# TRENDS IN SEROPREVALENCE OF TRANSFUSION TRANSMISSIBLE INFECTIONS AMONGST THE BLOOD DONORS IN TERTIARY CARE CENTRE

**By**

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**IN**

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**Under the Guidance of**

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# LIST OF ABBREVATIONS USED

| | |
|---|---|
| AIDS | Acquired Immune Deficiency Syndrome |
| DNA | Deoxyribonuclic Acid |
| EIA | Enzyme Immuno Assay |
| ELISA | Enzyme-linked immunosorbent assay |
| HBcAg | Hepatitis B Core Antigen |
| HBeAg | Hepatitis B ‘e’ Antigen |
| HBsAg | Hepatitis B Surface Antigen |
| HBV | Hepatitis B Virus |
| HCV | Hepatitis C Virus |
| HIV | Human Immunodeficiency Virus |
| ICTC | Integrated Counseling and Testing Centres |
| NACO | National AIDS Control Organisation |
| PCR | Polymerase Chain Reaction |
| RDT | Rapid Diagnostic Test |
| RNA | Ribose Nucleic Acid |
| RPR | Rapid Plasma Reagin |
| TTI | Transfusion Transmissible Infection |
| UNAIDS | United Nations Programme on HIV/AIDS |
| VCTC | Voluntary Counselling and Testing Centres |
| VDRL | Venereal Disease Research Laboratory test |
| WB | Western Blot |
| WHO | World Health Organization |

# ABSTRACT

**INTRODUCTION:** Blood transfusion is the most essential, lifesaving integral remedy in health care delivery system, but also carries the potential risk of transfusion transmissible infections (TTIs) which is one of the most dreaded complication of blood transfusion services.

**OBJECTIVE:** To study the trends in seroprevalence of HIV, HBV, HCV, Syphilis and Malaria amongst the blood donors over a period of 5 (3- retrospective and 2-prospective) years.

**MATERIALS AND METHODS:** All the prospective blood donors were examined for their preliminary health check-up and eligibility criteria for donation of blood. Screening tests were performed on all blood donors' samples. Retrospective blood donors' data was collected from the blood bank of BLDE (deemed to be university) University, Shri B.M. Patil Medical College, Hospital and Research Centre Vijayapura. Serum samples were tested for HIV-antibody, HCV-antibody and HBsAg using ELISA and Malarial parasite antigen by rapid diagnostic tests (RDT).

**RESULTS:** During the study total 20584 donors' blood unit were screened, out of which 369 blood units were found to be seropositive accounting for the seroprevalence of 1.8%. Seroprevalence of HIV, HBV, HCV, Syphilis and Malaria were 0.20%, 1.34%, 0.22%, 0.06% and 0% respectively. Out of total screened donors, voluntary donors were 79.4% and remaining 20.6% were replacement donors. Majority of the donors were in age group of 18 – 35 years. Majority of the donors were males (96.4%).

**CONCLUSION:** In the present study it has been established that prevalence of TTIs has decreased considerably after mandatory testing of blood Units for TTIs. With the implementation of strict donor selection criteria, eestablishment of strict

guidelines for blood transfusion and use of sensitive screening tests, it may be possible to reduce the incidence of TTI in Indian scenario.

**KEY WORDS:** Transfusion Transmissible Infection, HIV, HBV, HCV, Syphilis, Malaria, Seroprevalence.

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# 1. INTRODUCTION

Blood is considered as the living force of human body and is an essential element of human life and there are no substitutes for it.[1] WHO theme for 2000 AD was "Safe blood starts with me, blood saves lives." [1] Blood and its component form a substantial part of patient management treatment protocols in the present scenario but at the same time has life threatening hazards also as it carries the risk of transfusion transmitted infections (TTIs).[2] Every year with increase in population, urbanisation, life-expectancy, and increased demand for blood products, the burden of requirement of safe blood is increasing.[3]

TTIs can be caused by various microorganisms, the major globally prevalent TTIs are human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis and malarial parasite. As per guidelines it is mandatory to screen blood donors for HIV, HBV, HCV, Syphilis and Malaria. Screening strategies include starting from the medical history, clinical examination and screening for the markers of infections which are hepatitis B surface antigen (HBsAg), malarial antigens such as histidine and antibodies to HIV and HCV, and Syphilis.[4,5]

The evaluation of the data of the prevalence of these TTIs amongst the blood donors permits an assessment of epidemiology , acquisition of the infection and consequently the safety of the collected donations.[6]

The safety of the blood and its components depends on a proper donor selection by sensitive screening tests to exclude these infectious agents. Though the screening strategies have been effective, but transmission is still occurring, primarily because of the inability of test to detect the disease in the window period, immune silent carriers and immunologically variant viruses. [4]

So, this study was under taken to find out the trends in seroprevalence of transfusion transmissible infections amongst the blood donors from BLDE (Deemed to be University), Shri B.M. Patil Medical College, Hospital and Research Centre Vijayapura.

## 2. OBJECTIVE OF THE STUDY

To study the trends in seroprevalence of HIV, HBV, HCV, Syphilis and Malaria amongst the blood donors over a period of 5 (3 -retrospective and 2- prospective) years.

# 3.REVIEW OF LITERATURE

**Historical Background:**

Since as early as 2,500 B.C. blood has always held mysterious fascination for all and considered to be the living force of our body. Ancient Egyptians used to draw blood in an attempt to cleanse the body from diseases. In 500 B.C. Greeks used to perform human dissections in attempts to better understand how the blood flows through the body.[7]

In 1628, British physician William Harvey discovered blood circulation throughout the body and in the same year Wren, Wilkins, Boyle and Willis demonstrated that intravenous injection of substances was possible. Both of these discoveries opened the way for modern transfusion medicine.[7,8]

In 1665, the first recorded successful blood transfusion was performed in England by Physician Richard Lower who kept a dog alive by transfusing blood from another dog. It was published on November 19, 1666, in the Philosophical Transactions of the Royal Society Transactions in a short notation titled, "The Success of the Experiment of Transfusing the Blood of One Animal into Another." [7,8]

In 1818, James Blundell, a British obstetrician conducted the first successful human blood transfusion for the treatment of a postpartum hemorrhage **(Figure No.1).** Because of extensive experimental transfusion studies in both animals and humans, he is widely accepted as "Father of Blood Transfusion". [7,8]

**Figure No.1** [9]**: James Blundell's Gravitator from 'Observations in Transfusion of Blood'**

In 1869, Braxton-Hicks, using anticoagulated blood with phosphate solutions, performed a number of transfusions in women with obstetric bleeding. From 1873 to 1880, cow, goat, and even human milk were transfused as a blood substitute. Sodium citrate was widely considered to be the most effective and non-toxic anticoagulant.[10]

The 20th century was ushered by a truly monumental discovery-

In 1901, Karl Landsteiner, observed that sera of some persons agglutinated the red blood cells of others following which he discovered the first three human blood groups A, B, and C (subsequently group C renamed group O) and subsequently group AB discovered by Decastello and Sturli. [8]

In 1907, using blood typing and cross matching Reuben Ottenberg performed the first blood transfusion.[8]

During the World War I (1914-1918), Rous and Turner developed the anticoagulant solution and stored the blood for use.[9]

The phrase "Blood Bank" was first coined by Bernard Fantus for the operation because blood could be stored and saved for future use. He as the Director and Oswald Robertson as an advisor established the first blood bank at Cook County Hospital in Chicago in 1937. [9]

In 1939-1940, The Rh blood group system was discovered by Karl Landsteiner, Alexander Wiener, Philip Levine and R.E. Stetson. [9] In 1943, the anticoagulant preservative Acid-Citrate-Dextrose (ACD) was developed in Great Britain by Loutit and Mollison which extended the shelf life of whole blood to 21 days. It could be autoclaved and had the advantage of being easier to prepare, while requiring a smaller volume of solution relative to the amount of blood. Gibson group in 1950 used Citrate phosphate dextrose (CPD) solution as blood could be stored for up to 28 days with better red cell survival than ACD. [9]

By 1960s, preservative solution containing Adenine were shown to greatly extend shelf life of stored refrigerated blood as compared to ACD or CPD alone. The Food and Drug Administration in 1978 approved the addition of Adenine to CPD to create CPDA-1, which increased the shelf life of blood to 35 days.[11]

By 1983, the Food and Drug Administration (FDA) approved additive solutions containing saline, adenine and dextrose for RBC's, extending shelf life of this component to 42 days. [11]

## BLOOD DONATION PROCESS

The blood donation process mainly involves four basic stages:

1. Donor Registration
2. Medical history questionnaire and physical examination
3. Collection of blood
4. Recovery stage

**Donor registration**

During this stage blood bank staff documents about the basic information of donor like name, age, sex, address, and contact number. Educational material is given to donor in his/her local language and general idea about whole process of blood donation is given to the donor. Donor is also asked to show valid identification document to confirm his/her identity.[7]

**Questionnaire and physical examination**

The health history questionnaire helps determine donor eligibility and requires donor to reveal private health information as well as places they have travelled recently. The questionnaire also helps to determine the level of knowledge and attitude of donor towards blood donation process. This is also done to ensure the integrity and safety of the blood being donated. [7]

Physical examination includes measurement of temperature, pulse, blood pressure, pallor, clubbing, cyanosis and also for the multipuncture sites on forearm. [7]

**Collection of blood**

The donor is asked to lie down in a supine position. The skin at the puncture site is prepared using antibacterial scrub. This process minimizes the risk of bacterial contamination of blood. Whole blood is collected into sterilized bag sets containing anticoagulant and attached satellite bags to facilitate component separation. Phlebotomy is performed and typically 350ml of blood is collected for whole blood while 450ml is taken for component preparation. Another method of blood collection is the Apheresis technique, wherein after drawing whole blood, required component is taken and other components are returned back. The most common use of this technique is in collection of platelets known as “Single donor platelets”. However,

this method can also be used for collection of other components like plasma, red blood cells and leukocytes.

**Recovery stage**

This stage involves a recovery period of about 10-15 minutes. This time allows blood bank staff to observe the donor for any physical reaction or complication as a result of donation process. This recovery stage also allows the donor to receive refreshments in order to rehydrate their bodies due to fluid loss in the form of fruit juices. Blood bank staff can clear donors' misconceptions and doubts about the process of blood donation and can also get to know about the experience of the blood donation during this time. [7] This time also allows donor to understand the whole process of blood donation in a better way, which makes them more comfortable knowing what to expect, possibly positively increasing donor attitudes and future donating behaviors.

**Types of Donors** [12]

There are mainly three types of blood donors, namely:

- Voluntary (non-remunerated) donors- These are the donors who donate blood willingly, without any pressure or monetary benefit. Such type of donation is encouraged by a blood transfusion service as these donors belong to low risk category and are willingly participate in blood donation regularly. These donors also respond to appeals during emergencies.

- Replacement or relative donors- These donors are family members or relatives of the patient in need of blood, who donate their blood as replacement for the actual blood needed for the patient.

- Professional or commercial paid donors- These donors derive direct or indirect monetary benefit for the blood they donate. This type of donation is not encouraged and is prohibited by law in many countries including India.

## PRE-TRANSFUSION TESTING

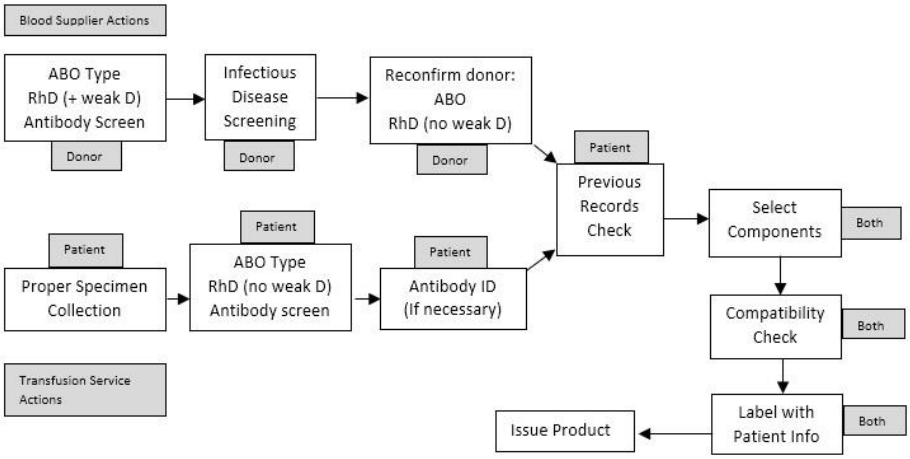
Samples for pre-transfusion testing should be collected no more than 3 days before transfusion. A unique number should be assigned to the pre-transfusion specimen and it should be affixed to an identification form that the patient must provide at the time of transfusion. Pre-transfusion samples must be retained until at least 7 days after each transfusion. [13]

Routine pre-transfusion testing includes ABO and Rh(D) typing, and screening for unexpected red cell antibodies. Antibody identification tests should be performed when the antibody screen is positive. Previous records, if available, must be cross checked with the results of current testing. Before the pre-transfusion testing can be concluded, any discrepancy in current testing with that of previous records must be resolved. [13]

The final step of pretransfusion compatibility testing is the cross match. Cross match is the final check of ABO compatibility and to a lesser extent detection of unidentified antibodies. A major cross match is between the recipient's serum or plasma and donors red cells. A minor cross match is done between recipient red cells and donor serum or plasma, which is usually not required. In the absence of unexpected red cell antibodies, a cross match can be performed by direct agglutination (immediate spin) for detection of ABO incompatibility. When unexpected red cell antibodies are present, the cross match should be performed by antiglobulin technique. [13,14]

This is explained in **(Figure 2).**

**Fig. 2: Diagram showing pre-transfusion testing algorithm.** [14]




## PRE-TRANSFUSION SCREENING FOR TRANSFUSION TRANSMISSIBLE DISEASES

Improvements in donor screening and testing have resulted in dramatic reductions in transfusion-transmitted disease risks in the past two decades. With each and every unit of blood there is risk of TTIs which includes Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Syphilis, Malaria and many more infections.[15]

Screening of donors first started in 1947. According to NACO guidelines screening for HIV, HBV, HCV, syphilis and malaria are mandatory tests. Indian Government has made mandatory to screen donated blood for HBV (since 1971), HIV (since 1989) and HCV (since 2001). [16]

Following are the markers:

- HIV-1 and HIV-2: screening for HIV antibodies.
- Hepatitis B: screening for hepatitis B surface antigen (HBsAg)
- Hepatitis C: screening for HCV antibodies
- Syphilis (Treponema pallidum): screening for specific treponemal antibodies.
- Malaria: screening for malarial parasite antigen.

## TRANSFUSION TRANSMISSIBLE INFECTIONS [17]

The microbial agents that are transmissible by blood transfusion the transfusion, are of prime importance to the blood transfusion services. These infectious agents, in order to be transmissible by blood, typically show the following characteristics:

- They are present in the blood for long periods, occasionally in high titres
- Show stability in blood stored at 4°C or lower
- They usually have long incubation period
- Asymptomatic or only mild symptoms in the blood donor, therefore not identifiable

  during the donor selection process.

After infection, several markers of infection appear at different times. Each TTI depending upon the infectious agent, has window period ranging from few days to months. During this period, the donor may be infectious but the particular screening marker is not yet detectable. First target to appear is the nucleic acid of native infectious agent, followed by antigen in few days, and subsequently by antibody as the immune response develops.

## HEPATITS B (HBV)

Hepatitis B is a life-threatening infection caused by the hepatitis B virus. It is one of the most serious type of viral hepatitis which constitutes a major global health problem. Many of these individuals progress to chronic liver disease resulting in high risk of death from cirrhosis of the liver and liver cancer.[18]

### Historical aspect of HBV

Hepatitis B epidemics have been seen throughout the history of mankind, dating back to antiquity and Hippocratic era. During World War II, first hint that hepatitis could be caused by blood transfusion came following the administration of yellow fever vaccine. Hepatitis B virus, the infectious agent responsible for these hepatitis outbreaks, identified by Dr. Baruch Blumberg while working at the NIH in the 1960s—a discovery that later earned him the Nobel Prize in Physiology or Medicine in 1976.[19, 9]

In the 1970s and 1980s, research on the natural history of HBV infection led to the preparation of the first hepatitis B vaccines based on heat-inactivated and blood plasma-derived viruses.[19]

### Epidemiology

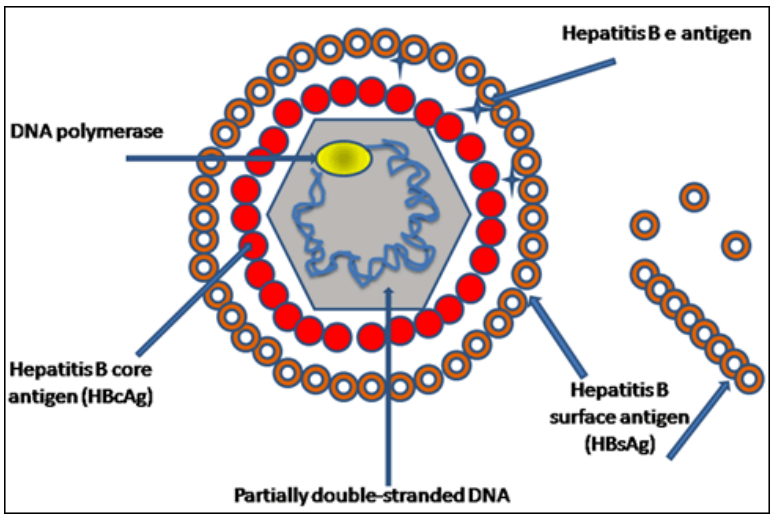
**Worldwide -** Hepatitis B continuous to remain a significant health problem all over the world. It is estimated by CDC that 257 million people are living with hepatitis B worldwide. Out of these only 1 in 10 had received a diagnosis and was aware of their infection. About 900,000 people die every year due to the acute or chronic consequences of hepatitis B. It is common in sub-Saharan Africa, Asia and the Pacific Islands, but also has increased rates in south America, Eastern and Central Europe and the Indian subcontinent.[20]

**India** - According to its endemicity and prevalence different areas of world have been divided into three groups, high (≥8%), intermediate (2-7%) and low (<2%). Among which India falls in the intermediate endemicity zone (prevalence of 2–7%, with an average of 4%), with a disease burden of about 50 million. Pockets of higher endemicity are seen in tribal areas where the high burden is maintained through illiteracy, intracaste marriages, tribal customs, and poor exposure to health care resources.[21]

**Hepatitis B Virus (HBV) –**

HBV is an enveloped DNA virus of Hepadanaviridae Family. The viral genome is partially double-stranded circular DNA with a short, single stranded piece of about 3.2 kilobase (kb) pairs, making it the smallest DNA virus known. The spheres and filaments are 22-nm particles composed of hepatitis B surface antigen (HBsAg) or protein coat. The infectious HBV virion of 42-nm diameter called "Dane particle" consisting of a lipid envelope containing HBsAg with a nucleocapsid core of 27-nm having hepatitis B core antigen (HBcAg) and the hepatitis B e antigen (HBeAg). [22]

**Figure No 3:- Structure of HBV virion** [23]



**Genotypes** [24]**-**

HBV genotypes represent naturally occurring strains and reflect the geographical distribution throughout the world. Up to now, eight different genotypes have been identified in different areas of the world.

Genotype A is mainly found in Northwestern Europe, North America and Africa, whereas genotypes B and C have been seen in South-Eastern Asian populations. Genotype E and F are seen in East Africa and the New World respectively. Genotype D is most often found in Africa, southern Europe, Middle East, India and parts of Central Asia. Genotype G is a recently determined genotype in France, America, and Germany while genotype H has been reported in patients from Central America.

**Transmission** [18] **-**

Transmitted through exposure to various body fluids containing the virus, with a high level of concentration in blood, serum and wound exudates, at moderate level in semen, saliva and vaginal fluids, and at low level in urine, feces, sweat, tears and breast milk. Virus remains infectious for up to 1 week on environmental surfaces. Transmission may occur by various routes like sexual, parenteral, or perinatal. Percutaneous transmission may occur through needle stick (drug abuse, acupuncture, occupational hazard or tattooing), hemodialysis and transfusion of unscreened blood or blood products.

**Serological tests** [17,18]**–**

Several antigens and proteins are present on HBV to which body can make antibodies. HBsAg a surface antigen protein is present on outer envelope of the virus. It can also be found floating free in the plasma. Also, antibodies can be formed to two core proteins hepatitis B core antigen (HBcAg) and hepatitis Be antigen (HBeAg).

Out of all these antigens HBsAg is used to screen the donor blood. It is detectable 2 to 12 weeks postexposure during the acute stage and becomes undetectable after development of anti-HBsAg by 12 to 20 weeks. This can also be used to monitor the stages of HBV from acute, active infection to recovery or a chronic infection.

First marker to appear is HBV DNA and can be detected by polymerase chain reaction testing before HBsAg reaches detectable levels. It reduces the risk of transmission through infected donated blood during the acute window period.

Recommendations as per WHO are: -

1. Highly sensitive and specific screening should be performed using HBsAg immunoassay (EIA)
2. Screening using a highly sensitive and specific HBsAg rapid assay may be performed in laboratories with small throughput or in emergency situations
3. As a routine screening for anti-HBc is not recommended. Based on the prevalence and incidence of HBV infection countries should determine the need for anti-HBc screening.

**Table No 1. Serology of Hepatitis B Virus** [18,9]

| Positive-serological tests | Interpretation | Appearance time |
|---|---|---|
| **1**.Hepatitis-B surface antigen | Indicates **Hepatitis B infection**<br><br>It is detected during acute and chronic Hepatitis B but does not differentiate between them. | Usually the earliest detectable serum marker for acute Hepatitis B infection |
| **2**.Hepatitis B surface antibodies | Indicates **immunity** to Hepatitis B infection or recovered infection or vaccination but does not differentiate between the last two. | It appears after the disappearance of antigen. |
| **3**.Hepatitis B **core** antibodies (IgM and IgG antibodies) | Indicates acute or chronic Hepatitis B infection<br>NOT produced after Hepatitis B immunization | Appears 6-8 weeks after exposure<br>Usually remains positive life-long, but may wane |
| **4**.Hepatitis B core **IgM** antibodies | Indicates recent acute Hepatitis B infection or in acute exacerbations of chronic Hepatitis B<br>Usually remains detectable for up to 6 months after acute Hepatitis B | Appears 4-8 weeks after exposure and remains in blood up to 36 weeks |
| **5**.Hepatitis B e antigen | Indicates **high infectivity or active replication of virus.** | Appears simultaneously with HBsAg. But may be negative in in mutant form of Hepatitis B- further tests, such as **HBV DNA**, may need to be performed to show high level on infectivity |
| **6**.Hepatitis B e antibody | Clinically of limited use. Indicates chronic infection | Appear after the disappearance of HBeAg. Level may become undetectable over time |

## Hepatitis C (HCV)

**Historical aspect** [25] **–**

Recognition of this agent was made around 60 years ago as a result of clinical picture different from hepatitis A and hepatitis B but ability to screen and detect was possible when it was discovered by Michael Houghton and colleagues in 1989 and henceforth, known to be the cause of post transfusion non-A, non-B hepatitis. Now it is considered to be the most frequent cause for chronic hepatitis, cirrhosis, and HCC.

