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(RESEARCH ARTICLE)

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Seroprevalence and molecular detection of cytomegalovirus and some viral STDs and their infection risk factors in male subjects in Onitsha, South-East, Nigeria

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Abstract

Viral infections including latent viruses have been known to affect semen quality of men negatively thereby affecting reproductive potentials. A cross-sectional study was done to assess the prevalence of CMV antigen/ antibody (IgG and IgM), HBV, HCV, HIV, confirm CMV molecularly and determine their risk factors for infection. Blood and semen samples were collected from 96 apparently healthy men aged between 24 – 65years in Onitsha, South-East, Nigeria. Ethical approval and informed consents were obtained. Data was collected using structured questionnaires and standard WHO tool for STDs.

Method: HBV and HCV antibodies were screened immunochromatographically; HIV tested by parallel method, confirmed using Western-blot; CMV IgG and IgM were screened using ELISA, confirmed molecularly with quantitative PCR. Data was assessed using SPSS version 23 set at 0.05 at 95% confidence interval.

Result: This revealed that CMV IgG had the highest prevalence of 68(70.8%) (p=0.04), HIV had 2(2.1%) (p=0.904) and HCV had 1(1.0%) (p=0.167). None of the subjects tested positive to CMV IgM and HBV. One out of 17 samples tested positive for CMV qPCR 1(5.9%). HIV was significantly associated with multiple partners (p=0.002) and unprotected anal sex (p=0.019).

Conclusion: Early screening and treatment should be done for a more quality health and reproductive life.

Keywords: Cytomegalovirus; Hepatitis B virus; Hepatitis C virus; Human Immunodeficiency virus; Males; Semen

1. Introduction

Viral diseases have plagued people since the beginning of humanity. Many viruses can affect the male reproductive system which has detrimental effects on male reproductive health, such as infertility [1]. Furthermore, some genital tract viral diseases can be passed on through sexual activity, which may have an effect on the health of the offspring. Too little attention has been given to male infertility when compared with female infertility. However studies show that infertility, in which male factors contribute to approximately 50%, is estimated to concern over 72 million people worldwide [2]. The meta-analysis review and the work by some researchers showed that fungal, viral and bacterial infections are risk factors that impair male fertility potential [3, 4]. Among the various viruses implicated in destructive impacts to male reproductive system are Cytomegalovirus (CMV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Human Immune Deficiency Virus (HIV) [3, 4, 5, 6]. Human Cytomegalovirus (HCMV) is a member of human herpesvirus family. CMV is the largest virus of the herpes family with a size of 190 nm [7]. Cytomegalovirus is a DNA enveloped virus, an infectious agent that is found everywhere in the world population. Hepatitis B is a viral infection that attacks the liver

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and can cause both acute and chronic disease. It is a member of the Hepadnaviridae family [8]. The virus is most commonly transmitted from mother to child during birth and delivery, as well as through contact with blood or other body fluids during sex with an infected partner, unsafe injections or exposures to sharp instruments [9]. In 2019, WHO estimated that 296 million people were living with chronic hepatitis B infection with 1.5 million new infections every year. Hepatitis C is RNA enveloped Flaviviridae virus which causes inflammation of the liver. The virus can cause both acute and chronic hepatitis, ranging in severity from a mild illness to a serious, lifelong illness including liver cirrhosis and cancer [9]. The Hepatitis C virus is mostly transmitted through exposure to blood from unsafe injection practices, unsafe health care delivery, unscreened blood transfusions and unprotected sexual practices with infected person. According to WHO, it is globally estimated that 58 million people have chronic hepatitis C virus infection, with about 1.5 million new infections occurring per year [9]. The Human Immunodeficiency Virus (HIV) belongs to the genus Lentivirus within the family of Retroviridae. It is a double stranded RNA virus that causes Acquired immunodeficiency syndrome (AIDS), a chronic, potentially life-threatening condition. HIV continues to be a major global public health issue. There were an estimated 38.4 million people living with HIV at the end of 2021, two thirds of whom (25.6 million) are in the WHO African Region [9]. The male reproductive system, particularly the testis, is very susceptible to most of these viruses, and the quality of semen can be impacted by them. The work aimed at determining some viral sexually transmitted diseases, conducting molecular identification of Cytomegalovirus and comparing the effects on semen quality of males in Onitsha, South-East, Nigeria

2. Material and methods

2.1. Study setting and design

The study area was at Onitsha which is located on the Eastern bank of the River Niger, in Anambra State, Nigeria. Onitsha is known for its river port and as an economic hub for commerce, industry, and education. The metro area population of Onitsha in 2023 is 1,623,000. The city spans up to 759 square miles (1,965km^2) [10]. A cross-sectional study was used to determine the prevalence of some viral sexually transmitted diseases on males in Onitsha, South-East, Nigeria. This study was conducted within a period of 4 months (from April - July, 2023). Subjects' recruitment and sample collection were done within Onitsha and its environ while analysis was carried out at New Hope Hospital, Onitsha and Molecular Research Foundation for Students and Scientists, Nnamdi Azikiwe University, Awka, Anambra State.

2.2. Study Population, sample size calculation and subject selection criteria

The sample population was comprised of men from Onitsha, South-East, Nigeria. Random sampling was used to recruit 96 willing men after adequate explanation on the significance of the study.

The sample size (n) was determined using standard Daniel's sample size determination formula (Daniel, 1999). $N=Z^2P(1-P)/d^2$ N = sample size required, Z = Statistic for a level of confidence (1.96 for 95% confidence level). P = Expected prevalence on semen of infertile men in area of study (6.1%) [4]. Q = (1-P) proportion of the population without the desired characteristics. d = Degree of precision i.e. the margin of error that is acceptable and will be taken at least 5% (0.05). Therefore, substituting the values we have; $n= (1.96)^2 \times 0.06 \times 0.94/(0.05)^2$. $n \approx 86.7$. Sample size is 86, but a total of 96 samples were done to correct for attrition.

2.3. Ethical considerations

Ethical approval was obtained from the ethical board committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State. Informed consents were obtained and duly signed by the participants. Permission was also obtained from the management of New Hope Hospital, Onitsha.

2.4. Sample Technique

A Random sampling technique was employed in the collection of blood and semen samples from subjects who met the inclusion criteria. The participation in the study was voluntary and the subjects were properly informed on the nature, merits and aim of the study before sample collection.

2.5. Data collection and demographic

Data for risk factor variables was collected using a structured questionnaire and observational checklist. The questionnaire was designed after reviewing relevant literature, national and international guidelines. The questionnaire was developed to gather data about personal social-demographic information, social economic information, reproductive and medical history of the subjects. Other data on the possible routes of transmission of the various STDs was obtained as recommended by Centre for Disease and Control [11].

2.6. Specimen and specimen collection

2.6.1. Semen

Subjects were informed to observe ejaculatory abstinence of minimum of 2days and maximum of 7days [12]. A sterile wide-necked, non-toxic plastic container was given to each subject for semen production through masturbation. Adherence to sample collection rules and submission time of within 30 minutes was strictly maintained. The samples were properly labeled with name, sex, number, and weight of the sample container, time of collection, and arrival, method of semen collection and number of days of abstinence.

2.6.2. Blood

Five (5) milliliters of blood were collected aseptically from the median cubital fore-arm after cleansing the area with 70% alcohol using a multi-sample needle attached to a vacutainer hub. Tourniquet was applied 3-4 inches above the selected puncture site before blood collection was made into a 5ml plain vacuum tube. Blood was allowed to clot and serum extracted after centrifugation at 1000rpm for 5 minutes. All sterile precautions and collection methods were observed as described by World Health Organization [12].

2.6.3. Sample storage

The serum samples were stored at 4^{o}C and all batches were analyzed at the same time. Each semen sample was immediately kept in incubator at 37^{o}C for 30 minutes to one hour after collection to allow for liquefaction in line with WHO standard [12]. Around 200*ul* of semen of each Subject reactive to CMV was stored at -80^{o}C for DNA extraction.

2.7. Laboratory analysis of samples

2.7.1. Blood serological analysis

Blood samples collected from 96 participants from age range of 24 – 65years were screened for HBV and HCV using immune chromatographic kits (Keytec). HIV test was done using three parallel immune chromatographic methods and confirmed with Western-blot technique. CMV IgG and CMV IgM were done with ELISA spectrophotometric method (Fortress Diagnostics Ltd, UK) and confirmed molecularly using quantitative PCR (Genesig standard kit, UK). Semen qualitative analysis was done according to WHO 6^{th} Edition manual for examination of human semen.

2.7.2. DNA extraction

Viral DNA was extracted using Quick-DNA™ Viral Kit (Zymo Research), according to recommended protocol.

2.7.3. Real time PCR (qPCR)

A Primerdesign genesig Kit for Human Herpes Virus 5 (Cytomegalovirus) (CMV) genomes designed for the in vitro quantification of CMV genomes (genesig standard kit, UK) was used according to recommended protocol.

2.7.4. Real time PCR Amplification

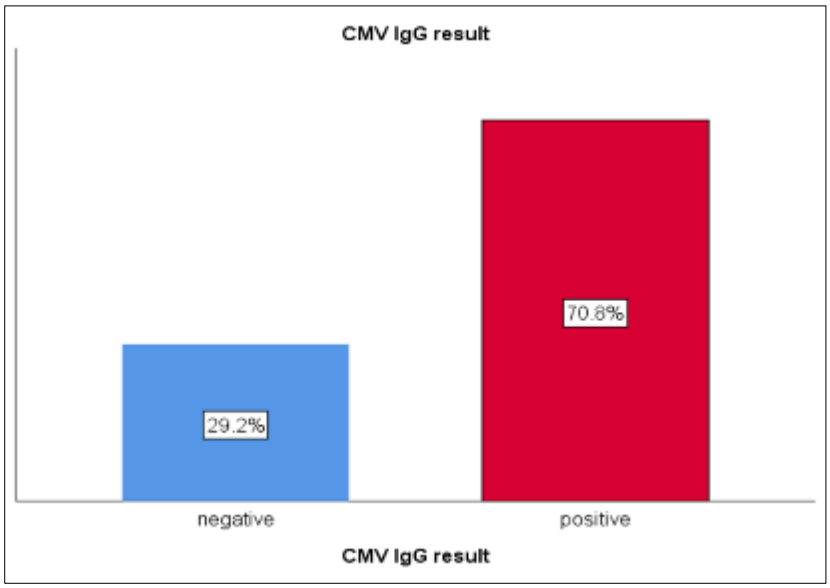
Amplification conditions for the qPCR were as follows: Enzyme activation for 2mins at 95^{o}C, followed by 50 cycles of denaturation for 10secs at 95^{o}C and DATA collection for 60s at 60^{o}C. Fluorogenic data was collected during this step through the FAM channel.

