**Improving the Screening of Blood Donors with Syphilis Rapid Diagnostic Test (RDT) and Rapid Plasma Reagin (RPR) in Low and Middle Income Countries (LMIC)**

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***Structured abstract***

**Background:** Syphilis testing conventionally relies on a combination of non-treponemal and treponemal tests. The primary objective of this study was to describe the positive predictive value (PPV) of a screening algorithm in a combination of treponemal rapid diagnostic test (RDT) and rapid plasma reagin (RPR) test at Komfo Anokye Teaching Hospital (KATH), Ghana.

**Materials and Methods:** From February 2014 to January 2015, five mL of venous blood sample were taken from 16016 blood donors and tested with a treponemal RDT. Five mL of venous blood were taken from a consenting 526 initial syphilis sero-reactive blood donors. These RDT reactive samples were confirmed with an algorithm applying Vitros®/Abbott-Architect® algorithm as gold standard.

**Results:** 478 of 526 RDT reactive donors were confirmed syphilis positive making a PPV of 90.9%. Of the 172 (32.7%) donors which were also RPR positive, 167 were confirmed resulting in a PPV of 97.1%. The PPV of the combined RDT and RPR (suspected active syphilis) testing algorithm was highest among donors at enhanced risk of syphilis: family/replacement donors (99.9 %) and among voluntary donors above 25 years (98.6 %).

**Discussion:** Screening of blood donors by combining syphilis RDT and RPR with relatively good PPV may provide a reasonable technology for LMIC that has limited capacity for testing and can contribute to improve blood safety with a minimal loss of donors.

***Introduction***

Syphilis screening is a big challenge in many low and middle-income countries (LMIC) that have limited capacity for testing. High syphilis prevalence among healthy blood donors in Africa aggravates the problem in this region. Techniques for syphilis testing are very problematic and conventionally rely on a combination of non-treponemal and treponemal tests. The non-treponemal antibody tests for donor screening include the venereal disease research laboratory (VDRL) ([Harris, Rosenberg et al. 1948](#_ENREF_7)) and the rapid plasma reagin (RPR) ([Larsen, Pope et al. 1998](#_ENREF_12)). The advantages are that, these tests are inexpensive, fast, simple to perform and more sensitive ([Montoya, Lukehart et al. 2006](#_ENREF_15)). They are able to identify the contaminated blood donors few days before the treponemal test and thus useful for acute infection. Moreover, quantifiable titers can establish a baseline to evaluate treatment response ([Sena, White et al. 2010](#_ENREF_27)), as they usually revert to negative after successful treatment ([Larsen 1989](#_ENREF_11), [Romanowski, Sutherland et al. 1991](#_ENREF_20), [Larsen, Pope et al. 1998](#_ENREF_12)). However, VDRL and RPR cannot be automated and are therefore time-consuming if used for large scale testing. In addition, results may be difficult to interpret and require sufficient training of health personnel to ensure correct testing and interpretation. Another major problem when using non-treponemal tests is the possibility of biological false positive reactions due to cross-reactivity with molecules in other conditions such as viral infections, pregnancy, malignant neoplasms, autoimmune diseases, and advanced age ([Larsen, Steiner et al. 1995](#_ENREF_13), [Sena, White et al. 2010](#_ENREF_28)).

Treponemal tests for donor screening classically included *T*. *pallidum* haemagglutination assay (TPHA), *T*. *pallidum* passive particle agglutination (TP-PA) assay, and the fluorescent treponemal antibody absorption (FTA-ABS) ([Hunter, Deacon et al. 1964](#_ENREF_8))*.* However newer automated treponemal tests have reduced running costs and provide objective readings, making them useful for large blood centres. Treponemal tests typically remain positive even after treatment ([Schroeter, Lucas et al. 1973](#_ENREF_26), [Gerber, Krell et al. 1997](#_ENREF_5), [Larsen, Pope et al. 1998](#_ENREF_12)), implying that a donor previously diagnosed for syphilis cannot be distinguished from a new or untreated case of syphilis.

