

Serological Profiling of Epstein Barr Virus Among Blood Donors Attending Hajia Gambo Sawaba General Hospital in Zaria, Kaduna, Nigeria

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Received on: April 2026

Revised and Accepted on: June, 2026

Published on: July, 2026

ABSTRACT

Epstein-Barr virus (EBV) is a ubiquitous human herpesvirus, and its serological profiling provides important insights into infection stages, immune response, and potential transmission risks. The aim of the research was to determine the serological profile of EBV blood donors attending Hajia Gambo Sawaba General Hospital in Zaria, Nigeria. Ninety (90) blood samples were collected and analyzed for anti-EBV antibodies (VCA-IgM, VCA-IgG, EBNA-IgG) using enzyme-linked immunosorbent assay (ELISA). Serological analysis showed antibody distributions of 4.4% for VCA-IgM, 90.0% for VCA-IgG, and 91.1% for EBNA-IgG. Based on antibody profiles, infection stages were classified as 8.8% primary infection, 4.4% recent active infection, 76.6% past infection, and 10% not classifiable. Real-time PCR with SYBR green analysis was carried out to confirm the presence of the EBNA-1 gene in recent active infections. PCR analysis confirmed the presence of the EBNA-1 gene in cases identified as recent active infections. The study demonstrates that EBV infection is widely distributed among apparently healthy blood donors in Zaria, with the majority presenting antibody profiles consistent with past infection and a small proportion showing asymptomatic recent infections, posing potential transfusion-related risks.

Keyword: Blood, Donors, Epstein Barr Virus, Hospital, Profiling and Serological

1.0 INTRODUCTION

Epstein-Barr virus (EBV), also known as Human Herpesvirus-4 (HHV-4), is a ubiquitous gammaherpesvirus infecting over 90% of the global population. While primary infection is often asymptomatic in childhood, it may manifest as infectious mononucleosis in adolescents and adults. EBV has been implicated in several malignancies, including nasopharyngeal carcinoma, Hodgkin lymphoma, and Burkitt lymphoma, as well as autoimmune disorders such as systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis (Iheukwumere *et al.*, 2025).

However, Due to the type of therapy intervention needed, the diagnostic approach of antibody detection involves a number of tests that are different for immunocompromised and immunocompetent individuals. As a result, the timing of intervention is crucial for immunocompromised individuals. In contrast, the primary diagnostic concern in immunocompetent patients is the identification or exclusion of a primary, previous, or no infection; serology has supplied criteria for interpreting the data. Therefore, VCA-IgM, VCA-IgG, and EBNA-IgG are the only three serological criteria that are necessary for the qualitative detection of

antibodies specific to EBV in immunocompetent individuals (Soldan & Lieberman, 2023).

Therefore, Transfusions of blood are essentially a medicinal procedure that has been used to save lives. The World Health Organization has advised that blood donation be voluntary. Nonetheless, there aren't many willing blood donors in Nigeria. The WHO advises that all blood for transfusion must be checked for transfusion-transmissible illnesses (TTIs), which include syphilis, HIV-1 and HIV-2, HBV, and HCV. Donors' blood will be allowed if they test negative for TTIs (Talukdar & Bhattacharya, 2025).

Furthermore, thorough information on EBV seroprevalence can support global health standards by guiding blood screening procedures and guaranteeing the security of the blood supply. Nigeria lacks effective EBV preventive and control measures (Anka *et al.*, 2024). The aim of this research was to determine the serological profile of EBV among blood donors attending Hajia Gambo Sawaba General Hospital Zaria.

2.0 MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Kaduna State, Nigeria's Zaria Local Government Area (LGA). One of the largest cities in Northern Nigeria, Zaria is a significant center for trade, culture, and education. It is located about 80 km northeast of Kaduna, the state capital, in the northern region of Kaduna State. Zaria is situated between latitudes 11°04'N and 11°12'N and longitudes 7°42'E and 7°52'E (Balarabe & Ikpe, 2025).

