doi.org/10.36721/PJPS.2022.35.1.SP.375-380.1

The effect analysis and prevention effects of nucleic acid testing combined with enzyme linked immunosorbent assay on blood-borne diseases

Ruming Zhou[1] and Jihuan Yan[2]*
[1]Hebei General Hospital, Shijiazhuang, China
[2]Hebei International Travel Health Care Center, Shijiazhuang, Hebei, China

Abstract: To investigate the effect analysis and preventive effects of nucleic acid testing combined with enzyme-linked immunosorbent assay (ELISA) on blood-borne diseases. This study included 72335 blood samples that were collected in our hospital from March 2019 to March 2020. All the samples were tested for anti HIV (AIDS antibody), anti HCV (hepatitis C antibody), HBsAg (hepatitis B surface antigen) and anti TP (syphilis antibody) in blood respectively with two manufacturers' reagents. The results of anti HIV, anti HCV, HBsAg and anti TP of all samples were analyzed, and blood samples with 0.7≤ sample test value / critical value (s / CO) ≤ 3.0 were tested by ELISA and negative blood samples were tested by ELISA and nucleic acid testing. Then we analyzed the results of nucleic acid testing. 610 blood samples failed to pass the test of anti HIV, anti HCV, HBsAg and anti TP ELISA, accounting for 0.84% of the total numbers, including 100 blood samples with 1.0 ≤s/Co ≤3.0, 172 blood samples with S/CO > 3.0, 338 blood samples with 0.7≤ s/CO <1.0 and 71725 blood samples were qualified. We used nucleic acid testing to test 71725 qualified samples tested by ELISA and then there were 50 samples with positive HBV-DNA , accounting for 0.07% (50 / 71725), no one with positive HIV-RNA and positive HCV-RNA, accounting for 0.00% (0/71725). The positive rate of blood samples with HBsAg 0.7 ≤s/CO <1.0 tested by ELISA and nucleic acid testing was 7 (5.83%), which was significantly lower than 9 (20.93%) of blood samples with HBsAg 1.0≤ s / Co ≤ 3.0 tested by ELISA (X^2= 8.15, P<0.01). In the clinical test of blood-borne disease, the joint test of nucleic acid testing and enzyme-linked immunosorbent assay reduces the occurrence of virus omission, and plays a positive role in preventing from spreading diseases through blood transfusion.

Keywords: Blood, infectious diseases, nucleic acid testing, enzyme linked immunosorbent assay, effect, prevention.

INTRODUCTION

As we all know, blood-borne diseases are one of the major safety issues of clinical blood transfusion (Bushman *et al*., 2020; Dalkan *et al*., 2020; Saushkin *et al*., 2019). HIV (AIDS virus), HCV (hepatitis C virus), HBV (hepatitis B virus) and TP (syphilis virus), etc. are the main pathogenic microorganisms that affect people's health, mostly spread through blood (Behnke *et al*., 2019). At present, most blood collection and supply institutions in China choose enzyme-linked immunosorbent assay (ELISA) to test anti-HIV, anti-HCV, HBsA and anti-TP in blood to confirm whether there are the above viruses in blood(Sun *et al*., 2020). Although the ELISA method features simple operation, specificity and sensitivity, it exists major defects for blood with virus mutations, infections in window phase and infections in immune silencing phase, which easily leads to omission and further poses a threat to blood transfusion. In recent years, with the continuous development of science and medical technology, nucleic acid testing has gradually been applied to clinical testing and we have achieved good clinical results (Chileshe *et al*., 2019; Gadermaier *et al*., 2019; Saravanan *et al*., 2020). This study aims to explore the effects and prevention effects of nucleic acid testing combined with ELISA on blood-borne diseases and provide an important reference for reducing the incidence of blood-borne diseases.