2.8. Data/Statistical Analysis

Statistical analysis was done using Statistical Package for Social Science (SPSS) version 23 for windows (IBM corp., Armok, Nig., USA) for simple prevalence. Variables were compared using Student T test and associations were done using Chi-square. Statistical significance was set at $P<0.05$ at 95% confidence interval

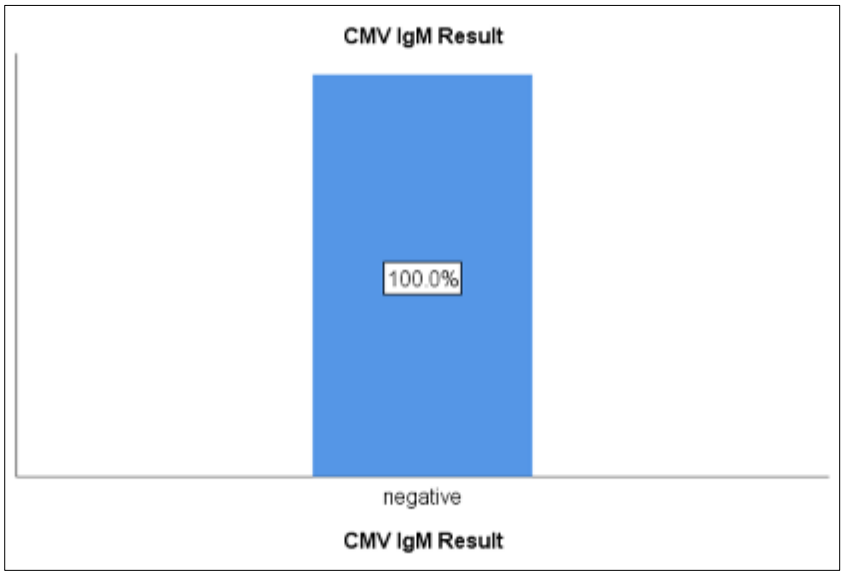
3. Results

The analysis of blood samples collected from 96 male participants from Onitsha with the age range of 24 – 65years revealed that CMV IgG had the highest prevalence of 68(70.8%) which was statistically significant (p=0.04). HIV had seroprevalence of 2(2.1%) (p=0.904) while HCV had 1(1.0%) (p=0.167). None of the subjects tested positive to CMV IgM and HBV 0(0%). One out of 17 samples tested positive for CMV qPCR 1(5.9%). HIV had significant association with risk factors of having multiple partners (p=0.002) and unprotected anal sex (p=0.019).



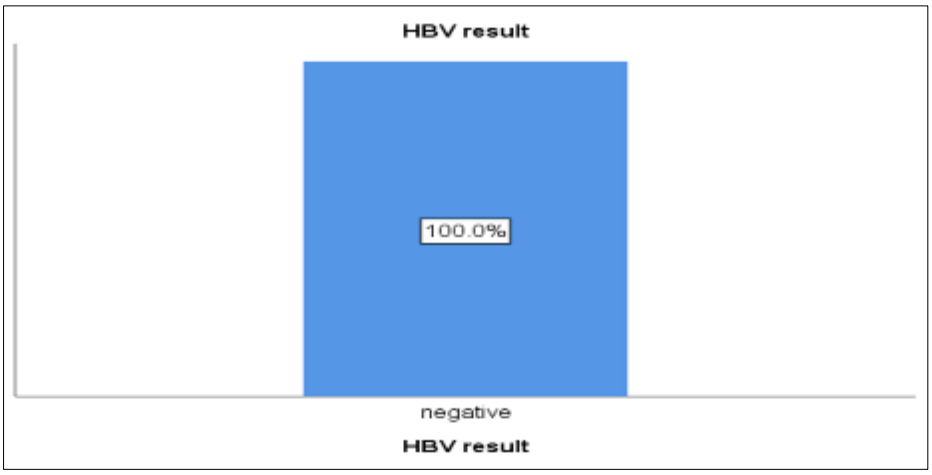
Key: CMV IgG: Cytomegalovirus Immunoglobulin G antibody

Figure 1 Graphical representation of seroprevalence of CMV IgG



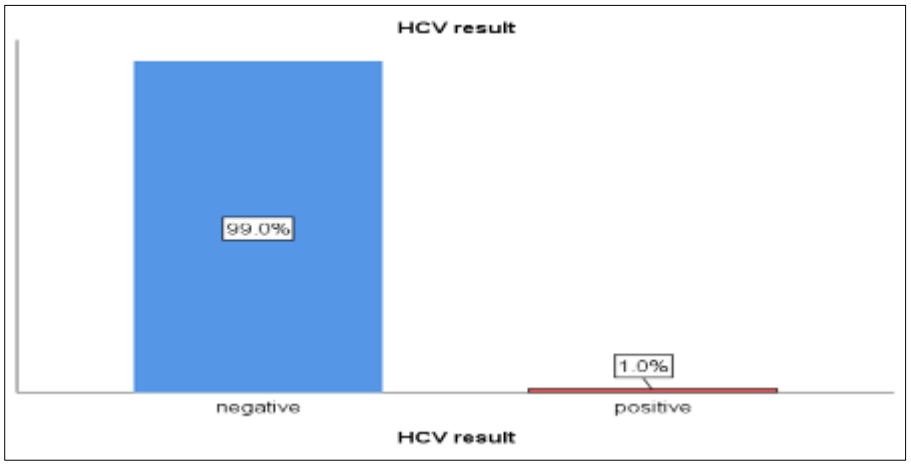
Key: CMV IgM: Cytomegalovirus Immunoglobulin M antibody

Figure 2 Graphical representation of seroprevalence of CMV IgM



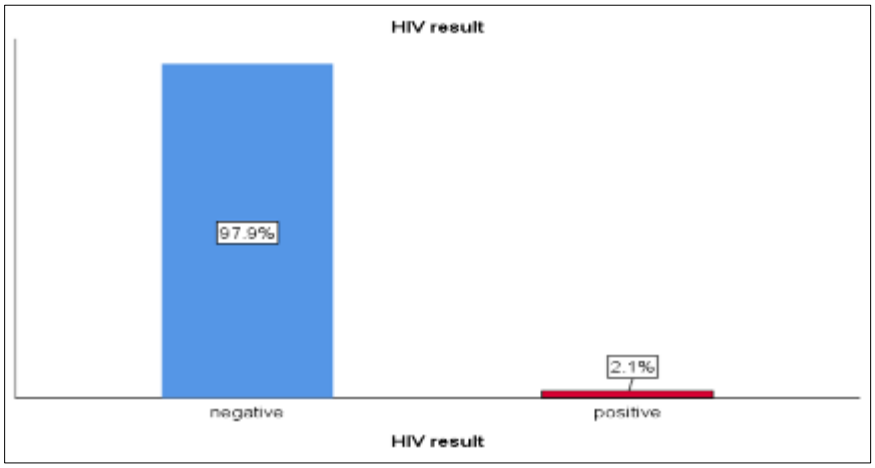
Key: HBV: Hepatitis B virus

Figure 3 Graphical representation of seroprevalence of Hepatitis B virus



Key: HCV: Hepatitis C virus

Figure 4 Graphical representation of seroprevalence of Hepatitis C virus



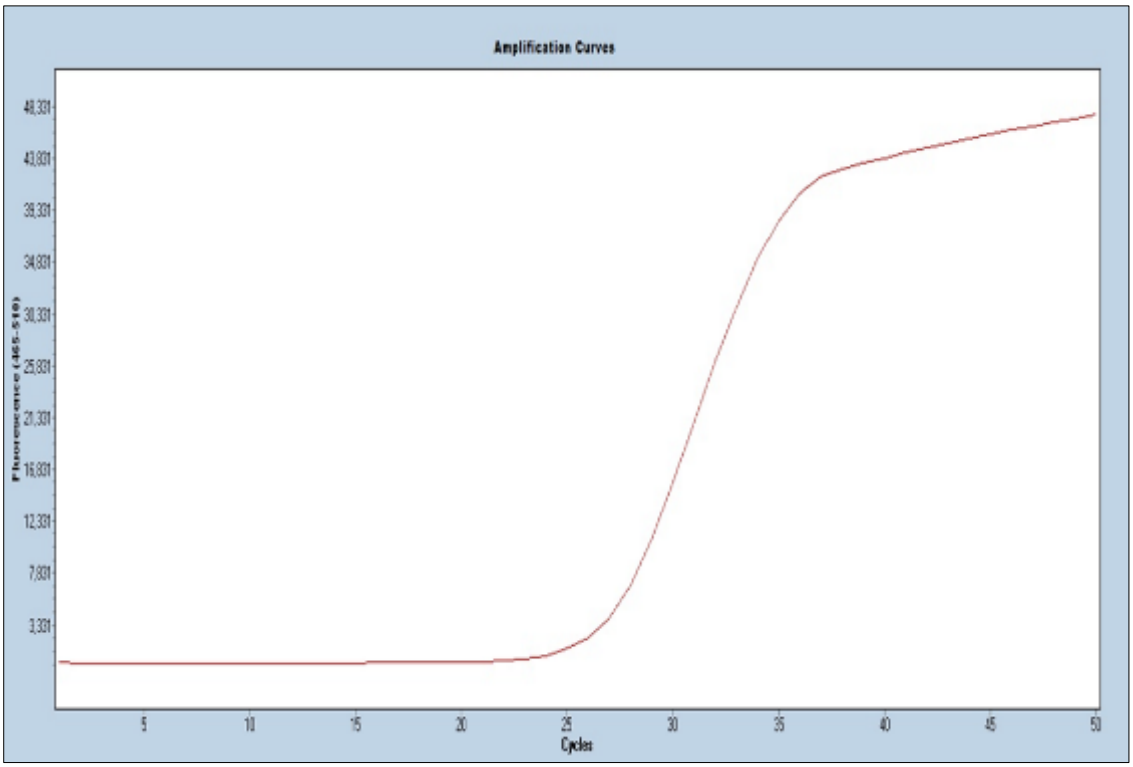
Key: HIV: Human immunodeficiency virus

Figure 5 Graphical representation of seroprevalence of Human immunodeficiency virus

Table 1 Seroprevalence of Cytomegalovirus (CMV) IgG and IgM, HBV, HCV and HIV antibodies in serum

