

## HIV – FORENSICS: IDENTIFICATION OF HIV ANTIGENS AND ANTIBODIES ON BLOOD-STAINED CLOTHES

Idris MA,<sup>1,3</sup> Onwumere GB,<sup>2</sup> Babadoko AA<sup>1,3</sup>, TB Bakare<sup>3,5</sup> Nasir U<sup>1</sup> Wada RY<sup>1</sup> and Abba G<sup>4</sup>

1 Department of Haematology & Blood Transfusion Services, Ahmadu Bello University Teaching Hospital, Zaria, Kaduna State, Nigeria

2 Department of Biological Sciences, Nigerian Defence Academy, Kaduna State, Nigeria

3 Antiretroviral Treatment Laboratory, ABU Teaching Hospital, Zaria, Kaduna state, Nigeria

4 Department of General & Applied Sciences, School of Health Technology, Makarfi, Kaduna state Nigeria

5 Department of Medical Microbiology, ABU Teaching Hospital, Zaria, Kaduna state, Nigeria

All Correspondence to: Muhammad Aminu Idris, Department of Haematology & Blood Transfusion Services, Ahmadu Bello University Teaching Hospital, Zaria,

Private Mail Bag, 1026. Post code; 810006, Kaduna State, Nigeria.

Telephone Numbers: +2348025698966 and +2347066768273

Email: [aminumed@yahoo.com](mailto:aminumed@yahoo.com).

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### ABSTRACT

**Background:** HIV is one of the sexually transmitted infections of viral origin that occurs by contact with or transfer of blood, pre-ejaculates, semen and vaginal fluids. It is possible to get it through sexual assault incidents such as rape. Detection and confirmation of HIV in infected human blood traces and bloodstains found on the bloodstained clothes of sexual assault survivors are critical in forensic analysis, especially in rape cases involving suspected HIV positive perpetrators and an HIV negative victim. Genetic relatedness between the HIV strain in the survivor and that in the suspect might match in criminal prosecutions as evidence of responsibility for HIV transmission to the survivor. **Objective:** To determine the extent to which HIV antigens and antibodies can be detected on different bloodstained clothes to develop a forensic diagnostic methodology for rape cases and early intervention for Prophylaxis, particularly in a no-suspect case. **Materials and Methods:** This is a case-control clinic-based study carried out on ten adult HIV positive patients on antiretroviral (ART) drugs as “subjects” and ten adult HIV negative individuals as “controls”. Blood samples were obtained from all participants, spotted on three fabrics (100% cotton, 50% cotton mixed with 50% polyester and 100% polyester), then tested consecutively after one month and after four months for the presence of HIV antibodies using Enzyme-Linked Immunosorbent Assay (ELISA). Also, HIV-1 RNA PCR (viral load) on all participants’ samples. Findings were subjected to statistical analysis to compare the Subject's and Control's results. **Results:** All the subject's HIV ELISA results were positive for HIV, and their Plasma HIV-1 RNA PCR was detectable in different copies. At the same time, that of controls was negative and undetectable. After one month, there was no statistical significance difference ( $p > 0.05$ ) between plasma at zero-days and whole blood absorbance in the three different fabrics. But after four months, a statistically significant difference ( $< 0.05$ ) was recorded between plasma absorbance and whole blood absorbance on both 100% polyester

and 50% plus 50% cotton cloth. **Conclusion:** HIV antigens and antibodies in bloodstains on clothes can be detected using the ELISA technique. It is possible on clothes found at room temperature in an open environment, but 100% cotton clothing material shows more accurate results as there was not much effect.

**Key Words:** HIV, Bloodstains, ELISA and HIV-1 RNA PCR

### INTRODUCTION

Human immunodeficiency virus (HIV) is a species of Lentivirus. It belongs to a subgroup of retroviruses that infect human beings and cause acquired immunodeficiency syndrome (AIDS) (Weiss, 1993). HIV can cause the failure of the immune system and can cause dangerous opportunistic infections and are times, cancers (Weiss, 1993 & Douek *et al.*, 2009). HIV can be transmitted sexually; it infects through contact with an infected person when exposed to infected blood or transfused, contact with seminal fluids, vaginal fluids and pre-ejaculate fluid. HIV is a sexually transmitted infection transmitted through sexual assaults such as rape. That is why it became imperative to screen every sexually assaulted victim and assailant for HIV to determine their HIV status and institute proper medical care as soon as possible. It is difficult to obtain a blood sample from the assailant, especially when he escaped. But having the assailant's traces of blood on the victim's cloth provides ample opportunity to test for HIV in it. Research has established that HIV might not infect if the HIV-positive person has a stable and undetectable viral load (Eisinger *et al.*, 1994 & Rodger *et al.*, 2019). The risk of transmitting HIV sexually varies, and it depends on the stage of the infection of the infected person or, in the case of rape, the perpetrator. The risks include; plasma RNA viral load, the virulence of the virus strain, clinical stage of the infection, mucosal damage and underlying genital tract infections (Gostinet *et al.*, 1994). There is an increased risk of HIV infection in persons with genital ulcer disease, from 1.5 to 7.0 % in both men and women. Bacterial infections could be related to a relative increase in the prevalence of HIV infection in men and women (Royce *et al.*, 1997). This relationship between genital tract infections and increased vulnerability to HIV infection is often high even after sexual behavior adjustment. There is a high chance of detection of HIV in genital secretions when