Hajiya Gambo Sawaba General facility, which was established and opened as a health center in Zaria on 3rd of March, 1975, was the facility chosen to carry out this study. In addition to physicians, nurses, chemists, and laboratory scientists, it is a secondary healthcare center.

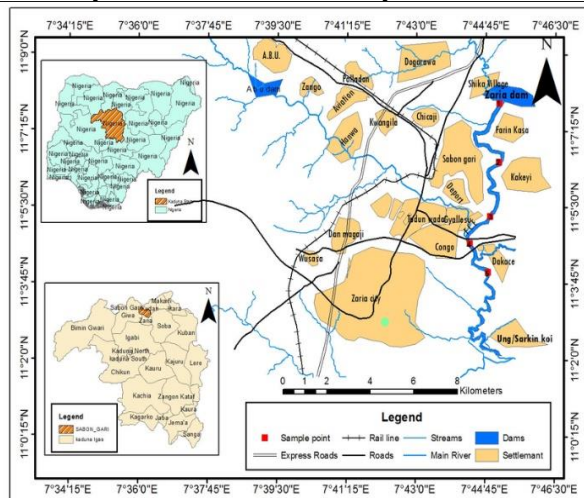


Fig. 1: Map of Zaria, Kaduna State, Nigeria showing location of Hajiya Gambo Sawaba General Hospital (Black dot) (Balarabe & Ikpe, 2025)

2.2 Study Population

The study populations are apparently healthy blood donors attending Hajiya Gambo Sawaba General Hospital Zaria. They commercial, relative and volunteer donors as adopted by Anka *et al.* (2024).

2.3 Sample Size Determination

The sample size was determined using a reported 6.6% prevalence of EBV-IgM antibodies among HIV patients as adopted by Coockey *et al.* (2023)

$$n = \frac{Z^2 p(1-p)}{d^2}$$

Where n = sample size; p = prevalence (6.6%); Z = standard normal distribution at 95% type 1 error = 1.96; q = 1-p; d = absolute desired precision (allowing error 0.05)

$$n = \frac{(1.96)^2 \times 0.06 \times (1-0.06)}{(0.05)^2}$$

$$= 86$$

(Coockey *et al.*, 2023)

However, based on the sample size determination, the minimum number of blood donors calculated for this study was 86, but a total number of 90 blood donors were selected.

2.4 Inclusion Criteria

Blood donors that were tested negative for Syphilis (VDRL), HIV, Hepatitis B (HBsAg), and Hepatitis C (HCV) as well as those who consented to participate in the study as adopted by Quintas *et al.* (2023).

2.5 Exclusion Criteria

Blood donors that were positive for either Syphilis (VDRL), HIV, Hepatitis B (HBsAg) and Hepatitis C (HCV) were excluded from this study as adopted by (Habibu *et al.*, 2025).

2.6 Ethical Approval and Consent

Approval for the research was obtained from the ethical committee of the Ministry of Health Kaduna State through the Hospital Secretary of Hajiya Gambo Sawaba General Hospital Zaria with reference number; MOH/ADM/744/VOL.1/731. The blood was obtained from the apparently healthy blood donors from those that consented by filling a consent form as adopted by Joshua *et al.* (2023).

2.7 Sample Collection

Three milliliters of blood were drawn from donors who appeared to be in good health. After the blood sample was collected and allowed to settle in the plain sample vial, it was centrifuged for 15 minutes at 3000 rpm. After being moved into a different sample bottle, the serum was chilled at -20°C (Coockey *et al.*, 2023).

After being properly labelled with the patient's identification, all samples were transported to the laboratory for further analysis in cold boxes with ice packs to keep the temperature between 2 and 8°C. Two distinct locations were used for the laboratory investigations:

1. Serological analysis (ELISA): At the Department of Heamatology, Ahmadu Bello University Teaching Hospital (ABUTH), Shika, Zaria, EBV was detected for viral capsid antigens (VCA IgM and VCA IgG) and nuclear antigen (EBNA-1 IgG) using a commercial ELISA kit in compliance with the manufacturer's instructions.

2. Molecular analysis (PCR and DNA extraction): At the Biomedical Precision Laboratory in Kaduna, PCR assays and DNA extraction were carried out strictly in accordance with accepted molecular biology procedures as adopted by Coockey *et al.* (2023).