In developing countries and areas with limited resources, laboratory facilities are often unavailable for standard automated syphilis tests. Given the barriers to automated testing, many resource-limited countries are resorting to syphilis rapid diagnostic tests (RDTs) for transfusion-transmitted infection (TTI) screening ([Kaur and Kaur 2015](#_ENREF_9)). Although quality RDTs hold potential for increasing the safety of the region's blood supply, uncertainty surrounding the performance of some RDTs in the field has increased debate regarding their application to TTI screening ([Scheiblauer, El-Nageh et al. 2006](#_ENREF_25), [Laperche and Francophone African Group for Research in Blood 2013](#_ENREF_10), [Mbanya 2013](#_ENREF_14)).

Some rapid tests are highly sensitive and specific ([Owusu-Ofori, Temple et al. 2005](#_ENREF_17), [Sena, White et al. 2010](#_ENREF_27)), but cannot differentiate between active and treated syphilis; others may give false positive reactions ([van Dommelen, Smismans et al. 2008](#_ENREF_29)). Most rapid tests detect IgM, IgG and IgA antibodies and involve immunochromatographic strips in which one or multiple *T.pallidum* recombinant antigens are applied to nitrocellulose strips as a capture reagent. Irrespective of the advantages of these rapid tests, if they have a low positive predictive value (PPV) - high false positive rate – and are used in blood banks, then apparently many donors will be deferred when they carry no risk to the blood supply. Conversely if the PPV is high (low false positive rate) then few donors with no risk to the blood supply will be deferred. This is really important in LMIC settings where blood supplies are critical coupled with a high burden of TTIs.

The Transfusion Medicine Unit (TMU) of the Komfo Anokye Teaching Hospital (KATH), has been practicing pre-donation screening with viral RDTs for blood donors since 2000 ([Owusu-Ofori, Temple et al. 2005](#_ENREF_17), [Owusu-Ofori, Temple et al. 2005](#_ENREF_18), [Allain, Sarkodie et al. 2010](#_ENREF_2)). KATH operates on two types of blood donors namely; voluntary non-remunerated blood donors (VNRBD) constituting 70% of blood collection, and family replacement donors (FRD) constituting 30% of blood collection for over a decade now. The decision to introduce syphilis screening as part of pre-donation screening was decided by the hospital transfusion committee in 2012. However, a loss of blood for transfusion of 7% by syphilis RDT reactive donors was a threat to blood supply and an algorithm which combined robustness, safety and minimal discard rates was needed. KATH implemented an algorithm consisting of syphilis testing by RDT before blood collection followed by RPR (IMMUTREP RPR, Omega Diagnostics – Scotland, UK) testing of syphilis RDT reactive donors ([Sarkodie, Ullum et al. 2016](#_ENREF_23)). The presumption was that by discarding only donors who were also positive for RPR only donors at risk of active syphilis were rejected whereas donors with previous well treated syphilis could continue to donate and their blood would be released for transfusion. One of the risks is that although combining two different syphilis tests the algorithm might still defer too many donors due to false reactivity in the two tests. In that case the algorithm would still compromise blood supply and it would expose donors to unneeded worries, stigma and therapy.

In order to study this aspect, the present study validates the PPV of the implemented algorithm by applying a golden standard retest algorithm combining two different automated anti-TP immune assays of 526 syphilis RDT reactive donors in improving blood donor screening.

***Materials and Methods***

We conducted a descriptive cross-sectional study between February 2014 and January 2015. 16016 blood donors were initially tested according to routine standard operational procedures with a treponemal RDT (Fortress® Diagnostics Limited – Antrim, UK). Sensitivity and specificity of the Fortress® RDT are stated to be 99.7% and 99.6% respectively - for the qualitative detection of antibodies (IgG and IgM) to *T. pallidum* in serum or plasma. Five ml of venous blood were taken from consenting 526 initial Fortress® RDT syphilis sero-reactive blood donors. All these samples were further tested according to routine standard procedures with RPR (BD Macro-VueTM Card test, New Jersey, USA) to identify potential active syphilis infections.