**Hepatitis C Virus**

HCV is a small 40-60 nm, lipid enveloped, single stranded RNA virus and belongs to Flaviviridae virus family. [18]

The genome of HCV is thought to encode at least ten different proteins including 3 structural (core, $E_1$, $E_2$), six nonstructural proteins ($NS_2$, $NS_3$, $NS_4A$, $NS_4B$, $NS_5A$, $NS_5B$) and a small protein p7 whose function has not yet been defined. It also has a core protein. Nonstructural proteins produce proteases, helicases, NTPase, RNA-dependent RNA polymerase. The envelope proteins ($E_1$, E2) are likely to form the principal target of antibody mediated neutralization of virus infectivity.[26]

Hepatitis C has six distinct genotypes with multiple subtypes in each genotype class. Genotypes are based on the genetic material in RNA strands of virus. Globally, genotype 1 being the most prevalent (46%), followed by genotype 3 in 22%, G2 (13%) and G4 (13%). [27]

**Epidemiology**

**Globally-** According to WHO 71 million individuals are living with chronic HCV infection globally accounting for 1% of population. The global prevalence of HCV is estimated to be up to 3% by WHO. Approximately 399000 people die each year

mostly from cirrhosis and hepatocellular carcinoma. The highest prevalence is reported in the Eastern Mediterranean region (2.3%) and European region (1.5%). [6,28]

**India** has an estimated prevalence of 15 million HCV positive people which ranges from 0.5 to 1.5%. In various studies genotype 1 found to be the most prevalent genotype in south India whereas genotype 1 predominant in northern and western India.[29]

**Transmission –**

HCV is a bloodborne virus with an incubation period of 2 weeks to 6 months. Most commonly it is transmitted through transfusion of unscreened blood and injecting IV drug use. Other different routes of transmission are needle stick injury, hemodialysis, transplant or transfusion, human bite, tattooing or piercing and sexual route. [28,18]

**Transfusion of blood products:** Transfusion of blood and blood products from unscreened donors is considered the most important type of transmission. However, a significant reduction in HCV transmission is observed after screening for HCV antibody was introduced.

After entering into the blood stream, the virus goes to the liver and there it replicates in hepatocytes, giving same picture as that of HBV. About 15 % of infected individual will show the resolution of acute hepatitis C while 85% will develop chronic infection leading to cirrhosis and hepatocellular carcinoma. [18]

**Serological tests** [17]**–**

The following screening targets are employed to identify the presence of HCV

- Serological markers:
  - HCV antibody
  - HCV antigen
- Viral nucleic acid: HCV RNA

HCV antibody is detectable after 30 to 60 days of infection while viral antigen appears normally between 0 to 20 days. For blood screening programmes anti- HCV has been used until recently however, HCV antigen can be detected early than antibody. To improve the overall effectiveness of serological HCV screening both antigens only and combined antigen- antibody are recommended.

HCV RNA is detectable within few weeks of infection and persists for 6-8 weeks. It further reduces the risk of HCV transmission by reducing the window period when results of HCV antigen- antibody assays are negative but HCV RNA is positive.

Recommendations as per WHO are: -

1) Highly sensitive and specific HCV antibody immunoassay or a combination HCV antigen-antibody immunoassay should be performed as a screening tool.
2) Also, highly sensitive and specific HCV antibody rapid assay may be performed where there is small throughput, emergency situation or in remote areas.

## Human Immunodeficiency virus (HIV)

**World History** [30]

- **1981 –** Internationally it was named and diagnosed as AIDS.
- **1984 –** HIV was identified as the cause of AIDS.
- **1985 -** FDA approved first enzyme linked immunosorbent assay (ELISA) test kit to screen for HIV antibodies.
- **1987 –** FDA approved the first drug AZT for the treatment of AIDS, and approved the first Western blot blood test kit- a more specific test.

**Historical Aspect of India** [31]

First case of HIV in India was diagnosed among sex workers in Chennai in 1986. Initial cases had occurred through heterosexual sex, but at the end of the 1980s, a rapid spread was observed among injecting drug users in Manipur, Mizoram and Nagaland. In 1987, a National AIDS Control Programme was launched and covered surveillance, blood screening and health education. In 1992, NACO (National AIDS Control Organization) was launched to oversee the formulation of policies and control programs related to HIV and AIDS.

**Epidemiology-**

**Worldwide** [32] **-** Since the commencement of epidemics, more than 70 million people have been infected with HIV virus and about 35 million people have died of HIV. Currently by the end of 2017, 36.7 million people are living with HIV. Most endemic part of the world which is most severely affected is Sub-Saharan Africa. Estimate of newly detected HIV infections is 1.8 million which has declined from 2.1 million new infection in the last years.

**Fig No 4.** [33] **Shows the global statistics of HIV in different region**

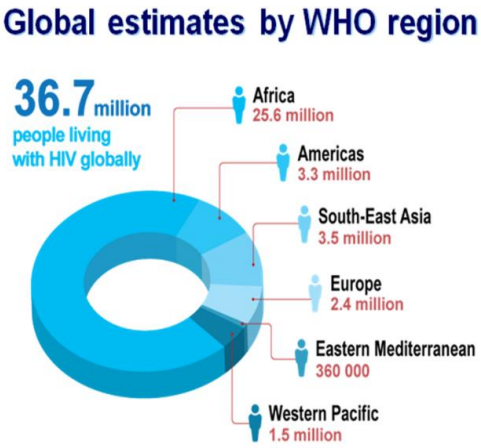



**Table No 2 : Global Epidemiology of HIV/AIDS** [32]

| | **Estimate** | **Range** |
|---|---|---|
| People living with HIV/AIDS by end of 2017 | 36.7 million | 31.1 - 43.9 million |
| Proportion of adults living with HIV/AIDS | 35.1 million | 29.6- 41.7 million |
| Proportion of children (<15 years) living with HIV/AIDS | 1.6 million | 1.3-2.4 million |
| People newly infected with HIV | 1.8 million | 1.4-2.4 million |
| AIDS deaths in 2017 | 94000 | 670000-1.3 million |

**Magnitude of HIV/AIDS in India** [34]

In world India has the third largest HIV epidemic, due to its large population size. The prevalence varies in different parts of the country and is concentrated among sex workers and homosexual men. Overall HIV prevalence currently is estimated to be 0.3%. There are 2.1 million people currently living with HIV by end of 2016 and with a death rate of 62,000 per year. HIV epidemic is now slowing down in India, with a 32% decline in new HIV infections (80,000 in 2016) and 54% decline in AIDS-

related deaths between 2007-2015. There was found to be significant decline of about 50% in new infections of HIV in states of Andhra Pradesh& Telangana, Karnataka, Maharashtra, Manipur and Odisha during 2007-2015.

**Fig no 5. Shows new HIV infections trends in India from (1998-2015).** [35]

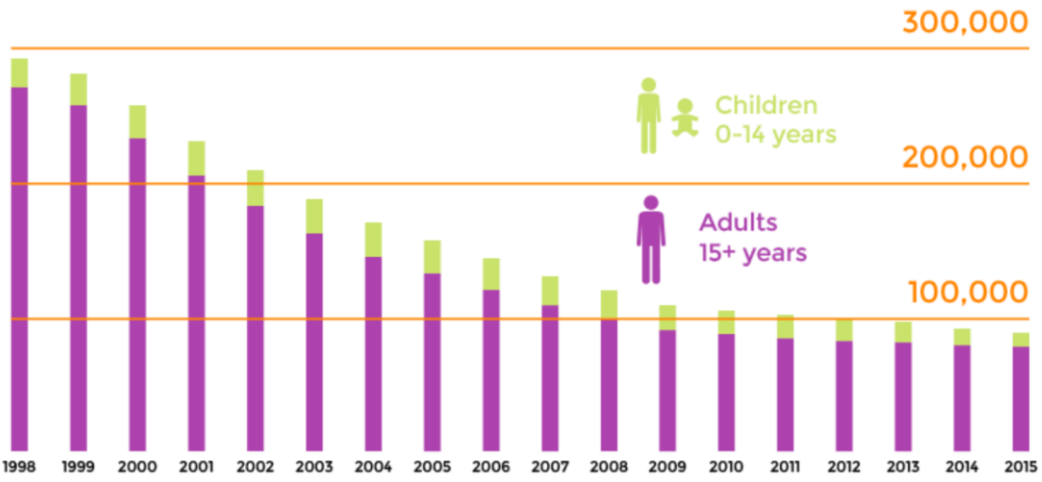
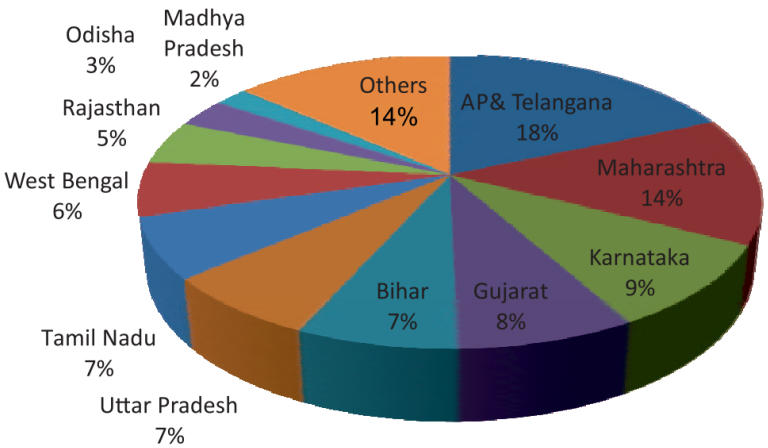



**Fig No 6. Shows prevalence of people living with HIV in India (2015).** [35]

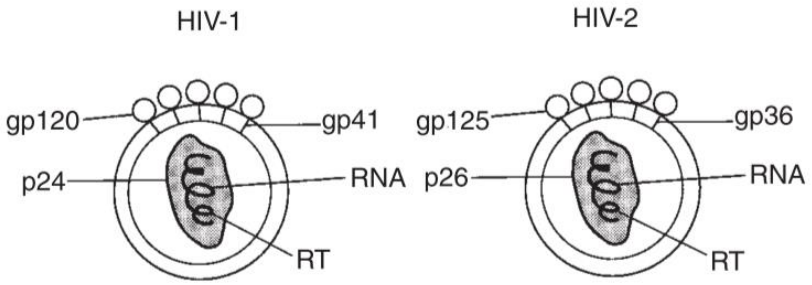


**Human immunodeficiency virus (HIV)** [10,17]

HIV is well recognized etiologic agent of AIDS. It belongs to the genus lentivirus of family retroviridae, so called because virus possess a reverse transcriptase (RT) enzyme to convert the viral RNA into DNA, which integrates in the cellular DNA to cause persistent infection. It also has envelope of glycoproteins and core proteins. This virus has more predilection for lymphocytes where it replicates.

Various groups and subtypes have been identified with significant difference in their antigen. The two major distinct virus types are HIV1 and HIV2 with similar structure but varying primarily in their envelope proteins **(fig 7).** In most of the parts in the world HIV1 is now endemic, although in some regions it has low incidence and prevalence. The prevalence of HIV2 is mainly restricted to countries like India and West Africa.

**Fig No.7.** [18]




HIV enters the cell by binding of the virus glycoprotein 120 with surface receptors on CD4+ lymphocytes, macrophages, and another antigen presenting cells. CD4 count decreases as the viral load increases and when it is less than 200 µl, the patient is said to have clinical AIDS.

**Transmission**[36,9] **–**

**A. Sexual Transmission**

Sexual route especially the heterosexual is the main driver of the epidemic in most of India, accounting for nearly 90% of the prevalence.

**B. Transmission by Blood and Blood products**

Blood transfusion is second recognized route of transmission after sexual route. High risk individuals are injecting drug users (IDUs), recipients of blood transfusions and hemophiliacs. HIV is stable at the temperatures at which blood and its components are stored. A single contaminated unit of whole blood and its components can transmit HIV infection.

**C. Occupational Exposure**

Exposure of blood to an individual with HIV infected blood as a result of percutaneous injury or contamination of mucous membranes.

**D. Maternal-fetal transmission of HIV**

Maternal-fetal HIV transmission is multifactorial, with increased risk associated with intrapartum events that increase fetal exposure to maternal blood, perinatally, or via breast-feeding.

**Serological tests**[10,37] **–**

The goal of most HIV diagnostic tests is to detect the HIV infection as early as possible, thereby decreasing the window period. The following screening targets are employed to identify the presence of HIV

- Serological markers:
  - anti-HIV 1 and HIV2

- HIV p24 antigen

- Viral nucleic acid: HIV RNA

HIV infectious process has been divided into three recognized stages. Immediately after viral infection there occurs the acute phase, also referred to as the diagnostic window or serological latency. At this stage, host is said to be seronegative as no antibodies can be detected but p24 antigen and viral nucleic acid are detectable in serum of host. HIV p24 antigen appears 10 to 21 days after infection while viral RNA detection can be positive approximately 7 to 11 days after infection. Detection of the latter reduces the window period by 3 to 7 days before antibody detection.

Since the mid-1980s, screening for anti-HIV has been the basis for blood screening. Antibodies detection in the host referred to as seroconversion and it marks the beginning of the second stage known as chronic phase. Antibodies are detected approximately three weeks after infection. Screening with both antibody and antigen is preferred as combinations identifies the vast majority of donations from infected donors. Anti- HIV assays have included specific antigen for both HIV-1 and HIV-2 since the early 1990s.

The final stage of HIV infection manifests as clinical AIDS, is distinguished by onset of clinical symptoms indicative of immunodeficiency, namely, opportunistic microbial infections like herpes, pneumonia and fungal infections. The commencement of clinical AIDS is usually marked by a CD4 T cell count $\leq$ 200 cells/mm3, which continues to decline and an increase in the number of HIV RNA copies in the blood.

Recommendations as per WHO are: -

1. Highly sensitive and specific anti-HIV 1 and anti-HIV 2 immunoassay or a combination HIV antigen- antibody immunoassay should be performed as a screening tool.

2. Also, highly sensitive and specific anti-HIV 1 and anti-HIV 2 rapid assay may be performed where there is small throughput, emergency situation or in remote areas.

# SYPHILIS

Sexually transmitted disease or venereal diseases are the infections caused by organisms that can be transmitted from one person to another through sexual activity. The causative agent of syphilis is a spirochete known as treponema pallidum. [17]

## History

In the preantibiotic era, syphilis plagued every major city. In the late 19th and early 20th centuries, Richard von Krafft-Ebing stated that "Civilization means syphilization," means modern life make men more susceptible to syphilis and other disease. Schauddin and Hoffmanon March 3, 1905 in Berlin demonstrated treponema pallidum under magnification**.**

Many hypotheses for the origin of syphilis were stated out of which the most popular one is the Columbian hypothesis. It states that original treponemal disease spread from Africa through Asia, entering North America when Columbus soldiers got infected and transmitted the disease to the old world after returning from new world in 1493.[38]

## Global epidemiology

Globally each year 6 million new cases of syphilis are estimated in persons aged 15 to 49 years and over 300,000 fetal and neonatal deaths occur annually.(39) The incidence of syphilis among blood donors is variable globally. As per WHO it is estimated to be 25.1 case per 100,000 adult population among the 55 countries with an annual incidence of about 5.6 million.[6]

**Syphilis in India**

The seroprevalence rates of syphilis varies extremely in different subpopulation of India. Various studies have shown prevalence rate ranging from 5.4% to 8.2% amongst STD clinics, 0.84% to 0.98% in antenatal groups and as high as 21.9% in long distance truck drivers.[40] The prevalence rate among Indian blood donors ranges from 0.85% to 3%.[4]

**Morphology of Treponema**

The spirochetes are helically coiled, motile, slender and flexible organisms. They have gram negative type cell wall composed of an outer membrane of peptidoglycan and a cytoplasmic membrane. The most distinguishing morphological property is presence of endoflagella which is polar and wound along the helical protoplasmic cylinder, situated between the two layer of cell wall. Based on the integrity of these endoflagella motility is of three types 1) flexion and extension 2) corkscrew like rotatory 3) translator. Treponema pallidum belongs to family of spirochetaceae and is the causative agent for syphilis.[41]

**Transmission** [3,10] **–**

Major route of transmission is sexual contact but can also be transmitted through blood transfusion. When blood and its components are stored at temperature below 20º C the risk of transmission is very low because of the fragility of treponemes at lower temperature whereas blood components stored at higher temperature above 20º C, such as platelet concentrates or those which are not stored at lower temperatures such as blood collected and used within 48 hours, presents with significantly higher risk of transmission.

It presents in stages; primary stage presents with painless chancre usually about three weeks after the exposure. These lesions may go unnoticed and disappear within 4-6

weeks. Secondary syphilis stage begins 2-8 weeks after the chancre first appears. It presents with constitutional symptoms and skin rashes, if left untreated, syphilis continues into a latent stage. Latent stage last for years, individual is infective during this time but is asymptomatic. About 15% of individuals develop the complications and present with tertiary stage of syphilis in which there is damage to eyes, heart, nervous system, bones and joints.

**Serological tests**[17]

The following screening or diagnostic targets are employed to identify the presence of Syphilis: -

1) Non-specific, non-treponemal assays: antibody to lipoidal antigen
2) Specific treponemal antibodies.

Treponema pallidum hemagglutination assays (TPHA), fluorescent treponemal antibody absorption (FTA-ABS) and enzyme immunoassay are specific assays commonly used for blood screening to detect specific treponemal antibodies. These tests identify donation from anyone who has ever been infected with syphilis, whether recent or past infection and whether treated or not.

Venereal Disease Research Laboratory (VDRL) and rapid plasma regain (RPR) tests are nonspecific assays and identifies those individuals who may have been infected recently. Recommendations as per WHO are: -

1) Highly sensitive and specific TPHA or enzyme immunoassay should be performed as a screening tool.
2) Non treponemal assay using VDRL or RPR should be performed in populations where there is a high incidence of syphilis.

Most recent and confirmatory test PCR which identifies organism by amplifying DNA or RNA sequence. It can be performed on lymph node aspirates, CSF, blood, amniotic fluid and on fixed or unfixed tissue sample.

# MALARIA

Malaria is caused by the protozoan parasite plasmodium. Human malaria is caused by one of the species of protozoan parasite: Plasmodium falciparum, P. vivax, P. malariae and P. ovale. Infection occurs typically with bite of an infected female anopheline mosquito and it is the most common parasitic complication of blood transfusion. It was always of concern in endemic countries but it is also a matter of concern to blood transfusion services in non-endemic countries. This is because of migration of blood donors from endemic to non- endemic areas. [17]

**Epidemiology:**

**Worldwide** [42]

In Tropical and sub-tropical countries of the world, malaria remains the most complex and overwhelming health problem with an estimated prevalence of 216 million cases (196-263 million) and death rate of 3-4 million per year. Most of the cases in the world (about 90%) occur in Africa. Other endemic areas include South East Asia, Eastern Mediterranean, Central and North America, Oceania and South America.

**Malaria in India**

Malaria continues to be one of India's leading public health problems, even a century after its discovery by Sir Ronald Ross in 1897. World Malaria Report suggested that India may not be able to reduce its Malaria burden by half by 2020. Of the total 216 million Malaria cases worldwide, India accounts for the 6 % proportion and 7% of malaria related deaths. In a list of 15 countries which contributed 80 percent of the global Malaria burden India stands third. In 2016, India was the one out of five countries who contributed 85% of estimated vivax Malaria cases.[42]

The rate of transfusion- transmitted malaria is estimated to be 0.25 cases per 1 million blood units. The prevalence of malaria in various parts of India ranges from 0.01-0.09%.[4]

**Transmission:**

**Blood transfusion (Transfusion malaria):**[10, 17]

Other than primarily route of transmission by mosquitoes, it can also be transmitted by blood transfusion through donations collected from asymptomatic, parasitaemic donors. Following an infection with Malaria, the donor may remain infective for years (1-3 years in P. falciparum, 3-4 years in P. vivax, and 15-50 years in P. malariae). During its life cycle, the parasite is released into the bloodstream and will therefor present in donated blood.

The Parasites are stable in whole blood and plasma for at least two and half weeks when stored at 4ºC or for prolonged period in frozen state. Frozen plasma is not known to transmit malaria.

**Mother to the growing fetus (Congenital malaria)[43]:**

Risk of Malaria is higher in pregnant women than nonpregnant women. Parasite infected RBCs enters the placenta. It is more common in primigravidae in their 2$^{nd}$ and 3$^{rd}$ trimesters.

**Needle stick injury:**

Rare route of transmission by drug addicts accidentally who share syringes and needles.

**Serological tests** [17]**-**

There are a number of potential targets for malaria screening and selection of that method depends on whether it is endemic in the country or not.

The following screening methods are employed to identify the presence of Malaria: -

1.Direct detection of parasite by thick and thin film

2. Serological markers:

-Antigen

-Antibody

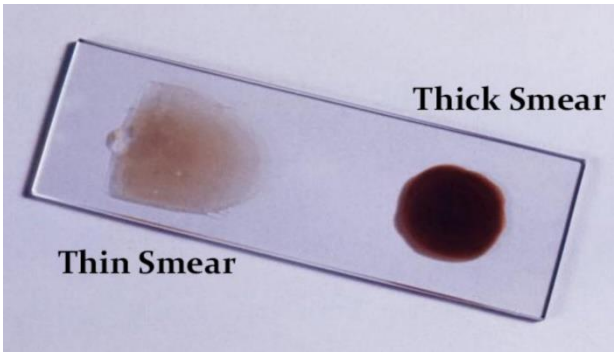
The gold standard test worldwide for the diagnosis of malaria is microscopy that is detection of parasite by thick film but sensitivity of this technique is low and its time consuming, needs high technical skills, rendering its unsuitability for large scale screening in endemic areas.

In malaria microscopy, two kinds of blood film are used: thick and thin film.

**The thick film** - Used to search for malaria parasites. The film consists of many layers of red and white blood cells. It is more sensitive to search for the parasite as large amount of blood can be examined easily and quickly.

**The thin film** - Used to confirm the malaria parasite species. A well-prepared thin film consists of a single layer of red and white blood cells spread over less than half the slide.

**Figure No.8. Freshly prepared Thick and Thin smear slides** [44]



High quality and sensitive antigen detection tests in form of rapid diagnostic tests (RDT) are now readily available.

There are also methods available for malarial antibody screening. Malarial antibody is not indicative of active infection as antibody persists up to several years after infection has been cured, resulting in false positive results. Moreover, antibodies are produced several days after infection thus a negative antibody test make the donor free of malaria.