2.8 Laboratory Analysis

2.8.1 Qualitative ELISA Testing of Anti-EBV Antibodies

Detection of Epstein–Barr virus (EBV) viral capsid antigen (VCA IgG and VCA IgM) and nuclear antigen EBNA-1 IgG antibodies was conducted using the AccuDiag™ ELISA kits (catalog#1405-2, catalog#1406-2 and catalog # 1425-11); Diagnostic Automation/Cortez Diagnostics, Inc., Woodland Hills, CA, USA) respectively (Ekşi *et al.*, 2022).

2.8.2 Assay Procedure of ELISA Testing of Anti-EBV Antibodies

The technique uses the indirect enzyme-linked immunosorbent assay principle, which involves pre-coating 96-well microplates with EBV VCA antigen. Prior to adding 100 µL of each diluted sample to the specified wells, patient sera were first diluted 1:100 in the supplied sample diluent. To enable particular anti-VCA IgG antibodies to attach to the immobilised antigen, the plate was incubated at room temperature for around 60 minutes. To get rid of unbound material, the wells were rinsed four or five times with 300 µL of diluted wash buffer after incubation. Each well was then filled with 100 µL of anti-human IgG coupled with horseradish peroxidase (HRP) and incubated for 20 to 30 minutes. The wells were then rinsed four to five times with wash buffer. After adding 100 µL of tetramethylbenzidine (TMB) substrate to each well, colour development was left to continue in the dark for ten to fifteen minutes. A steady yellow colour was produced when 50–100 µL of stop solution was added to halt the process. A microplate reader (THERMOmax; Molecular Devices Corporation, CA, USA) was used to detect optical density at 450 nm. The kit's positive and negative controls were included in every run, and assay validity was verified when control values fell within the manufacturer's designated ranges. The kit insert was used to calculate cutoff values for sample reactivity, and the results were qualitatively presented as either positive or negative. The manufacturer reported a sensitivity of 98.1% and specificity of 98.3%. The assay took about 60 minutes in total as adopted by Ekşi *et al.* (2022)

2.8.3 DNA Extraction

DNA was extracted from the sample in accordance with the Quick-DNA Miniprep Plus kit's manufacturer's instructions (Zymo Research, catalogue # D4068). The micro centrifuge tube was filled with 200µl of material, followed by 20ul of proteinase K, 200ul of biofluid, and cell buffer (red). After properly mixing it with a vortexing machine, it was incubated at 55°C for 10 minutes. The digested sample was combined with one volume of genomic binding buffer and carefully mixed.

The mixture was put into a Zymo-spin 11C x L column and centrifuged for one minute in a collection tube (> 12,000g). With the flow through, the collection tube was thrown away. A column in a fresh collection tube was filled with 400µl of DNA pre-wash buffer and centrifuged for one minute. After emptying the collecting tube, 700µl of g-DNA wash buffer was added, and the mixture was centrifuged. With the flow through, the collection tube was thrown away. After that, 200µl of g-DNA wash buffer was added, centrifuged for one minute, and discarded along with the flow through. The DNA was eluted by moving it to a sterile micro centrifuge tube, adding 50µl of DNA elution buffer, incubating it for five minutes, and centrifuging it for one minute as described by Miskimen and Miron (2023).

2.8.4 Polymerase Chain Reaction

The collection tube was discarded after the flow was completed. 400µl of DNA pre-wash buffer was added to a column in a brand-new collecting tube, and it was centrifuged for a minute. The mixture was centrifuged after the collection tube was emptied and 700µl of g-DNA wash buffer was added. The collection tube was discarded after the flow was completed. Following that, 200µl of g-DNA wash buffer was added, centrifuged for one minute, and the flow through was discarded. To elute the DNA, transfer it to a sterile micro centrifuge tube, add 50µl of DNA elution buffer, let it sit for five minutes, and was then centrifuge for one minute.