For golden standard confirmation of the Fortress® RDT, al1 526 RDT reactive samples were subsequently retested in an algorithm combining two automated treponemal immunoassays and a treponemal immunoblot. Initial retesting was performed by Vitros® Syphilis *Treponema Pallidum* Antibody (TPA) chemiluminescence immunoassay using the Vitros ECi/ECiQ Immunodiagnostic Systems described elsewhere ([Gonzalez, Fernandez et al. 2015](#_ENREF_6)). Briefly the Vitros® Syphilis TPA assay is a qualitative assay that detects total antibodies (IgG and IgM) to *Treponema pallidum* (TP) reacting with biotinylated and horseradish peroxidase (HRP)-labeled recombinant TP antigens TP15, TP17, TP47 and bound to streptavidin-coated wells. The illuminating reaction detected from the bound HRP-conjugates, is directly proportional to the concentration of anti-TP antibodies, and high signal samples (signal at Cutoff (S/CO) >100) was considered confirmed (figure 1). The assay was mainly validated in a western population (data not shown) with a specificity of 99.8% (CI 98.7-100%) and a sensitivity of 100% using Syphilis Mixed Titer Performance Panel PSS202 (BBI Diagnostics) and clinical samples from known Syphilis treated patients- of both Caucasian and African origin.

All Vitros low reactive samples (S/CO <100) were additionally tested with another qualitative anti-TP immunoassay Architect® Syphilis TP (Abbott Diagnostics, Wiesbaden, Germany) also detecting antibodies binding to the recombinant TP antigens TpN15, TpN17 and TpN47 (figure 1). A reactive Fortress® RDT sample was considered confirmed positive of specific anti-TP antibodies, if the Vitros Syphilis TPA was highly reactive (S/CO > 100) or if Vitros Syphilis TPA was low reactive (1< S/CO <100) and Architect® Syphilis TP reactive.

As a quality control measure 78 out of 526 syphilis RDT sero-reactive samples were further tested in a line immunoassay (LIA) (Furijebio, Ghent, Belgium) (Figure 2). The LIA detects individuals binding to the same recombinant TP antigens TpN15, TpN17 as well as to TpN47 and a synthetic peptide TmpA derived from *T. pallidum* proteins ([Ebel, Vanneste et al. 2000](#_ENREF_3)).

Syphilis sero-reactive donors were informed of the study and signed an informed consent form according to the study protocol approved by the Ethics Committees of Kwame Nkrumah University of Science and Technology (KNUST) Kumasi, Ghana (CHRPE/AP/423/13) and Liverpool School of Tropical Medicine, UK (18/02/2014).

Background data were recorded into a spreadsheet consisting of sex, age, number of donations, donor type and routine testing results. Data were then exported into STATA (STATACORP, Texas, version 12.0) for analysis. We estimated positive predictive values by calculating proportions and providing their respective confidence intervals. Multi-variable logistic regression was performed on syphilis reactivity as an outcome. Age, sex and donor type were included as independent variables with results presented as odd ratios and 95% Confidence Intervals. A p-value of <0.05 denoted a statistically significant difference in all statistical comparisons.

***Results***

A total of 599 out of 16016 blood donors reacted to the syphilis Fortress® test making an estimated sero-prevalence of 3.7% (95% [CI] 3.5% - 4.1%). Seventy-three (12.2%) blood donors who reacted with the Fortress syphilis test were excluded from the study (figure 1) because 41 (6.8%) of them were co-infected with HBV, 15 (2.8%) were co-infected with HIV and seven (1.2%) were co-infected with HCV, while ten (1.7%) did not consent. Thus, 526 (3.3%) syphilis sero-reactive blood donors were included in the study of which 199 (37.8%) of them were voluntary non-remunerated blood donors (VNRBD) (95% [CI] 33.8% - 42.1%). Generally, blood donors tested were aged 16 to 59 years with a mean age of 25 (SD=9.1) compared with syphilis sero-reactive donors who showed a range of 17 to 53 years with a mean age of 31 years (p<0.001).

*PPV of Syphilis RDT reactive samples according to donor type*

Of the 526 RDT syphilis sero-reactive samples tested with Vitros®, 478 were reactive and confirmed by the algorithm making, a PPV of 90.9% (table 1). Similarly, the proportion or PPV of sero-reactive FRD (309/327, 94.5%) who was confirmed (table 2) was statistically significantly higher than that of confirmed sero-reactive VNRBD (169/199, 84.9%), (p=0.001) although there was 100% PPV in some age groups. Of the total blood donors tested, 10218 (63.8%) were VNRBD, of which 199 (1.95%) were syphilis sero-reactive (95% [CI] 1.73% - 2.28%) as shown in table 2. Of the 5798 FRD tested, 327 were syphilis sero-reactive, which was significantly higher [5.64% (95% [CI] 5.08% - 6.26%)] p<0.001 compared to VNRBD.