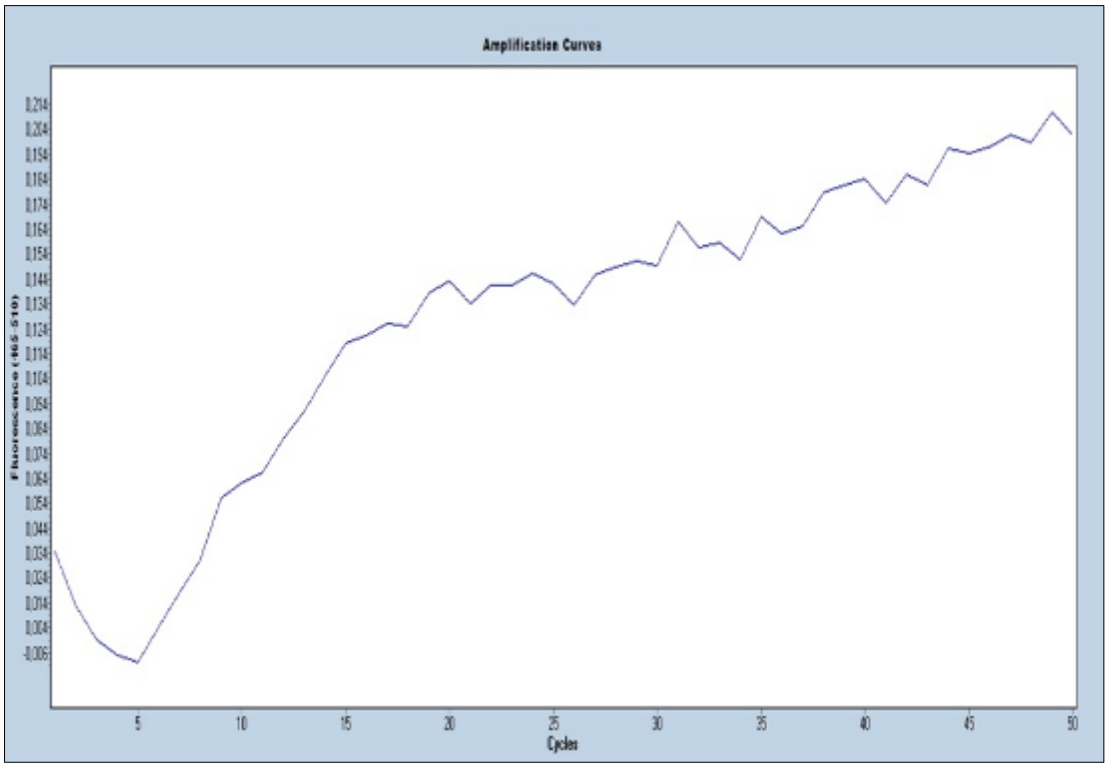
		Sexually transmitted diseases									
Variables	**Total**	**CMV IgG**		**CMV IgM**		**HBV**		**HCV**		**HIV**	
	n(%)	**NEG n(%)**	**POS n(%)**	**NEG n(%)**	**POS n(%)**	**NEG n(%)**	**POS n(%)**	**NEG n(%)**	**POS n(%)**	**NEG n(%)**	**POS n(%)**
Age range(years)											
24-30	4(4.2%)	0(0%)	4(5.9%)	4(4.2%)	0(0%)	4(4.2%)	0(0%)	4(4.2%)	0(0%)	4(4.3%)	0(0%)
31-37	33(34.4%)	16(57.1%)	17(25%)	33(34.4%)	0(0%)	33(34.4%)	0(0%)	33(34.7%)	0(0%)	32(34.0%)	1(50%)
38-44	23(24%)	3(10.7%)	20(29.4%)	23(24%)	0(0%)	23(24%)	0(0%)	23(24.2%)	0(0%)	22(23.4%)	1(50%)
45-51	11(11.5%)	2(7.1%)	9(13.2%)	11(11.5%)	0(0%)	11(11.5%)	0(0%)	10(10.5%)	1(100%)	11(11.7%)	0(0%)
52-58	24(25%)	7(25%)	17(25%)	24(25%)	0(0%)	24(25%)	0(0%)	24(25.3%)	0(0%)	24(25.5%)	0(0%)
59-65	1(1%)	0(0%)	1(1.5%)	1(1%)	0(0%)	1(1%)	0(0%)	1(1.1%)	0(0%)	1(1.1%)	0(0%)
Total	96(100%)	28(29.2%)	68(70.8%)	96(100%)	0(0%)	96(100%)	0(0%)	95(99.0%)	1(1.0%)	94(97.9%)	2(2.1%)
X^2		**11.556**		-		-		**7.809**		**1.574**	
p-value		**0.041**		-		-		**0.167**		**0.904**	
Profession											
Civil servant	23(24%)	7(25%)	16(23.5%)	23(24%)	0(0%)	23(24%)	0(0%)	23(24.2%)	0(0%)	22(23.4%)	1(50%)
Trader	60(62.5%)	18(64.3%)	42(61.8%)	60(62.5%)	0(0%)	60(62.5%)	0(0%)	59(62.1%)	1(100%)	59(62.8%)	1(50%)
Farmer	8(8.3%)	2(7.1%)	6(8.8%)	8(8.3%)	0(0%)	8(8.3%)	0(0%)	8(8.4%)	0(0%)	8(8.5%)	0(0%)
Student	1(1%)	0(0.0%)	1(1.5%)	1(1%)	0(0%)	1(1%)	0(0%)	1(1.1%)	0(0%)	1(1.1%)	0(0%)
Artisan	4(4.2%)	1(3.6%)	3(4.4%)	4(4.2%)	0(0%)	4(4.2%)	0(0%)	4(4.2%)	0(0%)	4(4.3%)	0(0%)
Total	96(100%)	28(29.2%)	68(70.8%)	96(100%)	0(0%)	96(100%)	0(0%)	95(99.0%)	1(1.0%)	94(97.9%)	2(2.1%)
X^2		**0.551**		**-**		**-**		**0.606**		**0.906**	
p-value		**0.968**		**-**		**-**		**0.962**		**0.924**	

Key: n: number of subjects; CMV IgG :Cytomegalovirus Immunoglobulin G; HCV: Hepatitis C virus; p- value: Probability value. CMV IgM: Cytomegalovirus Immunoglobulin M. HIV: Human Immunodeficiency virus; X^2: Chi-square test. HBV: Hepatitis B virus



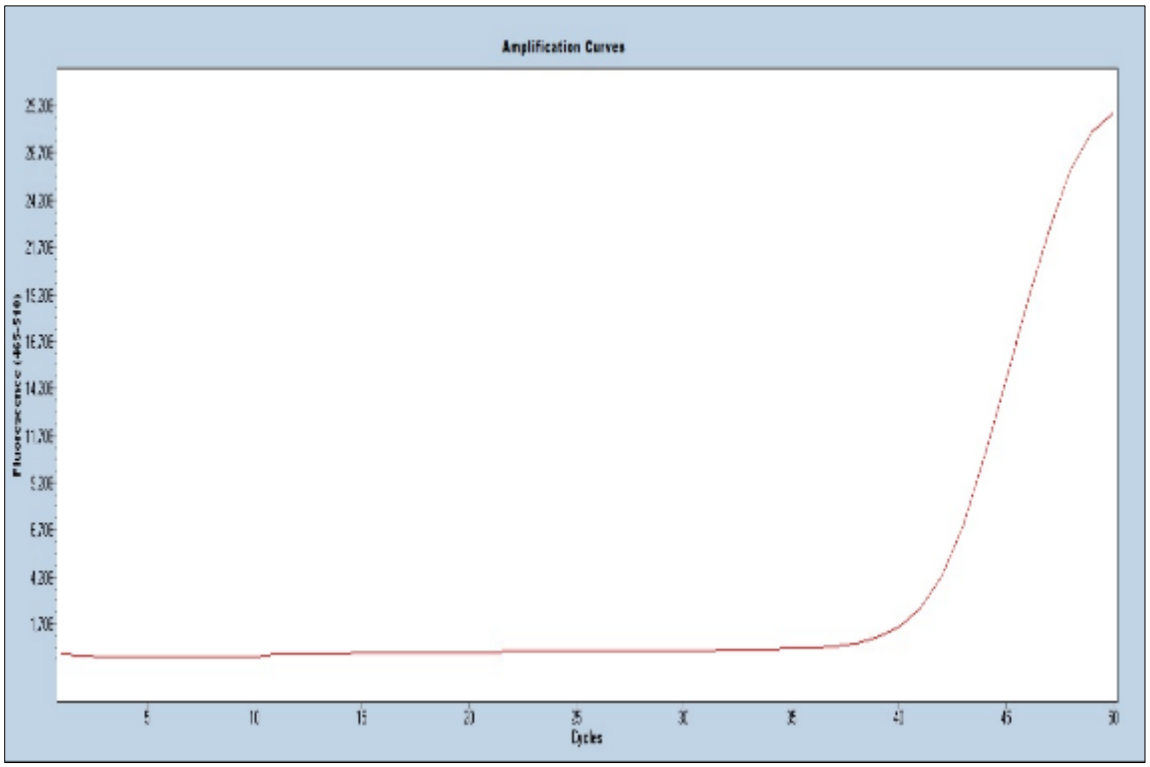
Key: y-axis: Fluorescence (465-510): x-axis: Cycles

Figure 6 Real- time PCR amplification plot of positive control for CMV DNA copies



Key: Y-axis: Fluorescence (465-510):X-axis: Cycles

Figure 7 Real- time PCR amplification plot of negative control for CMV DNA copies