As per NACO guidelines all blood units should be tested for malarial parasite by using a sensitive antigen test. Recommendations as per WHO are: -

1. All donations should be screened for parasite using thick blood films or for malarial antigen using a highly sensitive enzyme immunoassay.
2. All donors with a previous history of malaria should be temporarily deferred until six months after symptoms have ceased.
3. All donors with an identified exposure risk of malaria should be temporarily deferred for a period of six months from their last return from endemic areas.

## Screening assays

Over the past three decades several types of assay have been developed for use in blood screening. These assays detect antigen, antibodies or nucleic acid of the infectious agent. However, each assay has its limitation and selection of each depends upon the sensitivity, specificity, as well as cost and ease of use. The main types of assay are: -

- Immunoassays:
    - Enzyme immunoassays (EIA)
    - Chemiluminescent immunoassays
    - Hemagglutination /particle agglutination assays
    - Rapid/simple single use assays (rapid tests)
- Nucleic acid amplification technology (NAT) assays.

Amongst these most commonly and widely used ones are enzyme immunoassays that is enzyme linked immunosorbent assay (ELISA) and rapid single use assay test.

## ELISA

ELISA was first established in 1976 by Voller *et al.* It is a heterogenous type of EIA which involves multistep process and sequential addition of reagents.

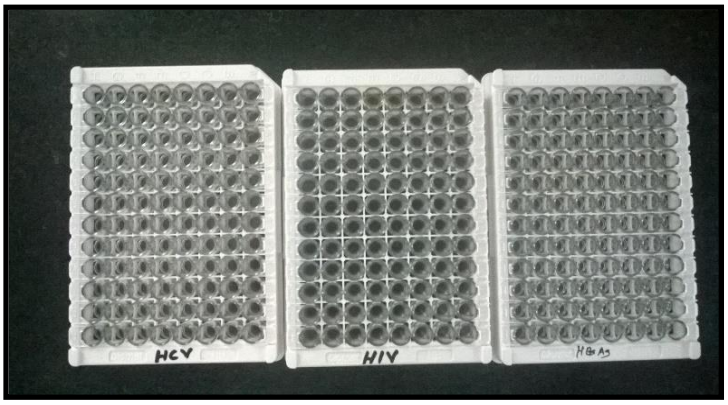
**Principle-** Test is performed using solid phase plated made up of polystyrene containing 96 well having 8 rows,12 columns with a volume of 350 µl each. Antigens or antibodies present in the testing serum sample are captured by the corresponding antigen or antibody coated on to the solid surface wells followed by washing with wash buffer to remove the unbound antigens, antibodies and serum, after this detection system having enzyme conjugated antibody or antigen is added which binds specifically to target antigen or antibody followed by addition of substrate which

detects the color produced on reaction of enzyme labelled antigen or antibody when it binds to its targets.[45]

Color intensity is a measure of concentrations of antigen or antibody present in the sample. Color Reaction is stopped finally by adding blocking reagents and finally measurement of optical density is done with the help of ELISA reader. Various enzyme conjugates used are alkaline phosphatase, horseradish peroxidase and β galactosidase and substrate used are Tetramethylbenzidine (TMB) and Diaminobenzidine (DAB). [45]

**Figure No.9 a), b), c): -**

**Solid phase ELISA plates-**




Total 96 wells having 8 rows and 12 columns.

5 wells are taken for control- Blank -1, Negative control- 3 & Positive control- 1

**Washer System & ELISA Plate Reader**

Done with buffered solution after each step.

**Spectrophotometer**


Measures the optical density using **spectrophotometers** at a specific wavelength.

**Types of ELISA**[45]

Divided into:

- Direct ELISA
- Indirect ELISA
- Sandwich ELISA
- Competitive ELISA

**Direct ELISA-** Enzyme linked primary antibody is added after adding sample of antigen to plate surface followed by addition of substrate.

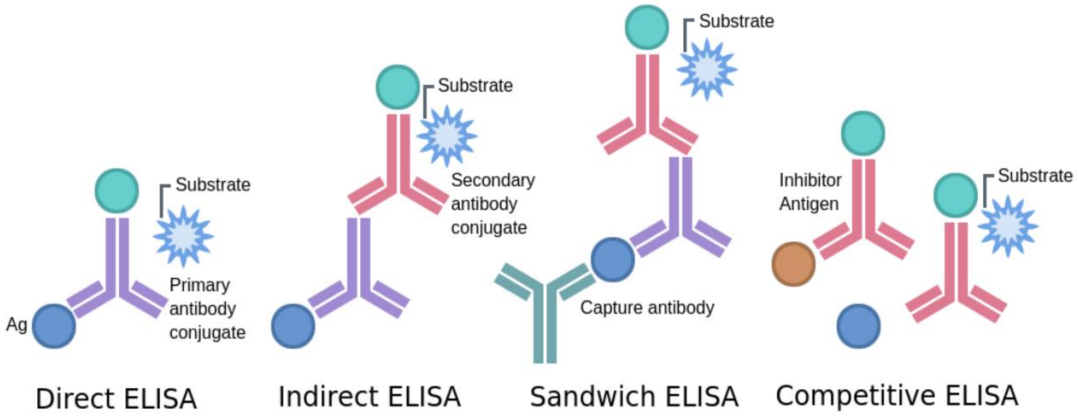
**Indirect ELISA-** Antigen is adsorbed to a well. Primary unlabelled antibody is added which binds to specific antigen followed by enzyme conjugated secondary antibody and substrate. Wide variety of labelled secondary antibodies are available commercially.

**Sandwich ELISA-** Plates are coated with suitable antibody, sample is added so antigen is bounded by capture antibody followed by addition of enzyme conjugate and substrate.

**Competitive ELISA-**Solid phase plate coated with antibody, unknown amount of unlabelled antigen and known amount of labelled antigen is added followed by addition of substrate.

In all types washing with buffer is done after each step of addition of antigen or antibody.

**Figure No.10. Shows different types of ELISA** [46]




**Different generations of ELISA** [47]

Different generations of ELISA were introduced and were improved further in terms of sensitivity and specificity. In first generation whole viral lysates were used as antigens which contained small amounts of host cell components giving rise to false positive reactions. Later, with the improvement 2nd and 3rd and 4th generation kits were developed using recombinant and synthetic peptides as antigens

First generation – In this kit viral lysate as antigen were used that is antigen derived from viruses grown in human lymphocytes. This had so many drawbacks like it detected only antibodies to HIV1 virus, not HIV2. Secondly, it detected only IgG antibody but not the IgM and thirdly, antigen purification was not very good leading to false positive results.

First generation HCV ELISA was introduced using recombinant proteins complementary to the NS4 region of the HCV genome as antigens. This generation is not in use anywhere.

Second generation- This generation incorporated HIV-2 antigen in addition to HIV-1 antigen. Also, antigen purification and production were improved leading to improved false positive rate. Synthetic peptide and recombinant antigen are used as antigen in this technique. For HCV it incorporated non-structural regions NS3 and NS4 resulted in marked improvement in sensitivity and specificity.

Third generation- In addition to 2nd generation, main advantage was detection of IgM and IgA Ab apart from IgG. For HCV it included NS5 region, allowing the detection of anti-HCV antibodies four to six weeks after infection with a sensitivity of more than 99%.

Fourth generation – Same as third generation. It detects simultaneously p24 Ag and HIV-1/HIV-2Ab. With this technique testing window period is even further reduced as p24Ag is produced even earlier than IgM antibody.

**Rapid assays** [48]

Rapid tests are screening tests that can be performed in less than 30 min and do not require any special training. They are preferred in resource poor areas, in emergency situations and for domestic home testing. This is because in resource-poor areas, best guarantee of quality performance is the simplicity of test. In high endemicity areas they can be used as predonation screening which limit blood bag wasting.

Wide range of rapid tests brands are available and have sensitivity and specificity that meets the standards of enzyme immunoassays. For e.g. TRI-DOT HIV (Mitra), Hepacard (Mitra), HCV TRI-DOT.

Their principle is based on three main technical categories: **1) agglutination, 2) Flow through (FT) and lateral flow (LF) assays**. Out of these, first and last are simple to

perform while second needs multiple reagents additions and incubations. **(Figure No.11)**

1)Agglutination- This technique utilizes a particular support (latex microparticles or red cells) on which antigen or antibody are coated for antibody or antigen capture, respectively.

2) FT assays- These assays immobilize the capture reagents on a porous membrane under which an absorbing pad is placed. Plasma or serum is added followed by conjugated anti-immunoglobulin IgG and substrate.

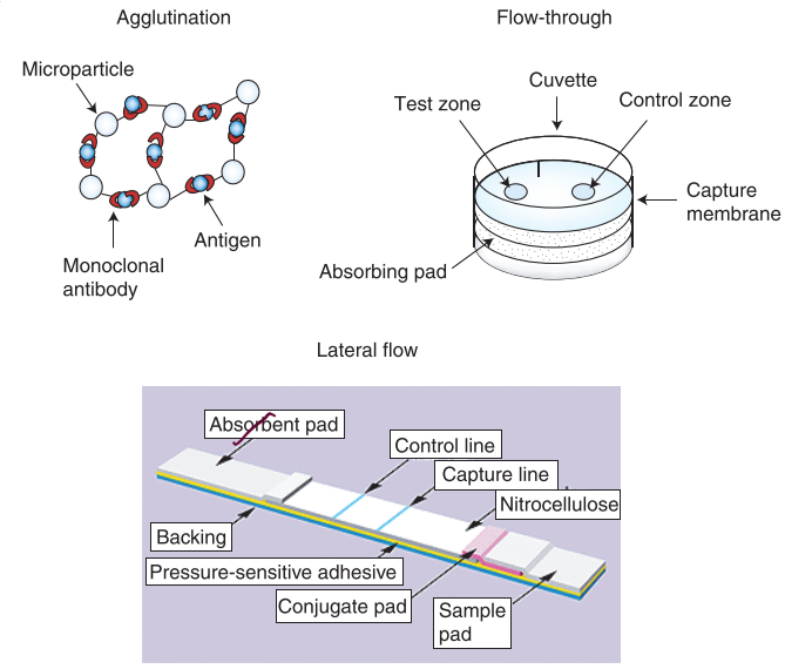
3) LF assays- It is the most common used technical method also called as immunochromatography (lateral flow technology). It involves dipsticks which are dipped in the sample (blood, serum or plasma) or fixed quantities (50-100µl) of sample is deposited at one end of strip. They give qualitative results.

Principle is same as that of sandwich ELISA, only difference is that immunologic reaction is carried out on chromatographic paper by capillary action.

**Method** - Two antibodies/antigen are used

1)One antigen/antibody is immobilized on nitrocellulose membrane which capture the test. 2) Other Ag/Ab labelled with colloidal gold (dye) and infiltrated into sample pad. This also involves an inbuilt procedural control which become visible as a single line when test is performed correctly.

**Figure No.11. Shows the schematic description of the three main types of rapid tests** [48]



# 4. MATERIALS AND METHODS

**Source of Data:**

All the blood donors of 3 years retrospective & 2 years prospective period from BLDE (Deemed to be University) Shri B.M. Patil Medical College, Hospital and Research Centre, Vijayapura who fulfil standard blood donor selection Criteria were included.

**Blood donor selection criteria (As per FDA guidelines) followed at our blood bank**

- Appearance: General healthy look
- Age: 18 to 60 years
- Weight: above 45 kilograms
- Normal vital signs (Pulse rate, blood pressure and respiratory rate)
- Hemoglobin or PCV: > 12.5 gms or >38%
- Minimum of 3 months interval between two consecutive blood donations
- No evidence or history of infections like hepatitis, malaria, HIV/AIDS, tuberculosis, typhoid and sexually transmitted diseases
- No evidence or history of diseases like heart diseases, lung diseases, kidney diseases, diabetes, jaundice, cancer/malignancy, epilepsy and abnormal bleeding tendency
- No history of medication within past 72 hour like antibiotics, aspirin, alcohol, steroids and vaccination.
- No history of any surgery/ blood transfusion in past 6 months.

Apart from above mentioned criteria for female donors there should be no evidence of pregnancy, abortion in the past 3 months, having child less than 1 year old, breast feeding and there should be minimum 3 days gap after menstruation.

**Study Period:**

All the data collected from 1st July 2013- 30th June 2018 (Five years study, three years retrospective and two years prospective) from blood bank of, Shri B.M. Patil Medical College, Hospital and Research Centre Vijayapura.

**Method of Collection of Data:**

All the prospective blood donors were examined for their preliminary health check-up and eligibility criteria for donation of blood. Screening tests were performed on all blood donors' samples. Retrospective blood donor's data was collected from blood bank of BLDE (Deemed to be University), Shri B.M. Patil Medical College, Hospital and Research Centre Vijayapura.

**A. SAMPLE COLLECTION:**

Informed consent was taken. 5ml of blood was collected into plain sterile bottle from donors. Blood samples were centrifuged and the sera separated were analysed by **ELISA 3rd generation (ELISA ERBA chemical kit)** for antibodies to HIV and HCV and for HBsAg. For testing syphilis **ALERE TRUELINE** Rapid TP Card was used. For malarial parasite antigen Rapid card (**ALERE TRUELINE**) test was used.

**B. SAMPLE SIZE:**

Sample size included all donors from the blood bank in 5-Year period from 1st July 2013- 30th June 2018.Total number of blood donors in 5-year study were 20,584 including 3-years retrospective (11,317) and 2- years prospective (9267) period.

## C. INCLUSION CRITERIA:

All blood donors of BLDE (Deemed to be University) University Shri B. M. Patil Medical College Hospital and Research Centre who fulfil standard blood donor selection criteria as per the drug controller of India (drugs and cosmetics act 1999) were included in the study.

## D. EXCLUSION CRITERIA:

Nil.

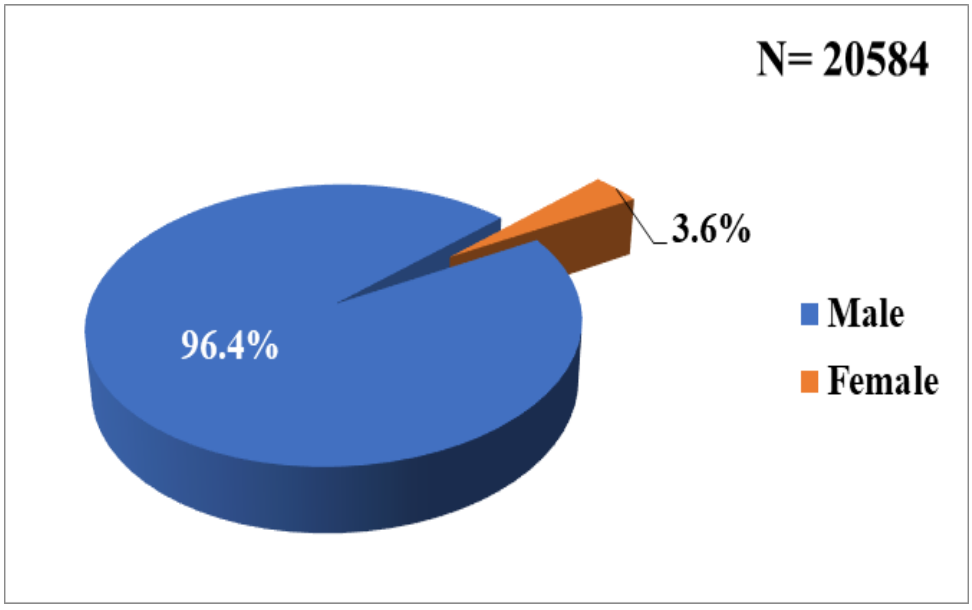
# 5. RESULTS

A total of 20,584 blood units were collected and screened for TTIs (HIV, HBsAg, HCV, Syphilis, and Malaria) during the study period of 5 years.

**Table No.3: Sex-wise distribution of total blood donors**

| Duration | Male | Female | Total |
|---|---|---|---|
| **5 years (July 2013 to June 2018)** | 19847 (96.4 %) | 737 (3.6%) | 20584 (100%) |

**Figure No.12: Pie diagram showing Sex-wise distribution of total blood donors**




Out of total majority were males accounting for 96.4% and remaining 3.6% were females. **(Table No 3, Figure No 12)**

**Table No.4: Shows year-wise distribution of total blood donors**

| Year | No. of donors | Percentage |
|---|---|---|
| July 2013 to June 2014 | 3010 | 14.6 % |
| July 2014 to June 2015 | 3632 | 17.6 % |
| July 2015 to June 2016 | 4675 | 22.7 % |
| July 2016 to June 2017 | 4484 | 21.9 % |
| **July 2017 to June 2018** | **4783** | **23.2 %** |
| **Total** | **20,584** | **100 %** |

**Figure No.13: Bar Diagram showing year-wise distribution of total blood donors**

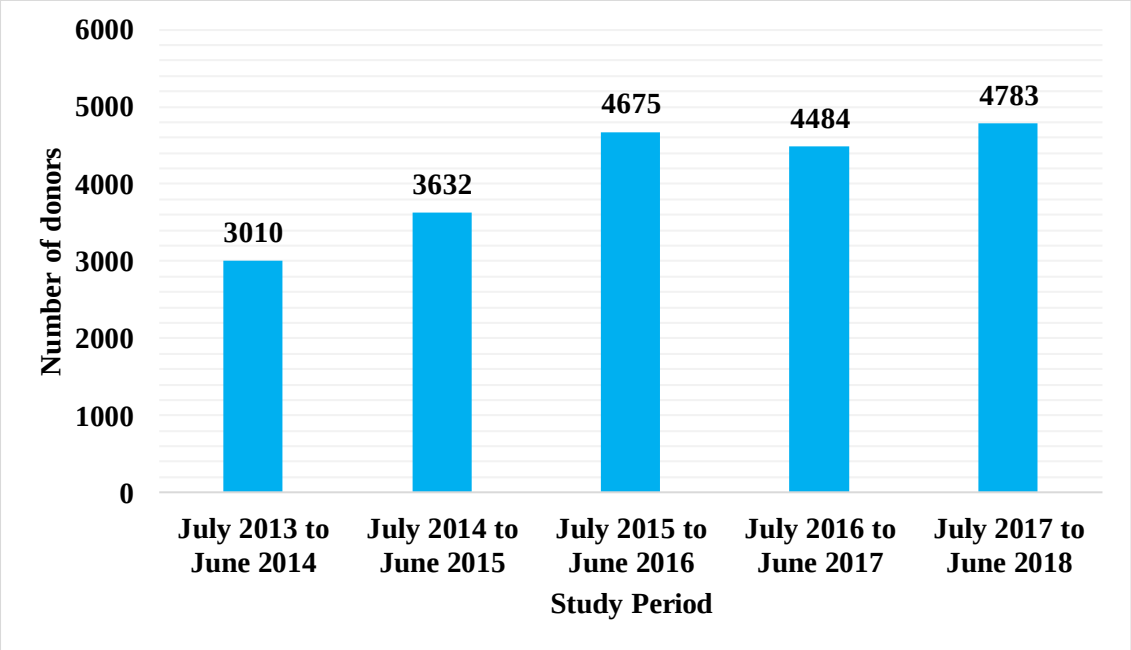



**Table 4 and Figure 13** shows the year-wise distribution of the total blood donors during the 5-year study period. Maximum donation of 4783 blood units were noted in the year 2017-2018.

**Table No.5: Year and sex wise distribution of blood donors**

| Year | Male | Female | Numbers of donors |
|---|---|---|---|
| **July 2013 to June 2014** | 2904 (96.5) | 106 (3.5) | 3010 |
| **July 2014 to June 2015** | 3480 (95.8) | 152 (4.2) | 3632 |
| **July 2015 to June 2016** | 4510 (96.5) | 165 (3.5) | 4675 |
| **July 2016 to June 2017** | 4270 (95.2) | 214 (4.8) | 4484 |
| **July 2017 to June 2018** | 4683 (97.9) | 100 (2.1) | 4783 |
| **Total five- year period** | **19847 (96.4)** | **737 (3.6)** | **20584** |

**Table No.6: Distribution of total voluntary and replacement blood donors**

| Duration | Voluntary | Replacement | Total |
|---|---|---|---|
| **5 years (July 2013 to June 2018)** | 16345(79.4) | 4239(20.6) | 20584 |

**FigureNo.14: Pie chart showing distribution of types of donor.**

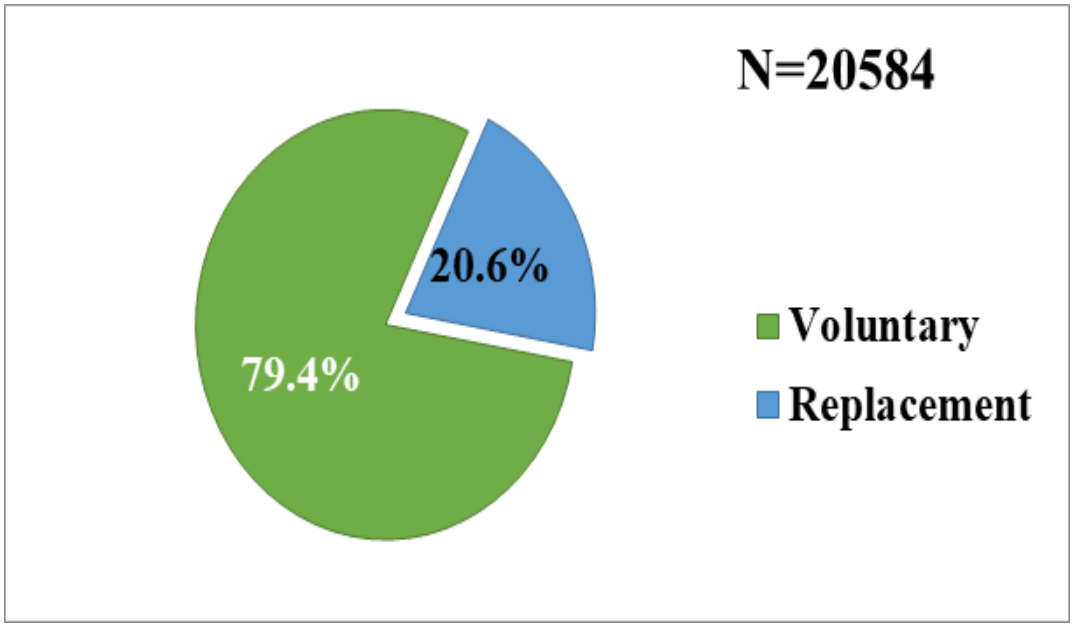



**Table No.6 and Figure No 14** shows the distribution of voluntary and replacement donors in 5 years period. Majority were voluntary donors accounting for 79.4%.

