

Level of Correlation of Opportunistic *Mycobacterium* Spp. Infection with Western Blot Confirmed Positive HIV-1 Infection in GOPD and S.T.C. Patients Attending National Hospital Abuja F.C.T. Nigeria

Ajobiewe JO.^{1*}, Yashim NA.¹, Sidi II¹, Ajobiewe HF.², Alexander P.², Umeji L.³, Ogundeji AA.⁴, Dangana A.⁵, Oguji C.⁵

¹National Hospital Abuja, Plot 132 Garki Central District, Nigeria

²Bingham University Kodape Km 5 Nasarawa State of Nigeria

³Defence Reference Laboratory, Asokoro, Abuja, Nigeria

⁴United State Department of Defence, Walter Reed Program-Nigeria US Embassy Nigeria

⁵University of Abuja Teaching Hospital, Gwagwalada, Nigeria

DOI: [10.36347/sjams.2021.v09i09.009](https://doi.org/10.36347/sjams.2021.v09i09.009)

| Received: 28.07.2021 | Accepted: 02.09.2021 | Published: 08.09.2021

*Corresponding author: Ajobiewe OJ

Abstract

Original Research Article

The sole aim of this study is to monitor the level of correlation between GOPD and STC Patients whose samples (sera and sputa) were tested and confirmed with Western Blot, for HIV-1 and Ziehl Nielsen, Z N Stain/ Lowenstein Jensen, L J slope culture for Sputa respectively. These samples were screened for HIV- 1 using three test principles- Determine, Capillus and Genie. Positive samples were confirmed with the Western Blot assay. Sputa samples of the same patients were collected and tested for *Mycobacterium* spp. infection using the Ziehl Nielsen staining technique and the Lowenstein Jensen Slope cultural technique. The Spearman's rank correlation coefficient was 0.466 (P<0.1) showing a relatively high level of concordance between Western Blot confirmed positive HIV-1 infection and *Mycobacterium* spp. infection in the same patients.

Keywords: *Mycobacterium*, Ziehl Nielsen Western Blot Lowenstein Jensen Slope.

Copyright © 2021 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC 4.0) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

STUDY BACKGROUND

Mycobacterium spp. the etiologic agent of *Mycobacterium tuberculosis* disease has been found associated with HIV-1 infection in several studies along this line of reasoning. Critical assessment of this sort of association is of utmost necessity in this context---as a sort of swift clinical intervention for Koch's disease [1]:

Tuberculosis was popularly known as consumption for a long time. Scientist knows it as an infection caused by *M. tuberculosis* in 1882, the microbiologist Robert Koch discovered the tubercle bacillus, at a time when one of every seven deaths in Europe was caused by TB. Because antibiotics were unknown, the only means of controlling the spread of infection was to isolate patients in private sanatoria or hospitals limited to patients with TB--- [2] a practice that continues to this day in many countries including Nigeria. The net effect of this pattern of treatment was to separate the study of tuberculosis from mainstream medicine. Entire organizations were setup to study not only the disease as it affected individual patients, but its

impact on the society as a whole. At the turn of the twentieth century more than 80% of the population in the United States were infected before age 20, and tuberculosis was the single most common cause of death. By 1938 there were more than 700 TB hospitals in some countries [3].

Mycobacterium tuberculosis which might be opportunistic in HIV-1 infection [1]. Screening for HIV- 1 could be done using three test principles- Determine, [4] Capillus [5] and Genie [6]. Positive samples were confirmed with the Western Blot assay. Sputa samples of the same patients were collected and tested for *Mycobacterium* spp. infection using the Ziehl Nielsen staining technique and the Lowenstein Jensen Slope cultural technique. And the Lowenstein Jensen Slope cultural technique [7].

Control Samples: (HIV-1 Assay) Control sera- both positive and negative controls as well as the internal controls were supplied by the kit manufacturers and were applied and used as they instructed.

Control Samples: (for *Mycobacterium* spp. Assay) Negative controls were obtained from known and pooled negative sputa of Patients while positive controls were obtained from known and pooled positive sputa of patients-using the ZN/L J *Mycobacterium* spp. Assays [7].