Key: Y-axis: Fluorescence (RFU): X-axis: Cycles.:RFU: Relative Fluorescence unit

Figure 8 Real- time PCR amplification plot 1for positive sample for CMV DNA

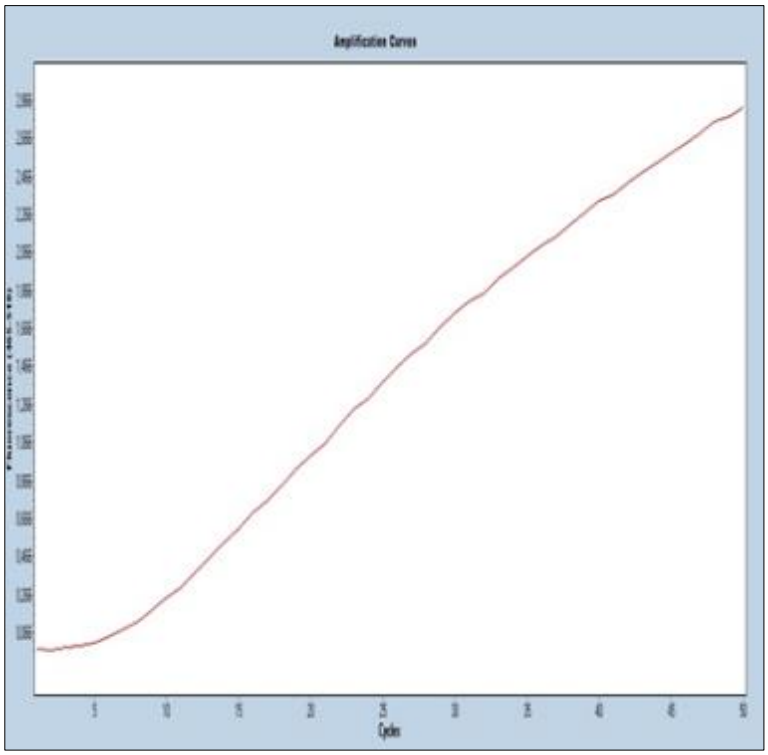


Figure 9 qPCR plot 1 for negative CMV DNA

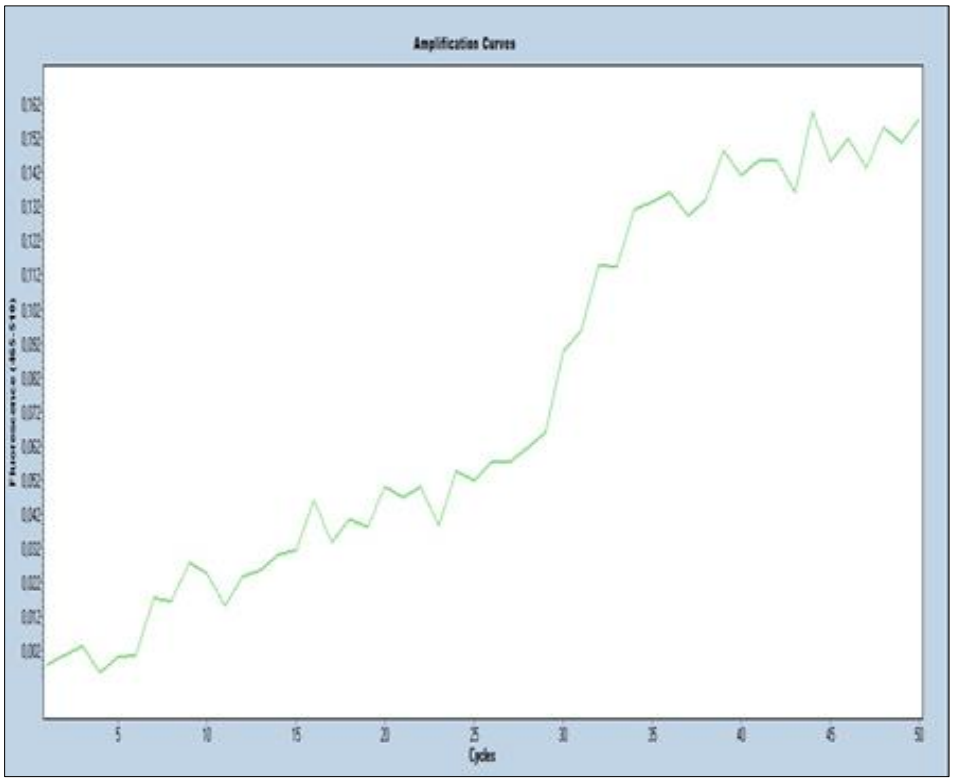


Figure 10 qPCR plot 2 for negative CMV DNA

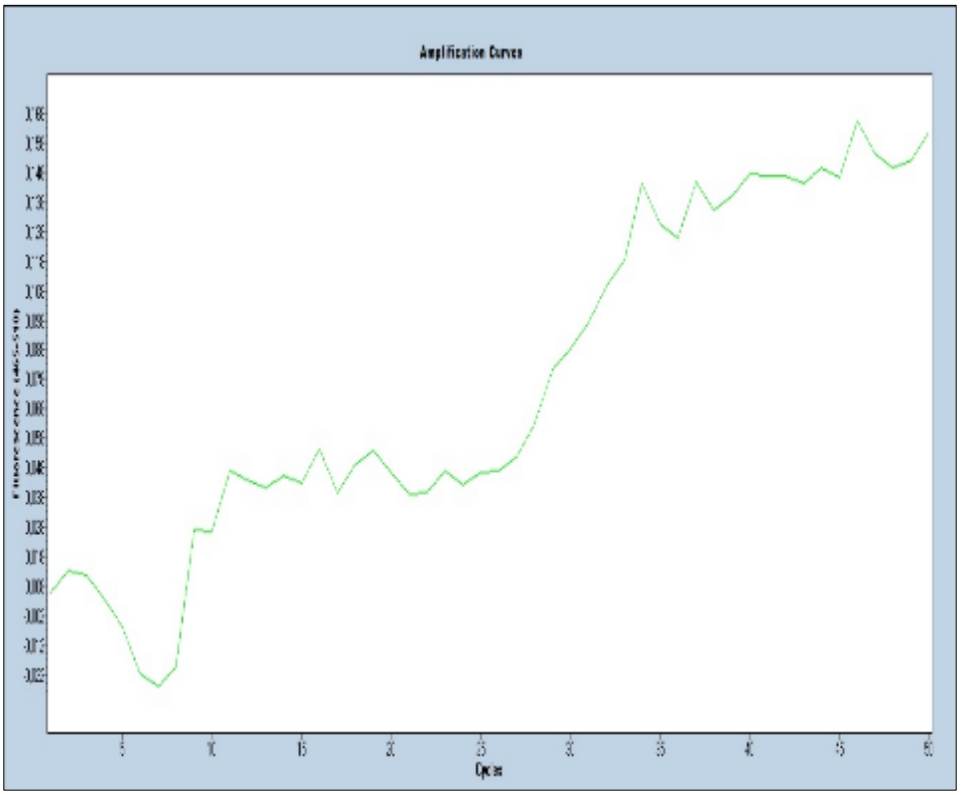


Figure 11 qPCR plot 3 for negative CMV DNA

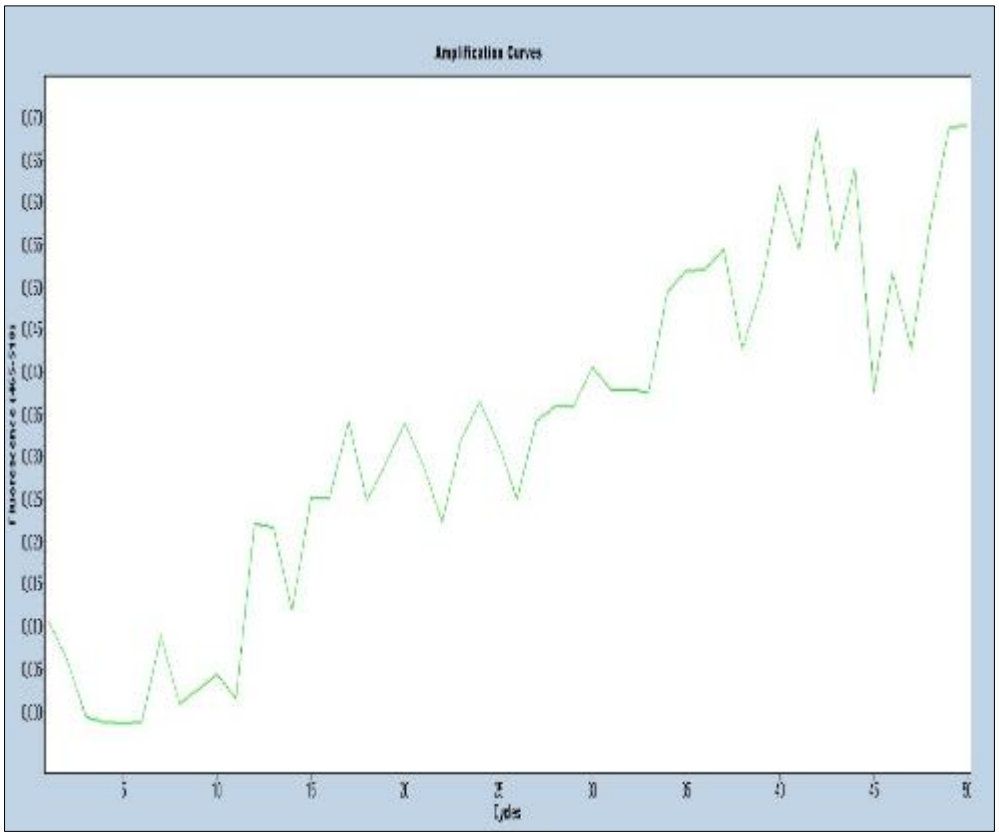


Figure 12 qPCR plot 4 for negative CMV DNA

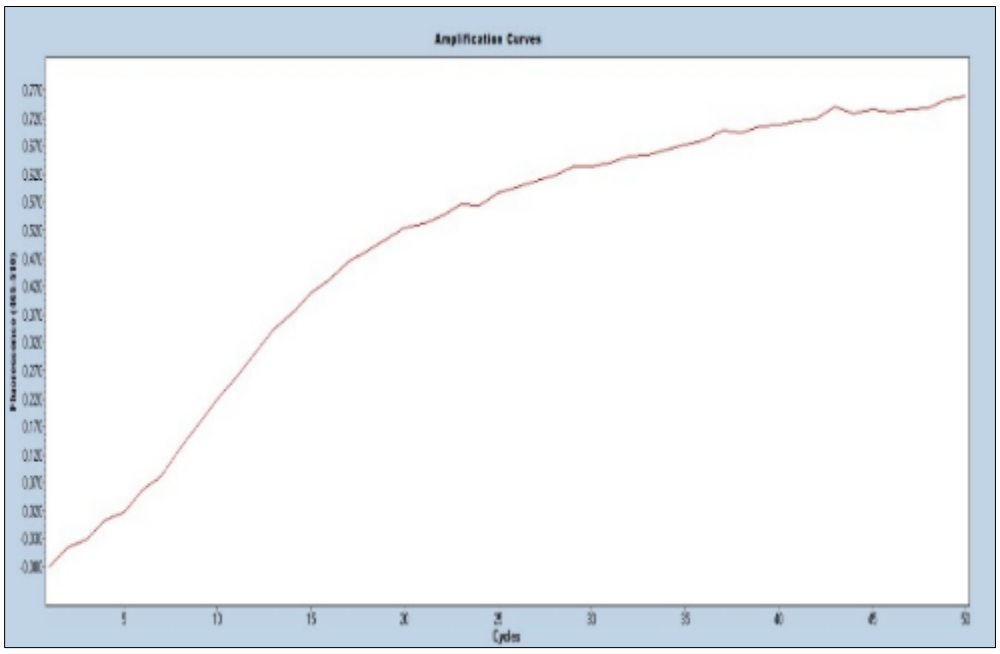


Figure 13 qPCR plot 5 for negative CMV DNA

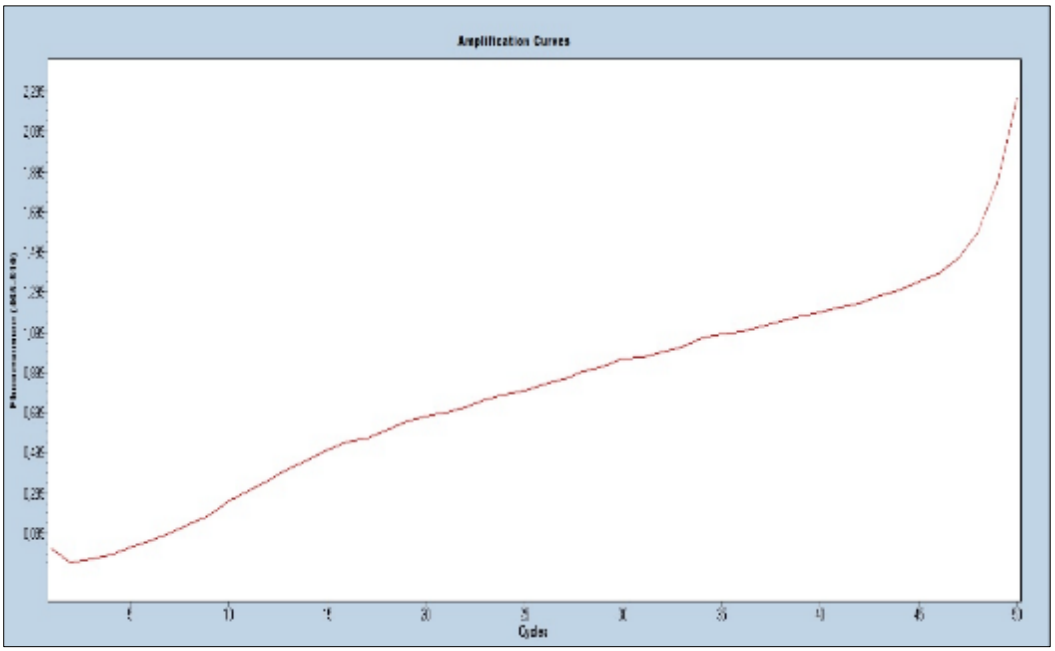


Figure 14 qPCR plot 6 for negative CMV DNA

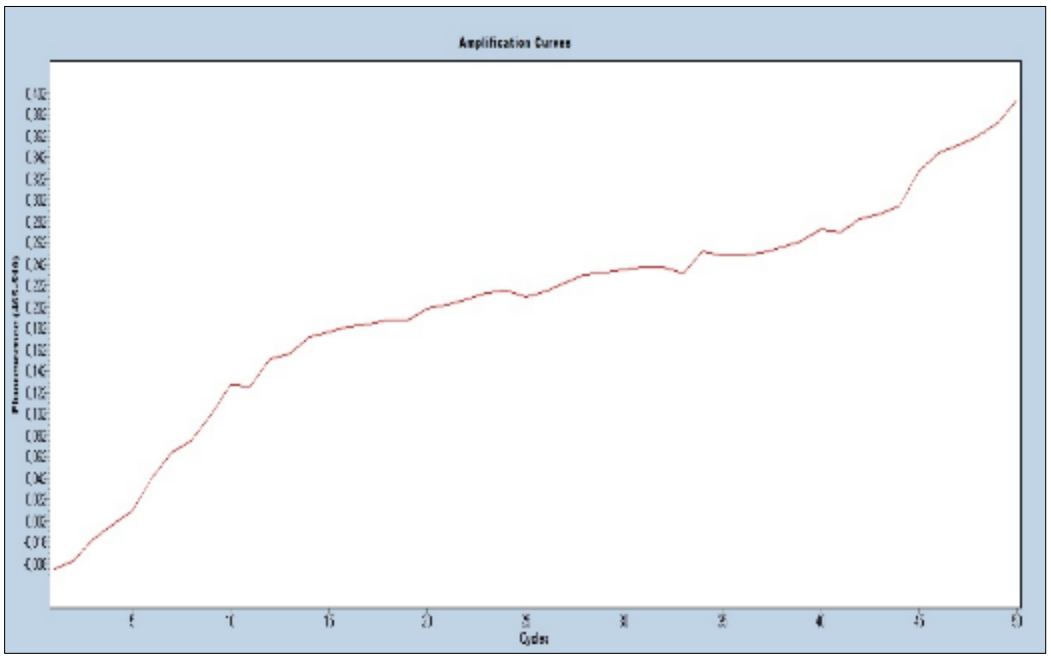


Figure 15 qPCR plot 7 for negative CMV DNA

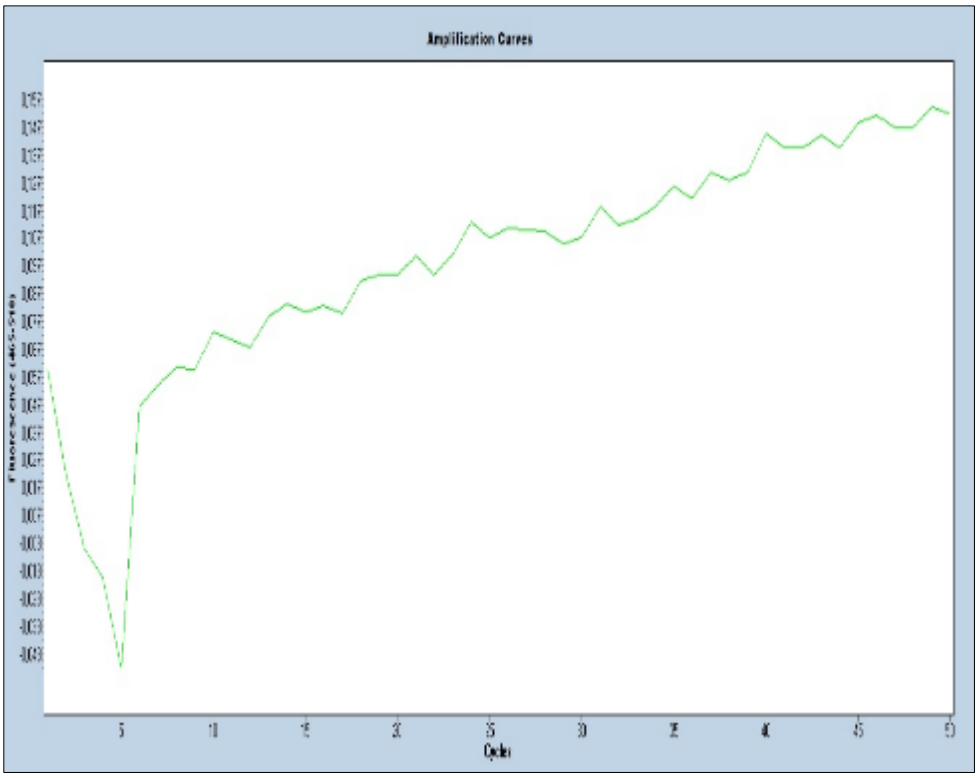


Figure 16 qPCR plot 8 for negative CMV DNA

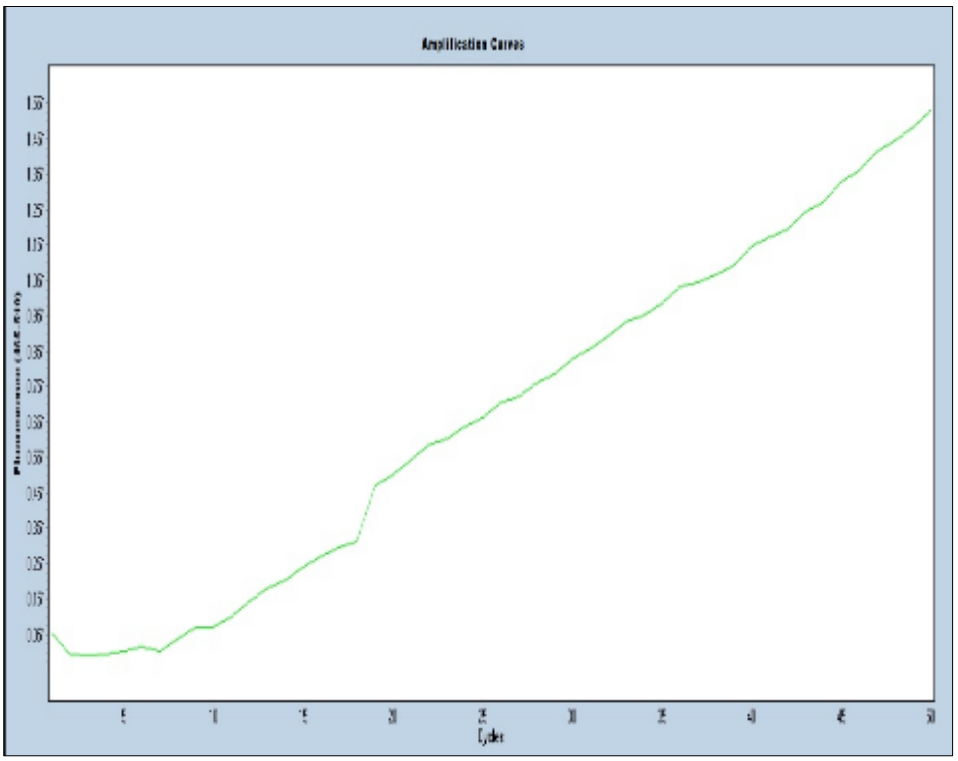


Figure 17 qPCR plot 9 for negative CMV DNA

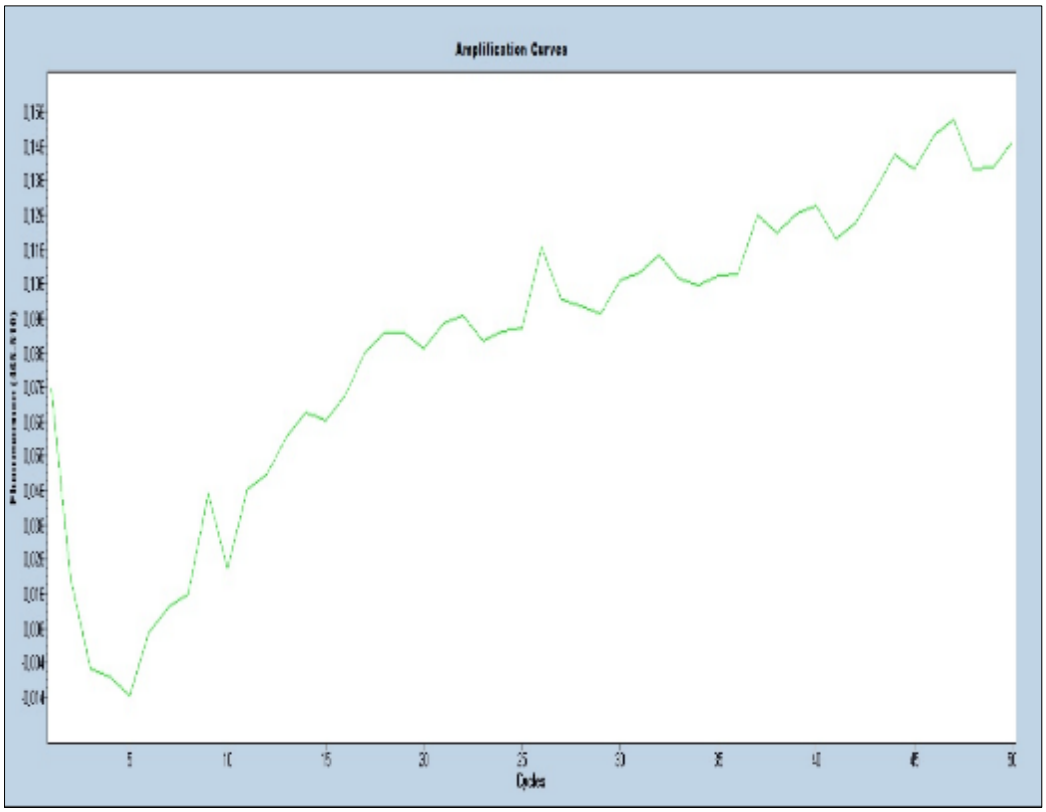


Figure 18 qPCR plot 10 for negative CMV DNA

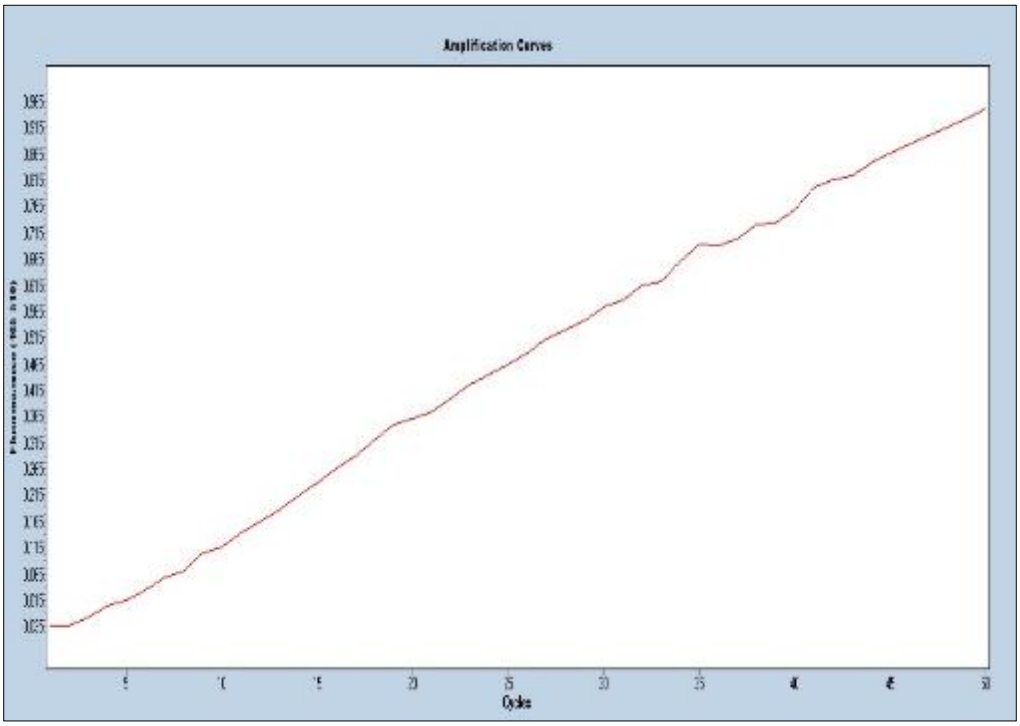


Figure 19 qPCR plot 11 for negative CMV DNA

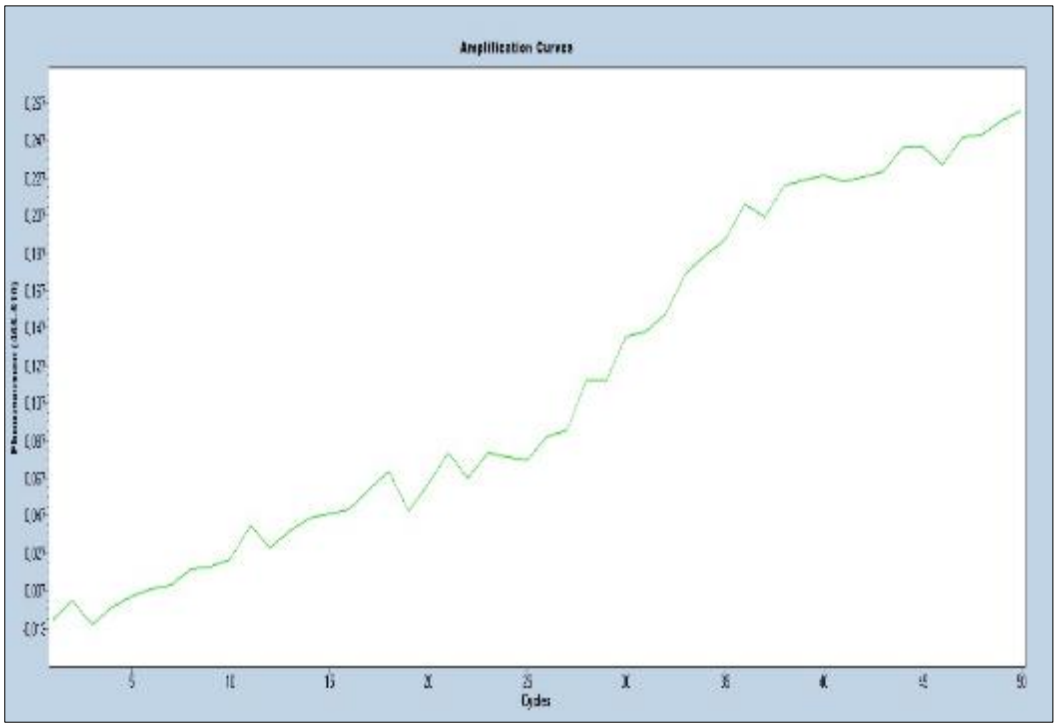


Figure 20 qPCR plot 12 for negative CMV DNA

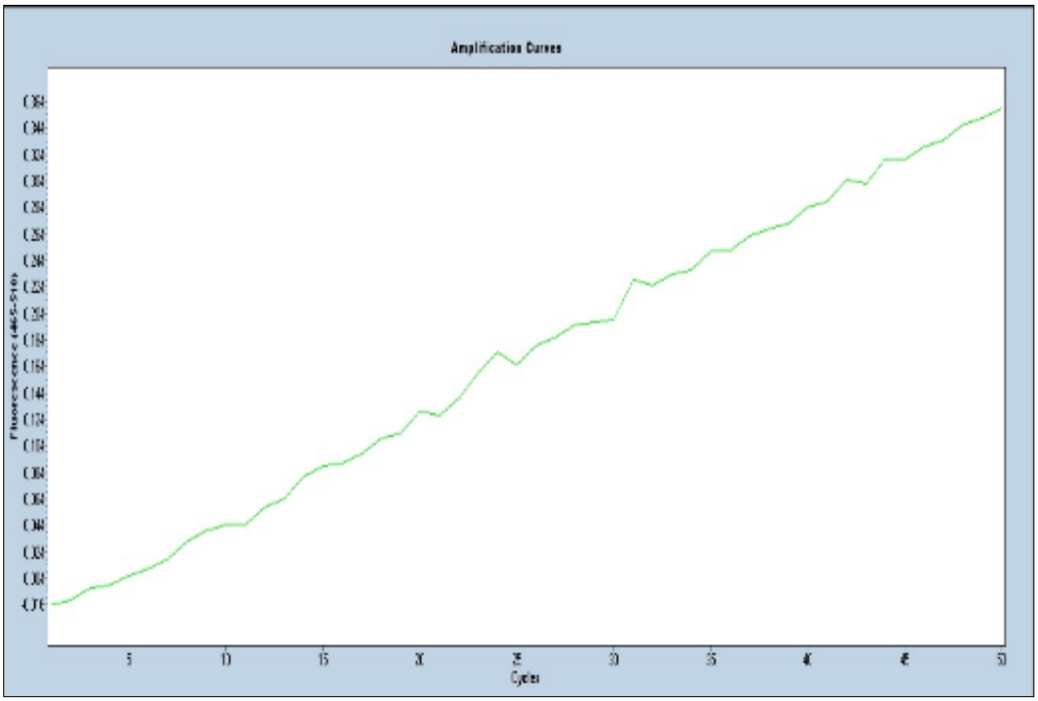


Figure 21 qPCR plot 13 for negative CMV DNA

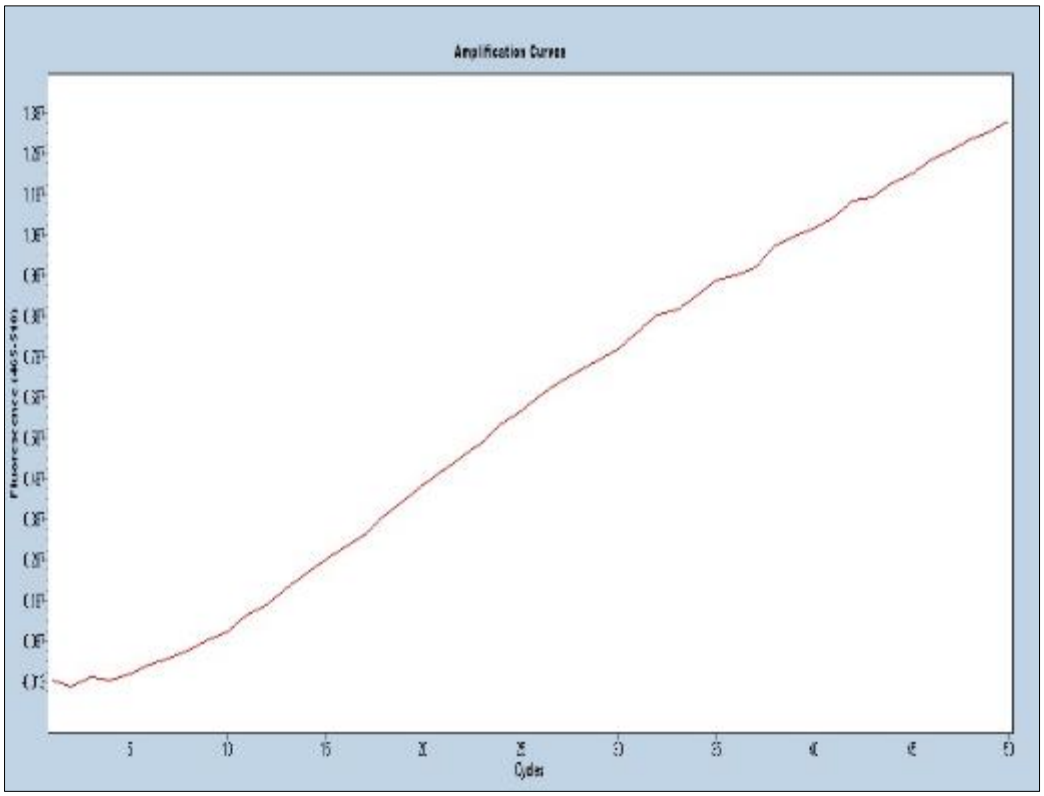


Figure 22 qPCR plot 14 for negative CMV DNA

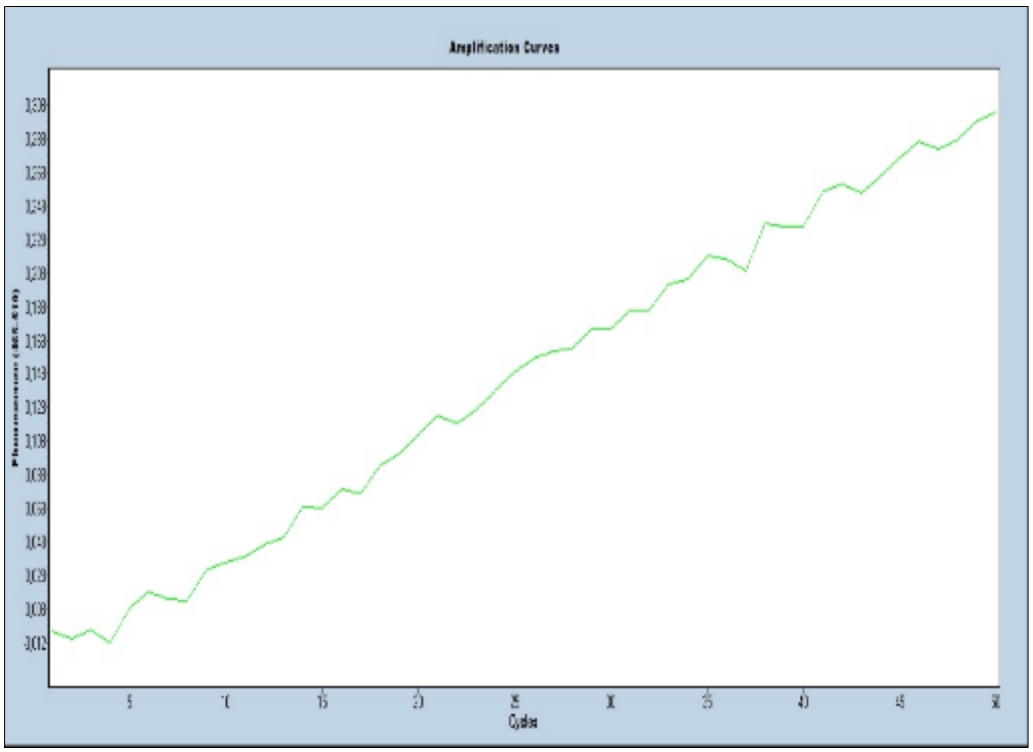


Figure 23 qPCR plot 15 for negative CMV DNA

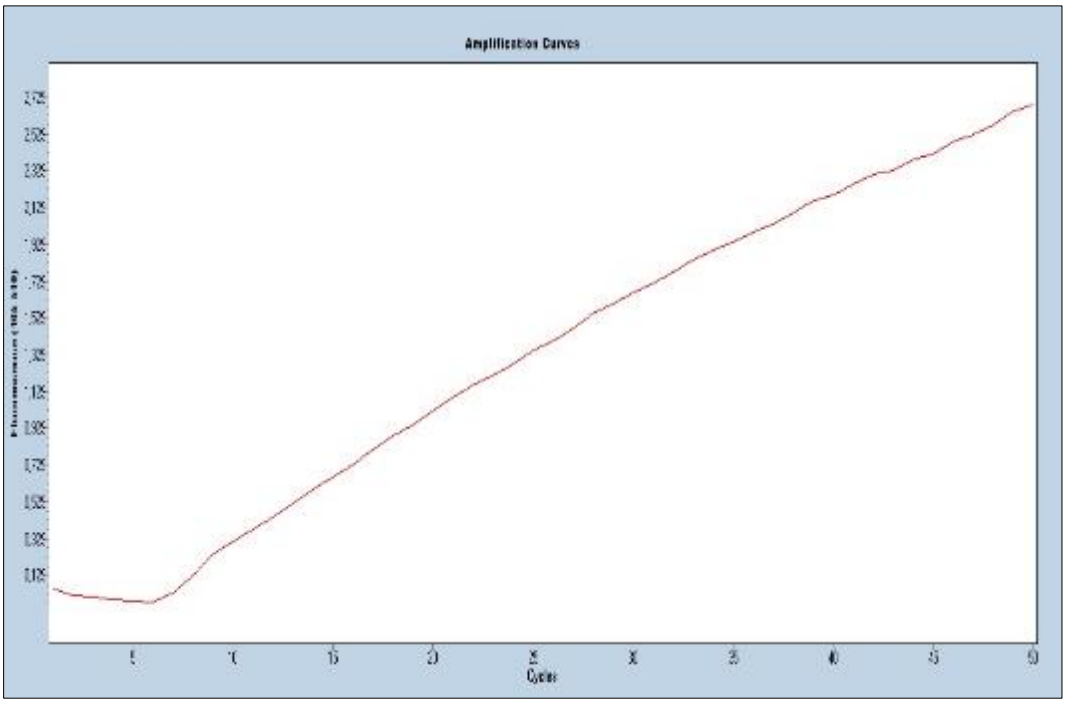


Figure 24 qPCR plot 16 for negative CMV DNA

Table 2a Association between CMV IgG status and risk factors variables

		CMV IgG status				
Risk factor	Category	Positive n(%)	Negative n(%)	Total	X^2	p-value
Multiple sex partner	Yes	10(14.7%)	7(25%)	17(17.7%)	1.442	0.230