there is a persistent sexually transmitted disease (STD) (Fong, 2001).

**Aim of the work:** To identify the presence of HIV antigens and or antibodies on bloodstained clothes by forensic methods to develop a diagnostic methodology for rape cases and subsequent early introduction of HIV post-exposure Prophylaxis.

### MATERIALS AND METHODS

We conducted this study at Nasara HIV treatment and care centre, Department of Haematology and blood Transfusion and Antiretroviral therapy Laboratories, ABUTH Shika in Zaria, over four months, from 24<sup>th</sup> February to 24<sup>th</sup> June 2020. Institutional ethical approval was sought and collected from the Research Ethics Committee. We administer informed consent of Subjects via Subject information consent/questionnaire forms before sample collection. HIV-1 RNA (viral load) was determined using COBAS AmpliPrep/COBAS Taqman HIV-1 test version 2.0 from Roche in the Antiretroviral Therapy Laboratory of ABUTH, Shika. We carried out HIV ELISA analyses at Haematology Laboratory ABUTH, Shika. HIV 1 and HIV 2 antigens and antibodies (HIV 1+2 Ag-Ab) were tested from the samples using Enzyme-Linked Immunosorbent Assay (ELISA) using RecombiLISA kit from CTK Biotech, USA.

### DATA COLLECTION

HIV ELISA was determined by EMax molecular devices microplate reader using HIV recombiLISA kit from CTK Biotech, USA, and HIV-1 RNA (viral load) was determined using COBAS AmpliPrep/COBAS Taqman HIV-1 test version 2.0 from Roche. The HIV ELISA kit has relative sensitivity of 100%, relative specificity of 99.9% and an overall agreement of 99.9%. The detection sensitivity of the kit is 0.05ng/mL when tested with recombinant p24 derived from *E. Coli* (CTK Biotech, 2018). Good laboratory research practices were adhered to to ensure reliable results. The testing kits manufacturer's instructions for conducting all investigations

were strictly followed, and optimum temperature for both samples and reagents were maintained.

#### Study Population

Ten HIV positive adults on antiretroviral therapy and ten HIV negative adults were recruited consecutively into this study. The HIV positive adults who are HIV seropositive constitute the study "subjects" while those that were seronegative were considered as the "control" group.

#### SAMPLE PREPARATION

2 Square centimetres of three materials, 100% cotton cloths, 50% cotton + polyester and 100% polyester, were cut into ten pieces to have 60 parts of clothing materials.

Each piece of cloth was stained with 100microliter of HIV positive whole blood and allowed to air dry

The dried bloodstained piece of cloth was transferred into sterile plain blood and left opened at room temperature

30 pieces of 100% cotton, 50% cotton + 50% polyester and 100% polyester bloodstained clothes were tested for HIV 1 &2 Ag-Ab after one month

The remaining 30 pieces of 100% cotton, 50% cotton + 50% polyester and 100% polyester bloodstained clothes were tested for HIV 1 & 2 Ag-Ab after four months

#### REAGENTS PREPARATION

All reagents were brought to room temperature (18-28°C).

The concentrated wash buffer was diluted to 30 folds with distilled water.

8.5gm of analytical grade Sodium Chloride (NaCl) was diluted with 1 litre of distilled water.

Each reagent was mixed thoroughly before transferring to the microtitre wells.

The number of microtitre wells required was determined and marked on the ELISA worksheet together with the required information.

#### ASSAY PROCEDURE

We dispensed 3mls of Normal Saline into bottles containing pieces of cloths stained with blood and centrifuged at 1000 rpm for five minutes to elude the sample.

The desired number of strips were removed from the reagent pack and secured in the microwell frame.