*PPV of syphilis RDT reactive samples according to donor age*

Syphilis RDT sero-reactive donors showed a range of 17 to 53 years with a mean age of 31 years. The PPV of the VNRBD ranges from 74.4% to 100% for the ages ranging between 17 and 55 years whilst that of FRD ranges from 91.2% to 100% for the same age difference (table 2). Generally, the PPVs of the syphilis confirmed reactive donors increase with age for all donor types except in VNRBD where those aged between 46 and 55 years have a relatively lower prevalence (3.17%) but higher PPV (100%). Although syphilis confirmed sero-prevalence of FRD was 5.33%, there was higher prevalence as the age increases. Similarly, prevalence for syphilis confirmed VNRBD although was 1.65%, there was a higher prevalence of 5.24% in the 36-45 age category.

*PPV of syphilis RDT and RPR reactive samples*

Of the 526 syphilis RDT reactive samples, 172 (32.7%) were RPR positive (95% [CI] 28.8% - 36.8%). Out of these, 167 were confirmed making a PPV of 97.1% (Table 1). Thus the PPV was higher among RDT and RPR reactives (97.1%) than in the total population of RDT reactives (90.9%) as shown in table 1. Conversely, the PPV was higher among FRD (99.1%) than in VNRBD (93.3%). More FRD and more donors at age 26 and over were RPR positive and increases with age as the PPV increases (Table 2). Similarly was the PPV of RDT and RPR dual reactive donors highest among FRD and among donors aged 26 or above (table 2). Out of the five RPR false positives, four were VNRBD out of which three aged 16 and 25 years and one aged 26 and 35 years, while the other one (FRD) was between 26 and 35 years. Additionally, 311 out of 354 (87.8%) RPR negative donors tested positive with Vitros®.

***The effect of age, sex and donor type on syphilis reactivity***

By multivariable logistic regression, we showed a positive association between syphilis reactivity and all included explanatory parameters: increased age, male sex and status as a family replacement donor (FRD) (Table 3). Effects of male sex and FRD status were similar whether a positive endpoint was defined as RDT+, RDT+ Vitros®+, RDT+ RPR+ or RDT+ Vitros®+ RPR+ (table 3). The effect of age was weaker for endpoints including RPR reactivity (table 3). Male sex was a stronger predictor of syphilis reactivity than status as a family replacement donor (table 3).

*Samples tested with INNO-LIA as quality control.*

Approximately 58% (28) of the samples which were negative with Vitros® test were tested with INNO-LIA. 25 of them were negative while the rest (3) were or Inconclusive. All 24 (~9%) samples which were Vitros® high reactives were confirmed with INNO-LIA and 25 of 26 low with Vitros® were confirmed with LIA, and one was inconclusive.

***Discussion***

Syphilis infection in blood donors continues to pose a major threat in many developing countries including Ghana ([Adjei, Kudzi et al. 2003](#_ENREF_1), [Sarkodie, Hassall et al. 2016](#_ENREF_22)). The Fortress syphilis RDT which was used for this study based on its performance characteristics has a sensitivity and specificity of 99.7% and 99.6% respectively according to the manufacturer. When compared to the gold standard in single testing, it gave a PPV of 90.9%. Similar syphilis RDTs in other studies gave a PPV of 95.2% with sensitivity and specificity as 93.6% and 92.5% respectively ([Sato, de Melo et al. 2003](#_ENREF_24)). As PPV relies on both test specification and disease prevalence it is not surprising that other studies have shown PPVs of some RDTs to be both lower and higher than this ([Pruett, Vermeulen et al. 2015](#_ENREF_19)). When combining a RDT with a nonspecific syphilis test in this case RPR, a much higher PPV was achieved (97.1%). Thus by combining the two tests both donors with confirmed but inactive TP infections and donors with unspecific RDT reactions could avoid deferral and they could therefore still contribute to blood supply. The key concept underlying blood safety especially in LMIC is the balance between blood supply and blood safety in the context of a poor blood supply, high prevalence of TTI compared and limited resources. As stated earlier, if there is a low PPV (high false positive rate) then many donors will be deferred when they carry no risk to blood supply. Furthermore, a screening test with a low PPV/high false positive rate has the potential to cause unnecessary harm to blood donors with fears and wrong information.