	No	58(85.3%)	21(75%)	79(82.3%)		
Multiple kissing partner	Yes	11(16.2%)	4(14.3%)	15(15.6%)	0.054	0.817
	No	57(83.8%)	24(85.7%)	81(84.4%)		
Share needle or other injection equipment	Yes	2(2.9%)	0(0%)	2(2.1%)	0.841	0.359
	No	66(97.1%)	28(100%)	94(97.9%)		
Received Tattoo or other piercing from an unlicensed artist	Yes	0(0%)	0(0%)	0(0%)	-	-
	No	28(100%)	68(100%)	96(100%)		
Had unprotected Anal sex	Yes	6(8.8%)	1(3.6%)	7(7.3%)	0.809	0.368
	No	62(91.2%)	27(96.4%)	89(92.7%)		
Had unprotected Oral sex with more than one partner	Yes	5(7.4%)	2(7.1%)	7(7.3%)	0.001	0.971
	No	63(92.6%)	26(92.9%)	89(92.7%)		
Injects hard drugs	Yes	1(1.5%)	0(0%)	1(1%)	0.416	0.519
	No	67(98.5%)	28(100%)	95(99%)		

Key: n: Number of subjects; X^2: Chi-square; p- value: Probability value; CMV IgG: Cytomegalovirus Immunoglobulin G antibodies

Table 2b Association between CMV IgM status and risk factors variables

Risk factor	**CMV IgM status**					
	Category	**Positive** n(%)	**Negative** n(%)	**Total**	**X^2**	**p-value**
Multiple sex partner	Yes	0(0%)	17(17.7%)	17(17.7%)	-	-
	No	0(0%)	79(82.3%)	79(82.3%)		
Multiple kissing partner	Yes	0(0%)	15(15.6%)	15(15.6%)	-	-
	No	0(0%)	81(84.4%)	81(84.4%)		
Share needle or other injection equipment	Yes	0(0%)	2(2.1%)	2(2.1%)	-	-
	No	0(0%)	94(97.9%)	94(97.9%)		
Received Tattoo or other piercing from an unlicensed artist	Yes	0(0%)	0(0%)	0(0%)	-	-
	No	0(0%)	96(100%)	96(100%)		
Had unprotected Anal sex	Yes	0(0%)	7(7.3%)	7(7.3%)	-	-
	No	0(0%)	89(92.7%)	89(92.7%)		
Had unprotected Oral sex with more than one partner	Yes	0(0%)	7(7.3%)	7(7.3%)	-	-
	No	0(0%)	89(92.7%)	89(92.7%)		
Injects hard drugs	Yes	0(0%)	1(1%)	1(1%)	-	-
	No	0(0%)	95(99%)	95(99%)		