TEST PRINCIPLES (HIV-1 SCREENING ASSAYS)

Determine: Determine HIV-1/2 is an immune chromatographic test for the qualitative detection of antibodies to HIV-1 and HIV-2. Sample is added to the sample pad. As the sample migrates through the conjugate pad, it reconstitutes and mixes with the selenium colloid-antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient's window site.

If antibodies to HIV-1 and / or HIV-2 are present in the sample, the antibodies bind to the antigen — selenium colloid at the patient window, forming a red line at the patient's site.

If antibodies to HIV-1 and / or HIV-2 are absent, the antigen selenium colloid flows past the patient window and no red line are formed at the patient's window site. To insure assay validity, a procedural control bar is incorporated in the assay device.

MATERIALS AND METHODS

Mycobacterium spp. infection may be associated with HIV-1 infection. Critical assessment of this sort of association is of utmost necessity in this context---as a sort of swift clinical intervention for Koch's disease: which might be opportunistic in HIV-1 infection. A total of 211 Patients had their blood samples prospectively collected between Januarys to October, 2003: while a total of 443 Patients had their blood samples similarly collected between Januarys to October 2004. These samples were screened for HIV- 1 using three test principles- *Determine*, *Capillus* and *Genie*. Positive samples were confirmed with the Western Blot assay. Sputa samples of the same patients were collected and tested for *Mycobacterium* spp. infection using the Ziehl Nielsen staining technique and the Lowenstein Jensen Slope cultural technique. A total of 211 Patients had their blood samples prospectively collected between Januarys to October, 2003: while a total of 443 Patients had their blood samples similarly collected between Januarys to October 2004. These samples were screened for HIV- 1 using three test principles- *Determine*, *Capillus* and *Genie*. Positive samples were confirmed with the Western Blot assay. Sputa samples of the same patients were collected and tested for *Mycobacterium* spp. infection using the Ziehl Nielsen staining technique and the Lowenstein Jensen Slope cultural technique. The total duration for the work was 20 months.

Control Samples: (HIV-1 Assay) Control sera- both positive and negative controls as well as the internal controls were supplied by the kit manufacturers and were applied and used as thy instructed.

Control Samples: (for *Mycobacterium* spp. Assay), Negative controls were obtained from known and pooled negative sputa of Patients while positive controls were obtained from known and pooled positive sputa of patients-using the ZN/L J *Mycobacterium* spp. assays

TEST PRINCIPLES (HIV-1 SCREENING ASSAYS)

Determine: Determine HIV-1/2 is an immune chromatographic test for the qualitative detection of antibodies to HIV-1 and HIV-2. Sample is added to the sample pad. As the sample migrates through the conjugate pad, it reconstitutes and mixes with the selenium colloid-antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient's window site.

If antibodies to HIV-1 and/or HIV-2 are present in the sample, the antibodies bind to the antigen — selenium colloid at the patient window, forming a red line at the patient's site.

If antibodies to HIV-1 and/or HIV-2 are absent, the antigen selenium colloid flows past the patient window and no red line are formed at the patient's window site. To insure assay validity, a procedural control bar is incorporated in the assay device

Capillus Assay Principles

The majority antigens from the envelope proteins of HIV-1 and HIV-2 have been identified and cloned using recombinant DNA technology. These HIV-1 and HIV-2 proteins have been expressed and purified. The Trinity Biotech Capillus, HIV- I and HIV- 2 employs these two proteins bound to polystyrene latex beads to form the basis of a direct latex aggregation assay for the detection of antibodies to HIV-I and HIV- 2 in the human serum, whole blood and plasma. The assay is performed on a patented capillary slide.