*The use of syphilis RDTs in resource-poor settings*

Pre-donation screening for TTIs with syphilis RDTs is a strategy that has been proposed for use in resource-poor, high-prevalence settings without access to a stable pool of low-risk donors ([Salawu and Murainah 2006](#_ENREF_21)). One of the reasons behind this was to reduce blood bag wastage and associated costs of consumables in collecting blood from donors which was not used because of positive screening tests. One study in Ghana demonstrated savings of more than $11,000 in blood bags and testing costs over a 1-year period using pre-donation screening ([Owusu-Ofori, Temple et al. 2005](#_ENREF_17)).

*Syphilis prevalence in Kumasi blood donors*

In this study, we found the prevalence of syphilis in Kumasi blood donor population with the use of RDT to be 3.7% which is not different from previous studies in Ghana ([Adjei, Kudzi et al. 2003](#_ENREF_1), [Erlichman, Hidalgo et al. 2006](#_ENREF_4), [Owusu-Ofori, Parry et al. 2011](#_ENREF_16)). Like other studies we found a higher rate of syphilis reactivity among FRD than among VNRBD. This was only partly explained through higher age and more males among FRD since FRD status was an independent positive predictor of syphilis reactivity in logistic regression analysis. There is an ongoing struggle to have 100% VNRBD in Ghana and elsewhere in Africa which if successful may reduce syphilis sero-reactivity. Despite this, family donations remain dominant on the African continent. The association between age and syphilis reactivity is most likely caused by a longer period of sexual exposure. However a cohort phenomenon with older donors being more exposed to yaws in childhood may also contribute. However the data confirms that younger first time donors are safer than older donors whereas the highest safety both with regards to infection risk and blood supply lies in a system of repeat donations as the main source of blood for transfusion ([Allain, Sarkodie et al. 2010](#_ENREF_2)).

*Syphilis seroreactivity and active syphilis in blood donors*

Our data suggest that a total of 167 or one percent of tested blood donors were confirmed syphilis RDT and RPR reactive. Our logistic regression data additionally indicate that there are independent effects of age, male sex and FRD donor type on syphilis seroreactivity. When considering RPR reactivity, the effect of age was smaller indicating that higher age is a stronger prediction of previous syphilis infections than of recent infections. In our previous published article ([Sarkodie, Ullum et al. 2016](#_ENREF_23)), the decrease in reactive samples from RDT to RPR is approximately 6 times compared to this study which is only approximately 3 times. This considerable discrepancy is probably due to changes in test kits. The RPR test kit used in the previous article, (IMMUTREP RPR, Omega Diagnostics – Scotland, UK) differs in sensitivity and specificity from the one used in this study (BD Macro-VueTM Card test – New Jersey, USA). Additionally, testing errors on the part of the laboratory scientists in both testing procedures could contribute to the discrepancy. Since a lot of blood is transfused without storage this may constitute a significant risk of syphilis transmission through transfusion as previously reported syphilis ([Owusu-Ofori, Parry et al. 2011](#_ENREF_16)). Our data thus support the combined use of RDT and RPR to detect active syphilis of potential blood donors, which would enable more focused, deferral of potential active syphilis cases for treatment. These cases of suspected active syphilis can be identified with a minimal loss of donors. It is important to repeat this study in other resource-poor settings where syphilis prevalence is high.

***Strengths and limitations***

The strength of this study is the description of real life performance with regards to PPV of a newly suggested combined syphilis testing algorithm combining a RDT and RPR for the identification of potential active syphilis. The algorithm used for golden standard confirmation was robust as it involved three different Treponema specific tests used sequentially to confirm weak and negative results. The INNO-LIA assay has been shown to provide highly reliable confirmatory diagnostic information of anti-TP antibodies ([Ebel, Vanneste et al. 2000](#_ENREF_3)) and was furthermore used as a quality control of the confirmation algorithm of anti-TP antibodies.

Three major limitations need to be mentioned. Firstly the assumption that RDT positive donors testing negative in RPR were without significant risk for transfusion was neither tested by recipient look back or by molecular testing of donors. Secondly the proportion of truly syphilis reactive donors missed by the initial RDT was not evaluated because of resource constraints.