75µL of the supernatant of the centrifuged samples was transferred to the microwells, excluding blank well.

72µL of HIV-1 Antibody, HIV-2 Antibody and p24 Antigen positive and negative controls were added into their respective wells.

25µL of Biotinylated p24 Ab was added into each well except the blank, and the plate was shaken gently for 20seconds.

The plate was covered with a plastic sealer and incubated at 37°C for 60 minutes.

The plate was washed using an automatic washing machine for five cycles.

100µL Horse Raddish Peroxidase (HRP) conjugate solution was transferred to every well except the blank.

The plate was covered with sealant and incubated for 30 minutes.

The plate was washed five times using the automatic washing machine.

50µL of Tetra Methyl Benzidine (TMB) substrate A and 50µL of TMB substrate B was added to every well together with the blank.

The plate was incubated in the dark for 10 minutes.

Stop solution was added into each well and mixed gently for 30 seconds to stop the reaction.

The absorbance was read immediately using a microplate reader at 450nm.

The cut-off value was calculated using this formula:  $C=0.10+N$ , where N= average of negative controls.

#### CALCULATION OF SPECIMEN ABSORBANCE RATIO

We calculate the absorbance ratio for each test sample by dividing its absorbance value by the cut-up value as follows:

$$\frac{\text{Sample absorbance}}{\text{Cut-off value}} = \text{ratio}$$

#### ASSAY VALIDATION

The average absorbance value of the p24 Ag positive control  $\geq 0.80$  nanometer

The average absorbance value of the HIV-1 Ab positive control  $\geq 0.80$  nanometer

The average absorbance value of the HIV-2 Ab positive control  $\geq 0.80$  nanometer

The average absorbance value of the HIV negative control  $\leq 0.10$  nanometer

Interpretation of the results

Sample absorbance ratio negative  $<1.00$  (Absorbance of a sample less than one nanometer is considered negative to HIV antigen/antibody).

Positive  $\geq 1.00$  (Absorbance of a sample higher than one nanometer is considered positive to HIV antigen/antibody) (CTK Biotech, 2018).

### STATISTICAL ANALYSIS

We analyzed data with SPSS version 23 statistical software. Findings presented as frequency distribution, means, standard deviation, bar charts and Student t-Test to compare the Subject's and Control's where appropriate, a p-value of  $\leq 0.05$  was considered significant at 95% confidence interval. Spearman's rank correlation was used to test the relationship.

### RESULTS

A total of 10 HIV positive samples from known adult patients with HIV positive on antiretroviral therapy (ART) drugs" subjects" and 10 HIV negative individuals" controls" were studied.

**Table (1)** shows the socio-demographics and Drug characteristics of the subjects. The ages range from 37 to 54. Six males and four females participated in the study. The person who commenced ART on 1<sup>st</sup> October 2005 has the most prolonged duration of ART treatment, while the subject who began ART on 18<sup>th</sup> February 2020 has the shortest treatment period. Eight subjects are on 1b antiretroviral drugs (ARDs), while one is on 2c and the remaining on 2c.

**Table (2)** shows HIV-1 ELISA Assay (Ag/Ab) on plasma and different fabrics stained with subjects' blood tested after one month and plasma RNA quantification. *All the subjects' HIV-1 ELISA assay results are positive for HIV.* There are five subjects with the least plasma RNA viral load of fewer than 20 copies per millilitre ( $<20$  copies/ml). Subjects with 70,800 copies/ml have the highest viral load. After four months, all HIV-1 ELISA Assay (Ag/Ab) results of the subject's blood tested on plasma and different stained fabrics remain positive (table: 3).

While all the results of controls blood tested came out negative (**table: 4& 5**).

**Table (6)** shows the mean  $\pm$ SD of whole Blood absorbance at one month after staining and plasma at zero days. There was no statistical significance difference ( $p>0.05$ ) between plasma at zero-days and whole blood absorbance in all the three different cloths materials after one month.

**Table (7)** shows absorbance median (interquartile range IQR), t- and p-values of plasma and whole Bloodstained clothes after four months. *After four months, a statistically significant difference ( $p<0.05$ ) between plasma absorbance and whole blood absorbance on both 100% polyester and 50% plus 50% cotton cloth was detected. At the same time, no statistically significant difference was detected on 100% cotton fabric.*

Also, there was a Statistically significant difference of *whole blood* ( $p<0.05$ ) *between one and four months*, detected only on 50% cotton material mixed with 50% polyester and on 100% polyester (**Table: 8**).