Target gene: EBNA-1

Primer sequence (5'-3')

Forward- GCC GGT GTG TTC GTA TAT GG

Reverse- CAA AAC CTC AGC AAA TAT ATG AG
(Shahi *et al.*, 2022)

2.9 Data Analysis

The prevalence of the Epstein–Barr virus in Blood donors was calculated using the following formulae below:

$$\text{Percentage Prevalence (\%)} = \frac{\text{number of donors positive}}{\text{number of donors diagnosed}} \times 100$$

3.0 RESULTS

3.1 Seroprevalence of Epstein -Barr Virus Among Apparently Healthy Blood Donors Attending Hajiya Gambo Sawaba General Hospital, Zaria.

Out of the ninety (90) samples used to determine the presence of the anti-EBV antibodies using ELISA, from the apparently healthy blood donors, the result showed seropositive for at least one of the mentioned anti- EBV antibodies (VCA-IgG, VCA-IgM and EBNA-IgG) indicating a prior exposure to the virus. It was found that for VCA-IgM, (4:4.44%) tested positive, (86:95.5%) were tested negative. (81:90.0%) were positive for VCA-IgG and (9:10.0%) tested negative. For EBNA-IgG,

(82:91.1%) were tested positive and (8:8.8%) were negative (Table 1).

Table 1: Seroprevalence of Epstein–Barr Virus (EBV) Antibodies Among Apparently Healthy Blood Donors at Hajiya Gambo Sawaba General Hospital, Zaria, Nigeria

EBV Antibodies	No. Tested	No. Positive (%)	No. Negative (%)
VCA-IgM	90	4.0 (4.4)	86 (95.5)
VCA-IgG	90	81.0 (90.0)	9 (10.0)
EBNA-IgG	90	82.0 (91.1)	8 (8.8)

Key: VCA-IgM and IgG =Viral Capsid Antigen IgM and IgG, EBNA-IgG =Epstein Barr Nuclear Antigen, HGSWGH = Hajiya Gambo Sawaba General Hospital, EBV= Epstein- Barr virus.

3.2 Serological Profile of EBV Among the Apparently Healthy Blood Donors Classified into 4 Possible Infectious Stages

The serological profile of EBV was classified into 4 possible infectious stages; Primary infection, recent active infection, past infection and not plausible (Table 2). Out of the 90 samples tested, (8:8.8%) were categorized to have primary infection (positive for VCA-IgM and VCA-IgM and negative for EBNA-IgG). (69:76.6%) were categorized as past infection (VCA-IgM negative and positive for VCA-IgG and EBNA-IgG). The other category was recent active infection, which appeared to be (4:4.4%) persons, which was positive all through against VCA-IgM, VCA-IgG and EBNA-IgG. And the last category was classified as not plausible, which had negative against VCA-IgM and VCA-IgG and positive against EBNA-IgG (9:10.0%).

Table 2: Serological Profile of EBV Among the Apparently Healthy Blood Donors Classified into 4 Possible Infectious Stages

Infection type	Frequency (%)	EBV Pattern of detection		
		VCA IgM	VCA IgG	EBNA IgG
Primary	8 (8.8)	-	+	-
Past	69 (76.6)	-	+	+
Recent active	4 (4.4)	+	+	+
Not plausible	9 (10.0)	-	-	+

Key: EBV= Epstein –Barr virus, VCA IgM and IgG =Viral Capsid Antigen IgM and IgG, EBNA IgG= Epstein- Barr Nuclear Antigen IgG, +=positive, -= negative

3.3 PCR Targeting EBNA-1 in Samples Classified as Recent Active Infection by Serology

Molecular confirmation of EBV infection was carried out by real-time PCR targeting EBNA-1 in samples classified as recent active infection by serology. The amplification curve (Fig. 2) demonstrated typical PCR kinetics with an exponential amplification phase, during which the amount of PCR product approximately doubled with each cycle, followed by a plateau phase where amplification slowed as reaction components became limited.