Finally, we cannot assume infectivity among all confirmed RPR reactive donors.

***Conclusion***

In a blood bank system like the one in Kumasi, Ghana with relatively high prevalence of syphilis and where infrastructure to support formal laboratory testing is often lacking, syphilis screening with RDTs may provide a reasonable technology. The combination of both RDT and RPR reduces loss of donors and blood for transfusion. The combined RDT and RPR testing has a satisfactory high PPV meaning that unneeded loss of blood for transfusion and false syphilis diagnoses of donors are minimized. The high PPV of a combined RDT and RPR algorithm suggests that further routine confirmation of a donor deferred with dual RDT and RPR reactivity is not needed. This adds to the robustness and cost efficiency of the suggested TP screening algorithm.

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***Statement of Conflict of Interest***

None of the authors declare any conflict of interest regarding this manuscript.

***Authorship contributions***

* Francis Sarkodie designed the study, performed the research, analysed the data and wrote the manuscript.
* Oliver Hassall contributed to the design, data analysis and manuscript writing.
* Ellis Owusu-Dabo contributed to the design, data analysis and manuscript writing.
* Shirley Owusu-Ofori contributed to the design and manuscript writing.
* Imelda Bates contributed to the design, data analysis and manuscript writing.
* Ib C. Bygbjerg contributed to the design, data analysis and manuscript writing.
* Alex Owusu-Ofori contributed to the design and manuscript writing.
* Lene Holm Harritshøj contributed to the confirmatory testing, analysis and manuscript writing.
* Henrik Ullum contributed to the design, confirmatory testing, analysis and manuscript writing.

**Fig. 1 Algorithm for syphilis confirmatory testing of sero-reactive blood donors, Transfusion Medicine Unit, Komfo Anokye Teaching Hospital**

**Test**  **Blood donors**

16016

**RDT Positive, consent Positive, dropout Negative**

15417 (96.2%)

73 (0.5%)

526 (3.3%)

**RPR Positive** **Negative**

172/526 (32.7%)

354/526 (67.3%)

**Confirmed**

127/172 (73.8%)

131/354 (37.0%)

**Vitros**® **(S/CO≥100)**

180/354 (50.8%)

**Vitros® (1< S/CO <100)**

40/172 (23.3%)

**Architect**® **Pos**

311/354 (87.8%)

167/172 (97.1%)

**Total confirmed**

43/354 (12.2%)

5/172

(2.9%)

**Total not confirmed**

RDT; - rapid diagnostic test, RPR; - rapid plasma reagin, S/CO-; sample over cut-off

N/B-Architect® was performed on specimen that were low reactive with Vitros® (1< S/CO <100)

**Fig. 2 Quality control with INNOLIA for syphilis testing of sero-reactive blood donors, Transfusion Medicine Unit, Komfo Anokye Teaching Hospital**

**Vitros**® **S/CO≥100 Vitros**® **S/CO<100 Vitros**® **Negative**

26 tested

24 tested

28 tested

**INNOLIA Positive Positive Negative Negative IC**

3

25

1

25

24

Approximately 15 % of the samples tested with Vitros® were further tested with INNO-LIA as a quality control of the validation algorithm.

IC; - Inconclusive, S/CO-; sample over cut-off

**Table 1: Syphilis RDT sero-reactive blood donors**

|  |  |  |  |
| --- | --- | --- | --- |
| **RDT reactive Blood donors** | **RPR** | | **TOTAL (%)** |
| **Positive (%)** | **Negative (%)** |
| **Confirmed** | **167 (97.1)** | **311 (87.8)** | **478 (90.9)** |
| **unconfirmed** | **5 (2.9)** | **43 (12.2)** | **48 (9.1)** |
| **TOTAL** | **172 (32.7)** | **354 (67.3)** | **526** |

