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Comparison of Rapid Diagnosis Test and Nested PCR for HIV Diagnosis in Kinshasa

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Abstract

Background: The need for appropriate methods for the diagnosis, monitoring and treatment of HIV infection is increasing in resource-poor settings. **Objective:** The main objective of this study was to compare the quality of the results of HIV diagnosis by Classical Nested PCR and Rapid Screening Tests (RDTs) in order to contribute to the improvement of the care of People Living with HIV (PLHIV). **Methods:** The present study is a cross-sectional study that was conducted in Kinshasa. Only people willing to voluntary testing for HIV were selected for this study. Our sample consisted of 100 individuals for voluntary testing for HIV, and of 50 PLHIV who came for their medical appointment. A minimum of 5.0 ml of blood was collected into tubes containing EDTA. After the RDT, the collected blood was transferred at the Laboratory for molecular analysis. The revelation of the amplification results was made under UV light after electrophoretic migration on agarose gel. **Results:** On D0, 60 samples were positive and 40 negative for HIV by RDT. After amplification by Nested DNA PCR, on D0, the gag region gave 65 positive and 35 negative; while the amplification on the pol region gave 63 positive and 37 negative. At the M6, for patients under treatment, 10 samples were positive and 40 negative for HIV using the RDT. After amplification, all the 50 samples collected were positive by Nested DNA PCR on the gag and pol regions. **Conclusion:** Nested DNA PCR is precise, specific and accurate that RDT especially for the cases of patients under treatment.

Subject Areas

HIV

Keywords

Nested DNA PCR, RDT, Diagnosis, HIV, Kinshasa

1. Introduction

The need for appropriate methods for the diagnosis, monitoring and treatment of HIV infection is increasing in Resource Limited Countries (RLC) [1]. Early detection of infection is necessary for proper monitoring of infected patients. Today, HIV infection is diagnosed by 5 means: 1) direct visualization by microscopy, 2) culture of lymphocytes, 3) measurement of serological responses specific to HIV, 4) detection of viral antigens, 5) detection of viral nucleic acids (Deoxyribose Nucleic Acid-DNA and Ribose Nucleic Acid-RNA) [2] [3]. To know a serological status, biologists currently have several techniques at their hands: Rapid Diagnosis Tests (RDTs), the ELISA test, Polymerase Chain Reaction (PCR), branched-DNA, Western Blot. These tools do not target the same elements [4].

PCR is a high-sensitivity molecular biology technique that detects fragments of DNA and RNA in viruses and bacteria and other microorganisms in the blood and tissue. It is this technique and its variants that are used in qualitative and quantitative HIV research. PCR has enabled the development of ultra-sensitive tests for HIV, thanks to which it is possible to diagnose this infection early, even before the appearance of antibodies specific to each of them. This technique has become essential in several fields of Biomedical Sciences [5] [6].

Thus, the objective of this study was to compare the quality of the results of HIV diagnosis by classical PCR and RDTs, in order to contribute to the improvement of the care of People Living with HIV (PLHIV).

2. Methods

2.1. Framework

The present study is a cross-sectional study that was conducted at the Molecular Biology Laboratory, at the Faculty of Medicine of the University of Kinshasa (UNIKIN) in collaboration with the Saint Alphonse Center and Pilot Health Center and Maternity, both are screening service and support for People Living with HIV (PLHIV) in Kinshasa. The study was conducted from January to June 2016.

2.2. Patients

The sample consisted of 100 individuals who have made voluntary testing for HIV by Rapid Diagnosis Test (RDT) selected randomly in the centers; of 50 PLHIV who came for their medical appointment during the period of study at both centers selected randomly. There was no sample calculation; the patients were recruited as they were coming until they reached the number for convenience. All were willing and signed consent for screening with the respective institutions.

2.3. Blood Samples

A minimum of 5.0 ml of blood was collected into tubes containing EDTA from

the respective centers by the technical team. After the RDT, the collected blood was transferred at the Laboratory of Molecular Biology in respect of the conservation temperature (5˚C to 10˚C). Once there, it was centrifuged at 3000 rotation per minute (rpm) for 10 minutes at room temperature to obtain a buffy coat. Five hundred microliters (500 µl) of buffy coat was collected in a pre-labeled tube and stored at 4˚C before molecular extraction and molecular analysis.

2.4. Extraction and DNA Amplification

The DNA extraction was made from 200 µl of buffy coat using the QIAamp DNA Mini Kit from QIAGEN ® for DNA extraction [7] [8]. The DNA extract was stored at −20˚C at the laboratory.

After extraction, a Classic PCR on HLA and Nested PCR on the *gag* and *pol* regions of the virus were performed to determine the proviral DNA. Nested PCR on the *env* region was performed about in cases of discordant results of *gag* and *pol* [8]. All the primers for amplification were previously described in the literature [8] and are presented in **Table 1**. PCR assays were carried out under the conditions previously described [8] and are presented in **Table 2**.