Genie Assay Principle

The (Gennie II HIV-1/HIV-2 is a dual recognition enzyme — immunoassay (EIA), based upon the specific detection of antibodies HIV-1 and HIV-2 antibodies by antigens that bind both antibody binding sites. The test incorporates a combination of immune — chromatography and immunoconcentration. The reaction device contains two ports, a circular specimen Port A for the addition of specimen: and a larger elliptical reaction Port B. Antigens derived from HIV-I and HIV-2 are immobilized in two separate spots on the reaction zone of port B. A third spot serves as the internal Control for monitoring the performance of the test.

The procedure followed was as given by BIO-RAD 3 Boulevard Raymond Poincare: 92430 MARNES COQUETTE-FRANCE [6].

Western blot confirmation assay

(New-Lav Blot-1) [8] Principle: The test is based on indirect ELISA technique on a nitrocellulose strip containing all the HIV — I constituent proteins and an internal anti IgG control. The band corresponding to the internal control is localized on the strip end without any number, before the P-18 reaction and allows validating the addition of the sample and reagents as well as the correct progress of the procedure. Inactivated HIV- 1 proteins are separated according to their molecular weights by polyacrylamide gel electrically transferred onto a nitrocellulose membrane sheet.

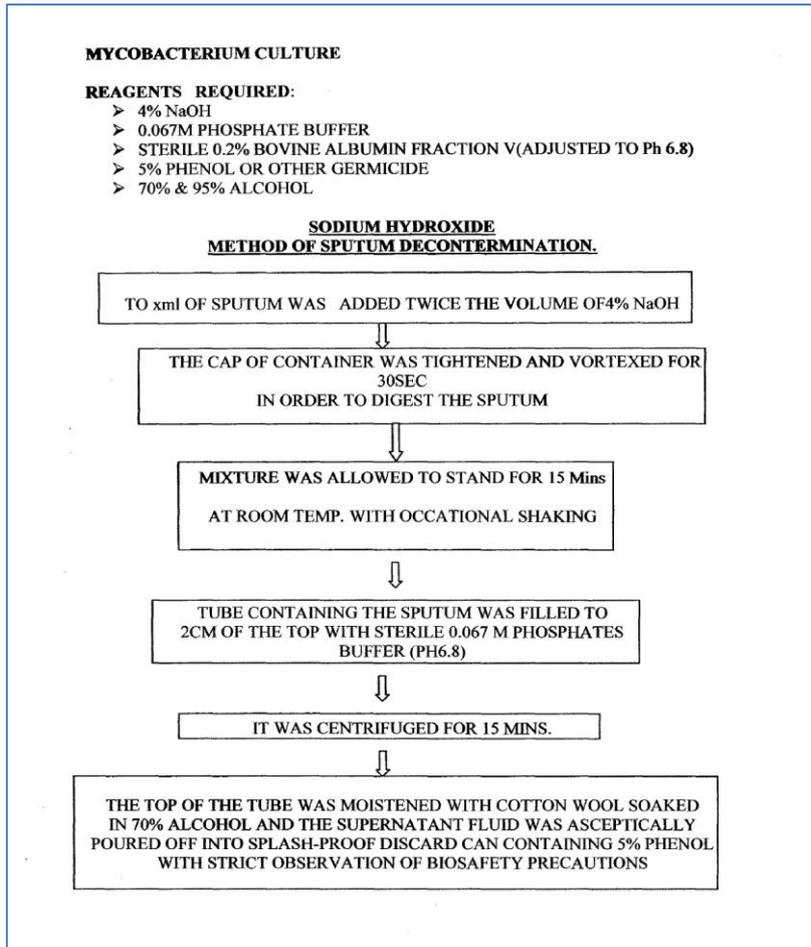
Reagents are first stabilized at room temperature for 30 min. Strip is dehydrated by adding 2ml of the reconstituted buffer solution into each cell for 5min. 20ul of each sample or control serum is added to the corresponding cell for 2hrs incubation and continuous slow shaking. The contents of each cell are completely drained using a vacuum pump. Each strip is washed 3times with the reconstituted buffer according to man.inst.2ml of conjugate is dispensed into each cell and then incubated for 1hr. at room temperature. They were washed as stated above. 2ml pf color

development solution was dispensed into each cell for 5min incubation at room temperature under slow shaking. The reaction is stopped by removing the development solution and rinsing the strips three times with distilled water. Strips are dried between 2 sheets of absorbent paper at room temperature. The bands on the strips are then validated and interpreted according to the manufacturer's instruction.