RDT;-rapid diagnostic test, RPR;-rapid plasma reagin, %;-percentage

**Table 2: Syphilis RDT and RPR sero-reactive blood donors confirmed with Vitros**® **TP stratified according to age**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Blood donors** | Total RDT tested  (%) | RDT +  (% ) | RDT+, Vitros®+  (%, PPV) | RDT+, RPR+  (%) | RDT+, RPR+, Vitros®+  (%, PPV) |
| **VNRBD**  **Age group (yrs.)**  **≤25**  **26-35**  **36-45**  **46-55**  **>56**  **GENDER**  **Females**  **Males** | 10218 (63.8)  7525 (73.6)  1750 (17.1)  687 (6.7)  221 (2.2)  35 (0.4)  3477 (34.0)  6741 (66.0) | 199 (1.95)  78 (1.04)  76 (4.34)  38 (5.53)  7 (3.17)  0  24 (0.69)  175 (2.60) | 169 (1.65, 84.9)  58 (0.77, 74.4)  68 (3.89, 89,5)  36 (5.24, 94.7)  7 (3.17, 100.0)  0  16 (0.46, 66.7)  153 (2.27, 87.4) | 60 (0.59)  27 (0.36)  23 (1.31)  9 (1.31)  1 (0.30)  0  6 (0.17)  54 (0.80) | 56 (0.55, 93.3)  24 (0.32, 88.9)  22 (1.26, 95.7)  9 (1.31, 100.0)  1 (0.30, 100.0)  0  6 (0.17, 100.0)  50 (0.74, 92.6) |
| **FRD**  **Age group (yrs.)**  **≤25**  **26-35**  **36-45**  **46-55**  **>56**  **GENDER**  **Females**  **Males** | 5798 (36.2)  1869 (32.2)  2405 (41.5)  1186 (20.4)  321 (5.5)  18 (0.4)  538 (9.3)  5260 (90.7) | 327 (5.64)  57 (3.05)  148 (6.15)  95 (8.01)  27 (8.41)  0  13 (2.42)  314 (5.97) | 309 (5.33, 94.5)  52 (2.78, 91.2)  139 (5.78, 93.9)  91(7.67, 95.8)  27 (8.41, 100.0)  0  13 (2.42, 100.0)  296 (5.63, 94.1) | 112 (2.12)  24 (1.28)  48 (2.00)  32 (2.70)  8 (2.50)  0  4 (0.74)  108 (2.05) | 111 (1.91, 99.1)  23 (1.23, 95.8)  48 (2.00, 100.0)  32 (2.70, 100.0)  8 (2.50, 100.0)  0  4 (0.74, 100.0)  107 (99.1) |
| **TOTAL** | 16016 | 526 (3.28) | 478 (2.98, 90.9) | 172 (1.07) | 167 (1.04, 97.1) |

RPR; - rapid plasma reagin, %;-percentage, VNRBD; - voluntary non-remunerated blood donors, FRD; - family replacement donors, RDT; - rapid diagnostic test, +; - positive, -; negative, PPV; - positive predictive value

**Table 3: Multiple variable logistic prediction of syphilis reactivity**

|  |  |  |  |
| --- | --- | --- | --- |
| **RDT +**  **526/16016 (3.28%)** | **Odds Ratio** | **P-values** | **[95% Conf. Interval]** |
| Age (years) | 1.04 | <0.001 | 1.03 1.05 |
| Gender (male) | 2.99 | <0.001 | 2.11 4.04 |
| Donor type (FRD) | 1.92 | <0.001 | 1.60 2.30 |
| **RDT+, Vitros®+**  **478/16016 (2.98%)** |  |  |  |
| Age (years) | 1.05 | <0.001 | 1.04 1.06 |
| Gender (males) | 3.41 | <0.001 | 2.29 5.08 |
| Donor type (FRD) | 1.75 | <0.001 | 1.44 2.14 |
| **RDT+, RPR+**  **172/16016 (1.07%)** |  |  |  |
| Age (years) | 1.03 | <0.001 | 1.02 1.05 |
| Gender (male) | 3.22 | <0.001 | 1.67 6.22 |
| Donor type (FRD) | 2.04 | <0.001 | 1.45 2.88 |
| **RDT+, RPR+, Vitros®+**  **167/16016 (1.04%)** |  |  |  |
| Age (years) | 1.03 | <0.001 | 1.02 1.05 |
| Gender (male) | 2.72 | <0.001 | 1.49 4.99 |
| Donor type (FRD) | 2.06 | <0.001 | 1.46 2.89 |

RPR; - rapid plasma reagin, %;-percentage, RDT; - rapid diagnostic test, +; - positive, FRD; family replacement donor

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