**Table No.7: Year-wise distribution of voluntary and replacement blood donors**

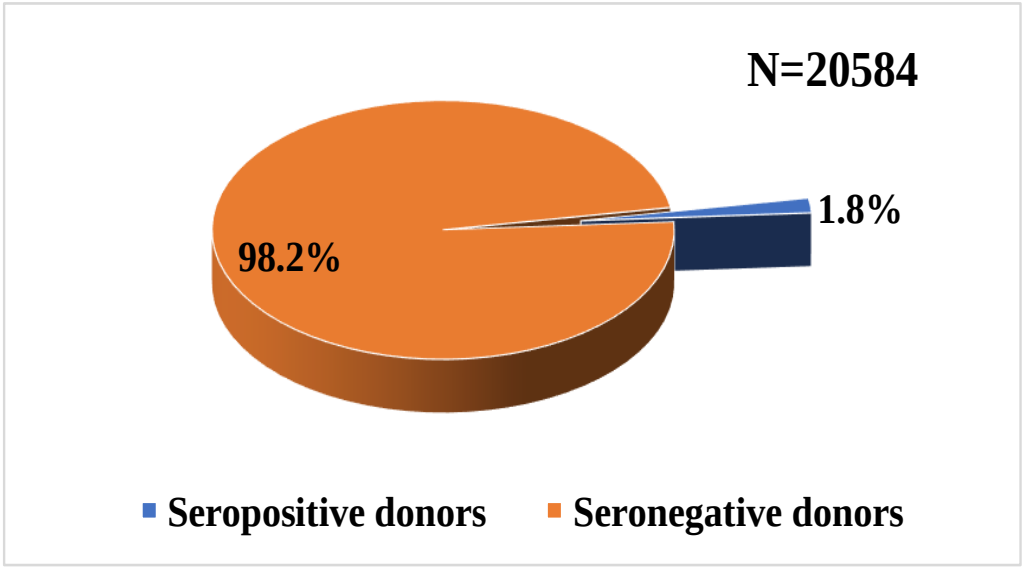
| Year | Voluntary | Replacement | No of donors |
|---|---|---|---|
| **July 2013 to June 2014** | 2287 (14) | 723 (17.1) | 3010 |
| **July 2014 to June 2015** | 2858 (17.4) | 774(18.3) | 3632 |
| **July 2015 to June 2016** | 3388 (20.7) | 1287 (30.4) | 4675 |
| **July 2016 to June 2017** | 3716 (22.7) | 768 (18.1) | 4484 |
| **July 2017 to June 2018** | 4096 (25.1) | 687 (16.2) | 4783 |
| **Total five-year period** | **16345 (79.4)** | **4239 (20.6)** | **20584** |

**Seroprevalence of Transfusion Transmitted Infections:**

**Table No.8: Seroprevalence of TTIs in total donors**

| **Total donors** | **No. of positive donors** | **Seroprevalence (%)** |
|---|---|---|
| **20584** | 369 | 1.8 % |

**Figure No.15: Shows the seroprevalence of seropositive donors.**



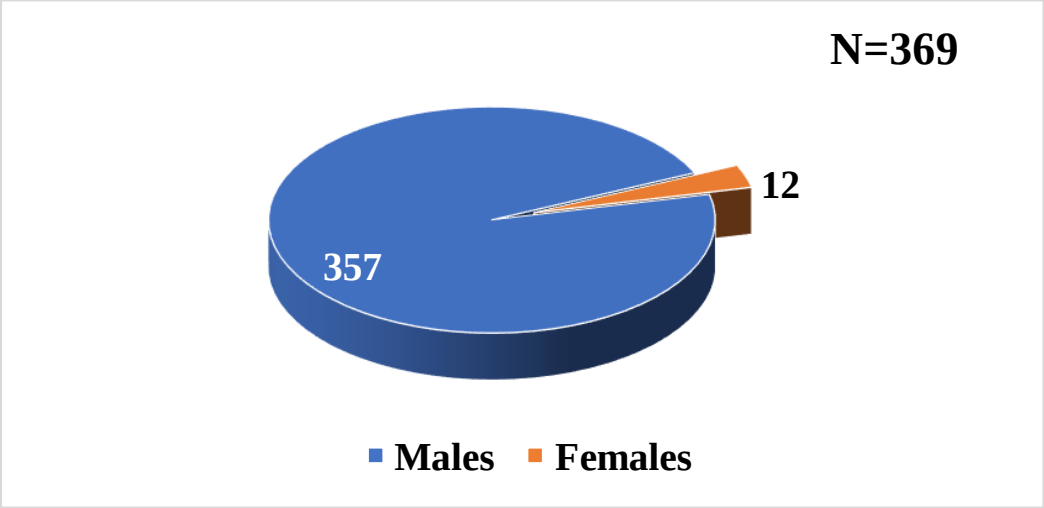

Out of total 20,584 screened blood units 369 units were seropositive for TTIs, giving prevalence rate of 1.8 %. **(Table No. 8 & Figure No. 15)**

**Sex distribution**

**Table No.9: Seroprevalence of TTIs in male and female donors**

| Sex | No. of donors | No. of positives | Seroprevalence |
|---|---|---|---|
| **Males** | 19847 | 357 | 1.8 % |
| **Females** | 737 | 12 | 1.6 % |
| **Total** | **20584** | **369** | **1.8 %** |

**Figure No.16: Shows sex distribution of seropositive donors. (N=369)**



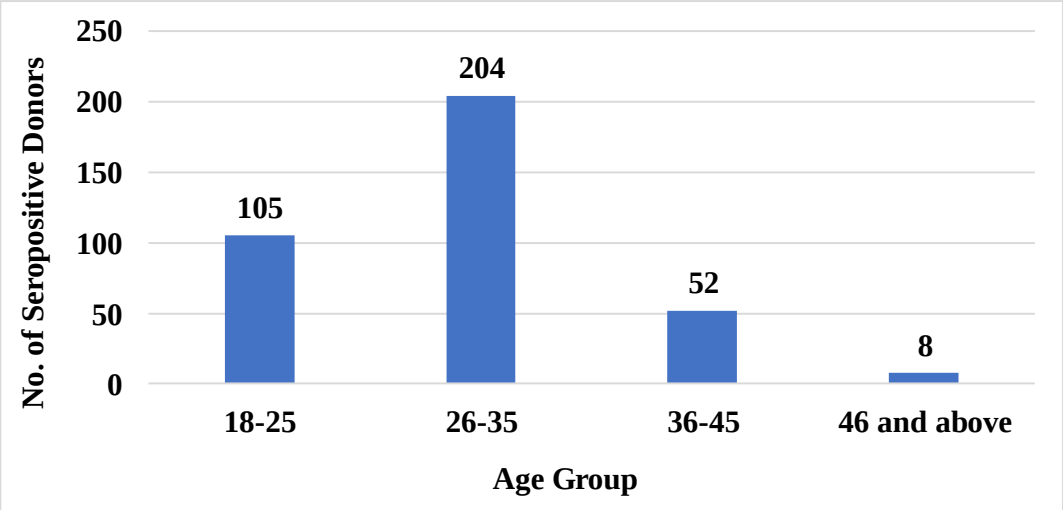

Out of total 357 were males and remaining 12 were females. Seroprevalence in male donors was noted to be 1.8% and in female donors 1.6% out of total males and females **(Table No 9 & Figure No.16).**

**Age-wise distribution**

**Table No.10: Age wise distribution of seropositive donors**

| Age (Years) | Number of donors (%) | Percentage % |
|---|---|---|
| **18-25** | 105 | 28.4 % |
| **26-35** | 204 | 55.3 % |
| **36-45** | 52 | 14.1 % |
| **46 and above** | 8 | 2.2 % |
| **Total** | 369 | 100% |

**Figure No .17: Distribution of Seropositive donors according to Age in 5-year period**



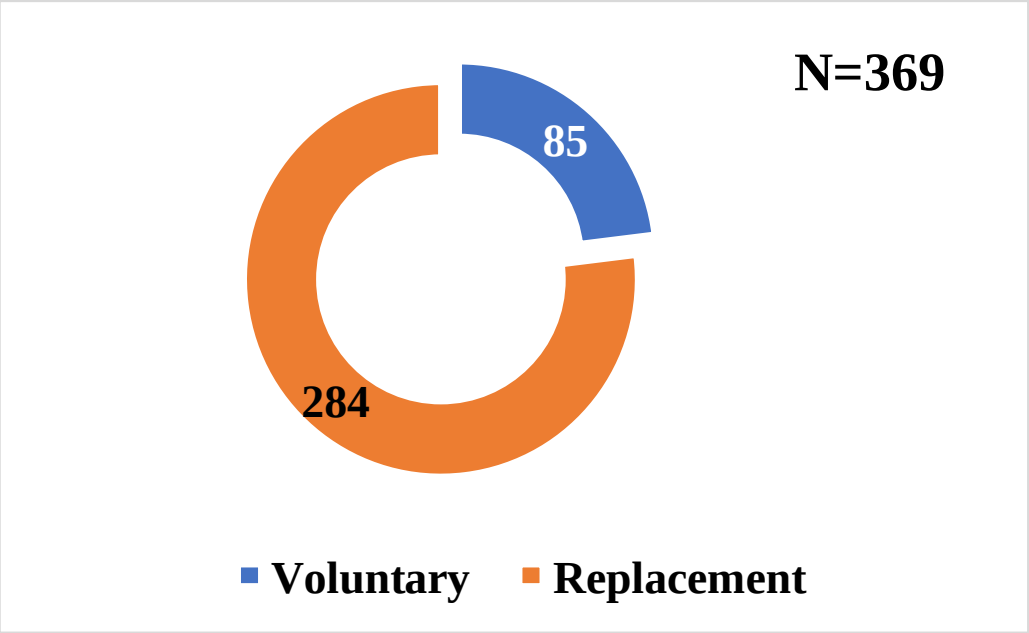

Out of total seropositive donors' majority were in age group of 26-35 accounting for 55.3% followed by age group of 18-25 accounting for 28.4%. **(Table No 10 & Figure No.17).**

**Types of donor**

**Table No.11: Seroprevalence in Voluntary and Replacement donors**

| Type of donor | No. of donors | No. of positives | Seropositivity |
|---|---|---|---|
| **Voluntary** | 16345 | 85 | 0.52% |
| **Replacement** | 4239 | 284 | 6.7 % |
| **Total** | **20584** | **369** | **1.79%** |

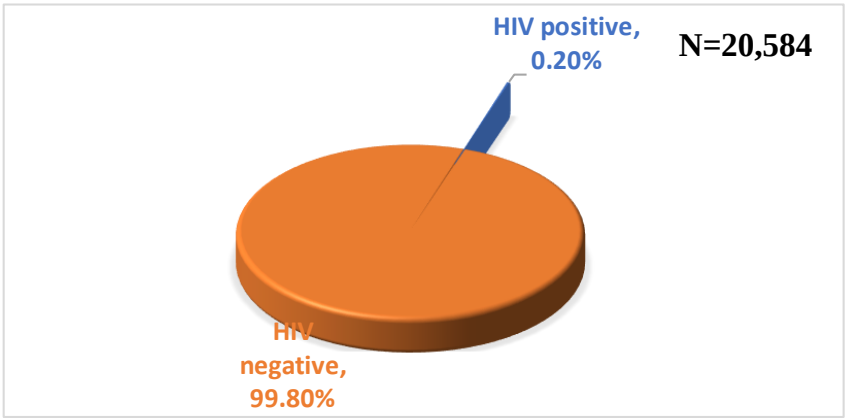
**Figure no.18: Shows distribution of seropositive donors in voluntary & replacement donors**




Out of total 369 seropositive donors 85 were voluntary donors and 284 were replacement donors. Seroprevalence in voluntary donors was noted to be 0.52% as compared to replacement donors 6.7% **(Figure No.18 & Table No.11).**

## SEROPREVALENCE OF HIV AMONG BLOOD DONORS

**Figure No.19: Seroprevalence of HIV amongst total screened donors (N=20,584)**



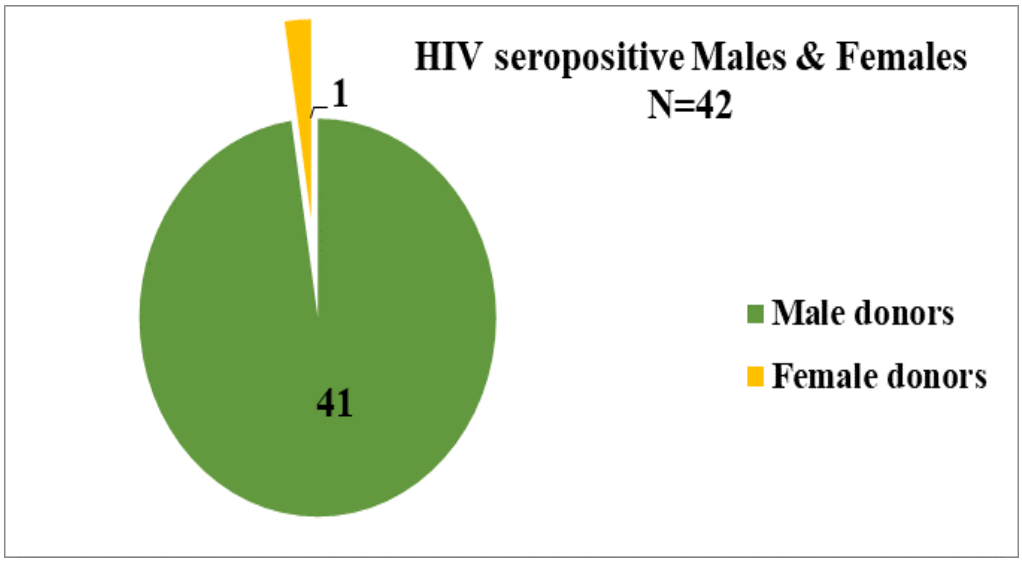

Among 20,584 units of total screened donors, 42 donors were HIV positive, accounting for 0.20% of seroprevalence (**Figure No.19).**

**Sex distribution-**

**Table No.12: Seroprevalence of HIV donors according to Sex.**

| Sex of donor | No. of donors | No. of HIV +ve | Seroprevalence |
|---|---|---|---|
| **Male** | 19847 | 41 | 0.21% |
| **Female** | 737 | 1 | 0.13 % |
| **Total** | **20584** | **42** | **0.20%** |

**Figure No.20: Distribution of HIV seropositive donors according to Sex**



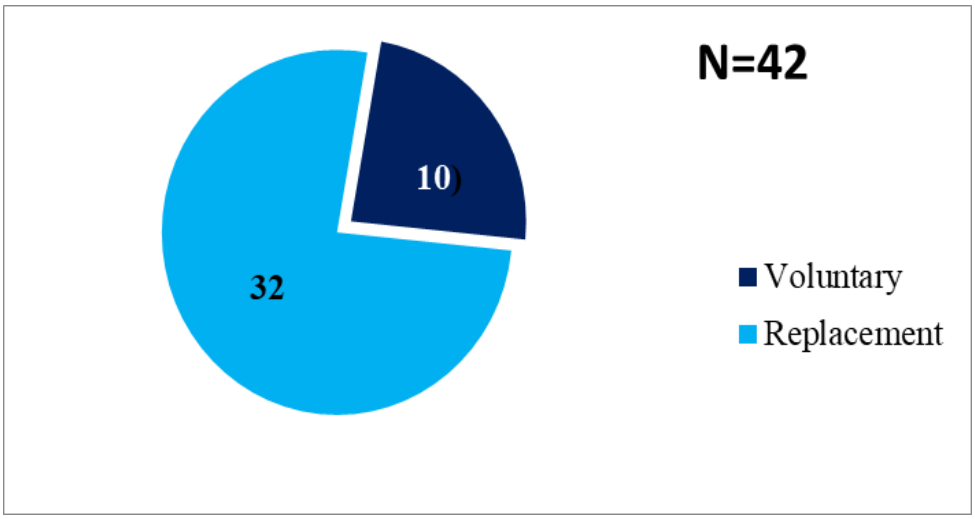

The seroprevalence in male HIV donors accounted for 0.21% and in females 0.13% among total male and female donors. **(Table No.12 & Figure No.20)**

**Types of Donor**

**Table No.13: seroprevalence of HIV among voluntary and replacement blood donors**

| Type of donor | No. of donors | No. of HIV +ve | Seroprevalence |
|---|---|---|---|
| **Voluntary** | 16345 | 10 | 0.06% |
| **Replacement** | 4239 | 32 | 0.75 % |
| **Total** | **20584** | **42** | **0.20%** |

**Figure No.21: Distribution of seropositive HIV donors according to Type of donation**



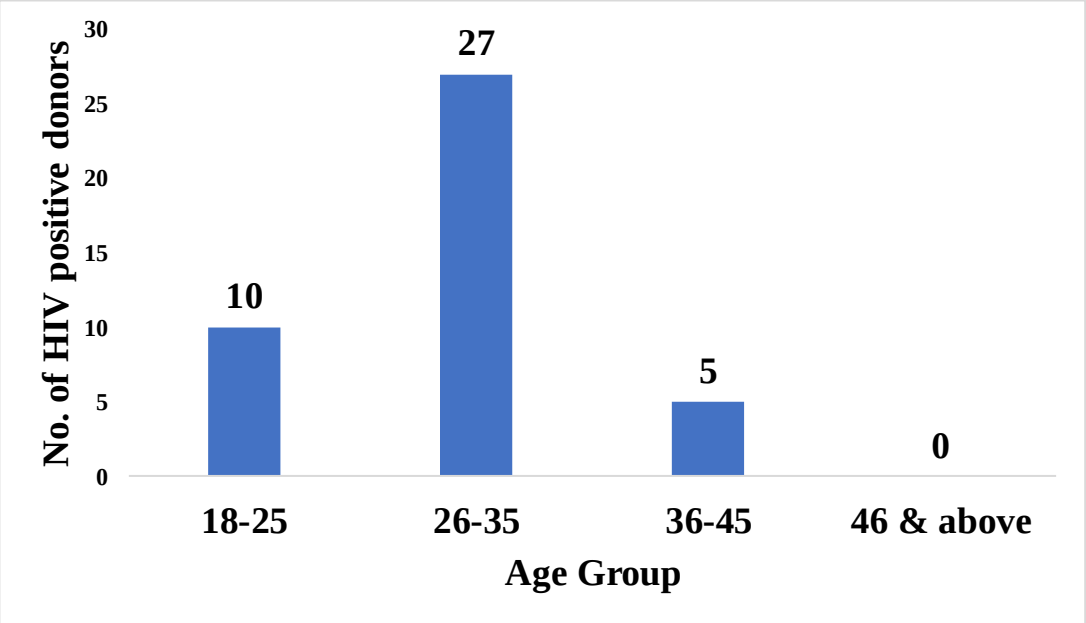

Out of total 42 seropositive HIV donors 32 were replacement donors accounting for majority of all seropositive HIV donors with seroprevalence of 0.75% **(Table No.13 & Figure No.21).**

**Age Distribution:**

**Table No.14: Age – wise distribution of HIV seropositive donors**

| Age | Number of positive donors | Percentage |
|---|---|---|
| **18-25** | 10 | 23.8 % |
| **26-35** | 27 | 64.3% |
| **36-45** | 5 | 11.9% |
| **46 and above** | 0 | 0% |
| **Total** | **42** | **100%** |

**Figure No.22: Age-wise distribution of HIV seropositive donors in 5-Year period.**



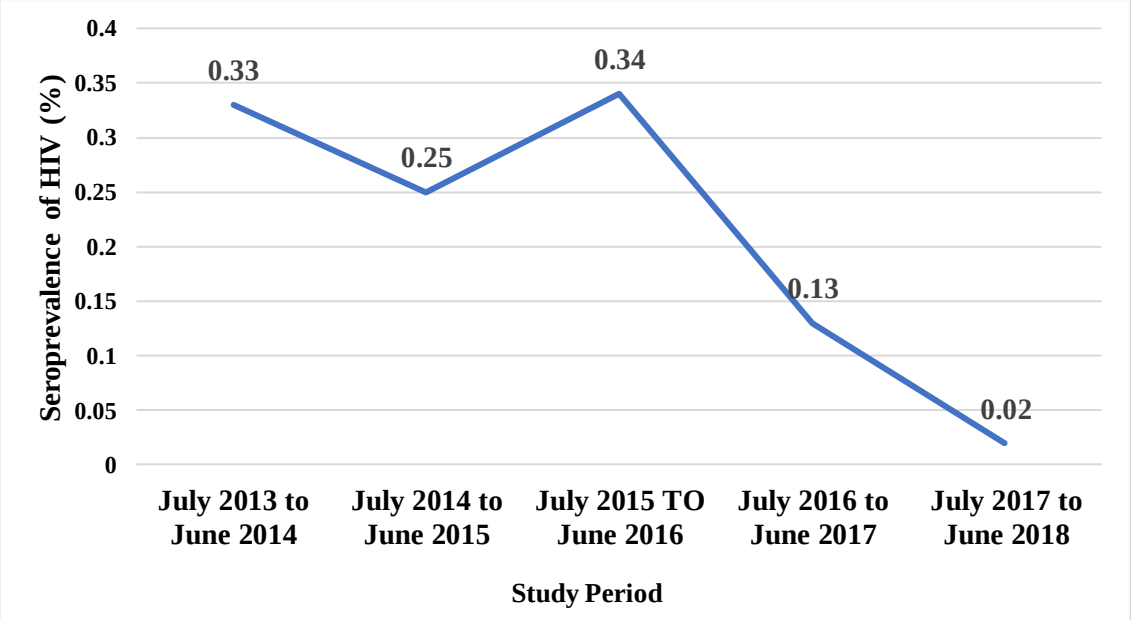

Out of total 42 seropositive HIV donor's majority were in age group of 26-35 years accounting for 64.3 % of all seropositive HIV donors (**Table No.14 & Figure No.22)**

## Yearly distribution and its Seroprevalence Trend-

**Table No.15: Shows the Yearly distribution of HIV seropositive donors**

| Year | Number of HIV seropositive donors | Total no of donors |
|---|---|---|
| **July 2013 to June 2014** | 10(0.33) | 3010 |
| **July 2014 to June 2015** | 9(0.25) | 3632 |
| **July 2015 to June 2016** | 16(0.34) | 4675 |
| **July 2016 to June 2017** | 6(0.13) | 4484 |
| **July 2017 to June 2018** | 1(0.02) | 4783 |
| **Total five-year period** | **42(0.20)** | **20584** |

