algorithm is strongly recommended. Point of care assays are under evaluation and could offer many advantages in near future (1-3, 33, 67).

Regarding breastfeeding, it is recommended that breastfeeding should not discontinue in order performing of diagnostic tests (51). A positive virological test in the presence of breastfeeding indicates infection and should prompt confirmatory tests, but negative virological tests

as long as exposure to HIV is continuing are not reliable and conduction of virological tests after 6 weeks of cessation of breastfeeding is indicative of the HIV status (46, 61, 69-71).

Another important point related to HIV virus is the diversity of HIV which means that molecular methods for identification of HIV DNA and RNA require constant monitoring and assessment. Commercial serological EIAs appear to detect most subtypes and groups of HIV and new rapid assays can also detect HIV1 and HIV2 with accuracy comparable with EIAs (3, 28). Cell culture is no longer recommended for diagnosis of HIV because of complexity, biosafety and costs (43).

Conclusion

Finally, it is emphasized that all laboratories performing HIV tests should follow available services provided by WHO or CDC for quality assurance programs. Both clinicians and staffs providing laboratory services need regular communications, well-defined SOPs and nationally validated algorithms for optimal use of laboratory tests. Non-commercial tests are not recommended for widespread use by national programs. Every country should use assays that have been validated by national reference laboratory.

Acknowledgements

The authors declare that there is no conflict of interests.

References

1. WHO; UNAIDS. Guidance on provider-initiated HIV testing and counselling in health facilities. Geneva: WHO; 2007. [09 December 2010]. http://whqlibdoc.who.int/publications/2007/9789241595568_eng.pdf.
2. Morss SS. Factors in the emergence of infectious diseases. *Emerg Infect Dis* [serial online] 1995 Jan-Mar

[cited 1996 Jun 5]; 1(1):[24 screens]. Available from: URL: <http://www.cdc.gov/ncidod/EID/eid.htm>

3. De Cock KM, Fowler MG, Mercier E, de Vincenzi I, Saba J, Hoff E, et al. Prevention of mother-to-child HIV transmission in resource-poor countries: translating research into policy and practice. *JAMA* 2000 1; 283(9):1175-82.

4. Sherman GG, Stevens G, Stevens WS. Affordable diagnosis of human immunodeficiency virus infection in infants by p24 antigen detection. *Pediatr Infect Dis J* 2004; 23(2):173-6.

5. Cassol S, Gill MJ, Pilon R, Cormier M, Voigt RF, Willoughby B, et al. Quantification of human immunodeficiency virus type 1 RNA from dried plasma spots collected on filter paper. *J Clin Microbiol* 1997; 35(11):2795-801.

6. Bertagnolio S, Soto-Ramirez L, Pilon R, Rodriguez R, Viveros M, Fuentes L, et al. HIV-1 drug resistance surveillance using dried whole blood spots. *Antivir Ther* 2007; 12(1):107-13.

7. Brambilla D, Jennings C, Aldrovandi G, Bremer J, Comeau AM, Cassol SA, et al. Multicenter evaluation of use of dried blood and plasma spot specimens in quantitative assays for human immunodeficiency virus RNA: measurement, precision, and RNA stability. *J Clin Microbiol* 2003;41(5):1888-93.

8. Isaakidis P, Raguenaud ME, Te V, Tray CS, Akao K, Kumar V, et al. High survival and treatment success sustained after two and three years of first-line ART for children in Cambodia. *J Int AIDS Soc* 2010 21;13:11.

9. Janssens B, Raleigh B, Soeung S, Akao K, Te V, Gupta J, et al. Effectiveness of highly active antiretroviral therapy in HIV-positive children: evaluation at 12 months in a routine program in Cambodia. *Pediatrics* 2007; 120(5):e1134-40.

10. Violari A, Cotton MF, Gibb DM, Babiker AG, Steyn J, Madhi SA, et al. Early antiretroviral therapy and mortality among HIV-infected infants. *N Engl J Med* 2008 20; 359(21):2233-44.

11. Chiappini E, Galli L, Tovo PA, Gabiano C, Gattinara GC, Guarino A, et al. Virologic, immunologic, and clinical benefits from early combined antiretroviral therapy in infants with perinatal HIV-1 infection. *AIDS*, 2006, 20(2):207–215.

12. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-

Ytter Y, Alonso-Coello P, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* 2008 26; 336(7650):924-6.

13. Reynolds SJ, Ndongala LM, Luo CC, Mwandagaliwa K, Losoma AJ, Mwamba KJ, et al. Evaluation of a rapid test for the detection of antibodies to human immunodeficiency virus type 1 and 2 in the setting of multiple transmitted viral subtypes. *Int J STD AIDS* 2002; 13(3):171-3.

14. Prendergast A, Mphatswe W, Tudor-Williams G, Rakgotho M, Pillay V, Thobakgale C, et al. Early virological suppression with three-class antiretroviral therapy in HIV-infected African infants. *AIDS* 2008, 22(11):1333–1343.

15. Lambert JS, Harris DR, Stiehm ER, Moye J Jr, Fowler MG, Meyer WA, et al. Performance characteristics of HIV-1 culture and HIV-1 DNA and RNA amplification assays for early diagnosis of perinatal HIV-1 infection. *J Acquir Immune Defic Syndr* 2003 15;34(5):512-9.

16. Kline MW, Lewis DE, Hollinger FB, Reuben JM, Hanson LC, Kozinetz CA, et al. A comparative study of human immunodeficiency virus culture, polymerase chain reaction and anti-human immunodeficiency virus immunoglobulin A antibody detection in the diagnosis during early infancy of vertically acquired human immunodeficiency virus infection. *Pediatr Infect Dis J* 1994; 13(2):90-4.

17. Kovacs A, Xu J, Rasheed S, Li XL, Kogan T, Lee M, Liu C, et al. Comparison of a rapid nonisotopic polymerase chain reaction assay with four commonly used methods for the early diagnosis of human immunodeficiency virus type 1 infection in neonates and children. *Pediatr Infect Dis J* 1995; 14(11):948-54.

18. Prasitwattanaseree S1, Lallemand M, Costagliola D, Jourdain G, Mary JY. Influence of mother and infant zidovudine treatment duration on the age at which HIV infection can be detected by polymerase chain reaction in infants. *Antivir Ther* 2004; 9(2):179-85.

19. Ribas SG, Ondo P, Schüpbach J, van der Groen G, Fransen K, et al. Performance of a quantitative human immunodeficiency virus type 1 p24 antigen assay on various HIV-1 subtypes for the follow-up of human immunodeficiency type 1 seropositive individuals. *J Virol Methods* 2003; 113(1):29-34.

20. Luo W, Yang H, Rathbun K, Pau CP, Ou CY, et al.

Detection of human immunodeficiency virus type 1 DNA in dried blood spots by a duplex real-time PCR assay. *J Clin Microbiol* 2005; 43(4):1851-7.

21. Lakshmi V, Sudha T, Bhanurekha M, Dandona L. et al. Evaluation of the Murex HIV Ag/Ab Combination assay when used with dried blood spots. *Clin Microbiol Infect* 2007; 13(11):1134-6.

22. Ou CY, Yang H, Balinandi S, Sawadogo S, Shanmugam V, Tih PM, et al. Identification of HIV-1 infected infants and young children using real-time RT PCR and dried blood spots from Uganda and Cameroon. *J Virol Methods* 2007; 144(1-2):109-14.

23. Sutcliffe CG, van Dijk JH, Bolton C, Persaud D, Moss WJ. et al. Effectiveness of antiretroviral therapy among HIV-infected children in sub-Saharan Africa. *Lancet Infect Dis* 2008; 8(8):477-89.

24. Gulia J, Kumwenda N, Li Q, Taha TE. HIV seroreversion time in HIV-1-uninfected children born to HIV-1-infected mothers in Malawi. *J Acquir Immune Defic Syndr* 2007 v 1; 46(3):332-7.

25. Beck IA, Drennan KD, Melvin AJ, Mohan KM, Herz AM, Alarcón J, et al. Simple, sensitive, and specific detection of human immunodeficiency virus type 1 subtype B DNA in dried blood samples for diagnosis in infants in the field. *J Clin Microbiol* 2001; 39(1):29-33.