2.5. Interpretation of Results by Electrophoresis

The revelation of the amplification results was made under UV light after electrophoretic migration on agarose gel 1% prepared in 1X TAE (TRIS/Acetate/EDTA).

Table 1. Primers for the PCR assays.

PCR assay	Primers	Sequences
HLA assay	GH26 Forward	5'-GTGCTGCAGGTGTAAACT-3'
	GH27 Reverse	5'-CACGGATCCGGT-3'
gag	GAG1 Forward	5'-GGTACATCAGGCCATATCACC-3'
	GAG4 Reverse	5'-ACCGGTCTACATAGTCTC-3'
pol	POLITG1 Forward	5'-CCCTACAATCCCCAAAGTCAAGG-3'
	POLITG4 Reverse	5'-TACTGCCCCTTCACCTTTCCA-3'
env	ENV1 Forward	5'-GAGGATATAATCAGTTTATGG-3'
	ENV4 Reverse	5'-AATTCCATGTGTACATTGTACTG-3'
Nested gag	GAG2 Forward	5'-GAGGAAGCTGCAGAATGGG-3'
	GAG3 Reverse	5'-GGTCCTTGTCTTATGTCC-3'
Nested pol	POLITG2 Forward	5'-TAAGACAGCAGACAAATGGCAG-3'
	POLITG3 Reverse	5'-GCTGTCCCTGTAATAAACCCG-3'
Nested env	ENV2 Forward	5'-GATCAAAGCCTAAAGCCATG-3'
	ENV3 Reverse	5'-CAATAATGTATGGGAATTGG-3'

Table 2. PCR cycles and temperatures.

	PCR *gag* and *pol*	PCR *env* and *Nested pol*	PCR *HLA* and *Nested gag*	*Nested env*
Denaturation	95˚C/9 minutes	95˚C/9 minutes	95˚C/9 minutes	95˚C/9 minutes
Denaturation	94˚C/1 minute	94˚C/1 minute	94˚C/1 minute	94˚C/1 minute
Hybridation	50˚C/1 minute	50˚C/1 minute	55˚C/1 minute	45˚C/1 minute
Elongation	72˚C/1 minute	72˚C/1 minute	72˚C/1 minute	72˚C/1 minute
Final Elongation	72˚C/10 minutes	72˚C/10 minutes	72˚C/10 minutes	72˚C/10 minutes
Hold	4˚C	4˚C	4˚C	4˚C
Number of cycles*	35	30	25	25

*Number of PCR cycle per assay.

For a sample to be count up positive for HIV by Nested DNA PCR, it should be positive for HLA amplification and then positive for amplifications of two regions (*gag*/*pol*, *gag*/*env* and/or *pol*/*env*).

3. Results

A total of 100 samples were collected for the inclusion (day zero-D0) for diagnosis by RDT and PCR, and 50 samples for the 6th month (M6) for PCR assay only. On D0, there was 70% of women while on M6 there was 65%.

On D0, 60 samples were positive and 40 negative for HIV by RDT. After amplification by Nested DNA PCR, on D0, the *gag* region gave 65 positive and 35 negative; while the amplification on the *pol* region gave 63 positive and 37 negative. The two discordant samples of *gag* and *pol* were reassessed by amplification on the *env* region as a confirmation method for the difference of these samples, the amplification of the two gave the two positive samples for HIV (Table 3 and Table 4).

At the M6, for patients under treatment, 10 samples were positive and 40 negative for HIV using the RDT. After amplification, all the 50 samples collected were positive by Nested DNA PCR on the *gag* and *pol* regions (Table 5). All data are presented in the following tables.

4. Discussion

The purpose of this study was to compare the results of HIV diagnosis by classical Nested DNA PCR and RDTs in order to contribute to improving the care of People Living with HIV (PLHIV) in Kinshasa. It was carried out on a population of 150 HIV infected patients of which 100 were newly diagnosed and naïve of treatment, and 50 were under treatment for 6 months.

On D0, 60 samples were diagnosed HIV positive by RDT and 40 negative. After amplification; 65 samples were confirmed HIV positive by PCR and 35 HIV negative. This difference is justified by the fact that the PCR catechizes the viral

DNA which, even when the Viral Load (VL) is undetectable. The 2 discordant samples of *gag* and *pol* were reevaluated by amplification on the *env* region as a method to confirm the discrepancy of these samples. The provirus integrated into the DNA of lymphocytes was detected. According to the Nested DNA PCR, there are 65 positive samples for HIV instead of 60 as presented by RDTs; meaning that 5% of positive samples in this group were not detected by RDTs. This difference of detection was presented in previous studies stating that PCR is more specific and precise than RDTs [8] [9].

Table 3. Confirmation of HIV diagnosis by RDT and PCR at D0.