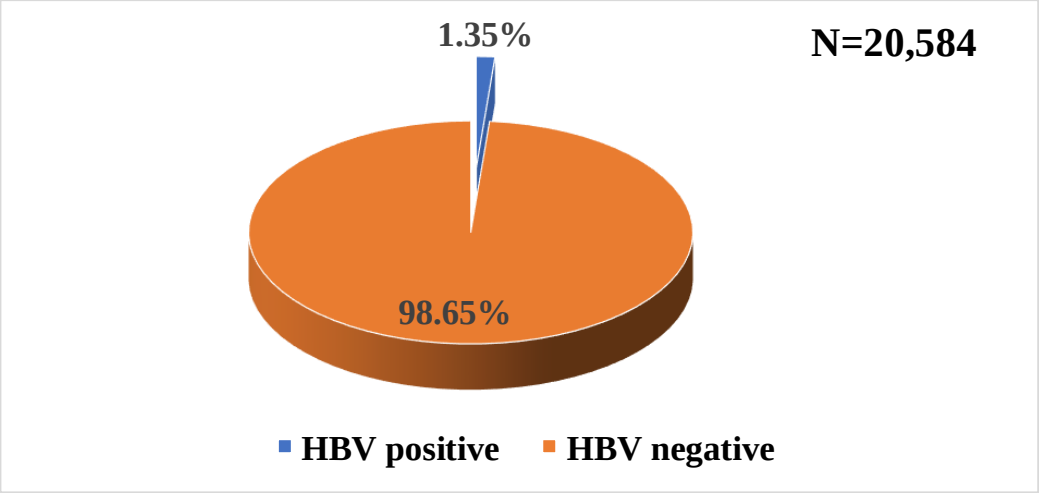
**Figure No.23: Shows the trend in seroprevalence of HIV in 5-Year period**




Seroprevalence of HIV was 0.33% in the year 2013-2014 and it decreased to 0.02% in the year 2017-2018. This showed a decreasing trend in the seroprevalence of HIV in 5 years which was found to be statistically significant with a **p value** of < 0.05 (**Table No.15 & Figure No.23)**

## SEROPREVALENCE OF HBV AMONG BLOOD DONORS

**Figure No.24: Seroprevalence of HBV amongst total screened donors (N=20,584)**



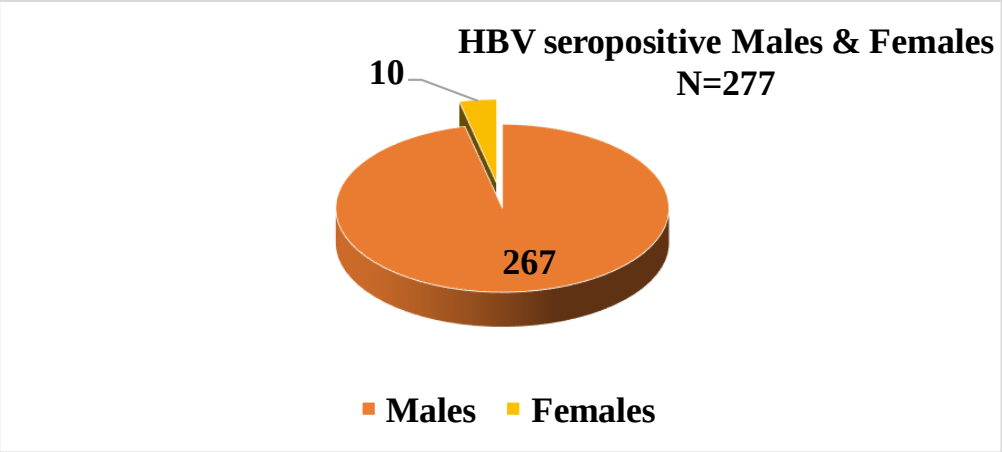

Among 20,584 units of total screened donors, 277 donors were HBV positive, accounting for 1.35% of seroprevalence **(Figure No.24).**

**Sex distribution**

**Table No.16: Seroprevalence of HBV donors according to Sex**

| Sex of donor | No. of donors | No. of HBV +ve | Seroprevalence |
|---|---|---|---|
| **Male** | 19847 | 267 | 1.34 % |
| **Female** | 737 | 10 | 1.35 % |
| **Total** | **20584** | **277** | **1.35 %** |

**Figure No.25: Distribution of HBV seropositive donors according to Sex**



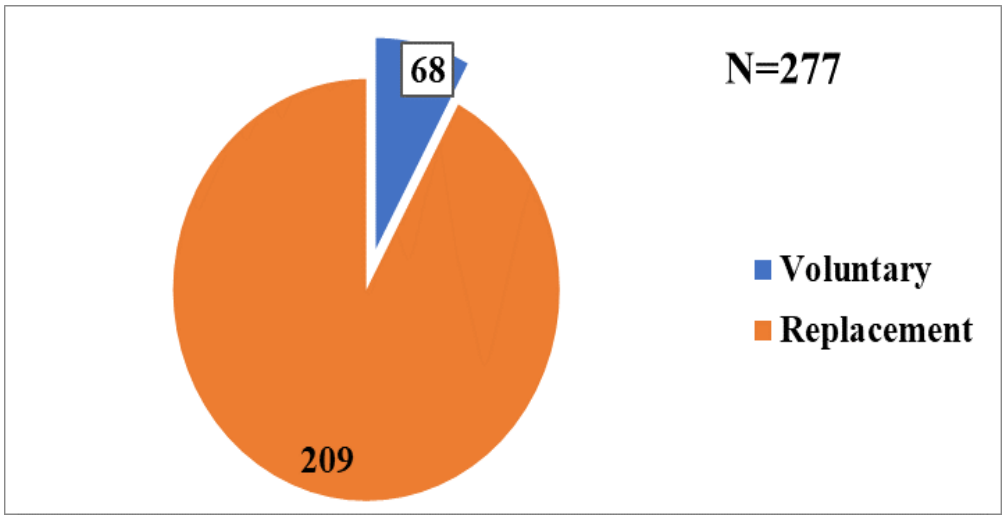

The seroprevalence in male HBV donors accounted for 1.34% and in females 1.35% among total male and female donors **(Table No.16 & Figure No.25)**

**Types of Donor-**

**Table No.17: Seroprevalence of HBV donors among voluntary & replacement blood donors**

| Type of donor | No. of donors | No. of HBV +ve | Seroprevalence |
|---|---|---|---|
| **Voluntary** | 16345 | 68 | 0.42% |
| **Replacement** | 4239 | 209 | 4.93 % |
| **Total** | **20584** | **277** | **1.35%** |

**Figure No.26: Distribution of seropositive HBV donors according to Type of donation**



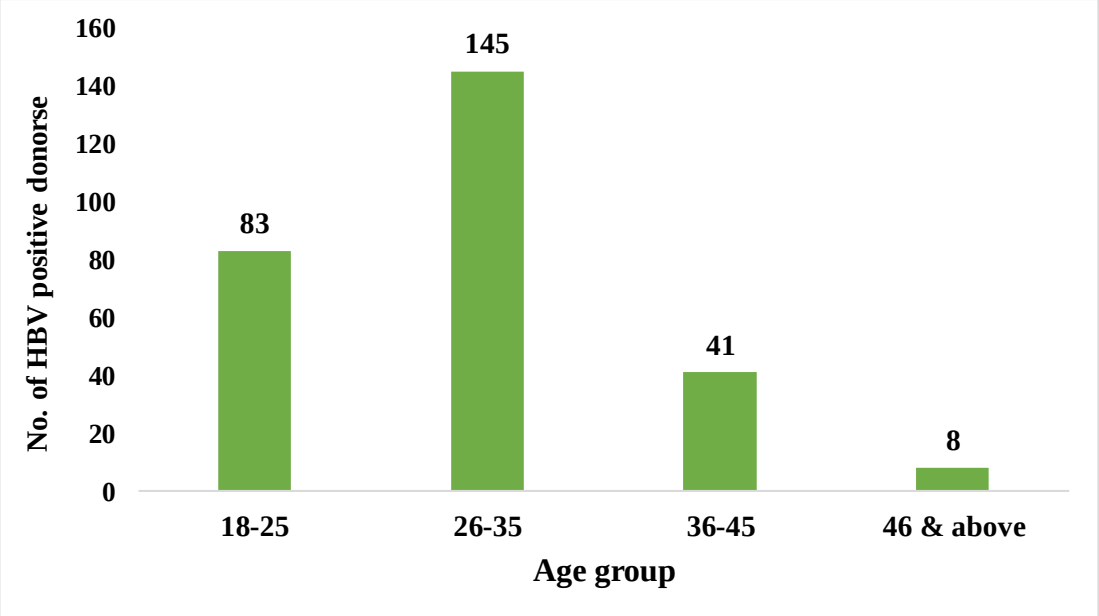

Out of total 277 seropositive HBV donors 209 were replacement donors accounting for majority of all seropositive HBV donors with seroprevalence of 4.93 % **(Table No.17 & Figure No.26).**

**Age Distribution**

**Table No.18: Age – wise distribution of HBV seropositive donors**

| Age | Number of positive donors | Percentage % |
|---|---|---|
| **18-25** | 83 | 30 % |
| **26-35** | 145 | 52.3 % |
| **36-45** | 41 | 14.8 % |
| **46 and above** | 8 | 2.9 % |
| **Total** | **277** | **100%** |

**Figure No.27: Age-wise distribution of HBV seropositive donors in 5-Year period.**



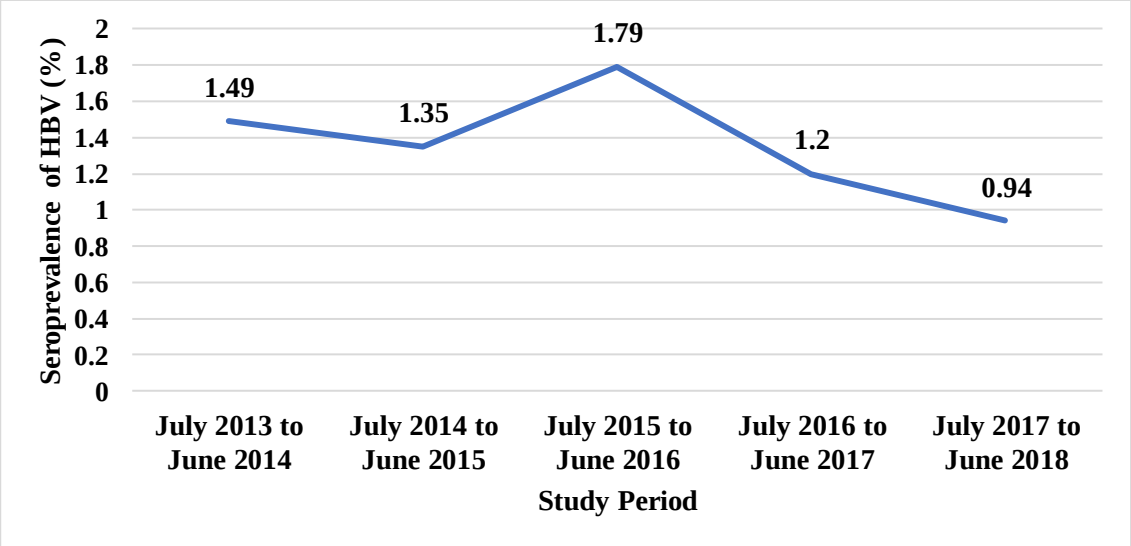

Out of total 277 seropositive HBV donor's majority were in age group of 26-35 years accounting for 52.3 % of all seropositive HBV donors (**Table No.18 & Figure No.27).**

## Yearly distribution and its Seroprevalence Trend-

**Table No.19: Shows the Yearly distribution of HBV seropositive donors**

| Year | Number of HBsAg positive donors | Total |
|---|---|---|
| **July 2013 to June 2014** | 45(1.49) | 3010 |
| **July 2014 to June 2015** | 49(1.35)) | 3632 |
| **July 2015 to June 2016** | 84(1.79) | 4675 |
| **July 2016 to June 2017** | 54(1.20) | 4484 |
| **July 2017 to June 2018** | 45(0.94) | 4783 |
| **Total five-year period** | **277(1.35)** | **20584** |

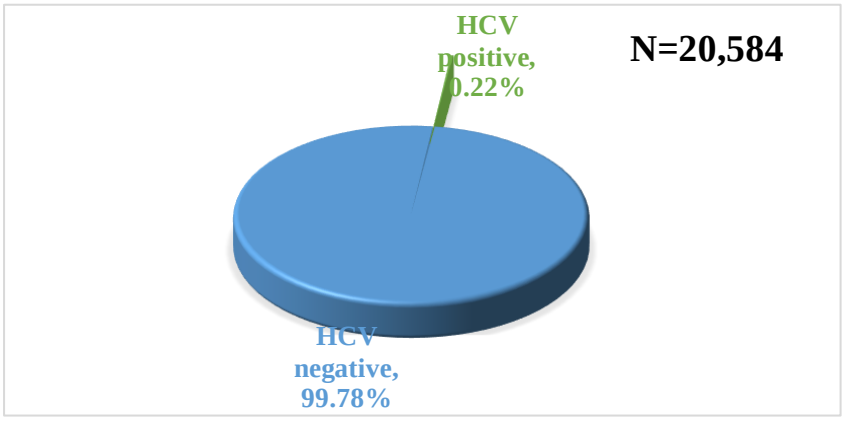
**Figure No.28: Shows the trend in seroprevalence of HBV in 5-Year period**




Seroprevalence of HBV was 1.49 % in the year 2013-2014 and it decreased to 0.94 % in the year 2017-2018. This showed a decreasing trend in the seroprevalence of HBV in 5 years but was not found to be statistically significant with a **p value** of > 0.05 (**Table No.19 & Figure No.28).**

## SEROPREVALENCE OF HCV AMONG BLOOD DONORS

**Figure No.29: Seroprevalence of HCV amongst total screened donors (N=20,584)**



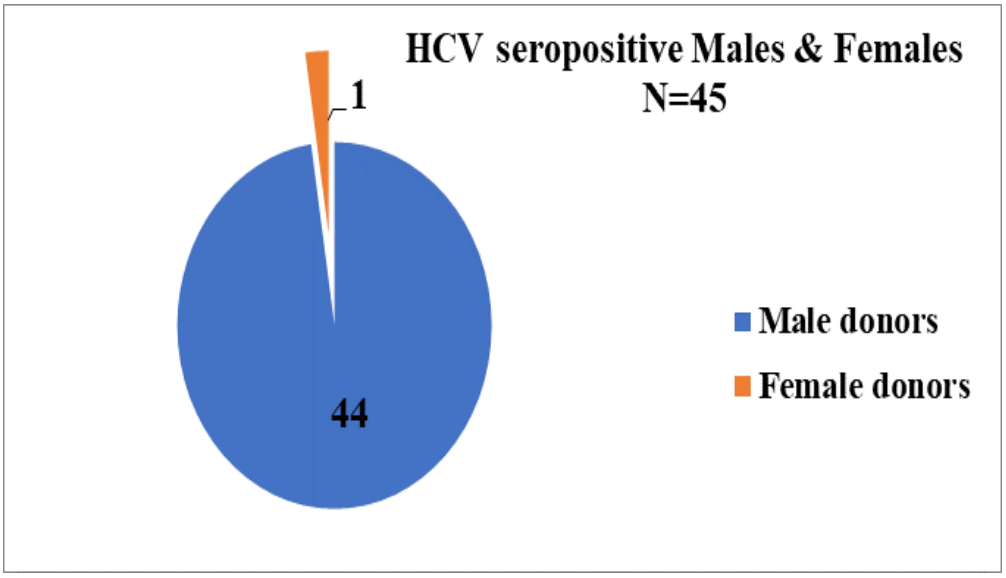

Among 20,584 units of total screened donors, 45 donors were HCV positive, accounting for 0.22% of seroprevalence **(Figure No.29).**

**Sex distribution-**

**Table No.20: Seroprevalence of HCV donors according to Sex**

| Sex of donor | No. of donors | No. of HCV +ve | Seroprevalence |
|---|---|---|---|
| **Male** | 19847 | 44 | 0.22% |
| **Female** | 737 | 1 | 0.13 % |
| **Total** | **20584** | **45** | **0.22%** |

**Figure No.30: Distribution of HCV seropositive donors according to Sex**



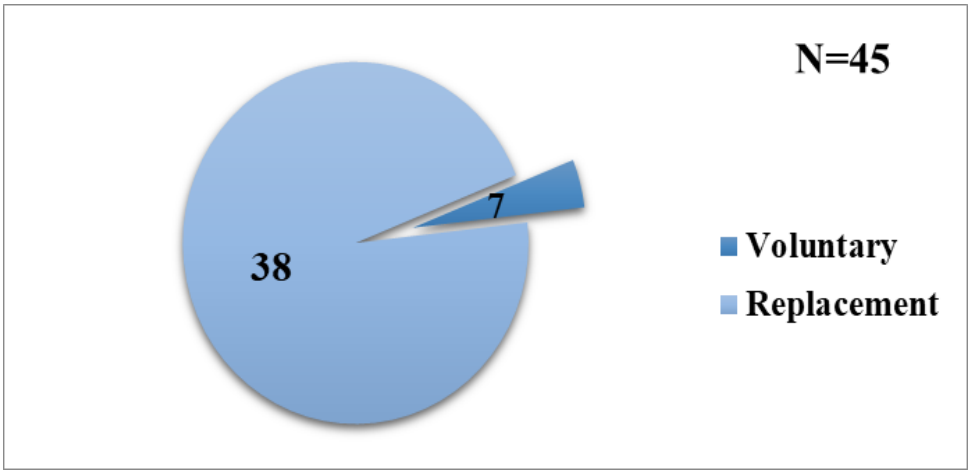

The seroprevalence in male HCV donors accounted for 0.22% and in females 0.13% among total male and female donors **(Table No.20 & Figure No.30)**

**Types of Donor**

**Table No.21: Seroprevalence of HCV among voluntary & replacement blood donors**

| Type of donor | No. of donors | No. of HCV +ve | Seroprevalence |
|---|---|---|---|
| **Voluntary** | 16345 | 7 | 0.04% |
| **Replacement** | 4239 | 38 | 0.89 % |
| **Total** | **20584** | **45** | **0.22%** |

**Figure No.31: Distribution of seropositive HCV donors according to Type of donation**



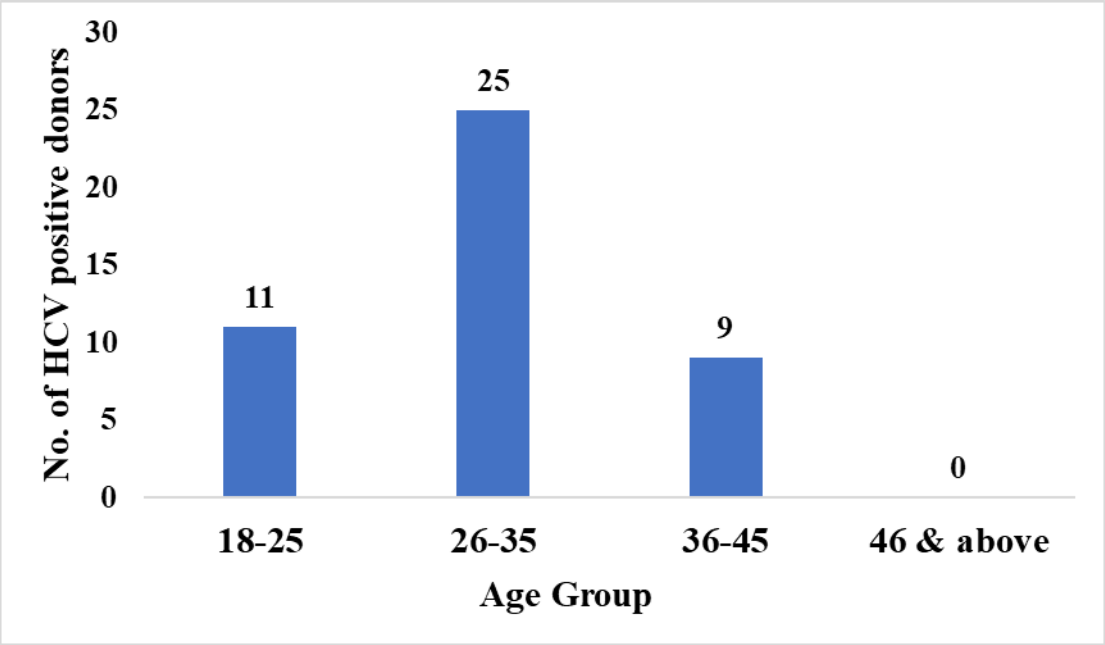

Out of total 45 seropositive HCV donors 38 were replacement donors accounting for majority of all seropositive HCV donors with seroprevalence of 0.89% **(Table No.21 & Figure No.31)**

**Age Distribution**

**Table No.22: Age – wise distribution of HCV seropositive donors**

| Age | Number of positive donors | Percentage % |
|---|---|---|
| **18-25** | 11 | 24.4 % |
| **26-35** | 25 | 55.6 % |
| **36-45** | 9 | 20.0 % |
| **46 and above** | 0 | 0 % |
| **Total** | **45** | **100%** |

**Figure No.32: Age-wise distribution of HCV seropositive donors in 5- Year period**



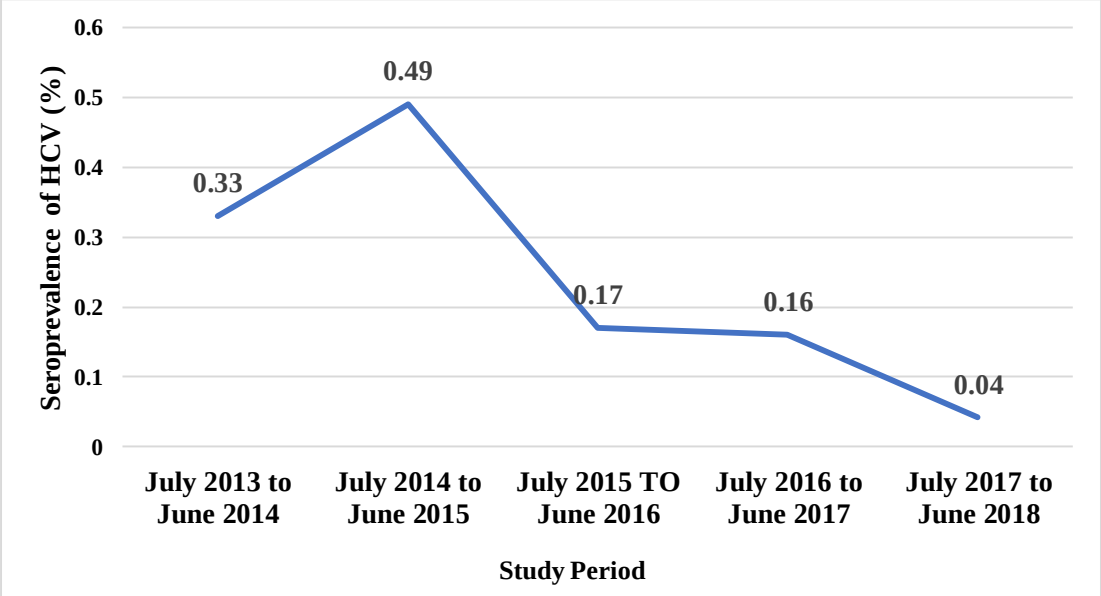

Out of total 45 seropositive HCV donor's majority were in age group of 26-35 years accounting for 55.6 % of all seropositive HCV donors **(Table No.22 & Figure No.32).**