26. Berhan Z, Abebe F, Gedefaw M, Tesfa M, Assefa M, Tafere Y. Risk of HIV and associated factors among infants born to HIV positive women in Amhara region, Ethiopia: a facility based retrospective study. *BMC Res Notes* 2014 4;7(1):876.

27. Divala O, Michelo C, Ngwira B. Morbidity and mortality in HIV-exposed under-five children in a rural Malawi setting: a cohort study. *J Int AIDS Soc* 2014 2; 17(4 Suppl 3):19696.

28. Hatcher AM, Woollett N, Pallitto CC, Mokoatle K, Stöckl H, MacPhail C, et al. Bidirectional links between HIV and intimate partner violence in pregnancy: implications for prevention of mother-to-child transmission. *J Int AIDS Soc* 2014 3; 17:19233.

29. Odeny TA, Newman M, Bukusi EA, McClelland RS, Cohen CR, Camlin CS. Developing content for a mHealth intervention to promote postpartum retention in prevention of mother-to-child HIV transmission programs and early infant diagnosis of HIV: a qualitative study. *PLoS*

One 2014 2; 9(9):e106383.

30. Blumental S, Ferster A, Van den Wijngaert S, Lepage P. HIV transmission through breastfeeding: still possible in developed countries. *Pediatrics* 2014; 134(3):e875-9.

31. Sohn AH, Thanh TC, Thinh le Q, Khanh TH, Thu HK, Giang le T, et al. Failure of human immunodeficiency virus enzyme immunoassay to rule out infection among polymerase chain reaction-negative vietnamese infants at 12 months of age. *Pediatr Infect Dis J* 2009; 28(4):273-6.

32. Chantry CJ, Cooper ER, Pelton SI, Zorilla C, Hillyer GV, Diaz C. Seroreversion in human immunodeficiency virus-exposed but uninfected infants. *Pediatr Infect Dis J* 1995; 14(5):382-7.

33. Rakusan TA, Parrott RH, Sever JL. Limitations in the laboratory diagnosis of vertically acquired HIV infection. *J Acquir Immune Defic Syndr* 1991; 4(2):116-21.

34. Makuwa M, Souquière S, Niangui MT, Rouquet P, Apetrei C, Roques P, et al. Reliability of rapid diagnostic tests for HIV variant infection. *J Virol Methods* 2002 16; 103(2):183-90.

35. Phillips S, Granade TC, Pau CP, Candal D, Hu DJ, Parekh BS. Diagnosis of human immunodeficiency virus type 1 infection with different subtypes using rapid tests. *Clin Diagn Lab Immunol* 2000; 7(4):698-9.

36. Rouet F, Montcho C, Rouzioux C, Leroy V, Msellati P, Kottan JB, et al. Early diagnosis of paediatric HIV-1 infection among African breast-fed children using a quantitative plasma HIV RNA assay. *AIDS* 2001 28;15(14):1849-56.

37. Souza IE, Azevedo ML, Succi RC, Machado DM, Diaz RS. RNA viral load test for early diagnosis of vertical transmission of HIV-1 infection. *J Acquir Immune Defic Syndr* 2000 1; 23(4):358-60.

38. Alvarez-Muñoz MT, Zaragoza-Rodríguez S, Rojas-Montes O, Palacios-Saucedo G, Vázquez-Rosales G, Gómez-Delgado A, et al. High correlation of human immunodeficiency virus type-1 viral load measured in dried-blood spot samples and in plasma under different storage conditions. *Arch Med Res* 2005;36(4):382-6.

39. Fischer A, Lejczak C, Lambert C, Servais J, Makombe N, Rusine J, et al. Simple DNA extraction method for dried blood spots and comparison of two PCR

assays for diagnosis of vertical human immunodeficiency virus type 1 transmission in Rwanda. *J Clin Microbiol* 2004; 42(1):16-20.

40. Nesheim S, Palumbo P, Sullivan K, Lee F, Vink P, Abrams E, et al. Quantitative RNA testing for diagnosis of HIV-infected infants. *J Acquir Immune Defic Syndr* 2003 1; 32(2):192-5.