*Concerning longevity of antiretroviral therapy (ART), viral load and fabrics used*, Subject number one started ART on first October 2005. It shows that he has been on medication for the past 15 years, yet the *viral load is 2730copies/ml*. His sample on 100% polyester presented the highest absorbance of 3.675nm and the *least absorbance of 1.152nm on 50% cotton mixed 50% polyester*. Subject number two commenced ART on 7<sup>th</sup> June 2006 had a viral load of less than 20copies/ml. This is *the least HIV RNA copies* the machine we used can detect. He has the *highest absorbance of 2.986nm on 50% cotton mixed with 50% polyester* and the least absorbance of 1.187 in his plasma. This shows direct proportionality between viral load and viral antigen/antibodies (**fig. 1**).

Subject number three, who started ART on 23<sup>rd</sup> October 2008, has a viral load of 144copies/ml and the highest absorbance of 3.231nm on 50% cotton plus 50% polyester and the least absorbance of 1.885nm in plasma. Subject number four has been on ARVs since 23<sup>rd</sup> January 2009 and has a viral load of  $<20$ copies/ml. He has the highest absorbance of 2.399nm on 100% polyester and the least absorbance of 1.194nm in 50% cotton plus 50%

polyester. Subject number five, who started ART on 17<sup>th</sup> June 2010, has a viral load of 158,00copies/ml after ten years of treatment. The highest absorbance of 1.592nm was detected on 100% cotton material and the least absorbance of 0.839nm on 100% polyester. Subject number six commenced ART on 1<sup>st</sup> September 2010 has a viral load of <20copies/ml. There is high absorbance of 3.104nm on 100% polyester and the least absorbance of 1.106nm on 100% cotton material. Subject number seven, who started treatment with ARVs on 7<sup>th</sup> March 2011, also has a viral load of <20copies/ml. The highest absorbance of 2.532nm was detected in plasma, and the least of 1.852nm was on 50% cotton plus 50% polyester. On 1<sup>st</sup> April 2011, Subject number eight started ART and has a viral load of 70,800copies/ml. Highest absorbance of 3.763nm on 100% polyester and the least of 0.962nm on 50% cotton plus 50% polyester. Subject number nine began ART on 11<sup>th</sup> June 2012 and has a viral load of <20copies/ml. This patient has the highest absorbance of 1.932nm

on 100% polyester and the least absorbance of 1.202nm in plasma.

The last patient, number ten, started ART on 18<sup>th</sup> February 2020 and has a viral load of 1250copies/ml. Despite starting ART recently, his viral load is less than patients number one, five and eight. He has the highest absorbance of 2.292nm on 100% polyester and the least 1.053nm on 50% cotton plus 50% polyester.

**Table (9)** shows the correlation coefficient of Antiretrovirals (ARVs) longevity—and the fabrics used(100% cotton material, 100% polyester and 50% cotton mixed with 50% polyester). There was a non-significant correlation between ARVs and viral load and100% cotton, and there was a weak and positive relationship *between 100% polyester and viral load*. There were strong negative relationships between 50% cotton mixed with 50% polyester and viral load.

**Table (1):** socio-demographics and Drug characteristic of the participants

Sample No.	Age of participants (years)	Gender of participants	ART regimen	Date of ART initiation
1	50	Male	2d	1 <sup>st</sup> October, 2005
2	50	Male	1b	7 <sup>th</sup> June, 2006
3	42	Male	1b	23 <sup>rd</sup> October, 2008
4	40	Male	1b	23 <sup>rd</sup> January, 2009
5	46	Female	1b	17 <sup>th</sup> June, 2010
6	44	Female	1b	1 <sup>st</sup> September, 2010
7	48	Male	2c	7 <sup>th</sup> March, 2011
8	37	Female	1b	1 <sup>st</sup> April, 2011
9	38	Female	1b	11 <sup>th</sup> June, 2012
10	54	Male	1b	18 <sup>th</sup> February, 2020

1b=Tenofovir/Lamivudine/Rfavirenz  
 2c=Tenofovir/Lamivudine/Atazanavir/Ritinoivir  
 2d=Tenofovir/Lamivudine/Lopinavir/Ritinovir

**Table (2):** HIV-1 ELISA Assay (Ag/Ab) on plasma and on different fabrics of subjects after one month and RNA quantification