Key: n: Number of subjects: X2: Chi-square: p- value: Probability value: CMV IgM: Cytomegalovirus Immunoglobulin M antibodies

Table 2c Association between HBV status and risk factors variables

Risk factor	**HBV status**					
	Category	**Positive** n(%)	**Negative** n(%)	**Total**	**X^2**	**p-value**
Multiple sex partner	Yes	0(0%)	17(17.7%)	17(17.7%)	-	-
	No	0(0%)	79(82.3%)	79(82.3%)		
Multiple kissing partner	Yes	0(0%)	15(15.6%)	15(15.6%)	-	-
	No	0(0%)	81(84.4%)	81(84.4%)		
Share needle or other injection equipment	Yes	0(0%)	2(2.1%)	2(2.1%)	-	-
	No	0(0%)	94(97.9%)	94(97.9%)		
Received Tattoo or other piercing from an unlicensed artist	Yes	0(0%)	0(0%)	0(0%)	-	-
	No	0(0%)	96(100%)	96(100%)		
Had unprotected Anal sex	Yes	0(0%)	7(7.3%)	7(7.3%)	-	-
	No	0(0%)	89(92.7%)	89(92.7%)		
Had unprotected Oral sex with more than one partner	Yes	0(0%)	7(7.3%)	7(7.3%)	-	-
	No	0(0%)	89(92.7%)	89(92.7%)		
Injects hard drugs	Yes	0(0%)	1(1%)	1(1%)	-	-
	No	0(0%)	95(99%)	95(99%)		

Key; n: Number of subjects; X2: Chi-square; p- value: Probability value; HBV: Hepatitis B virus

Table 2d Association between HCV status and risk factors variables

Risk factor		**HCV status**				
	Category	**Positive n(%)**	**Negative n(%)**	**Total**	**X^2**	**p-value**
Multiple sex partner	Yes	0(0%)	17(17.9%)	17(17.7%)	0.217	0.641
	No	1(0%)	78(82.1%)	79(82.3%)		
Multiple kissing partner	Yes	0(0%)	15(15.8%)	15(15.6%)	0.187	0.665
	No	1(100%)	80(84.2%)	81(84.4%)		
Share needle or other injection equipment	Yes	0(0%)	2(2.1%)	2(2.1%)	0.022	0.883
	No	1(100%)	93(97.9%)	94(97.9%)		
Received Tattoo or other piercing from an unlicensed artist	Yes	0(0%)	0(0%)	0(0%)	-	-
	No	1(100%)	95(100%)	96(100%)		
Had unprotected Anal sex	Yes	0(0%)	7(7.4%)	7(7.3%)	0.079	0.778
	No	1(100%)	88(92.6%)	89(92.7%)		