## Yearly distribution and its Seroprevalence Trend-

**Table No.23: Shows the Yearly distribution of HCV seropositive donors**

| Year | Number of HCV positive donors | Total no of donors |
|---|---|---|
| July 2013 to June 2014 | **10(0.33)** | **3010** |
| July 2014 to June 2015 | **18(0.49)** | **3632** |
| July 2015 to June 2016 | **8(0.17)** | **4675** |
| July 2016 to June 2017 | **7(0.16)** | **4484** |
| July 2017 to June 2018 | **2(0.04)** | **4783** |
| Total five-year period | **45(0.22)** | **20584** |

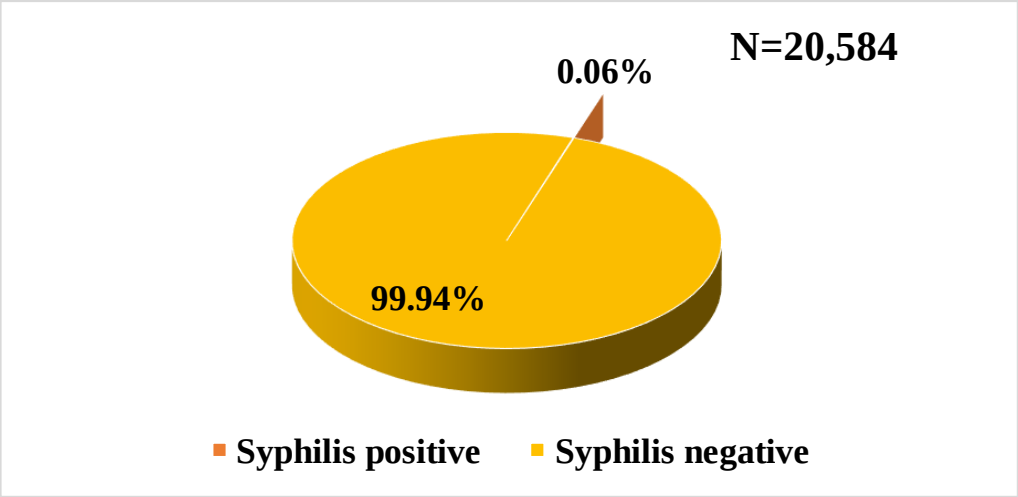
**Figure No.33: Shows the trend in seroprevalence of HCV in 5-Year period**




Seroprevalence of HCV was 0.33% in the year 2013-2014 and it decreased to 0.04% in the year 2017-2018. This showed a decreasing trend in the seroprevalence of HCV in 5 years which was found to be statistically significant with a **p value** of < 0.05 **(Table No.23 & Figure No.33)**

## SEROPREVALENCE OF SYPHILIS AMONG BLOOD DONORS

**Figure No.34: Seroprevalence of Syphilis amongst total screened donors (N=20,584)**




Among 20,584 units of total screened donors, 12 donors were Syphilis positive, accounting for 0.06 % of seroprevalence (**Figure No.34)**

**Sex distribution**

**Table No.24: Seroprevalence of Syphilis donors according to Sex**

| Sex of donor | No. of donors | No. of Syphilis +ve | Seroprevalence |
|---|---|---|---|
| **Male** | 19847 | 12 | 0.06 % |
| **Female** | 737 | 0 | 0 % |
| **Total** | **20584** | **12** | **0.06 %** |

All of the seropositive Syphilis donors were males with no females having seroprevalence of 0.06 % among total male donors (**Table No.24)**

**Types of Donor-** Out of total 12 seropositive Syphilis donors all were replacement donors with no voluntary donors with seroprevalence of 0.28 % **(Table No.25).**

**Table No.25: Seroprevalence of Syphilis donors according to Type of donor**

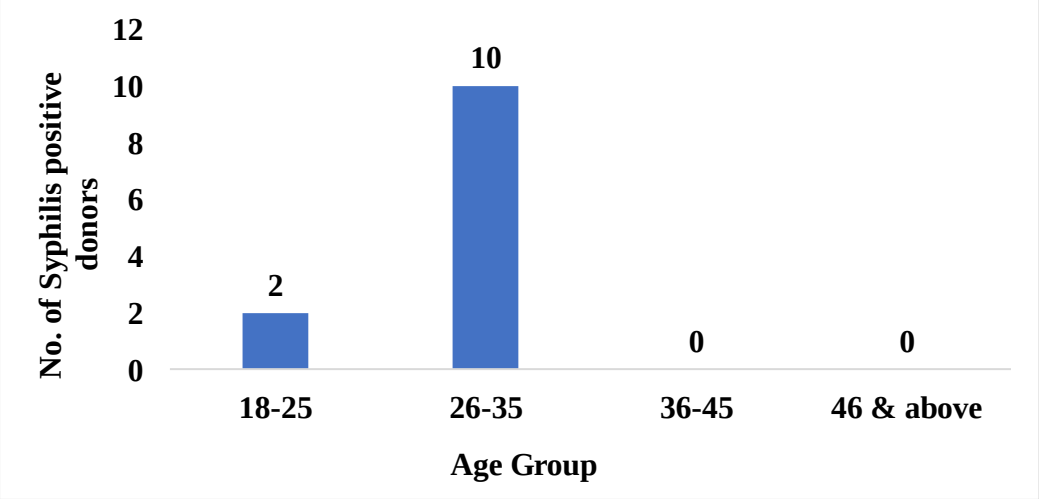
| Type of donor | No. of donors | No. of Syphilis +ve | Seroprevalence |
|---|---|---|---|
| **Voluntary** | 16345 | 0 | 0 % |
| **Replacement** | 4239 | 12 | 0.28 % |
| **Total** | **20584** | **12** | **0.06 %** |

**Age Distribution:**

**Table No.26: Age – wise distribution of Syphilis seropositive donors**

| Age | Number of Syphilis positive donors | Percentage % |
|---|---|---|
| **18-25** | 2 | 16.7 % |
| **26-35** | 10 | 83.3 % |
| **36-45** | 0 | 0 % |
| **46 and above** | 0 | 0 % |
| **Total** | **12** | **100 %** |

**Figure No.35: Age-wise distribution of Syphilis seropositive donors in 5-Year period**



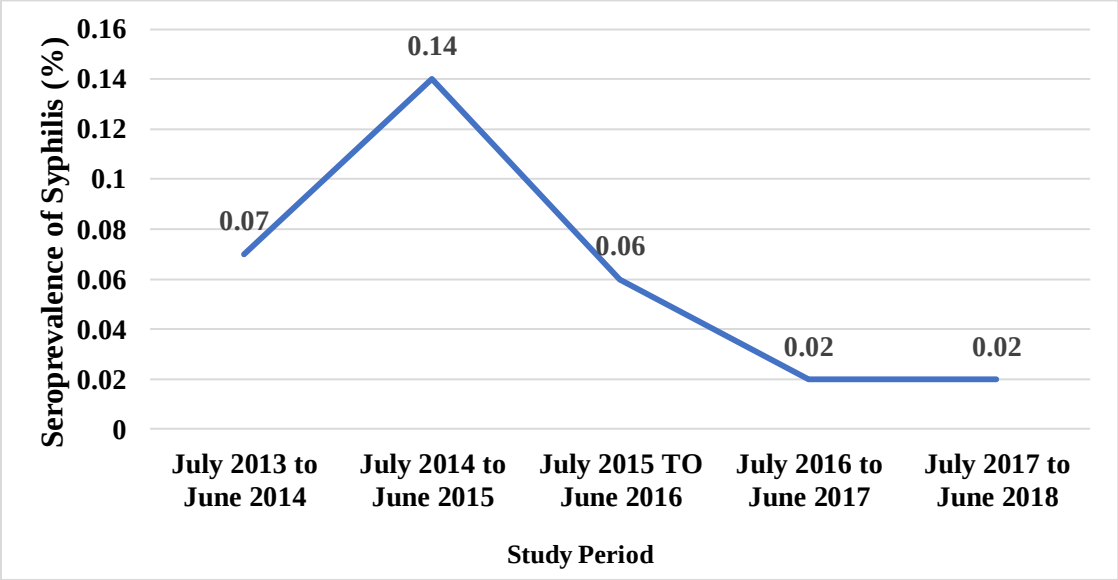

Out of total 12 seropositive Syphilis donors majority were in age group of 26-35 years accounting for 83.3 % of all seropositive Syphilis donors. **(Table No.26 & Figure No.35)**

## Yearly distribution and its Seroprevalence Trend-

**Table No.27: Shows the Yearly distribution of Syphilis seropositive donors**

| Year | Number of Syphilis positive donors | Total |
|---|---|---|
| **July 2013 to June 2014** | 2 (0.07) | 3010 |
| **July 2014 to June 2015** | 5 (0.14) | 3632 |
| **July 2015 to June 2016** | 3(0.06) | 4675 |
| **July 2016 to June 2017** | 1(0.02) | 4484 |
| **July 2017 to June 2018** | 1(0.02) | 4783 |
| **Total five-year period** | **12(0.06)** | **20584** |

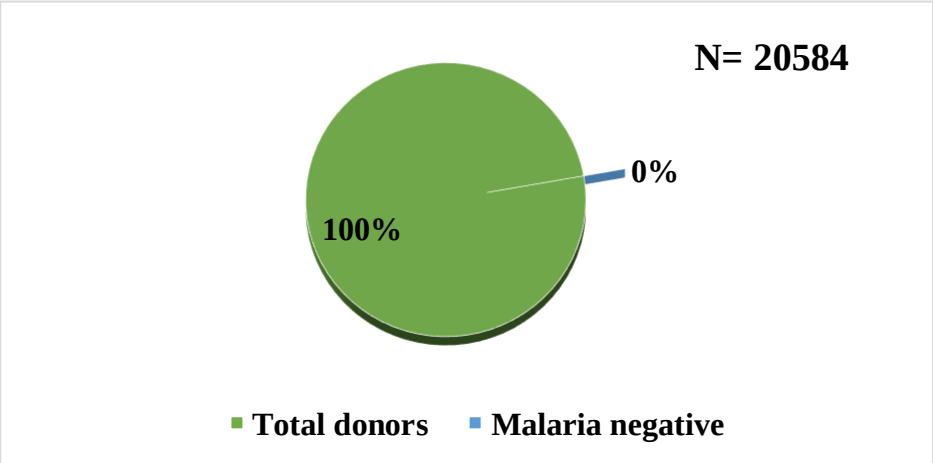
**Figure No.36: Shows the trend in seroprevalence of Syphilis in 5-Year period**




Seroprevalence of Syphilis was 0.07% in the year 2013-2014 and it decreased to 0.02% in the year 2017-2018. This showed a decreasing trend in the seroprevalence of Syphilis in 5 years but was not found to be statistically significant with a **p value** of >0.05 **(Table No.27 & Figure No.36**

## SEROPREVALENCE OF MALARIA AMONG BLOOD DONORS

**Figure No.37: Seroprevalence of Malaria amongst total screened donors (N=20,584)**

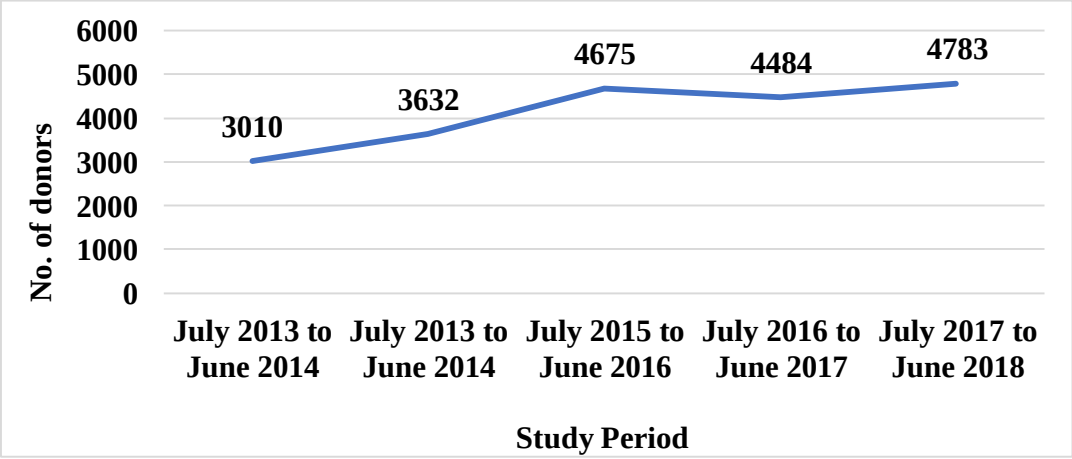



Among 20,584 units of total screened donors, none of the donors were Malaria positive, accounting for 0 % of seroprevalence. (**Figure No.37)**

## TRENDS IN BLOOD DONORS

### Trend in Total No of blood donors

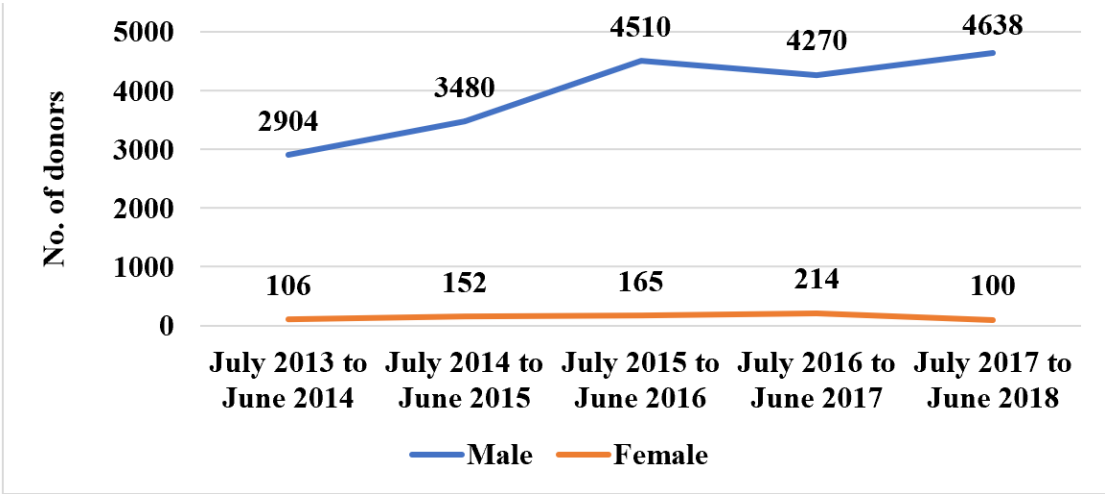
**Figure No.38: Shows the trend of total screened donors in 5-Year period**




In 5 years, total blood donations increased from 3010 (year 2013-2014) to 4783 (year 2017-2018), showing an increase of 58.9% in blood donation **(Figure No.38)**

### Sex distribution trend

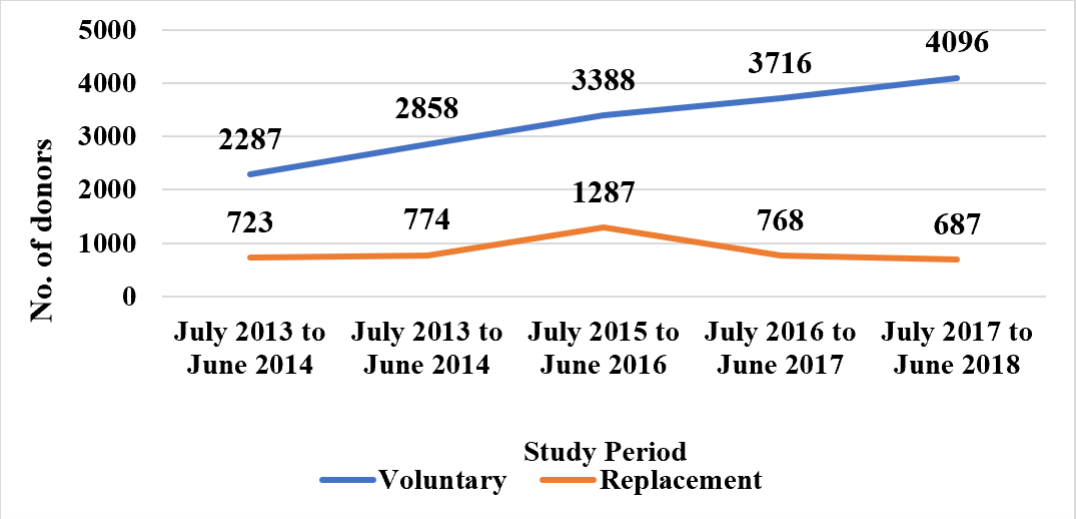
**Figure No.39: Shows the trend of total Male and Female donors in 5-Year period**




An increasing trend in the number of male blood donors was noted during the 5-year period, whereas this trend was almost static in case of female blood donors **(Figure No.39)**

## Trends in Type of donation

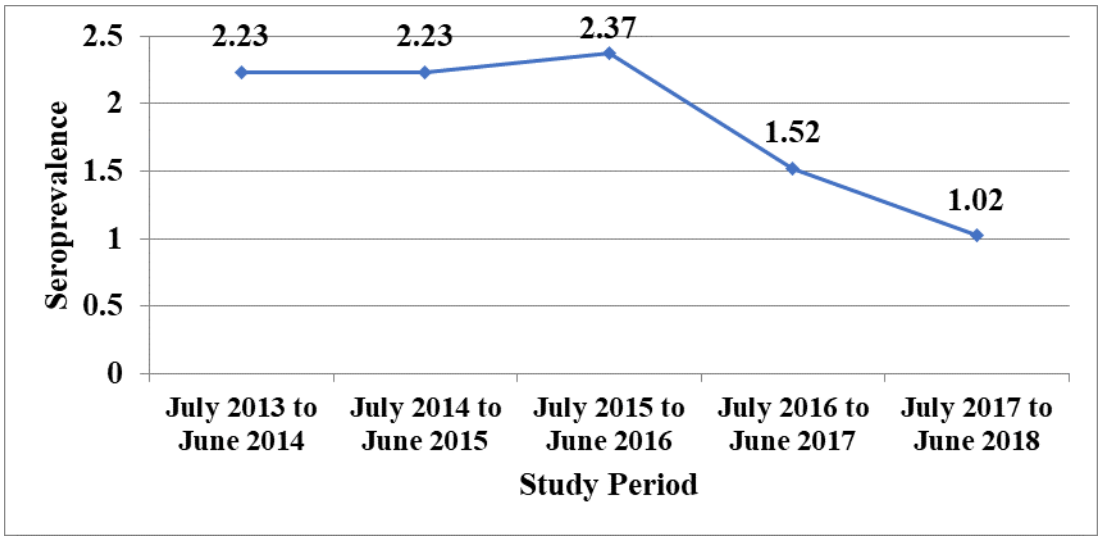
**Figure No.40: Shows the trend of voluntary and replacement donors in 5-Year period**




An increasing trend in the number of voluntary blood donors was noted during the 5-year period, whereas this trend was almost static in case of replacement blood donors **(Figure No.40)**

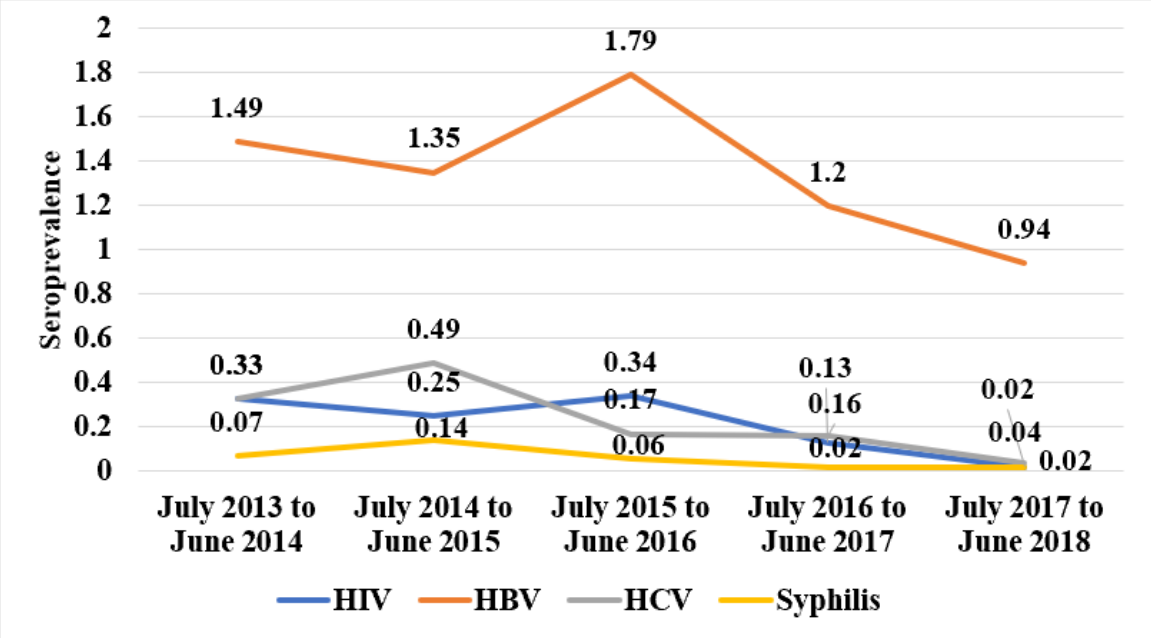
## Trend of TTIs

**Figure No.41: Shows the trend of all TTIs in 5-Year period**




Seroprevalence of all TTIs showed the decreasing trend from 2.23 in first year to 1.02 in last year which was found to be statistically significant with a p value of <0.05 **(Figure No.41)**

**Figure No.42: Shows the trend of individual TTIs in 5-Year period**




Trend in seroprevalence of individual TTIs during 5-year period is shown in **(Figure No.42)** The high seroprevalence of HBV can be seen compare to the other TTIs. A decreasing trend is noted in the seroprevalence of HBV, HIV and HCV with the last two infections showing a statistically significant decrease.