Samples	Plasma	Whole blood on		Whole blood on 50% cotton		Whole blood on		Plasma	
	Viral load	100% cotton		mixed with 50% polyester		100% polyester			
In	Absorbance	HIV	Absorbance	HIV	Absorbance	HIV	Absorbance	HIV	
copies/ml	(nm)	Status	(nm)	Status	(nm)	Status	(nm)	status	
1	2730	1.299	+	1.152	+	3.675	+	1.362	+
2	< 20	2.649	+	2.986	+	1.359	+	1.187	+
3	144	2.205	+	3.231	+	1.192	+	1.885	+
4	< 20	2.288	+	1.194	+	2.399	+	1.530	+
5	15,800	1.592	+	1.279	+	0.839	+	1.304	+
6	< 20	1.106	+	1.462	+	3.104	+	1.892	+
7	< 20	1.877	+	1.852	+	2.361	+	2.532	+
8	70,800	1.613	+	0.962	+	3.763	+	2.466	+
9	< 20	1.286	+	1.698	+	1.932	+	1.202	+
10	1250	1.092	+	1.053	+	2.292	+	2.173	+

+ = positive and Nm = nanometer

**Table (3):** HIV-1 ELISA Assay (Ag/Ab) on plasma and on different fabrics of subjects after four months

Sample s	Absorbance of Whole blood on 100% cotton material		Absorbance of Whole blood on 50% cotton mixed with 50% polyester (nm)		Absorbance of Whole blood on 100% polyester (nm)	
	Absorbance(nm)	HIV Status	Absorbance(nm)	HIV Status	Absorbance(nm)	HIV status
1	0.380	+	3.667	+	3.430	+
2	3.812	+	0.920	+	3.337	+
3	2.383	+	3.993	+	4.000	+
4	0.932	+	3.529	+	3.998	+
5	3.602	+	3.770	+	0.890	+
6	3.954	+	3.230	+	3.700	+
7	3.610	+	0.659	+	3.749	+
8	1.484	+	3.498	+	3.841	+
9	0.371	+	3.895	+	3.476	+
10	3.742	+	3.760	+	3.581	+

+ = positive and Nm = nanometer

**Table (4):** HIV-1 ELISA Assay (Ag/Ab) on plasma and on different fabrics for controls after one month

Samples	Whole blood on100% cotton material		Whole blood on 50% cotton mixed with50%polyester (nm)		Whole blood on100%polyester (nm)		Plasma (nm)	
	Absorbance(n m)	HIV Status	Absorbance(n m)	HIV Status	Absorbance(n m)	HIV Status	Absorbance(n m)	HIV status
1	0.087	-	0.055	-	0.077	-	0.021	-
2	0.089	-	0.035	-	0.057	-	0.033	-
3	0.073	-	0.067	-	0.059	-	0.076	-
4	0.076	-	0.078	-	0.061	-	0.053	-
5	0.052	-	0.061	-	0.041	-	0.045	-
6	0.090	-	0.054	-	0.055	-	0.066	-
7	0.021	-	0.081	-	0.045	-	0.062	-
8	0.039	-	0.043	-	0.088	-	0.056	-
9	0.056	-	0.033	-	0.099	-	0.037	-
10	0.077	-	0.031	-	0.031	-	0.022	-

-= Negative and nm=nanometer



**Table (5):** HIV-1 ELISA Assay (Ag/Ab) on plasma and on different fabrics for controls after four months

Samples	Whole blood on 100% cotton material		Whole blood on 50% cotton mixed with 50% polyester (nm)		Whole blood on 100% polyester (nm)		Plasma (nm)	
	Absorbance (nm)	HIV Status	Absorbance (nm)	HIV Status	Absorbance (nm)	HIV Status	Absorbance (nm)	HIV status
1	0.097	-	0.035	-	0.047	-	0.041	-
2	0.079	-	0.075	-	0.077	-	0.023	-
3	0.083	-	0.057	-	0.069	-	0.066	-
4	0.066	-	0.098	-	0.081	-	0.073	-
5	0.072	-	0.071	-	0.091	-	0.035	-
6	0.050	-	0.094	-	0.045	-	0.096	-
7	0.051	-	0.091	-	0.075	-	0.072	-
8	0.099	-	0.053	-	0.098	-	0.036	-
9	0.076	-	0.073	-	0.089	-	0.047	-
10	0.087	-	0.061	-	0.051	-	0.052	-

-= negative and nm= nanometer

**Table (6):** Mean ±SD of whole Blood at 1 month after staining and that of plasma at zero days

	One Month		
	Mean ±SD	t-value	P-value
Plasma	1.75 ±0.51	0.175	0.756
Whole Blood on 100% Cotton material	1.70 ±0.54	0.197	0.846
Whole Blood on 50% cotton mixed with 50% polyester material	1.70 ±0.82	0.167	0.869
Whole Blood on 100% Polyester material	2.29 ±1.00	-1.529	0.144