**Corresponding author:* e-mail: shajiaodui125@126.com

MATERIALS AND METHODS

General materials

72,335 blood samples that were collected in our hospital from March 2019 to March 2020 were selected in this study, including 37,833 male blood donors and 34,502 female blood donors aging from 21 to 58 years old, with an average age of (28.44±6.73) years old; and their weight is from 45kg to 81kg, with an average weight of (58.32±6.28)kg.

Inclusion criteria

(1) All blood donors met the standards of *Physical Examination Requirements for Blood Donors*;
(2) Blood donors aged ≥18 years old;
(3) This study had been approved by the ethics committee of our hospital and the blood donors and their families were informed and signed a consent form letter.

Instruments and reagents

FIA8000 automatic enzyme immunoassay analyzer was purchased from Vedeng and the automatic biochemical

analyzer was purchased from Beckman Coulter; the automatic nucleic acid testing analysis system was purchased from Rendu Biotech, and the HBsAg kit was purchased from Merck Biotech; anti-HIV kits were purchased from Jianlun biology Technology Co., Ltd; anti-HCV kits were purchased from Boxbio and HBsAg kits were purchased from ZhenKe Biological Technology Co., Ltd; anti-TP kits are purchased from Jining Industrial Co., Ltd.

Testing Methods

Blood samples collection

Two blood samples were collected from a blood donor, with 5ml each. One was tested for the nucleic acid testing and collected into a separating gel EDTA-K2 anticoagulant vacuum tube; the other was used for blood type and ELISA and collected into an EDTA-K2 anticoagulant vacuum tube. The collected samples were kept in a storage box at 2-8℃ and then we completed the centrifugation experiment within 3h and the test within 24h.

ELISA testing

Two ELISA kits were used to test the collected blood samples and we strictly followed the implementation in process of the operation.

Nucleic acid testing

We took further nucleic acid testing on negative blood samples with ELISA test and blood samples with 0.7≤S/CO≤3.0DE; Auto SAT automatic nucleic acid testing and analysis system was used to implement HIV-RNA, HCV-RNA and HBV-DNA triplet examination on 6 mixed samples; if the result of the mixed test was not reactive, it proved that 6 samples are all qualified; if the result of the mixed test showed positive, we tested these 6 samples one by one. Positive nucleic acid and negative nucleic acid were respectively reactive and non-reactive.

Evaluation Indexes

ELISA evaluation: if anti-HIV, anti-HCV, HBsAg, anti-TP tested S/CO<0.7 without any reactivity, it was negative; 0.7≤S/CO<1 was the gray zone, and if S/CO≥1.0 was reactive, it was positive; when the single reagent was reactive and the double and single reagent were reactive, we performed double-hole retest for samples in the corresponding braided tube and samples in the original tube. The retest tube without any reactivity was qualified, and the optional tube with reactivity was unqualified.

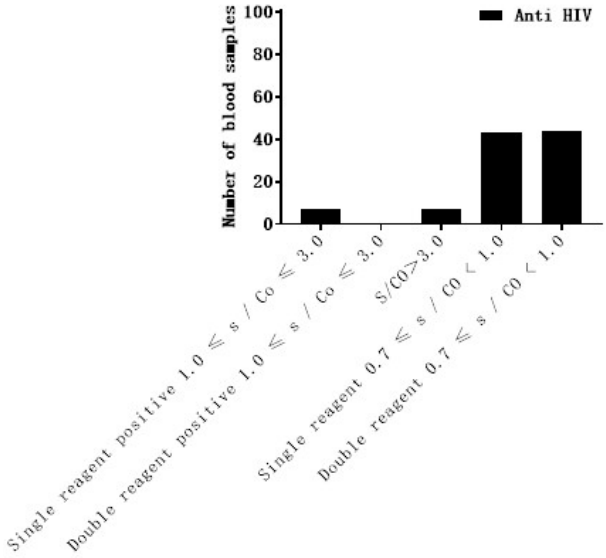
STATISTICAL ANALYSIS

In this study, all the data were processed and analyzed by software SPSS19.0. The enumeration data were represented by [n(%)] with X^2 test(P<0.05 indicates that there is statistical significance).

