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Technique for detecting HIV-1 proviral DNA by PCR: The case of newborns

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INTRODUCTION

Polymerase chain reaction (PCR)testing for integrated viral DNA (proviral DNA) is the standard method used for early detection of HIV in newborns. In developing countries, this approach has been made more accessible through the dried blood spot (DBS) technique. The medical challenge is to identify infants infected via vertical transmission of HIV so that they can receive antiretroviral (ARV) treatment early. In resource-limited countries, there are many advantages to using DBS samples rather than whole blood for PCR testing to detect DNA.

MATERIALS AND METHODS

Extraction of proviral DNA using DBS: Cutting: Prepare AW1 and AW2 buffers according to the manufacturer's instructions; Cut 3 mm diameter pellets from a drop of dried blood using scissors; Transfer the pellet pieces from the blotting paper containing the dried blood into a 1.5 ml tube and add 180 µl of ATL buffer;

Lysis: Pipette 180 µl of ATL buffer into the tubes containing 100 µl of plasma RNA pellet; Incubate for 10 min at 85°C. Add 20 µl of Proteinase K solution, mix by vortexing, then incubate for 1 hour at 56°C. Centrifuge briefly to recover the droplets accumulated in the cap. Add 200 µl of AL buffer to the sample, mix vigorously by vortexing, then incubate for 10 min at 70°C. Centrifuge briefly;

Fixation: Add 200 µl of absolute ethanol to the sample and mix vigorously by vortexing. Centrifuge briefly; Carefully place the mixture obtained in step 6 into the QIAmp column without wetting the edge. Close the cap and centrifuge for 1 min at 8000 rpm. Transfer the QIAmp column to a 2 ml collection tube and discard the tube containing the effluent.

Washing: Add 500 µl of AW1 buffer. Centrifuge for 1 minute at 8000 rpm; transfer the QIAmp column to a 2 ml collection tube and discard the tube containing the effluent; add 500 µl of AW2 buffer. Close the cap and centrifuge for 3 minutes at 14,000 rpm. Place the QIAMP column in a new 2 ml collection tube and discard the old collection tube containing the effluent. Centrifuge for 1 min at 14,000 rpm; Transfer the QIAmp column to a

clean 1.5 ml tube. Discard the old collection tube containing the effluent;
Elution: Add 70µl of elution buffer. Incubate for 1 min at room temperature, then centrifuge for 1 min at 8,000 rpm.
Preparation of qualitative PCR: Polymerase chain reaction (PCR) was initially conducted using the DNA samples. The Generic DNA Cell Kit (Biocentric, Bandol, France) was employed for this procedure. A second PCR of the DBS spot DNA was performed to confirm the real-time PCR results for samples with an indeterminate (doubtful) serological status, which proved negative in the qualitative real-time PCR. The amplification reaction mixture is described in Table 1 below:

Table 1: PCR Reaction Mixture

MIX components	Volume µl
qPCR mix with platinum Taq 60U/ml + Rox	25
Primer A	1
Primer B	1
Probe C	1
Sterile water	2
Total	30 µl

PCR was performed in a 9700 thermal cycler (Applied Biosystems, USA) in a reaction volume of 50 µl comprising 30 µl of mix and 20 µl of DNA according to the amplification program described in Table 2.

Table 2: PCR amplification program

Stages		**Time**	**Temperature**
Initial Denaturation		2 min	50°C
Enzyme activation	50 cycles	10 min	95°C
Denaturation 50 cycles		15 s	95°C
Hybridization		1 min	60°C

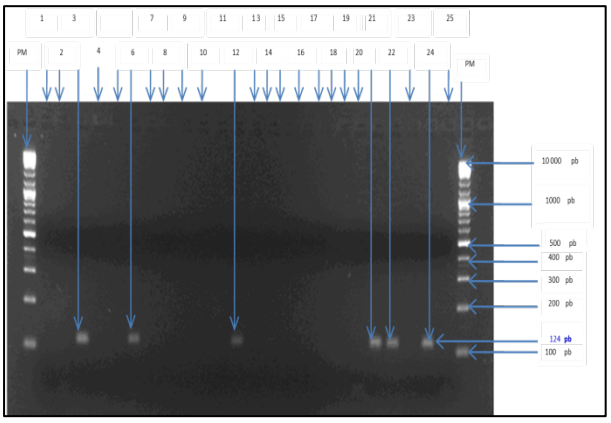

Figure 1: PCR product on agarose gel, under UV light Figure 2: Preparation of DNA extraction
Legend: MW = Molecular weight marker (Fermentas: GeneRuler™ DNA Ladders "GeneRuler™ DNA Ladder Mix," ready-to-use, ≠ SM 0333; 100-10,000 bp).
Well 24 = positive control; Well 25 = negative control
In wells 1, 2, 4, 5, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, we have visible band s: these samples are PCR positive. Wells 3, 6, 12, 21, 22, and 23 correspond to PCR-negative samples. The expected DNA bands formed between 100 and 200 base pairs (bp), which corresponds to the expected fragment size of approximately 110 to 120 bp.

Advantages and limitations of the technique

Advantages	Limitations
Less expensive technique Rapid detection of proviral DNA Limited use of sophisticated equipment	Requires venous blood sampling from the newborn. Viral load cannot be estimated using this technique, as a positive result only indicates the presence of the virus

Conclusion: Early diagnosis of HIV plus early treatment are the cornerstones of successful paediatric HIV/AIDS care as they reduce morbidity and mortality. This means that more newborns are diagnosed early and t remain healthier. Promoting PCR as a routine technique for the early diagnosis of HIV infection in newborns is now widely recognized as best practice.

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