**Table (7):** Median (IQR) t and p-values of whole Blood after four months

	Four Month		
	Median (IQR)	t-value*	P-value
Plasma	1.68(0.97)	23.01	0.06
Whole Blood on 100% cotton material	2.99(2.97)	38.00	0.364 <sup>a</sup>
Whole blood on 50% cotton mixed with 50% polyester material	3.60(1.15)	20.00	0.023
100% Polyester material	3.64(0.47)	10.00	0.002

\*Mann-Whitney U test, <sup>a</sup>Insignificant

**Table (8):** Median (IQR) t and p-values of whole Blood after one and four months

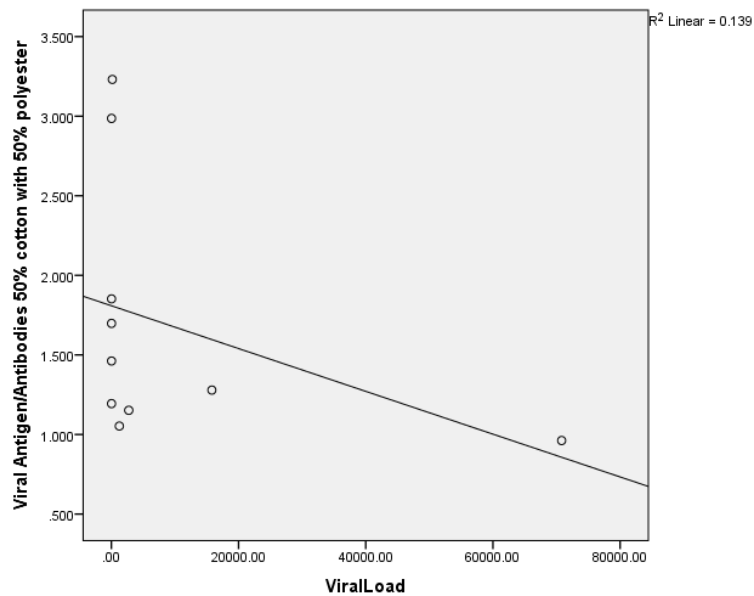
	One Month	Four Month	t	P-value*
	Median (IQR)	Median (IQR)		
Whole Blood on 100% Cotton material	1.60(0.99)	2.99(2.97)	-1.274	0.203 <sup>a</sup>
Whole blood on 50% cotton mixed with 50% polyester material	1.37(1.01)	3.60(1.15)	-2.293	0.022
Whole Blood on 100% Polyester material	2.33(1.93)	3.64(0.47)	-2.191	0.028

\*Wilcoxon sign rank, <sup>a</sup>Insignificant

**Table (9):** the correlation coefficient of Anti Retrovirals (ARVs) longevity (from 2005-2020), on 100% cotton material, 100% polyester, and 50% cotton mixed with 50% polyester

Variables	Viral Load	
	<i>rho</i>	P-value
ARV Longevity	-0.026	0.943
100% Cotton	-0.226	0.530
50% Cotton with 50% Polyester	-0.614	0.059
100% Cotton Polyester	0.123	0.735

*rho* = Spearman rank correlation coefficient



**Figure (1):** a plot between viral antigens/antibodies on 50% cotton mixed with 50% polyester material versus viral load

## DISCUSSION

Serological evidence of infection with HIV may be established by testing for the presence of HIV antigens or antibodies in the blood of subjects suspected of having HIV infection. HIV can be present in cellular and cell-free components of human blood because the main path of infection with HIV involves sexual transmission (Barr *et al.*, 1987).

This paper designed a diagnostic model where HIV antigens/antibodies can be detected in bloodstains on different fabrics forensically using HIV ELISA methods over some time. There are methods for the detection of human blood in which specific proteins in antigen/antibody interactions are detected. (Schweers *et al.*, 2008). Some studies have shown that immunoassays for bloodstain analysis are much more sensitive than the usual precipitin method (Gomes *et al.*, 2001 & Yamamoto *et al.*, 1989). In the present study, all the subject's HIV ELISA results were positive for HIV. Their Plasma HIV-1 RNA PCR was detectable in different copies, from less than 20 copies per millilitre to 70 800 copies per millilitre.