RESULTS

Anti-HIV, anti-HCV, HBsAg and anti-TP ELISA test results of 72335 samples

Among 72,335 blood samples, there were 610 samples failing to pass anti-HIV, anti-HCV, HBsAg and anti-TP ELISA tests, accounting for 0.84%, including 100 blood samples with 1.0≤S/CO≤3.0, 172 blood samples with S/CO>3.0, 338 blood samples with 0.7≤S/CO<1.0 and 71725 qualified blood samples. See figs. 1 to 5.



Note: The X-axis from left to right indicates positive single reagent 1.0≤S/CO≤3.0, positive double reagents 1.0≤S/CO≤3.0, S/CO＞3.0, single reagent 0.7≤S/CO＜1.0, and double reagents 0.7≤S/CO＜1.0; the Y-axis represents the unqualified samples. It is seen from fig. 1 that there are 7 unqualified samples with positive single reagent for anti-HIV 1.0≤S/CO≤3.0, 0 unqualified samples with positive double reagents 1.0≤S/CO≤3.0, 7 unqualified samples with S/CO>3.0, 43 unqualified samples with single reagent 0.7≤S/CO<1.0, and 44 samples with double reagents 0.7≤S/CO<1.0.

Fig. 1: Analysis of unqualified samples with anti HIV test

Nucleic acid test results of 71725 blood samples qualified by ELISA

We applied the nucleic acid testing to 71,725 qualified samples tested by ELISA. There are 50 blood samples with positive HBV-DNA, accounting for 0.07% (50/71725) and zero blood samples with positive HIV-RNA and HCV-RNA, accounting for 0.00% (0/71725) (table 1).

Blood samples with HBsAg 0.7≤S/CO≤1.0 tested by ELISA and blood samples with 1.0≤S/CO≤3.0 tested by nucleic acid testing

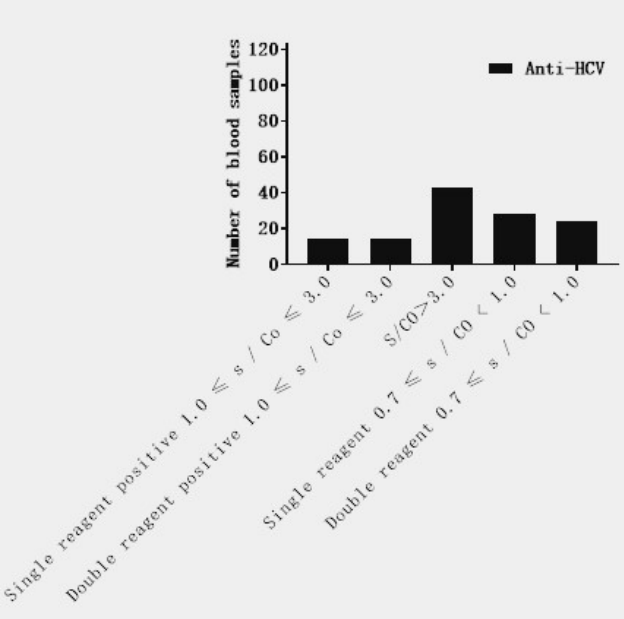
The positive rate of blood samples with HBsAg 0.7≤S/CO<1.0 tested jointly by ELISA and nucleic acid testing was 7 (5.83%), which was greatly lower than 9(20.93%) of blood samples with HBsAg1.0≤S/CO≤3.0 tested by ELISA (X2= 8.15, P<0.01) (table 2).