Had unprotected Oral sex with more than one partner	Yes	0(0%)	7(7.4%)	7(7.3%)	0.079	0.778
	No	1(100%)	88(92.6%)	89(92.7%)		
Injects hard drugs	Yes	0(0%)	1(1.1%)	1(1%)	0.011	0.918
	No	1(100%)	94(98.9%)	95(99%)		

Key: n: Number of subjects: X2: Chi-square: p- value: Probability value: HCV: Hepatitis C virus

Table 2e Association between HIV status and risk factors variables

Risk factor		**HIV status**				
	Category	**Positive n(%)**	**Negative n(%)**	**Total**	**X^2**	**p-value**
Multiple sex partner	Yes	2(100%)	15(16%)	17(17.7%)	9.492	0.002
	No	0(0%)	79(84%)	79(82.3%)		
Multiple kissing partner	Yes	1(50%)	14(14.9%)	15(15.6%)	1.831	0.176
	No	1(50%)	80(85.1%)	81(84.4%)		
Share needle or other injection equipment	Yes	0(0%)	2(2.1%)	2(2.1%)	0.043	0.835
	No	2(100%)	92(97.9%)	94(97.9%)		
Received Tattoo or other piercing from an unlicensed artist	Yes	0(0%)	0(0%)	0(0%)	-	-
	No	2(100%)	94(100%)	96(100%)		
Had unprotected Anal sex	Yes	1(50%)	6(6.4%)	7(7.3%)	5.511	0.019
	No	1(50%)	88(93.6%)	89(92.7%)		
Had unprotected Oral sex with more than one partner	Yes	0(0%)	7(7.4%)	7(7.3%)	0.161	0.699
	No	2(100%)	87(92.6%)	89(92.7%)		
Injects hard drugs	Yes	0(0%)	1(1.1%)	1(1%)	0.022	0.883
	No	2(100%)	93(98.9%)	95(99%)		

Key; n: Number of subjects; X2: Chi-square; p- value: Probability value; HIV: Human Immunodeficiency virus

4. Discussion

Male fertility and semen quality have been demonstrated to be negatively impacted by viral STDs. These viruses have such detrimental effects on the reproductive systems and overall health of those affected and these have in fact continued to be issues of interest and discussion among researchers. In this study, we accessed the prevalence of some viral sexually transmitted diseases and their infection risk factors on males in Onitsha Anambra State.