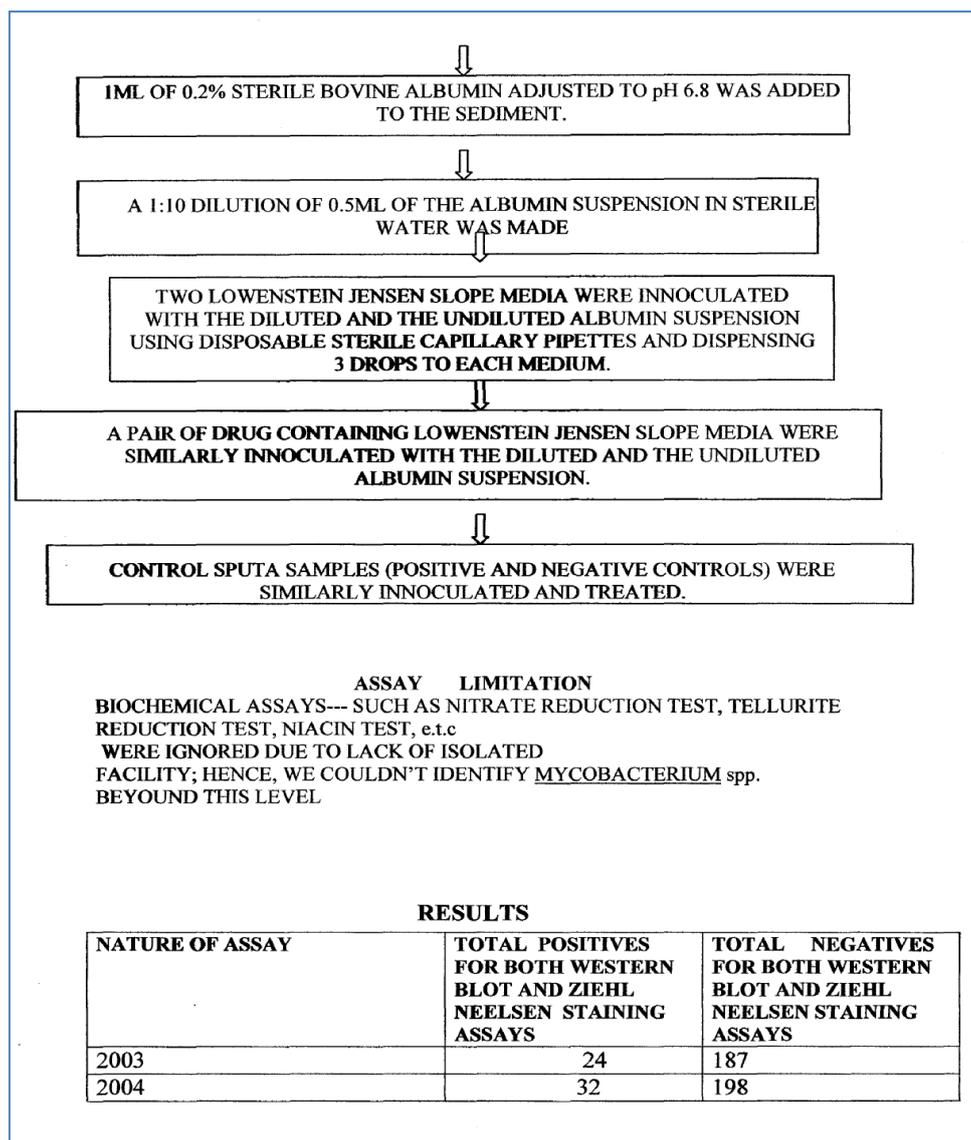
Negative Control Strip

Ziehl- Nielsen staining Procedure [7]. The reagents used were Carbol Fuchin: a saturated solution of basic fuchin(3g of basic fuchin in 100ml of 95% ethyl alcohol),5% aqueous solution of phenol,3% acid alcohol, 0.3% aqueous; the detailed procedure followed was-a smear (1cm x 2cm) was prepared on a labeled, grease-free slide. The slide was placed on a hot plate at 85°C for 15 mins. in a p.3 safety cabinet. The smear was flooded with Carbol fusion and was allowed to stand for 15 mins at room temperature. It was steamed gently with flame from the underside for some minutes (* but it wasn't allowed to boil).The stain was allowed to stand for 5min. on the slide and then washed with distilled water and tilted to drain. The stained slide was completely decolorized with 3% acid alcohol. The slide was rinsed with distilled water and tilted to drain. The slide was flooded with methylene blue for one minute. The slide was rinsed and allowed to air dry. The slide was then examined under X100 oil immersion lens.

For clarity and future safety of those using this Journal, the procedure would be clearly outlined



MONTH	TOTAL PATIENTS CONFIRMED POSITIVE FOR BOTH WESTERN BLOT & ZN ASSAYS			TOTAL PATIENTS CONFIRMED POSITIVE FOR BOTH WESTERN BLOT AND ZN ASSAYS				
	2003	%	RANK	2004	%	RANK	di	di ²
JANUARY	1/22	4.5	1	0/22	0	1.5	-0.5	0.25
FEBRUARY	1/11	9.1	5.5	0/21	0	1.5	1.0	1
MARCH	1/18	5.6	2.0	3/21	13.6	5.0	-3	9
APRIL	1/15	6.6	3.0	3/16	18.8	7.0	-4	16
MAY	2/17	11.7	7.0	1/15	6.7	3.0	4	16
JUNE	6/33	18.2	9.0	7/28	25.0	10.0	-1	1
JULY	2/22	9.1	5.5	3/20	15.0	6.0	-1	1
AUGUST	4/18	22.2	10.0	6/31	19.4	9.0	1	1