Table 1: Nucleic acid test results of 71725 blood samples qualified by ELISA

Nucleic acid test indexes	Positive numbers	Positive rate
HBV-DNA	50	0.07%
HIV-RNA	0	0.00%
HCV-RNA	0	0.00%

Table 2: Blood samples with HBsAg 0.7≤S/CO≤1.0 tested by ELISA and blood samples with 1.0≤S/CO≤3.0 tested by nucleic acid testing

HBsAg S/CO value tested by ELISA	ELISA testing numbers	Positive rate of nucleic acid testing	Negative rate of nucleic acid testing
0.7≤S/CO<1.0	120	7(5.83%)	113(94.17%)
1.0≤S/CO≤3.0	43	9(20.93%)	34(79.07%)
X^2 value		8.15	8.15
P value		0.00	0.00

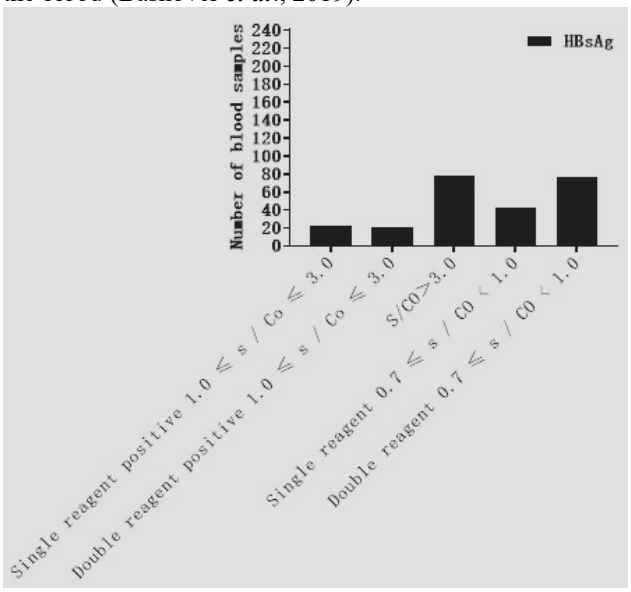


Note: The X-axis from left to right indicates positive single reagent 1.0≤S/CO≤3.0, positive double reagents 1.0≤S/CO≤3.0, S/CO＞3.0, single reagent 0.7≤S/CO＜1.0, double reagents 0.7≤S/CO＜1.0; the Y-axis represents the unqualified samples. It is seen from fig. 2 that there are 14 unqualified samples with positive single reagent for anti-HCV 1.0≤S/CO≤3.0, 14 unqualified samples with positive double reagents 1.0≤S/CO≤3.0, 43 unqualified samples with S/CO＞3.0, 28 unqualified samples with single reagent 0.7≤S/CO<1.0, and 24 unqualified samples with double reagents 0.7≤S/CO<1.0.

Fig. 2: Analysis of unqualified anti HCV samples

DISCUSSION

Today there are test items mainly including anti-HIV-ELISA, anti-HCV-ELISA, HBsAg-ELISA and syphilis test, etc. for blood-borne diseases and the sample size for each test cannot be less than 10,000 (Bhatia *et al*., 2019; El-Hage *et al*., 2019; Teka *et al*., 2018). According to relevant clinical studies, HCV, HIV and HBV seriously threaten the safety of blood transfusion, especially HBV. Through statistics, the probability of HBV infection in China is as high as about 10%, which has seriously threatened the safety of clinical blood transfusion. Therefore, it is extremely important to strengthen the clinical screening for HIV, HCV, HBV, syphilis, etc. in the blood (Bashover *et al*., 2019).