In comparison, that of controls was negative and undetectable. Subject number 8 has the highest viral load of 70,800 copies per ml of whole blood, while subjects 2, 4, 6, 7 and 9 have the lowest plasma RNA viral load of fewer than 20 copies per millilitre of plasma. In general, five patients have less than twenty copies of viral load per millilitre of plasma. Three of them have a minor plasma Ag/Ab absorbance than all the patients. Tyramide signal-amplification boosted Enzyme Immuno Assay for quantification of p24 antigen (HIV core antigen) reportedly has equivalent sensitivity to viral RNA reverse transcriptase-polymerase chain reaction (RT-PCR) amplification at 200-400 RNA copies/mL (Coombs, 2010). Comparative analysis of early versions of fourth-generation ELISA tests revealed p24 detection limits in the range of 125pg/mL across several HIV-1 subtypes, which should detect many infections with relatively high viremia (greater than 500,000 copies/mL plasma) (Francesco *et al.*, 2015).

100% polyester cloth material showed high Ag/Ab retention capacity because 60% of samples were greater than 2.1nm absorbance, followed by 100% cotton cloth material with 30% samples having greater than 2.1nm absorbance. At one month, 100% polyester and cotton show high Ag/Ab retention capacity than 50% cotton mixed with 50% polyester. The highest absorbance was recorded after four months among the three different cloth materials used for one month. Only one patient had an absorbance of greater than 3.1nm on 50% cotton mixed 50% polyester at one month. Only three had greater than 3.1nm on 100% polyester and none in 100% cotton cloth material. But after four months, five patients had greater than 3.2nm on 100% cotton cloth, eight on 50% cotton plus 50% polyester cloth and nine on 100% polyester. Thus, the highest antibody longevity is recorded on 100% polyester cloth material at this level. Competitive ELISA was able to detect the presence of human blood from stains on a variety of surfaces. In addition, the assay gave positive results with both fresh and older stains, including bloodstains up to 1 year old, indicating that the target epitopes for the polyclonal antisera are not significantly degraded over time, at least within the time-span tested (Ian *et al.*, 2009).

After one month, there was no statistical significance difference ( $p > 0.05$ ) between plasma at zero-days and whole blood absorbance in all three different clothes materials. This shows that HIV Ag/Ab can be detected accurately as in plasma as in bloodstains found on 100% cotton, 100% polyester and 50% cotton plus 50% polyester for forensic purposes. *But after four months*, a statistically significant difference ( $< 0.05$ ) between plasma absorbance and whole blood absorbance on 100% polyester and 50% plus 50% cotton cloth was recorded. This shows that HIV Ag/Ab is likely to be *detected precisely on 100% cotton cloth, and their longevity is more assured on this material than the others*. Ian et al. tested bloodstains of human origin found on clothes and got precise positive results with anti-human IgG ELISA. The assay gave identification of bloodstains that were 6 or 12 months old (Ian *et al.*, 2009).

Concerning the correlation between ARVs longevity and the fabrics used, the present study showed a strong and negative correlation in cloth of 50% cotton mixed with 50% polyester. While the other materials used showed insignificant correlations, which could be due to the sample size.

### CONCLUSION

HIV antigens or antibodies are detectable using the ELISA technique on 100% cotton, 50% cotton mixed with 50% polyester and 100% polyester clothing materials stained with HIV positive blood after four months found at room temperature in an open environment. But 100% cotton clothing material produced more accurate results as not much effect was noticed.

### RECOMMENDATIONS

We recommend bloodstains found on rape victims be tested for HIV antigens or antibodies using the ELISA technique to commence HIV prophylaxis on the victim on time to prevent infection.

We recommend this study be carried out on a large sample size and in different temperatures.

We also recommend this study be carried out on Hepatitis and other sexually transmitted infections that can be found in blood.

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## الملخص العربي

### فيروس نقص المناعة البشرية - الطب الشرعي: تحديد مسببات ومضادات فيروس نقص المناعة البشرية على الملابس الملوثة بالدم

إدريس محمد أمين، 1,3 أونومير جودوين بريان، 2، بابادوكو علي أحمد 1,3 ، توكت بولا بكري 5,3 ناصر عثمان ، 1 ودا رابع يونس 1 وأبا جمبو 4

1. قسم أمراض الدم وخدمات نقل الدم ، مستشفى جامعة أحمدو بيلو التعليمي ، زاريا ، ولاية كادونا ، نيجيريا
2. قسم العلوم البيولوجية ، أكاديمية الدفاع النيجيرية ، ولاية كادونا ، نيجيريا
3. معمل العلاج بمضادات الفيروسات القهقرية ، مستشفى جامعة أحمد بالو التعليمي ، زاريا ، ولاية كادونا ، نيجيريا
4. قسم العلوم العامة والتطبيقية ، كلية التكنولوجيا الصحية ، مكافري ، ولاية كادونا ، نيجيريا
5. قسم الأحياء الدقيقة الطبية ، مستشفى جامعة أحمد بللو التعليمي ، زاريا ، كادونا

**جميع المراسلات مع:** محمد أمين إدريس ، قسم أمراض الدم وخدمات نقل الدم ، مستشفى جامعة أحمدو بيلو التعليمي ، زاريا ، حقيبة بريد خاصة ، 1026 الرمز البريدي ؛ 810006 ، ولاية كادونا ، نيجيريا.