SEPTEMBER	3/37	8.10	4.0	5/26	19.2	8.0	4	16
OCTOBER	3/18	16.7	8.0	4/30	13.3	4.0	4	16



$R_s = 0.466 (P < 0.1)$

The Spearman's rank correlation coefficient was 0.466($P < 0.1$) showing a relatively high level of concordance between Western Blot confirmed positive HIV-1 infection and *Mycobacterium* spp. infection in the same patients.

CONCLUSION

Mycobacterium spp. which may be reliably tested in Sputa samples of HI V-I positive patients using the above techniques-appears to be a strong indicator of opportunistic infection in HIV- I infected patients. It is suggested that early diagnosis of this, shall boost

REFERENCES

- McGraw, H. (2002). Concise Dictionary of Modern Medicine @ 2002 by McGraw Hill
- Ushananthini, A. S. (2012). *Assessment of DNA Damage in Pulmonary Tuberculosis Patients by Single Cell Gel Electrophoresis/Comet Assay* (Doctoral dissertation, Swamy Vivekanandha College of Pharmacy, Tiruchengode).
- Koch, R. (1881). "Zur Ätiologie des Milzbrandes" On the etiology of anthrax]. Mittheilungen aus dem Kaiserlichen Gesundheitsamte. (2010). Berlin: Robert Koch-Institut, 174–206
- Abott, L. (2000). ABOtt Laboratories Diagnostic Division SEPT.2000, 100, Abott Park IL60064 USA
- Trinity Biotech; Trinity Biotech 2005 www.Trinity biotech.com
- Bio, R. (2000). Biorad 3 Boulevard Raymond Pointcare 92430 MARNES COQUETTE FRANCE.
- Monica, C. (2001). Regional or Central Microbiology Laboratory for pathogen identification. The 3rd edition, 2001; Principles of Medicine in Africa.3. 462 pages

8. Mahmood, T., & Yang, P. C. (2012). Western blot: technique, theory, and trouble shooting. *North*

American journal of medical sciences, 4(9), 429.