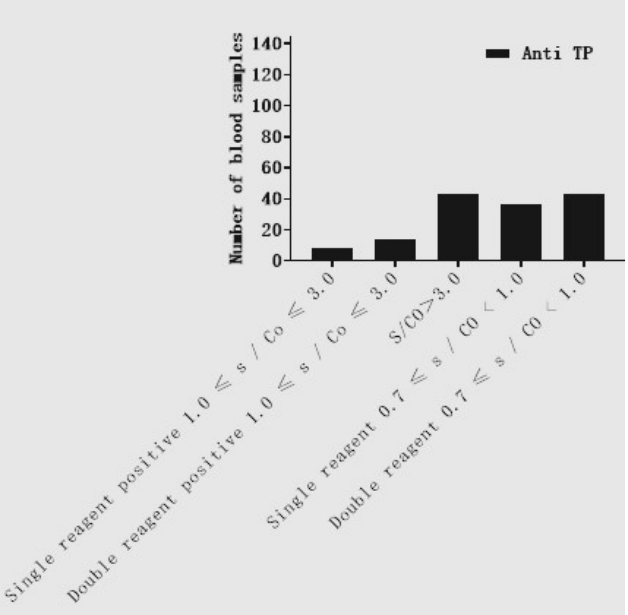


Note: The X-axis from left to right indicates positive single reagent 1.0≤S/CO≤3.0, positive double reagents 1.0≤S/CO≤3.0, S/CO>3.0, single reagent 0.7≤S/CO<1.0 and double reagent 0.7≤S/CO＜1.0; the Y-axis represents the unqualified numbers. It is seen from fig. 3 that there are 22 unqualified samples with positive HBsAg single reagent 1.0≤S/CO≤3.0, 21 unqualified samples with positive double reagents 1.0≤S/CO≤3.0, 79 unqualified samples with S/CO>3.0, 43 unqualified samples with single reagent 0.7≤S/CO<1.0 and 77 unqualified samples with double reagents 0.7≤S/CO<1.0.

Fig. 3: Analysis of unqualified HBsAg samples

Nowadays, the continuous advancement of test technology and biomedicine greatly improves the safety of blood transfusion, but there is still a high risk in blood-

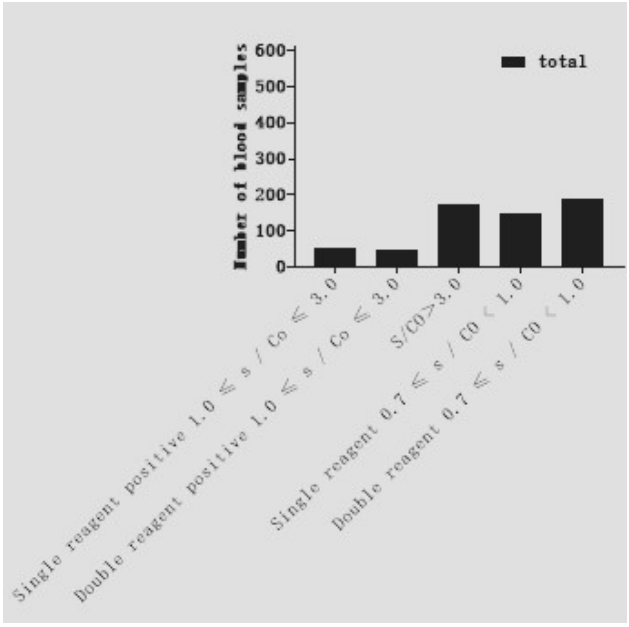
borne diseases (Belanger *et al*., 2020; Ba *et al*., 2018; Sisti *et al*., 2020). The immunological test method is mainly based on the immune response mediated by antigens and antibodies. But when the body is infected, there will be a window phase and it is influenced by individual differences at the same time. Some patients are not against immunological testing, and also affected by force factors, sensitivity, etc., resulting in omission.



Note: The X-axis from left to right indicates positive single reagent 1.0≤S/CO≤3.0,positive double reagents 1.0≤S/CO≤3.0, S/CO>3.0, single reagent 0.7≤S/CO<1.0, double reagents 0.7≤S/CO<1.0; the Y-axis represents unqualified numbers. It is seen from fig. 4 that there are 8 unqualified samples with positive anti-TP single reagent 1.0≤S/CO≤3.0, 14 unqualified samples with positive double reagents 1.0≤S/CO≤3.0, 43 unqualified samples with S/CO>3.0, 36 unqualified samples with single reagent 0.7≤S/CO<1.0, and 43 unqualified samples with double reagents 0.7≤S/CO＜1.0.