41. Rouet F, Sakarovich C, Msellati P, Elenga N, Montcho C, Viho I, et al. Pediatric viral human immunodeficiency virus type 1 RNA levels, timing of infection, and disease progression in African HIV-1-infected children. *Pediatrics* 2003;112(4):e289.

42. Cachafeiro A, Sherman GG, Sohn AH, Beck-Sague C, Fiscus SA. Diagnosis of human immunodeficiency virus type 1 infection in infants by use of dried blood spots and an ultrasensitive p24 antigen assay. *J Clin Microbiol* 2009; 47(2):459-62.

43. Report of the WHO Technical Reference Group, Paediatric HIV/ART Care Guideline Group Meeting. Geneva, World Health Organization, 2008 (http://www.who.int/hiv/pub/paediatric/WHO_Paediatric_ART_guideline_rev_mreport_2008.pdf).

44. Antiretroviral therapy of HIV infection in infants and children: towards universal access: recommendations for a public health approach Geneva, World Health Organization, 2007 (http://whqlibdoc.who.int/publications/2007/9789241594691_eng.pdf).

45. WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. Geneva, World Health Organization, 200 (http://whqlibdoc.who.int/publications/2007/9789241595629_eng.pdf).

46. Guidance on provider-initiated HIV testing and counselling in health facilities. Geneva, World Health Organization, 2007 (http://whqlibdoc.who.int/publications/2007/9789241595568_eng.pdf).

47. Gleaves CA, Welle J, Campbell M, Elbeik T, Ng V, Taylor PE, et al. Multicenter evaluation of the Bayer VERSANT HIV-1 RNA 3.0 assay: analytical and clinical performance. *J Clin Virol* 2002;25(2):205-16.

48. World Health Organization and Joint United Nations Programme on HIV/AIDS (UNAIDS). HIV assays: operational characteristics. Geneva, World Health Organization, 2009 (http://www.who.int/diagnostics_

laboratory/evaluations/hiv/en/index.html accessed on 18 December 2009).

49. Antiretroviral Therapy for HIV infection in infants and children: Toward universal access Recommendations for a public health approach, 2010 Revision .WHO.

50. Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection National Institutes of Health, USA, August 16, 2010.

51. Garrido C, Zahonero N, Corral A, Arredondo M, Soriano V, de Mendoza C, et al. Correlation between human immunodeficiency virus type 1 (HIV-1) RNA measurements obtained with dried blood spots and those obtained with plasma by use of Nuclisens EasyQ HIV-1 and Abbott Real Time HIV load tests. *J Clin Microbiol* 2009; 47(4):1031-6.

52. Mwaba P, Cassol S, Nunn A, Pilon R, Chintu C, Janes M, et al. Whole blood versus plasma spots for measurement of HIV-1 viral load in HIV-infected African patients. *Lancet* 2003 20; 362(9401):2067-8.

53. Braun J, Plantier JC, Hellot MF, Tuillon E, Gueudin M, Damond F, et al. A new quantitative HIV load assay based on plasma virion reverse transcriptase activity for the different types, groups and subtypes. *AIDS* 2003 14; 17(3):331-6.

54. Crowe S, Turnbull S, Oelrichs R, Dunne A. Monitoring of human immunodeficiency virus infection in resourceconstrained countries. *Clin Infect Dis* 2003 1; 37(Suppl 1):S25-35.

55. Sutthent R, Gaudart N, Chokpaibulkit K, Tanliang N, Kanoksinsombath C, Chaisilwatana P. p24 Antigen detection assay modified with a booster step for diagnosis and monitoring of human immunodeficiency virus type 1 infection. *J Clin Microbiol* 2003; 41(3):1016-22.

56. Maliwichi M, Rosenberg NE, Macfie R, Olson D, Hoffman I, van der Horst CM, et al. CD4 count outperforms World Health Organization clinical algorithm for point-of-care HIV diagnosis among hospitalised HIV-exposed Malawian infants. *Trop Med Int Health* 2014 Aug;19(8):978-87.

57. Selik RM, Gebo KA, Borkowf CB, Whitmore SK, Espinoza L. CD4 T-lymphocyte percentages corresponding to CD4 T-lymphocyte count thresholds in a new staging system for HIV infection. *J Acquir Immune Defic Syndr* 2014 Aug 1;66(4):e92-4.