أرقام الهاتف + 2348025698966 و: 2347066768273

بريد إلكتروني: aminumed@yahoo.com

الخلفية: فيروس نقص المناعة البشرية هو أحد الأمراض المنقولة جنسيا ذات المنشأ الفيروسي والتي تحدث عن طريق الاتصال أو نقل الدم ، والقذف ، والسائل المنوي والسوائل المهبلية. من الممكن الحصول عليه من خلال حوادث الاعتداء الجنسي مثل الاغتصاب.

يعتبر الكشف عن فيروس نقص المناعة البشرية وتأكيده في آثار الدم البشرية المصابة وبقع الدم الموجودة على الملابس الملطخة بالدماء للناجين من الاعتداء الجنسي أمراً بالغ الأهمية في تحليل الطب الشرعي ، لا سيما في حالات الاغتصاب التي تشمل مرتكبي فيروس نقص المناعة البشرية المشتبه بهم وضحية سلبية لفيروس نقص المناعة البشرية.

قد تتطابق العلاقة الجينية بين سلالة فيروس نقص المناعة البشرية في الناجي وتلك الموجودة في المشتبه به في الملاحظات الجنائية كدليل على المسؤولية عن انتقال فيروس نقص المناعة البشرية إلى الناجية.

الهدف:

تحديد إلى أي مدى يمكن اكتشاف مستضدات فيروس نقص المناعة البشرية والأجسام المضادة على ملابس مختلفة ملطخة بالدماء لتطوير منهجية تشخيص الطب الشرعي لحالات الاغتصاب والتدخل المبكر للوقاية ، خاصة في حالة غير مشتبه فيها. المواد والطرق: هذه دراسة مستندة إلى العيادة أجريت على حالة ضابطة أجريت على عشرة مرضى بالغين مصابين بفيروس نقص المناعة البشرية يتناولون الأدوية المضادة للفيروسات القهقرية (ART) بوصفهم "أشخاصاً" وعشرة أفراد بالغين سلبيين لفيروس نقص المناعة البشرية "ك" ضوابط. تم الحصول على عينات الدم من جميع المشاركين. تم رصدها على ثلاثة أقمشة (100) % قطن ، 50 % قطن ممزوج بـ

50% بوليستر و100% بوليستر (، ثم تم اختبارها على التوالي بعد شهر واحد وبعد أربعة أشهر لوجود الأجسام المضادة لفيروس نقص المناعة البشرية باستخدام فحص ممتص مناعي مرتبط بالإنزيم (ELISA) أيضًا، HIV-1 RNA PCR الحمل الفيروسي (على عينات جميع المشاركين. تم إخضاع النتائج للتحليل الإحصائي لمقارنة نتائج الموضوع والتحكم. النتائج: كانت جميع نتائج ELISA الخاصة بفيروس نقص المناعة البشرية إيجابية لفيروس نقص المناعة البشرية، وكان من الممكن اكتشاف Plasma HIV-1 RNA PCR في نسخ مختلفة. في الوقت نفسه، كانت الضوابط سلبية وغير قابلة للكشف. بعد شهر واحد، لم يكن هناك فرق ذو دلالة إحصائية ( $p > 0.05$ ) بين البلازما عند صفر يوم وامتصاص الدم الكامل في الأقمشة الثلاثة المختلفة. ولكن بعد أربعة أشهر، تم تسجيل فرق معتمد به إحصائياً ( $< 0.05$ ) بين امتصاص البلازما وامتصاص الدم الكامل لكل من 100% بوليستر و50% زائد 50% قماش قطني.

خاتمة

يمكن اكتشاف مستضدات فيروس نقص المناع البشرية والأجسام المضادة في بقع الدم على الملابس باستخدام تقنية ELISA. من الممكن على الملابس الموجودة في درجة حرارة الغرفة في بيئة مفتوحة، لكن مادة الملابس القطنية 100% تظهر نتائج أكثر دقة حيث لم يكن هناك تأثير كبير.

الكلمات المفتاحية: HIV، Blood Stains، ELISA and HIV-1 RNA PCR،