SAMPLES	TDR	HLA		*Gag*		*Pol*		*env**	
		+	−	+	−	+	−	+	−
Positive	60	60	0	60	0	58	2	2	--
Negative	40	40	0	5	35	5	35	--	--
Indeterminate	0	0	0	0	0	0	0	--	--
Total	**100**	**100**	**0**	**65**	**35**	**63**	**37**	**2**	**--**

**env* region Nested DNA PCR to verify discordance between *gag* and *pol* region PCR.

Table 4. Confirmation of results by Nested DNA PCR at D0.

	Total
RDT+ => PCR+	60
RDT+ => PCR−	0
RDT− => PCR+	5
RDT− => PCR−	35
RDT± => PCR+	0
RDT± => PCR−	0
Total	**100**

Legends: RDT+ = positive results with Rapid Diagnostic Test; RDT− = negative results with Rapid Diagnostic Test; RDT± = undetermined results with the Rapid Diagnostic Test; PCR+ = positive results with PCR assay; PCR− = negative results with PCR assay.

Table 5. Confirmation of HIV diagnosis by RDT and PCR at M6.

SAMPLES	TDR	HLA		*gag*		*pol*	
		+	−	+	−	+	−
Positive	10	10	0	10	0	10	0
Negative	40	40	0	40	0	40	0
Undeterminate	0	0	0	0	0	0	0
Total	**50**	**50**	**0**	**50**	**0**	**50**	**0**

On M6, of the 50 samples received, 10 and 40 were respectively positive and negative for HIV by RDT. This could be explained by the limit of the test when the patient is taking properly his treatment to reach viral suppression; after 6 months of ARV treatment, the virus should be undetectable if the treatment is respected. However, after amplification by Nested DNA PCR, all 50 samples were positive. This is because the Nested DNA PCR digs out the provirus in the human genome of the infected cells, where it is hiding. Therefore, even under undetectable condition of the virus in the plasma, the retro-transcribe virus in the lymphocytes can still be detected.

These results emphasize the great specificity and precision of the Nested DNA PCR over the RDTs even in limited resources setting. What was indeterminate with the RDTs was revealed positive with the PCR which gives more accurate results. These findings are similar to those presented in previous studies [8] [9].

5. Conclusion

The results of the RDTs and those of the Nested DNA PCRs made it possible to evaluate and compare the accuracy and precision of the HIV diagnosis technics in Kinshasa. Nested DNA PCR is precise, specific and accurate that RDT especially for the cases of patients under treatment.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Aghokeng, A.F., Mpoudi-Ngole, E., Dimodi, H., Atem-Tambe, A., Tongo, M., Butel, C., Delaporte, E. and Peeters, M. (2009) Inaccurale Diagnosis of HIV-1 Group M and O Is a Key Challenge for Ongoing Universal Access to Antiretroviral Treatment and VIH Prevention in Cameroon. *PLoS ONE*, **4**, e7702. https://doi.org/10.1371/journal.pone.0007702

[2] Tang, Y.W. and Ou, C.Y. (2012) Past, Present and Future and Molecular Diagnosis and Characterization of Human Immunodeficiency Virus Infections. *Emerging Microbes and Infections*, **1**, e19. https://doi.org/10.1038/emi.2012.15

[3] Branson, B.M. (2007) State of the Art for Diagnostics of HIV Infection. *Clinical Infectious Diseases*, **45**, S221-225. https://doi.org/10.1086/522541

[4] Dépistage et confirmation de la séropositivité (2008) https://www.actupparis.org/spip.php?article3375

[5] Programme National de Lutte contre le VIH/SIDA et les Infections Sexuellement Transmissible (PNLS) (2018) Ministère de la Santé Publique, République Démocratique du Congo. Rapport Annuel.

[6] Gyllensten, U.B. and Erlich, H.A. (1998) Generation of Single-Stranded DNA by the Polymerase Chain Reaction and Its Application to Direct Sequencing of the HLA-DA Locus. *Proceedings of the National Academy of Sciences USA*, Vol. 85, 7652-7656. https://doi.org/10.1073/pnas.85.20.7652

[7] QIAGEN (2010) QIAamp® DNA Mini and Blood Mini Handbook. 3rd Edition,

Germany, 27-29.

[8] Kamangu, N.E., Mayemba, C., Mbikayi, S., Ndarabu, A., Kalala, L.R., Mvumbi, L.G. and Vaira, D. (2014) Implementation of a Classic Nested PCR DNA for HIV Diagnostic in Kinshasa. *International Journal of Collaborative Research on Internal Medicine & Public Health*, **6**, 145-150.

[9] Kleinmans, S., Bush, M.P., Hall, L., Thomson, R., Glynn, S., Gallahan, D., Ownby, H.E. and Williams, A.E. for the Retrovirus Epidemiology Donor Study (1998) False Positive HIV-1 Test Results in Low-Risk Screening Setting of Voluntary Blood Donation. *JAMA*, **280**, 1080-1085. https://doi.org/10.1001/jama.280.12.1080