This study showed variations in seroprevalence of Cytomegalovirus (CMV) IgG, CMV IgM, Hepatitis B virus (HBV), Hepatitis C virus (HCV), and Human immunodeficiency virus (HIV) antibodies among the subjects. Highest prevalence observed with CMV IgG 68(70.8%) in this research could be attributed to previous exposure of the subjects to the virus or presence of similar antigen cross reacting with the antibody. CMV is a latent virus and positive CMV IgG result indicates past CMV infection which could have been reactivated at the time of the study due to stress or weakened immune system. The finding is at par with the CMV IgG prevalence of 78.35% recorded in a study on serostatus of CMV among population in Jazan region, Saudi Arabia [13]. However, our finding is lower than the overall CMV prevalence of 91.3%, reported in Western Brazil [14]. It is also lower than CMV prevalence of 87.9% observed in Ekiti, Nigeria [15]. On the other hand, prevalence observed was higher than CMV seroprevalence of 67% observed from another study among Adult residents from Manaus [16].

The reason for the negative CMV IgM status 0(0%) in this study could be that though many subjects had been previously exposed at the time of our study, none had an active CMV infection. A positive CMV IgM status confirms an acute systemic reactivation of CMV infection while a positive CMV IgG suggests previous exposure to CMV [17]. Sensitivity of the method used may also be a fact. This finding does not correlate with the findings that had higher CMV IgM prevalences of (32.7%) and (7.8%) respectively [15, 24].

No subject tested positive to Hepatitis B Virus. The method used in the present study was immunochromatographic method which is a lesser sensitivity method than PCR. This may have been the main factor asides the probability of the subjects being negative due to lack of exposure to the virus, probable negative window phase or protective effects of HBV immunization program from the state in the population group area a couple of years before this study. This finding is in agreement with the finding that recorded a zero percentage of HBV in a study in Nnewi, Nigeria [4]. However, our finding differs from the study in Abuja and Nnewi, Anambra State, Nigeria where HBV prevalence of 20.5% was observed [18]. It also disagrees with the work done in Niger-Delta region of Nigeria where HBV prevalence was observed to be 4.6% [19]. Another study also had HBV prevalence of 10.1% with patients at tertiary health institute in Bayelsa State, Nigeria [20].

A low seroprevalence of 2(2.1%) observed with HIV infection in this research could be associated with diverse lifestyles, less sensitivity of test method used (Immunochromatography), probability of latency exhibited by the virus and geographical area where the study was conducted. This correlates with the 2.2% HIV prevalence observed in Anambra state [21]. This HIV prevalence was also similar with that of 2.6% observed when impact of HIV was compared on fertility variables of commercial motorcyclist and non-motorcyclists in Nnewi, South-East, Nigeria [4]. However, the HIV prevalence observed in this work is much lower than the 13.7% and 31% observed in Abuja and Onitsha, Nigeria respectively [22, 23].

Variations in prevalence of different viral infections across age ranges and professions were noted. CMV IgG with the highest positive case observed in the age group 38-44 years 20(29.4%) could have been associated with chronic infection with this age group who are known to be sexually active due to marital status and relationships. They may also have sexual habits such as unprotected sex, multiple sexual partners and kissing habits or other experimental sexual adventures which increase the risk of CMV infection. This finding does not agree with the study in Edo State, Nigeria that had highest CMV prevalence among age group <19 years (51.0%) [24]. The significant difference observed in age in this study ($p=0.041$) does not correlate with the studies in Ado-Ekiti and Edo States, Nigeria respectively that did not observe a statistical significant difference of CMV infection in relation to age [15, 24].

A single positive status of HCV in age range of 45-51 years was not statistically significant ($p=0.167$). This could be due to expected difference in sexual life styles associated with different age groups. This does not agree with the study on Co-infection of HCV among HIV infected persons in Anambra that had a highest prevalence with age-group 31-40 with significant age difference [25]. It disagrees also with the findings in Awka, Anambra State where the age group of 26-35 had the highest significant HCV prevalence [26]. HIV prevalence is observed in age groups of 31-37 and 38-44 years old respectively, with each having one positive case 1(50%). These age groups are early adults that are sexually active and are likely to have had sex several times in their lives with multiple partners or may have been exposed to risk factors for HIV such as unprotected sexual intercourse, shared needles, etc., at least once in their lifetimes.

The highest prevalence of CMV IgG 18(61.8%) exhibited by traders among other professions in this study could be as a result of person to person contact which traders experience daily with people via body fluids, money contact or even sweaty contaminated hand shake. CMV has been found to be shed in multiple body fluids including saliva, urine and semen for months and years [27]. Subjects could be asymptomatic carriers of vibrant infectious agent that establishes latency. Students showed no positive CMV IgM, HBV, HCV and HIV case. This could probably be due to methods not sensitive enough or they were not exposed to the risk factors. It has been noted that poor level of education, inadequate sanitary conditions, cultural characteristics, and households with a significant number of persons are key variables that are associated with elevated CMV prevalence rates [14]. This perhaps explains why traders had highest prevalent rate and students had no positive case in this study. The finding in this work correlates with that in Edo state where the prevalence of HCMV was more pronounced in urban locales as opposed to rural regions [24]. The high levels of HCMV infection in urban areas may be due to significant growth in urban population, inadequate basic infrastructure, racial disparities amongst the populations, declining socioeconomic standards, inadequate hygiene procedures, higher rates of social interaction, commercial sex activities, multiple sexual partners and other factors which predispose the residents to CMV infection. Our finding also agrees with the study in Western Brazilian Amazon where they discovered that students had low CMV prevalence, thus being a student was directly connected with decreased susceptibility to CMV infection [14].

The traders and civil servants had single positive case each for HIV 2(100%) which could be because the traders are usually on business trips that take them away from their wives. They are confronted with temptation of patronizing commercial sex workers at different business locations, thereby exposing themselves to contracting HIV infection. No significant variation is observed with profession (p=0.968). Other parameters, including CMV IgM, HBV, HCV, and HIV, did not show significant variations with age or profession. This agrees with the work in Ado-Ekiti and Edo States, Nigeria respectively that did not have significant difference of CMV prevalence associated with age groups [15, 24].

In the present study, only 1 out of 17 samples from highest titre values for CMV IgG and IgM tested positive (5.9%) with real-time PCR (qPCR). Reasons for high positive IgG and IgM antibodies not correlating positively with qPCR detection could be associated with presence of false positive IgG and IgM antibodies (Rheumatoid factor), presence of cross-reacting antibodies that could have accumulated to the high serological positivity or latency state of the virus as at the time of the study. PCR that was designed and adopted depends on picking the major immediate early viral particles replicating at that time. More so, because the samples had been stored prior to testing, the virus may be at latent stage, hence the low positive qPCR detection. This is supported with the findings in Italy which observed that a qPCR, which is a gold standard for detecting CMV DNA, when positive in an infectious sample signifies viral replication going on at that time [28]. Storage factor can also affect time lag between sero-conversion as storage in a lowered freezing temperature could have slowed down activity of various seroconverting proteins. Our finding is consistent with the finding in Sudan which detected only (29.7%) of CMV DNA with qPCR method as against (91.3%) positive ELISA IgG antibodies comparing both methods in CMV detection [29]. Similarly, another research revealed that out of 8 (100%) sample that tested CMV IgG positive, only 5(62.5%) samples were positive with real-time PCR [30]. Real time PCR is found to be more specific, sensitive, accurate and reliable than ELISA in the detection of CMV infection [29].

The association between CMV IgG status and various risk factors was analyzed to determine any significant correlation. The highest prevalence of abnormal status was observed in subjects that had multiple kissing partners 11(16.2%), followed by multiples sex partners 10(14.7%), those that had unprotected anal sex 6(8.8%), unprotected oral sex with more than one partner 5(7.4%), shared needle and other injection equipment 2(2.9%) and those that injected hard drugs 1(1.5%). None of the subjects received tattoo or piercing from unlicensed artist 0(0%). However, no significant association was observed among the risk factors with CMV IgG status (p>0.05). The reason could be because CMV is readily transmitted through exposures to saliva sharing behaviours such as kissing, sharing drinks, oral sex, etc., and other risky behaviours including multiple sexual partners. The Centers for Disease Control and Prevention noted that direct contact with infectious body fluids (blood, tears, urine, saliva, blood, semen, and breast milk), sexual intercourse, organ transplants, blood transfusions and contact with the mother's vaginal secretions after childbirth can all transmit CMV [31]. This finding correlates with the work in United States that found CMV not to be significantly associated with multiple sexual partners among other risk factors [32].

The no significant associations between CMV IgM and HBV with the risk factors may reflect low statistical power due to no positive participants. The negative associations between HCV status and the risk factors may be an indicative of its mode of transmission. It has been observed that HCV is primarily transmitted through parenteral exposure to infectious blood or body fluid that contains blood, most commonly through injection drug use [33]. Thus sexual transmission of HCV is possible but much less efficient as compared to HIV. This finding agrees with the findings that discovered no significant association between risk factors and HCV positive status [34]. It also correlates with another work that found no significant association between HCV and socio-demographic traits [25]. However, our finding disagrees with the

findings in Lagos State, Nigeria that had significant association between HCV and subjects that had multiple sexual partners [35]. Meanwhile, they had no significant associations with other risk factors which agree with our findings.

The significant associations between HIV status and the subjects that had multiple sex partners (p=0.002) and those that had unprotected anal sex (p=0.019) in this finding is an indication that HIV can be transmitted through the exchange of body fluids from people living with HIV, such as blood, breast milk, semen, vaginal secretions, pregnancy and delivery as noted by WHO [36]. This finding is in agreement with the research where HIV had a significant association with risky sexual practices such as unprotected vaginal/anal sex [37]. It also correlates with the study involving female sex workers that associated HIV with multiple sex violence among other factors [38]. It however disagrees with the study in Iran that found HIV to be significantly associated with injection drug use [39].

5. Conclusion

The finding that CMV IgG was statistically significant among the subjects (p=0.04) indicates a higher exposure rate to Cytomegalovirus (CMV) in this group. CMV IgG positivity reflects past exposure to the virus, which can remain dormant and potentially reactivate under certain conditions. The observation that only 1 out of 17 CMV IgG positive samples tested positive for CMV qPCR (5.9%) suggests that real-time PCR (qPCR) is more sensitive and specific compared to ELISA for detecting CMV infection. This highlights the importance of using appropriate diagnostic tools for accurate detection of viral infections.

The association of HIV with having multiple sex partners (p=0.002) and engaging in unprotected anal sex (p=0.019) underscores the link between high-risk sexual behaviours and increased HIV prevalence. This finding could inform targeted prevention and education strategies to reduce HIV transmission.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Contributions of authors

CNO and OBO devised and planned the study. CNO wrote the first draft of the manuscript and reviewed literature along with OBO and NRA. CNO and OBO collected clinical samples from subjects. CNO performed laboratory analysis while OBO and NRA supervised the work. CNO, OBO and NRA reviewed the manuscript. All authors read and approved the final manuscript.

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Statement of ethical approval

Ethical approval was obtained from institutional ethical board committee on 14/07/2023.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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