## SEROPREVALENCE OF CO-INFECTION IN BLOOD DONORS

**Table No.28: Shows the seroprevalence of co-infection among blood donors**

| Total donors | Co-infection units | Seroprevalence |
|---|---|---|
| **20584** | 07 | 0.03% |

Out of total 20584 blood donors 7 were having coinfection (more than one seromarker positivity), accounting for 0.03% of seroprevalence of co-infection amongst blood donors. **(Table No.28)**

**Table No.29: Shows the no of blood donors with co-infection**

| Co-infections | No of blood donors |
|---|---|
| HIV-HBV | 2 |
| HIV-Syphilis | 1 |
| HBV-HCV | 4 |

There were found be 2 HIV-HBV, 1 HIV-Syphilis and 4 HCV-HBV co-infection in 5-year period **(Table No.29)**

# 6. DISCUSSION

India is the 2nd most populous nation in the world, having current population of 1.3 billion with 2.1 million HIV, 50 million HBV and 15 million HCV positive cases. The prevalence of these TTIs in Indian blood donors ranges from 2-7 % in HBV, 0.22-0.32% in HIV, 0.5 %- 1.5 % in HCV, 0.14 -1 % in syphilis and 0.01- 0.09% in Malaria. This seroprevalence data varies widely according to high risk areas, socioeconomic conditions and types of donor. [21,49,50,51]

**Table No. 30: Sex distribution in comparison to other studies**

| Authors | Male Donors | Female Donors |
|---|---|---|
| Giri PA *et al* [51] | 95.28 % | 4.72 % |
| Sundaramurthy *et al* [2] | 99.23 % | 0.76 % |
| D.C Sharma *et al* [52] | 96.2 % | 3.8 % |
| Yadav *et al* [53] | 94% | 6 % |
| **Present study** | **96.4 %** | **3.6 %** |

In the present study out of the total 20584 donors, majority were males (96.4 %). The overall turnout of female donors was low which might be due to that females in India are generally anaemic and underweight hence, unable to meet the donor selection criteria.[52] Similar findings were seen in various studies shown in **(Table No. 30).**

**Table No. 31: Distribution of total voluntary and replacement donors in various studies.**

| **Authors** | **Voluntary Donors** | **Replacement Donors** |
|---|---|---|
| Sundaramurthy *et al* [2] | 68.7 % | 31.3 % |
| **Deshpande *et al*** [54] | **79.33 %** | **20.67 %** |
| Makroo *et al* [4] | 3.06 % | 96.93 % |
| Chattoraj *et al* [55] | 14.33 % | 85.67 % |
| **Present study** | **79.4 %** | **20.6 %** |

In the present study majority of the donors were voluntary (79.4%). This was in concordance with study done by Deshpande *et al* [54] where 79.33 % were voluntary donors.

Voluntary blood donors are the safest, non- remunerated donors from low-risk populations and are the cornerstone of a safe and adequate supply of blood and blood products. In country like India, where comprehensive laboratory tests are not possible, voluntary blood donation should be switch over to 100 %. As in the present study voluntary donors were more this reflects presence of awareness about voluntary donation in general population. [56]

## TRANSFUSION TRANSMITTED INFECTIONS

**Table No. 32: Comparison of seroprevalence of TTIs with other studies.**

| Authors | Total screened donors | Seropositive donors | Seroprevalence of TTIs |
|---|---|---|---|
| **Yadav UC *et al*** [53] | **1336156** | **25224** | **1.75%** |
| Dobariya *et al* [16] | 40971 | 550 | 1.34% |
| Kulkarni *et al* [57] | 19135 | 883 | 4.5% |
| **Present study** | **20,584** | **369** | **1.8%** |

The seroprevalence of TTIs, accounting for a prevalence rate of 1.8% which was in concordance with study done by Yadav UC *et al* [53] and Dobariya *et al* [16] while higher seroprevalence was seen in study by Kulkarni *et al* [57]. This could be attributed to a greater number of replacement donors in the latter study. **(Table No. 32)**

**Table No. 33: Comparison of seroprevalence of TTIs in voluntary & replacement donors with other studies**

| Studies | HIV | | HBV | | HCV | | Syphilis | | Malaria | |
|---|---|---|---|---|---|---|---|---|---|---|
| | V | R | V | R | V | R | V | R | V | R |
| **Gupta *et al*** [59] | 0.51% | 1.53% | 1.22% | 2.59% | 0.34% | 0.51% | 0.27% | 0.85% | - | - |
| **Kaur *et al*** [60] | 0.15% | 0.44% | 0.65 % | 1.07% | 0.3% | 0.5% | 0.19% | 0.48% | - | - |
| **Present study** | **0.06%** | **0.75%** | **0.42%** | **4.93%** | **0.04%** | **0.89%** | **0%** | **0.28%** | **0%** | **0%** |

**V- Voluntary R- Replacement**

In our study highest seroprevalence was of HBV in replacement donors 4.93% and least was for Malaria that is 0%. Other infections also had higher seroprevalence in replacement donors similar to other studies shown in **(Table No. 33).**

Out of total seropositive donors 284 were replacement donors and remaining 85 were voluntary donors, so maximum seroprevalence of TTIs was found amongst replacement donors. This can be compared with study by Chandra *et al* [58] where prevalence of TTIs was higher in replacement in comparison to voluntary donors. Similarly, a study by Kulkarni *et al* [57] 100% replacement donors showed seropositivity and none of the voluntary donor was found to be seropositive.

## HUMAN IMMUNOIDEFICIENCY VIRUS (HIV)

Though the prevalence of HIV is low (0.3%) in India as per 2016 estimates but India has third largest burden of people living with HIV. By the end of 2016 there were 2.1 million people living with HIV.

**Table No. 34: Comparison of HIV seroprevalence among blood donors in different studies.**

| Authors (Yrs.) | Seroprevalence of HIV |
|---|---|
| Jasani *et al* (2012) [61] | 0.25% |
| Leena MS *et al* (2012) [62] | 0.27% |
| **Mythreyee *et al* (2011)** [63] | **0.19%** |
| Sunderam *et al* (2015) [64] | 0.08% |
| Dobariya GH *et al* (2016) [16] | 0.08% |
| Sundaramurthy *et al* (2018) [2] | 0.13% |
| **Present study** | **0.20%** |

The seroprevalence ranged from 0.08% to 0.27% in various studies in India. According to New estimates released by National AIDS Control Organization (NACO), 0.24% of seroprevalence was found in Vijayapura district of Karnataka which is in concordance with our study (0.20%)[65] which is comparable with study done by Mythreyee *et al.*[63] **(Table No. 34)**

In the present study majority of HIV seropositive donors were males accounting for the seroprevalence of 0.21% and 0.13% in females, which is similar to study done by Makroo *et al* [4] having seroprevalence of 0.24% in males and 0.16% in females.

In the present study, age group of 26-35 (64.3% ) accounted for majority of the HIV seropositive donors, which is in concordance with study done by Leena MS *et al* [62] where majority (68.4% ) were in age group of 21-30 yrs.

In India presently, strategy I / algorithm I is followed for HIV testing – This strategy is used to ensure blood transfusion / donation safety. Serum of the donor is subjected to one of the sensitive tests and is considered as free of HIV if the test is non- reactive and positive if the test is reactive. After testing, he /she will be notified about the results and will be sent to Integrated Counselling and Testing Centres (ICTC)/ Voluntary Counselling and Testing Centres (VCTC) for confirmation of result and proper counselling. [66] The later should be provided as soon as practicable after the test results are available by a trained health-care professional who is able to explain the result, counsel with understanding and empathy. The donor should be given sufficient time to comprehend the test results and to ask questions or raise any concern.[67]

In present study also, we follow the same strategy. The positive donors were given post-test counselling and were referred to ICTC/VCTC for further investigations and management.

## HEPATITIS B VIRUS (HBV)

Worldwide Hepatitis B is a common life-threatening disease caused by HBV. India occupies the intermediate endemicity zone (2-7%) having prevalence of an average 4% and with a disease burden of about 50 million.

**Table No. 35: Comparison of HBV seroprevalence among blood donors in different studies.**

| Authors (Yrs.) | Seroprevalence of HBV |
|---|---|
| Arora *et al* (2010) [68] | 1.7% |
| Kulkarni *et al* (2012) [57] | 3.2% |
| **Jasani *et al* (2012)** [61] | **1.35 %** |
| Sastry JM *et al* (2014) [69] | 1.23% |
| Dobariya GH *et al* (2016) [16] | 0.98% |
| Yadav UC *et al* (2018) [53] | 1.16 % |
| **Present study** | **1.34%** |

The seroprevalence in various other studies ranged from 0.98% - 3.2% in India. According to recent NACO data the seroprevalence in Vijayapura district was found to be 1.18% which is in concordance with our study. [65] The comparison of seroprevalence with various other studies shown in **(Table No.35).**

The seroprevalence of HBV was 1.34% in males and 1.35% in females in the present study. Majority of the seropositive donors were males, which is similar to study done by Dobariya GH *et al* [16]. While a study by Makroo *et al* [4] showed seroprevalence of 1.21% in males and 0.58% in females.

In the present study, age group of 26-35 (52.3%) accounted for majority of the HBV seropositive donors ,which is in concordance with study done by Leena MS *et al* [62] where majority (69.4% ) were in age group of 21-30 yrs. Similar finding was seen in study done by Mandal R *et al* [70] where maximum TTIs were seen in the age group of 26-35 yrs.

Despite the introduction of universal immunisation since 1985, the HBsAg prevalence in India remains high. The cause of high seroprevalence is attributed to many factors like high titre of infectious virus, immunologic variants, many immune silent carriers and inability of test to detect the disease in window period. Finally factors like non-adherent, costly, long term treatment modality and the lack of widespread vaccination programme.

One of the reasons for high prevalence in our study may be due to use of 3$^{rd}$ generation ELISA as a screening method which is less sensitive than 4$^{th}$ generation ELISA. Another problem with HBV detection is the fact that, the screening method employed for HBV detects HBsAg, which can't differentiate between active infection and chronic carrier state. Secondly, HBsAg cannot be detected during the window period, while IgM anti-HBc and HBV DNA studies can detect even during window period.

## HEPATITIS C VIRUS (HCV)

Worldwide HCV known to be the most common cause of post transfusion non-A, non-B hepatitis. The high risk of chronicity and its progression to cirrhosis or hepatocellular carcinoma in 50-80% cases emphasize its public health importance. India has an estimated seroprevalence 15 million people living with HCV which ranges from 0.5 -1.5%.

**Table No. 36: Comparison of HCV seroprevalence among blood donors in different studies.**

| **Authors (Yrs.)** | **Seroprevalence of HCV** |
|---|---|
| Arora *et al* (2010) [68] | 1.0% |
| Jasani *et al* (2012) [61] | 0.16% |
| **Deshpande *et al* (2012)** [54] | **0.22%** |
| Sastry JM *et al* (2014) [69] | 0.41% |
| Dobariya GH *et al* (2016) [16] | 0.09% |
| Sundaramurthy *et al*(2018) [2] | 0.56% |
| **Present study** | **0.22%** |

The seroprevalence in various other studies ranged from 0.09 -1% in India. According to recent NACO data the seroprevalence in Karnataka was found to be 0.22% which is in concordance with our study. [65] The comparison of seroprevalence with various other studies shown in **(Table No.36).**

The seroprevalence of HCV was 0.22% in males and 0.13% in females in the present study. Majority of the seropositive donors were males, this is similar to study done by Makroo *et al* [4] which showed seroprevalence of 0.44% in males and 0.20% in females. While studies conducted by Sundaramurthy *et al* [2] and Mandal R *et al* [70] showed comparatively higher seroprevalence of HCV in females.

In the present study, age group of 26-35 (55.6%) accounted for majority of the HCV seropositive donors. Similar findings were seen in studies done by Mandal R *et al* [70], Sundaramurthy *et al* [2] whereas study done by Leena MS *et al* [62] showed majority of HCV seropositive donors in age group of 31-40 yrs.

In recent years, there has been a significant reduction in the prevalence of HCV, probably due to increased awareness among blood donors and availability of improved diagnostic kits like 4$^{th}$ generation ELISA which detect both capsid antigen and the antibodies resulting in reduced window period.

## SYPHILIS

Syphilis continues to be the cause of increased mortality and morbidity worldwide. While strategies followed by WHO led to elimination of mother to child transmission in several countries but it continues to remain endemic in some selected groups which led to its variable incidence in India.

**Table No. 37: Comparison of Syphilis seroprevalence among blood donors in other studies.**

| Authors (Yrs.) | Seroprevalence of Syphilis |
|---|---|
| Jasani *et al* (2012) [61] | 0.9% |
| **Giri P A *et al* (2012)** [51] | **0.07%** |
| Leena MS *et al* (2012) [62] | 0.1% |
| Sastry JM *et al* (2014) [69] | 0.008% |
| Dobariya GH *et al* (2016) [16] | 0.16% |
| Sundaramurthy *et al*(2018) [2] | 0% |
| **Present study** | **0.06%** |

The seroprevalence in various other studies ranged from 0% -0.9% in India. According to recent NACO data the seroprevalence in Karnataka was found to be 0.07% which is in concordance with our study. [65] The comparison of seroprevalence with various other studies shown in **(Table No.37).**

In the present study all of the Syphilis seropositive donors were males having seroprevalence of 0.06 % which is similar to study done by Giri P A *et al* (2012) [51] with 100% male donors and none of the female showed seropositivity, while study done by Makroo *et al* [4] showed seroprevalence of 0.24% in males and 0.02% in females. This sex-wise difference in seroprevalence might be due to difference in the risk behaviour.[51]

In the present study, age group of 26-35 (83.3%) accounted for majority of the Syphilis seropositive donors. Similar findings were seen in studies done by Mandal R *et al* [70] and Leena MS *et al* [62] which showed majority of Syphilis seropositive donors in age group of 21-30 yrs.

In our study seroprevalence of Syphilis is lower as compared to studies done in other parts of India which indicates variable prevalence in different group of population. Transfusion transmitted Syphilis is not worrisome in modern blood transfusion practice but its presence designates donors' indulgence in "high risk" behaviour and subsequently high risk of co- infection with hepatitis and HIV.

## MALARIA

Malaria is one of the most common parasitic infection that is transmitted by blood. In Malaria endemic world Transfusion Transmitted Malaria is one of the overwhelming health problems in tropical and sub-tropical countries. Worldwide India carries a high burden of Malaria cases.

**Table No. 38: Comparison of Malaria seroprevalence among blood donors in other studies.**

| Authors (Yrs.) | Seroprevalence of Malaria |
|---|---|
| Chattoraj *et al* (2008)[55] | 0% |
| Pallavi P *et al* (2011)[71] | 0% |
| Leena MS *et al* (2012) [62] | 0.12% |
| Sastry JM *et al* (2014) [69] | 0% |
| Dobariya GH *et al* (2016) [16] | 0.02% |
| Mandal R *et al* (2016) [70] | 0.004% |
| Yadav UC *et al* (2018) [53] | 0.04% |
| **Present study** | **0%** |

Malaria in the present study was not found to be positive in any blood unit accounting for 0% of the seroprevalence. This can be compared with various other studies where seroprevalence ranged from 0%- 0.12% shown in **(Table No. 38)**

Data provided by NACO [65] shows 0% Malaria seroprevalence in Karnataka state which is in concordance with our study. This might be due to deferral of blood donors in their preliminary health check-up during blood donation process.

Although in India it is mandatory by NACO and CDC to screen for the Malaria but there are no definite guidelines for the test to be followed. Screening of donors for parasite by microscopy involves high technical skills and large manpower which is not possible on daily basis and also Malaria antibody screening does not indicate active infection as it persists for years after infection. The most common method employed in all blood banks is histidine rich protein (HRP2) based immunochromatographic assays which detects the Malarial antigen.

**TRENDS OF TTIs**

Our study showed increasing trend of voluntary donation with male predominance. In the present study seroprevalence of TTIs showed a significant decreasing trend from July 2013 to June 2018. Among individual TTIs, HIV and HCV showed a statistically significant decreasing trend whereas HBV continues to be prevalent among blood donors in our area. Syphilis showed low prevalence while none of the donors were found to have Malaria.

Similar study conducted by D.C. Sharma *et al* [52] showed decreasing trend of HIV and HCV whereas HBV showed the increasing trend. Other study by Makroo *et al* [4] showed decreasing trend of HIV, HBV and Syphilis while increase in HCV trend.

## PATTERN OF CO- INFECTION AMONG BLOOD DONORS

Donors with a combination of ≥ 2 TTIs were labelled as co-infection. It is important to know and identify the co-infected blood donors as it carries epidemiological significance. Most of the studies have not mentioned about the prevalence of co-infection in blood donors. Overall prevalence of co-infection is very low in India.

In the present study out of total 20584 blood donors, 7 blood units were having co-infection, accounting for 0.03% of seroprevalence. In 5- year period, there were 2 HIV-HBV, 1 HIV-Syphilis and 4 HCV-HBV co-infected blood units.

In comparison, a study by Sastry JM *et al* [69] showed seroprevalence of 0.04% with 3 HIV and HBV, 1 HIV and HCV and 1 HBsAg and HCV and similarly another study by Kaur *et al* [60] showed seroprevalence of 0.05% which is in concordance with our study.

# 7. CONCLUSION

In the present study the overall prevalence of TTIs and their trend in the last 5-years has been decreased considerably. This is a reassuring sign supporting the growing awareness among general population about the blood donation. But seroprevalence of TTIs was found to be significantly more in replacement donors hence a healthy voluntary donor base is the need of the hour which can be achieved by still more increase in public awareness and knowledge thus influencing donor behaviour and attitude towards voluntary blood donation. Stringent and comprehensive donor screening**,** sensitive and standard laboratory screening tests should be used to decrease the risk of TTIs . NAT is recommended to reduce the risk of TTI during window period to accomplish near zero risk. Thus, will lead to good clinical practices to ensure the safe blood donation for the welfare of the community.

# 8. SUMMARY

- A five (3 yr. retrospective + 2 yr. prospective) year study to evaluate the trend in seroprevalence of HIV, HBV, HCV, Syphilis and Malaria in blood donors was undertaken from 1st July 2013 to 30$^{th}$ June 2018.
- During the study period total 20584 donor blood units were screened.
- 96.4% were Males and remaining 3.6% donors were Females.
- Voluntary donors were 79.4% and remaining 20.6% were replacement donors.
- Seroprevalence rate of Transfusion Transmitted Infections was 1.8% out of total screened donors.
- Out of total 19847 male and 737 female donors, seroprevalence in male donors was found to be 1.8% and 1.6% in female donors.
- Seroprevalence of HIV, HBV, HCV and Syphilis infections in blood donors was found to be 0.20%, 1.35%, 0.22%. and 0.06% respectively.
- None of the blood donor unit was found to be positive for Malaria.
- The seroprevalence of TTIs was higher in replacement donors as compared to voluntary donors.
- Significantly decreasing trend was observed in HIV and HCV infections while HBV infection continuous showed the same trend.
- Seroprevalence of co-infection (more than one seromarker positivity), accounted for 0.03% (7 cases). Out of these, HIV-HBV, HIV-Syphilis, HCV-HBV co- infection was found in, 2, 1 and 4 blood units respectively.

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## ANNEXURE I


*B.L.D.E. UNIVERSITY'S*

**SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103**

INSTITUTIONAL ETHICAL COMMITTEE

### *INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE*

*The Ethical Committee of this college met on* 04/10/2016 *at* 3-00pm *to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.*

*Title* "Trends in seroprevalence of transfusion transmissible infections amongst the blood donors in tertiary care centre

*Name of P.G. student* Neha kathpal

Dept in pathology

*Name of Guide/Co-investigator Dr* S.B. Thippara:

Professor of Pathology

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization

1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

## ANNEXURE II

## BLOOD DONOR QUESTIONNAIRE AND CONSENT FORM/VOLUNTARY DONOR SELECTION/REJECTION FORM

**LICENCE NO.: KTK/28C-56/97** **BLOOD UNIT NO.:** **BLOOD GROUP AND Rh TYPE:**

**DATE OF COLLECTION:** **EXPIRY DATE:**

**CONFIDENTIAL**

**Name:** **Age:** **Sex:** **D.O.B.:** **Contact No.:**

**Occupation:** **Address for communication:**

a. Have you donated previously? YES/NO

If yes, then on how many occasions? Last donation on:

b. Your blood group: Time of last meal:

**Did you have discomfort during donation?**

1. Do you feel well today? YES/NO

2. Did you have something to eat in the last 4hrs? YES/NO

3. Did you sleep well last night? YES/NO

4. Have you any reason to believe that you may be infected by either hepatitis, malaria, HIV/AIDS and/or venereal disease? YES/NO

5. In the last 6months have you had any history of following?

Unexplained weight loss: Repeated diarrhea: Swollen glands:

6. In the last 6 months have you had any?

Tattooing: Ear Piercing: Dental extraction:

7. Do you suffer from or have suffered from any of the following diseases?

| | | | |
|---|---|---|---|
| Heart disease: | Lung disease: | Kidney disease: | STD: |
| Diabetes: | Tuberculosis: | Jaundice: | Malaria: |
| Hepatitis B/C: | Cancer: | Epilepsy: | Fainting Spells: |

Allergic Disease: Abnormal Bleeding Tendency: Typhoid:

Are you taking or have taken any of these in the past 72 hrs.?

Antibiotics: Aspirin: Alcohol: Steroids: Vaccination: Dog Bite:

8. Is there any history of surgery or blood transfusion in the past 6 months?

Major surgery: Minor surgery: Blood transfusion:

9. Women Donors:

a. Are you pregnant?

YES/NO

b. Have you had an abortion in the last 3months?

YES/NO

c. Do you have a child less than 1yr old?

YES/NO

d. Are you having your periods today?

YES/NO

10. Would you like to be informed about any abnormal test result at the address furnished by you? YES/NO

11. Have you read and understood all the information presented and answered all the questions truthfully, as any incorrect statement or concealment may affect your health so may harm the recipient. YES/NO

**I UNDERSTAND**

A. Blood donation is a totally voluntary act and no inducement or remuneration has been offered.
B. Donation of blood /component is a medical procedure and that by donating voluntarily, I accept the risk associated with the procedure.
C. My blood will be tested for Hepatitis B, Hepatitis C, Malarial parasite, HIV/AIDS and venereal diseases in addition to any other screening tests required to ensure blood safety.

I prohibit any information provided by me or about my donation to be disclosed to any individual or government agency without my prior permission.