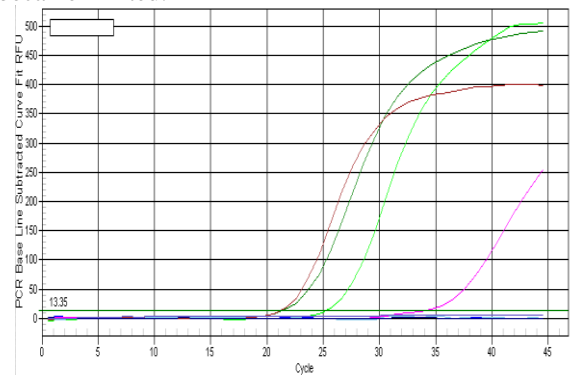


Fig. 2: Real-Time PCR amplification curve graphic for EBNA-1 detection on EBV recent active infection samples.

4.0 DISCUSSION

EBV is widespread throughout the world's population and is connected to a number of illnesses that are linked to cancer. Most of these cancers are caused by infections of epithelial cells and B or T cells. EBV can be found using serological and molecular methods, which is essential for illness surveillance, prognosis evaluation, and the use of preventive interventions (Wong *et al.*, 2022).

The genes examine in this research is in conformity with that of of Du *et al.* (2023), who conducted a population-based nested case-control study in southern China on the relationship between EBV antibody and the risk of stomach cancer. He reported among the 444 matched controls, 22 (5.0%) were EBNA1-IgA positive and 26 (5.9%) were VCA-IgA positive. This result is also in conformity with that of population-based nested case-control research conducted in southern China by Ji *et al.* (2025) who reported that seropositive individuals had higher risks of total cancer (HR 4.88, 95% CI: 2.84-8.37), lung cancer (1.76, 1.23-2.54), liver cancer (1.70, 1.10-2.63), nasopharyngeal carcinoma (26.05, 11.77-57.65), and lymphoma (3.20, 1.46-6.99) compared to seronegative individuals.

However, Teshome *et al.* (2023), who worked on the molecular and serological detection of Epstein Barr virus from lymphoma patients in Ethiopia, reported that 31.6% and 69.4% of the study participants were HL and NHL

patients, respectively. EBV DNA was detected in 99% of study participants through real-time quantitative polymerase chain reaction (qPCR) and 96% serologically. He concluded that there is a high EBV prevalence among lymphoma patients in Ethiopia and represents the first study in this field that can be utilized as a baseline for future studies.

The result obtained is in disagreement with that Ekşi *et al.* (2022) who worked on investigation of EBV antibodies in Turkey reported that Positive Anti-VCA IgM results were 3% by ELISA and 25% by the IFA, positive Anti-VCA IgG results were 96.3% by ELISA and 98% by IFA. ELISA Anti-VCA IgG sensitivity was found to be 96.3% while ELISA Anti-VCA IgM sensitivity was found to be 12.9%. However, he concluded that serological diagnosis of acute EBV infection or reactivation of latent infection EBV Anti-VCA IgM, Anti-VCA IgG, Anti-EBNA IgG, Anti-EA IgG, and Anti-VCA IgG avidity antibodies should be evaluated together.

The result of acute infection is similar with the result obtained by Nishio *et al.* (2024) who worked on Clinical significance of anti-Epstein-Barr virus antibodies in systemic chronic active Epstein-Barr virus disease and concluded that Anti-EBNA antibody titers were significantly lower and anti-VCA-IgG antibody titers significantly higher in patients with sCAEBV than those in healthy controls. However, interpreting profiles with conflicting antibody results may require additional tests like immunoblotting, IgG avidity, and molecular methods to resolve diagnostic challenges as reported by Chen *et al.* (2023).

Consequently, due to different treatment requirements, immunocompromised and immunocompetent people require different diagnostic techniques; early detection is especially important for immunocompromised patients. VCA IgG, VCA IgM, and EBNA-1 IgG are crucial markers for qualitative detection in immunocompetent people (Savitz & Yolken, 2022).

5.0 CONCLUSION

This study demonstrates a high prevalence of EBV exposure among blood donors in Zaria, with most individuals exhibiting markers of past infection. The presence of recent active infections highlights a potential risk for transfusion-related transmission. These findings underscore the need for further evaluation of EBV screening policies in blood transfusion services in Nigeria

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