58. Frizzera Dias C, Moreira-Silva SF, Reis MA, Ribeiro Patrício L, Biancardi Gavioli CF, Miranda AE. Late diagnosis and HIV infection in children attending a service of specialized care for pediatric AIDS in Brazil. *Rev Soc Bras Med Trop* 2014 Jan-Feb;47(1):93-6.
59. Smit PW, Sollis KA, Fiscus S, Ford N, Vitoria M, Essajee S, et al. Systematic review of the use of dried blood spots for monitoring HIV viral load and for early infant diagnosis. *PLoS One* 2014 Mar 6;9(3):e86461.
60. Gupta R, Praveen R, Sharma M. Can we prevent pediatric HIV? An experience at a tertiary care hospital. *Med J Armed Forces India* 2013 Jul;69(3):218-21.
61. Corró G, Crudeli CM, Rocco CA, Marino SA, Sen L. High levels of anti-Nef antibodies may prevent AIDS disease progression in vertically HIV-1-infected infants. *J Int AIDS Soc* 2014 Feb 14;17:18790.
62. Motswere-Chirwa C, Voetsch A, Lu L, Letsholathebe V, Lekone P, Machakaire E, et al. Follow-up of infants diagnosed with HIV - Early Infant Diagnosis Program, Francistown, Botswana, 2005-2012. *MMWR Morb Mortal Wkly Rep* 2014 Feb 21;63(7):158-60.
63. Shiao S, Kuhn L, Strehlau R, Martens L, McIlleron H, Meredith S, et al. Sex differences in responses to antiretroviral treatment in South African HIV-infected children on ritonavir-boosted lopinavir- and nevirapine-based treatment. *BMC Pediatr* 2014 Feb 12; 14:39.
64. Gebremedhin A, Gebremariam S, Haile F, Weldearegawi B, Decotelli C. Predictors of mortality among HIV infected children on anti-retroviral therapy in Mekelle Hospital, Northern Ethiopia: a retrospective cohort study. *BMC Public Health* 2013 Nov 6;13:1047.
65. Shiao S, Kuhn L. Antiretroviral treatment in HIV-infected infants and young children: novel issues raised by the Mississippi baby. *Expert Rev Anti Infect Ther* 2014 Mar; 12(3):307-18.
66. HIV Paediatric Prognostic Markers Collaborative Study. Predictive value of absolute CD4 cell count for disease progression in untreated HIV-1-infected children. *AIDS* 2006, 20(9):1289–1294.
67. Joint United Nations Programme on HIV/AIDS (UNAIDS) and World Health Organization. AIDS epidemic update: December 2009. Geneva, Joint United Nations Programme on HIV/AIDS (UNAIDS), 2009 (http://whqlibdoc.who.int/unaid/2009/97892911738328_eng.pdf).
68. Patton JC, Sherman GG, Coovadia AH, Stevens WS, Meyers TM. Ultrasensitive human immunodeficiency virus type 1 p24 antigen assay modified for use on dried whole-blood spots as a reliable, affordable test for infant diagnosis. *Clin Vaccine Immunol* 2006; 13(1):152-5.
69. Schüpbach J. Measurement of HIV-1 p24 antigen by signal-amplification-boosted ELISA of heat-denatured plasma is a simple and inexpensive alternative to tests for viral RNA. *AIDS Rev* 2002; 4(2):83-92.
70. Zijenah LS, Tobaiwa O, Rusakaniko S, Nathoo KJ, Nhembe M, Matibe P, et al. Signal-boosted qualitative ultrasensitive p24 antigen assay for diagnosis of subtype C HIV-1 infection in infants under the age of 2 years. *J Acquir Immune Defic Syndr* 2005 1; 39(4):391-4.
71. Poulsen AG, Aaby P, Gottschau A, Kvinesdal BB, Dias F, Mølbak K, et al. HIV-2 infection in Bissau, West Africa, 1987–1989: incidence, prevalences, and routes of transmission. *J Acquir Immune Defic Syndr* 1993; 6(8):941-8.

How to cite this article:

Abdollahi A, Saffar H. The Diagnosis of HIV Infection in Infants and Children. *Iran J Pathol.* 2016;11(2):89-96.