DATE: TIME: DONOR SIGNATURE:

General physical examination:

Weight: Pulse: Hb: Temperature: BP:

Accept: Defer: Reason:

Signature of Medical officer

## ANNEXURE III

## PROFORMA FOR STUDY

Sample No: Bag no:-

Date:-

1.Demographic Details: -

1. Age:-
2. Sex: M/F:-
3. Blood Group:-
4. Haemoglobin:-

2.Type of donor:-

Voluntary:-

Replacement:-

Others:-

3.Investigations:

| Parameters | Status |
|---|---|
| HIV | |
| HCV | |
| HBV | |
| SYPHILIS | |
| MALARIA | |

## 11. MASTER CHART

| S. No. | Age | Sex | Voluntary | Replacement | Blood Group | HIV | HBsAg | HCV | Syphilis | Malaria |
|---|---|---|---|---|---|---|---|---|---|---|
| **JULY 2013 - JUNE 2014** | | | | | | | | | | |
| 1 | 23 | M | | Replacement | B Positive | Negative | Negative | Positive | Negative | Negative |
| 2 | 25 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 3 | 28 | M | | Replacement | B Positive | Negative | Negative | Positive | Negative | Negative |
| 4 | 22 | M | Voluntary | | A Positive | Negative | Positive | Negative | Negative | Negative |
| 5 | 31 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 6 | 35 | M | | Replacement | O Positive | Positive | Negative | Negative | Positive | Negative |
| 7 | 38 | M | | Replacement | B Negative | Negative | Positive | Negative | Negative | Negative |
| 8 | 25 | M | | Replacement | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 9 | 44 | M | Voluntary | | B Positive | Negative | Positive | Negative | Negative | Negative |
| 10 | 35 | M | | Replacement | O Positive | Positive | Negative | Negative | Negative | Negative |
| 11 | 24 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 12 | 35 | M | | Replacement | AB Positive | Positive | Negative | Negative | Negative | Negative |
| 13 | 22 | M | | Replacement | O Positive | Negative | Negative | Negative | Positive | Negative |
| 14 | 34 | M | | Replacement | A Positive | Negative | Negative | Positive | Negative | Negative |
| 15 | 26 | M | | Replacement | AB Positive | Negative | Positive | Positive | Negative | Negative |
| 16 | 34 | M | Voluntary | | O Positive | Negative | Positive | Negative | Negative | Negative |
| 17 | 25 | M | Voluntary | | A Positive | Negative | Positive | Negative | Negative | Negative |
| 18 | 19 | M | | Replacement | A Negative | Negative | Positive | Negative | Negative | Negative |
| 19 | 36 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 20 | 42 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 21 | 42 | M | Voluntary | | O Positive | Negative | Positive | Negative | Negative | Negative |
| 22 | 21 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 23 | 22 | M | Voluntary | | O Positive | Negative | Positive | Negative | Negative | Negative |
| 24 | 25 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 25 | 22 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 26 | 37 | M | | Replacement | B Positive | Negative | Negative | Positive | Negative | Negative |
| 27 | 46 | M | Voluntary | | A Positive | Negative | Positive | Negative | Negative | Negative |
| 28 | 42 | M | | Replacement | O Positive | Negative | Positive | Positive | Negative | Negative |
| 29 | 31 | M | Voluntary | | AB Positive | Negative | Negative | Positive | Negative | Negative |
| 30 | 28 | M | | Replacement | A Negative | Positive | Negative | Negative | Negative | Negative |
| 31 | 36 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 32 | 26 | M | Voluntary | | AB Positive | Positive | Negative | Negative | Negative | Negative |
| 33 | 25 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 34 | 22 | M | Voluntary | | A Positive | Negative | Positive | Negative | Negative | Negative |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 40 | M | | Replacement | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 36 | 32 | M | | Replacement | A Positive | Negative | Negative | Positive | Negative | Negative |
| 37 | 27 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 38 | 26 | M | Voluntary | | A Positive | Negative | Positive | Negative | Negative | Negative |
| 39 | 21 | M | Voluntary | | B Positive | Negative | Positive | Negative | Negative | Negative |
| 40 | 26 | M | | Replacement | B Positive | Negative | Positive | Positive | Negative | Negative |
| 41 | 44 | M | | Replacement | AB Positive | Positive | Positive | Negative | Negative | Negative |
| 42 | 30 | M | Voluntary | | B Positive | Negative | Positive | Negative | Negative | Negative |
| 43 | 22 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 44 | 31 | M | Voluntary | | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 45 | 22 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 46 | 23 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 47 | 35 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 48 | 35 | M | Voluntary | | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 49 | 38 | M | Voluntary | | B Positive | Negative | Positive | Negative | Negative | Negative |
| 50 | 19 | M | Voluntary | | A Positive | Negative | Positive | Negative | Negative | Negative |
| 51 | 36 | M | | Replacement | A Positive | Positive | Negative | Negative | Negative | Negative |
| 52 | 36 | M | Voluntary | | B Positive | Negative | Positive | Negative | Negative | Negative |
| 53 | 30 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 54 | 28 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 55 | 42 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 56 | 40 | M | | Replacement | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 57 | 35 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 58 | 28 | M | | Replacement | B Positive | Negative | Negative | Positive | Negative | Negative |
| 59 | 32 | M | Voluntary | | O Negative | Negative | Positive | Negative | Negative | Negative |
| 60 | 27 | M | | Replacement | B Positive | Positive | Negative | Negative | Negative | Negative |
| 61 | 31 | M | | Replacement | O Positive | Positive | Negative | Negative | Negative | Negative |
| 62 | 34 | M | | Replacement | B Positive | Positive | Negative | Negative | Negative | Negative |
| **JULY 2014 – JUNE 2015** | | | | | | | | | | |
| 1 | 30 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 2 | 27 | M | | Replacement | AB Positive | Positive | Negative | Negative | Negative | Negative |
| 3 | 28 | M | | Replacement | O Positive | Negative | Negative | Negative | Positive | Negative |
| 4 | 26 | M | | Replacement | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 5 | 24 | M | Voluntary | | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 6 | 22 | M | Voluntary | | O Positive | Positive | Negative | Negative | Negative | Negative |
| 7 | 35 | M | | Replacement | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 8 | 30 | M | Voluntary | | B Positive | Negative | Positive | Negative | Negative | Negative |
| 9 | 26 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 10 | 36 | M | | Replacement | O Positive | Negative | Negative | Positive | Negative | Negative |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 11 | 38 | F | | Replacement | A Negative | Positive | Negative | Negative | Negative | Negative |
| 12 | 35 | M | Voluntary | | B Positive | Negative | Negative | Positive | Negative | Negative |
| 13 | 28 | F | | Replacement | B Positive | Negative | Negative | Positive | Negative | Negative |
| 14 | 32 | M | Voluntary | | O Positive | Positive | Negative | Negative | Negative | Negative |
| 15 | 22 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 16 | 24 | M | | Replacement | AB Negative | Negative | Positive | Negative | Negative | Negative |
| 17 | 21 | M | | Replacement | B Positive | Negative | Negative | Positive | Negative | Negative |
| 18 | 37 | M | Voluntary | | O Positive | Negative | Positive | Negative | Negative | Negative |
| 19 | 29 | M | | Replacement | O Positive | Positive | Negative | Negative | Negative | Negative |
| 20 | 19 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 21 | 32 | M | | Replacement | B Positive | Negative | Negative | Positive | Negative | Negative |
| 22 | 34 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 23 | 28 | M | Voluntary | | A Positive | Negative | Positive | Negative | Negative | Negative |
| 24 | 20 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 25 | 38 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 26 | 21 | M | Voluntary | | A Negative | Negative | Positive | Negative | Negative | Negative |
| 27 | 40 | M | Voluntary | | A Positive | Negative | Positive | Negative | Negative | Negative |
| 28 | 22 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 29 | 23 | M | Voluntary | | O Positive | Negative | Positive | Negative | Negative | Negative |
| 30 | 45 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 31 | 48 | F | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 32 | 51 | M | | Replacement | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 33 | 35 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 34 | 38 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 35 | 31 | M | | Replacement | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 36 | 34 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 37 | 31 | M | Voluntary | | A Positive | Negative | Positive | Negative | Negative | Negative |
| 38 | 47 | M | | Replacement | O Positive | Negative | Negative | Positive | Negative | Negative |
| 39 | 25 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 40 | 24 | M | | Replacement | A Positive | Negative | Negative | Positive | Negative | Negative |
| 41 | 33 | M | Voluntary | | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 42 | 23 | M | | Replacement | O Negative | Negative | Positive | Negative | Negative | Negative |
| 43 | 28 | M | | Replacement | A Negative | Positive | Negative | Negative | Negative | Negative |
| 44 | 23 | M | Voluntary | | O Positive | Negative | Positive | Negative | Negative | Negative |
| 45 | 22 | M | | Replacement | A Positive | Negative | Negative | Positive | Negative | Negative |
| 46 | 24 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 47 | 34 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 48 | 28 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 49 | 45 | M | Voluntary | | A Positive | Positive | Negative | Negative | Negative | Negative |

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| 50 | 20 | M | | Replacement | AB Positive | Negative | Negative | Negative | Positive | Negative |
| 51 | 26 | M | | Replacement | O Positive | Negative | Negative | Positive | Negative | Negative |
| 52 | 23 | M | | Replacement | A Positive | Negative | Negative | Positive | Negative | Negative |
| 53 | 35 | M | Voluntary | | B Positive | Negative | Negative | Positive | Negative | Negative |
| 54 | 32 | M | | Replacement | O Negative | Negative | Negative | Positive | Negative | Negative |
| 55 | 35 | M | | Replacement | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 56 | 25 | M | | Replacement | AB Positive | Negative | Negative | Negative | Positive | Negative |
| 57 | 27 | M | Voluntary | | O Positive | Negative | Positive | Negative | Negative | Negative |
| 58 | 24 | M | | Replacement | B Positive | Negative | Negative | Negative | Positive | Negative |
| 59 | 40 | M | Voluntary | | A Positive | Negative | Positive | Negative | Negative | Negative |
| 60 | 27 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 61 | 35 | M | | Replacement | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 62 | 34 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 63 | 33 | M | | Replacement | O Positive | Positive | Negative | Negative | Negative | Negative |
| 64 | 35 | M | | Replacement | O Positive | Negative | Negative | Negative | Positive | Negative |
| 65 | 33 | M | Voluntary | | AB Negative | Negative | Negative | Positive | Negative | Negative |
| 66 | 28 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 67 | 45 | M | Voluntary | | O Positive | Negative | Positive | Negative | Negative | Negative |
| 68 | 42 | M | | Replacement | B Positive | Negative | Negative | Positive | Negative | Negative |
| 69 | 34 | M | | Replacement | A Positive | Negative | Negative | Positive | Negative | Negative |
| 70 | 33 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 71 | 35 | M | | Replacement | O Positive | Negative | Negative | Positive | Negative | Negative |
| 72 | 59 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 73 | 23 | M | Voluntary | | A Positive | Positive | Negative | Negative | Negative | Negative |
| 74 | 27 | M | | Replacement | O Positive | Negative | Negative | Positive | Negative | Negative |
| 75 | 51 | M | Voluntary | | O Positive | Negative | Positive | Negative | Negative | Negative |
| 76 | 32 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 77 | 35 | M | Voluntary | | B Positive | Negative | Positive | Negative | Negative | Negative |
| 78 | 34 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 79 | 31 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 80 | 36 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 81 | 32 | M | | Replacement | O Positive | Negative | Negative | Positive | Negative | Negative |
| **JULY 2015 – JUNE 2016** | | | | | | | | | | |
| 1 | 30 | M | Voluntary | | A Positive | Negative | Positive | Negative | Negative | Negative |
| 2 | 35 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 3 | 24 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 4 | 35 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 5 | 26 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 6 | 24 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |

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| 7 | 35 | M | | Replacement | A Negative | Negative | Positive | Negative | Negative | Negative |
| 8 | 38 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 9 | 24 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 10 | 21 | M | Voluntary | | B Positive | Negative | Positive | Negative | Negative | Negative |
| 11 | 32 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 12 | 40 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 13 | 23 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 14 | 23 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 15 | 44 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 16 | 22 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 17 | 32 | M | Voluntary | | A Positive | Negative | Positive | Negative | Negative | Negative |
| 18 | 24 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 19 | 26 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 20 | 24 | M | Voluntary | | O Positive | Negative | Positive | Negative | Negative | Negative |
| 21 | 30 | M | | Replacement | O Negative | Negative | Positive | Negative | Negative | Negative |
| 22 | 32 | M | | Replacement | A Positive | Positive | Negative | Negative | Negative | Negative |
| 23 | 45 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 24 | 26 | M | Voluntary | | B Positive | Negative | Positive | Negative | Negative | Negative |
| 25 | 35 | M | | Replacement | A Negative | Negative | Positive | Negative | Negative | Negative |
| 26 | 33 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 27 | 28 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 28 | 31 | M | | Replacement | O Positive | Negative | Negative | Negative | Positive | Negative |
| 29 | 23 | M | Voluntary | | B Positive | Negative | Positive | Negative | Negative | Negative |
| 30 | 32 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 31 | 27 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 32 | 20 | M | | Replacement | A Positive | Negative | Negative | Positive | Negative | Negative |
| 33 | 35 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 34 | 30 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 35 | 25 | M | Voluntary | | B Positive | Negative | Positive | Negative | Negative | Negative |
| 36 | 32 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 37 | 22 | M | | Replacement | A Positive | Positive | Negative | Negative | Negative | Negative |
| 38 | 22 | M | | Replacement | O Positive | Negative | Negative | Positive | Negative | Negative |
| 39 | 20 | M | Voluntary | | B Positive | Positive | Negative | Negative | Negative | Negative |
| 40 | 34 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 41 | 25 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 42 | 33 | M | | Replacement | AB Positive | Negative | Negative | Positive | Negative | Negative |
| 43 | 28 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 44 | 28 | F | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 45 | 22 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |

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| 46 | 22 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 47 | 26 | M | Voluntary | | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 48 | 34 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 49 | 28 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 50 | 32 | M | | Replacement | A Positive | Positive | Negative | Negative | Negative | Negative |
| 51 | 29 | M | | Replacement | A Positive | Negative | Negative | Negative | Positive | Negative |
| 52 | 23 | M | | Replacement | A Positive | Positive | Negative | Negative | Negative | Negative |
| 53 | 42 | M | Voluntary | | B Positive | Negative | Positive | Negative | Negative | Negative |
| 54 | 22 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 55 | 25 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 56 | 30 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 57 | 35 | M | | Replacement | B Positive | Negative | Negative | Negative | Positive | Negative |
| 58 | 28 | M | Voluntary | | O Positive | Negative | Positive | Negative | Negative | Negative |
| 59 | 35 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 60 | 40 | M | | Replacement | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 61 | 31 | F | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 62 | 26 | M | Voluntary | | O Positive | Negative | Positive | Negative | Negative | Negative |
| 63 | 34 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 64 | 19 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 65 | 35 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 66 | 40 | M | | Replacement | AB Positive | Positive | Negative | Negative | Negative | Negative |
| 67 | 53 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 68 | 26 | M | Voluntary | | b Negative | Negative | Positive | Negative | Negative | Negative |
| 69 | 25 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 70 | 24 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 71 | 30 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 72 | 43 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 73 | 40 | M | | Replacement | B Positive | Positive | Negative | Negative | Negative | Negative |
| 74 | 21 | M | Voluntary | | B Positive | Positive | Negative | Negative | Negative | Negative |
| 75 | 23 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 76 | 28 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 77 | 30 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 78 | 32 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 79 | 40 | M | | Replacement | B Positive | Negative | Negative | Positive | Negative | Negative |
| 80 | 23 | M | Voluntary | | O Positive | Negative | Positive | Negative | Negative | Negative |
| 81 | 40 | M | | Replacement | A Positive | Positive | Negative | Negative | Negative | Negative |
| 82 | 26 | M | | Replacement | A Positive | Positive | Positive | Negative | Negative | Negative |
| 83 | 22 | M | | Replacement | A Positive | Positive | Negative | Negative | Negative | Negative |
| 84 | 22 | M | Voluntary | | A Positive | Negative | Negative | Positive | Negative | Negative |

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| 85 | 29 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 86 | 31 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 87 | 35 | M | Voluntary | | O Positive | Negative | Positive | Negative | Negative | Negative |
| 88 | 28 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 89 | 24 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 90 | 30 | M | | Replacement | A Positive | Positive | Negative | Negative | Negative | Negative |
| 91 | 32 | M | | Replacement | AB Negative | Negative | Negative | Positive | Negative | Negative |
| 92 | 42 | M | | Replacement | O Negative | Negative | Positive | Negative | Negative | Negative |
| 93 | 38 | M | Voluntary | | B Positive | Negative | Positive | Negative | Negative | Negative |
| 94 | 25 | M | Voluntary | | B Positive | Positive | Negative | Negative | Negative | Negative |
| 95 | 26 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 96 | 28 | M | | | O Positive | Negative | Positive | Negative | Negative | Negative |
| 97 | 22 | M | | Replacement | A Negative | Positive | Negative | Negative | Negative | Negative |
| 98 | 43 | M | Voluntary | | A Positive | Negative | Negative | Positive | Negative | Negative |
| 99 | 30 | M | | Replacement | B Positive | Positive | Negative | Negative | Negative | Negative |
| 100 | 22 | M | | Replacement | B Negative | Negative | Positive | Negative | Negative | Negative |
| 101 | 25 | M | Voluntary | | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 102 | 26 | M | | Replacement | B Negative | Negative | Positive | Negative | Negative | Negative |
| 103 | 28 | M | Voluntary | | B Positive | Positive | Negative | Negative | Negative | Negative |
| 104 | 40 | M | | Replacement | O Positive | Negative | Positive | Positive | Negative | Negative |
| 105 | 28 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 106 | 25 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 107 | 30 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 108 | 32 | M | Voluntary | | A Positive | Negative | Positive | Negative | Negative | Negative |
| 109 | 22 | M | Voluntary | | A Positive | Negative | Positive | Negative | Negative | Negative |
| **JULY 2016 – JUNE 2017** | | | | | | | | | | |
| 1 | 30 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 2 | 40 | M | | Replacement | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 3 | 26 | M | Voluntary | | B Positive | Negative | Positive | Negative | Negative | Negative |
| 4 | 22 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 5 | 22 | M | | Replacement | O Positive | Negative | Negative | Positive | Negative | Negative |
| 6 | 29 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 7 | 31 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 8 | 35 | F | | Replacement | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 9 | 24 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 10 | 21 | M | Voluntary | | B Positive | Negative | Positive | Negative | Negative | Negative |
| 11 | 32 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 12 | 40 | M | | Replacement | A Positive | Negative | Negative | Positive | Negative | Negative |
| 13 | 23 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |

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| 14 | 24 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 15 | 30 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 16 | 32 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 17 | 42 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 18 | 38 | M | Voluntary | | O Positive | Negative | Positive | Negative | Negative | Negative |
| 19 | 25 | M | | Replacement | A Positive | Negative | Negative | Positive | Negative | Negative |
| 20 | 26 | M | | Replacement | O Positive | Negative | Negative | Positive | Negative | Negative |
| 21 | 28 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 22 | 22 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 23 | 43 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 24 | 30 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 25 | 22 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 26 | 33 | M | Voluntary | | O Negative | Negative | Positive | Negative | Negative | Negative |
| 27 | 28 | F | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 28 | 31 | M | | Replacement | O Positive | Negative | Negative | Positive | Negative | Negative |
| 29 | 23 | M | | Replacement | B Positive | Negative | Negative | Negative | Positive | Negative |
| 30 | 32 | M | Voluntary | | A Positive | Positive | Negative | Negative | Negative | Negative |
| 31 | 27 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 32 | 20 | M | | Replacement | O Positive | Positive | Negative | Negative | Negative | Negative |
| 33 | 35 | M | | Replacement | AB Positive | Positive | Negative | Negative | Negative | Negative |
| 34 | 30 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 35 | 25 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 36 | 32 | M | Voluntary | | O Positive | Negative | Positive | Negative | Negative | Negative |
| 37 | 22 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 38 | 22 | M | Voluntary | | B Positive | Negative | Negative | Positive | Negative | Negative |
| 39 | 20 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 40 | 34 | M | | Replacement | AB Positive | Positive | Negative | Negative | Negative | Negative |
| 41 | 25 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 42 | 40 | M | Voluntary | | A Positive | Negative | Positive | Negative | Negative | Negative |
| 43 | 28 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 44 | 28 | M | Voluntary | | B Positive | Negative | Positive | Negative | Negative | Negative |
| 45 | 22 | M | | Replacement | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 46 | 22 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 47 | 26 | F | Voluntary | | B Positive | Negative | Positive | Negative | Negative | Negative |
| 48 | 34 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 49 | 28 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 50 | 32 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 51 | 29 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 52 | 23 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |

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| 53 | 25 | M | Voluntary | | B Positive | Negative | Positive | Negative | Negative | Negative |
| 54 | 24 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 55 | 30 | M | Voluntary | | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 56 | 43 | M | | Replacement | B Positive | Negative | Negative | Positive | Negative | Negative |
| 57 | 40 | M | | Replacement | B Positive | Positive | Negative | Negative | Negative | Negative |
| 58 | 21 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 59 | 23 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 60 | 28 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 61 | 30 | M | | Replacement | B Negative | Negative | Positive | Negative | Negative | Negative |
| 62 | 32 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 63 | 40 | M | | Replacement | B Negative | Negative | Positive | Negative | Negative | Negative |
| 64 | 23 | M | | Replacement | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 65 | 40 | M | Voluntary | | A Positive | Negative | Positive | Negative | Negative | Negative |
| 66 | 26 | M | | Replacement | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 67 | 53 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 68 | 26 | M | | Replacement | O Positive | Positive | Negative | Negative | Negative | Negative |
| **JULY 2017 – JUNE 2018** | | | | | | | | | | |
| **1** | 25 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 2 | 32 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| **3** | 28 | F | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 4 | 33 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| **5** | 23 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 6 | 32 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| **7** | 27 | M | Voluntary | | A Positive | Negative | Positive | Negative | Negative | Negative |
| 8 | 20 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| **9** | 33 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 10 | 21 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| **11** | 25 | M | Voluntary | | O Positive | Negative | Positive | Negative | Negative | Negative |
| 12 | 32 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| **13** | 30 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 14 | 22 | M | Voluntary | | B Positive | Negative | Positive | Negative | Negative | Negative |
| **15** | 29 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 16 | 34 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| **17** | 25 | M | | Replacement | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 18 | 40 | M | Voluntary | | AB Positive | Negative | Positive | Negative | Negative | Negative |
| **19** | 28 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 20 | 25 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| **21** | 22 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 22 | 31 | M | | Replacement | O Positive | Negative | Negative | Negative | Positive | Negative |

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| **23** | 26 | F | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 24 | 34 | M | | Replacement | O Positive | Positive | Negative | Negative | Negative | Negative |
| **25** | 28 | M | | Replacement | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 26 | 40 | F | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| **27** | 29 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 28 | 23 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| **29** | 25 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| 30 | 24 | M | Voluntary | | B Positive | Negative | Positive | Negative | Negative | Negative |
| **31** | 30 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 32 | 43 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| **33** | 40 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 34 | 21 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| **35** | 23 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 36 | 28 | M | | Replacement | O Negative | Negative | Positive | Negative | Negative | Negative |
| **37** | 30 | M | | Replacement | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 38 | 32 | M | Voluntary | | O Negative | Negative | Positive | Negative | Negative | Negative |
| **39** | 40 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 40 | 23 | M | | Replacement | B Positive | Negative | Positive | Negative | Negative | Negative |
| **41** | 36 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 42 | 26 | M | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 43 | 48 | M | | Replacement | O Positive | Negative | Negative | Positive | Negative | Negative |
| 44 | 26 | M | Voluntary | | A Positive | Negative | Positive | Negative | Negative | Negative |
| 45 | 27 | F | | Replacement | O Positive | Negative | Positive | Negative | Negative | Negative |
| 46 | 30 | M | | Replacement | AB Positive | Negative | Positive | Negative | Negative | Negative |
| 47 | 24 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |
| 48 | 22 | M | | Replacement | A Positive | Negative | Negative | Positive | Negative | Negative |
| 49 | 25 | M | | Replacement | A Positive | Negative | Positive | Negative | Negative | Negative |