Fig. 4: Analysis of unqualified samples of anti TP test

ELISA is a special reagent analysis method that developed on the basis of immunoenzymes (Kumar *et al*., 2019; Oakes *et al*., 2020). It is mainly to combine some enzyme complexes with antibodies and display them through color development, which keeps the activities of their immunity and enzyme. In general, the test steps are to centrifuge the blood, extract the serum, dilute it with the enzyme complex and add the serum for comparative analysis, and then perform operations such as incubation, adding substrates, etc., and then we evaluate the result with the obtained values. The reagents used in ELISA must be enzyme substrates, immunoadsorption and conjugate. The main test techniques include one-step method, double antibody sandwich technique, capture technique, indirect technique, etc. to detect antibodies so as to effectively detect the low levels of HIV, HBV and HCV virus infection and clarify the virus mutation, occult infection, immune silencing, virus subtypes, etc. (Zhang *et al*., 2020).



Note: The X-axis from left to right indicates the positive single reagent 1.0≤S/CO≤3.0, positive double reagents 1.0≤S/CO≤3.0, S/CO>3.0, single reagent 0.7≤S/CO<1.0 and double reagents 0.7≤S/CO<1.0; the Y-axis represents the unqualified numbers. It is seen from fig. 5 that there are 50 unqualified samples with positive single reagent 1.0≤S/CO≤3.0, 49 unqualified samples with positive double reagents 1.0≤S/CO≤3.0, 172 unqualified samples with S/CO>3.0, 150 unqualified samples with single reagent 0.7≤S/CO<1.0, and 180 unqualified samples with double reagents 0.7≤S/CO<1.0.

Fig. 5: Analysis of total unqualified samples

Nucleic acid testing is also called NAT that is widely present in microorganisms and animal and plant cells and has high sensitivity. However, nucleic acid testing shows that there is no reactivity in a positive single reagent because of its window phase, with the high-quality laboratory (Jehn *et al*., 2020). This study aims to explore the effects and prevention effects of nucleic acid testing combined with ELISA on blood-borne diseases. The results showed that 610 blood samples failed to pass the test of anti HIV, anti HCV, HBsAg and anti TP ELISA, accounting for 0.84% of the total numbers, including 100 blood samples with 1.0≤ s/Co ≤3.0, 172 blood samples with S/CO > 3.0, 338 blood samples with 0.7≤ s/CO <1.0 and 71725 blood samples were qualified.

This result is similar to the research by Osman Yaşar (Yaşar *et al*., 2019) and others, which indicates that ELISA is applied to the screening and test of blood-borne diseases. This study also found that 71,725 qualified samples tested by ELISA method were conducted using nucleic acid testing. There are 50 samples with positive HBV-DNA, accounting for 0.07% (50/71725), 0 samples with positive HIV-RNA, 0 samples with positive HCV-

RNA, both accounting for 0.00% (0/71725). This shows that ELISA in blood sample screening cannot eliminate all the HBV infections in blood. Nucleic acid testing effectively avoids loopholes in ELISA screening, thereby increasing the test rate of HBV. In this study, we believe that nucleic acid testing is to accept PCR amplification of viral nucleic acids in blood, and them shorten their window phase and remedy the limitation of ELISA testing, which increases the test rate of blood-borne diseases. In addition, the results of this study showed that the positive rate of blood samples with HBsAg 0.7≤S/CO<1.0 tested by ELISA combined with nucleic acid testing was 7(5.83%), which was significantly lower than 9(20.93%) of blood samples with HBsAg 1.0≤S/CO≤3.0 tested by ELISA (X2=8.15, P<0.01), which indicates that nucleic acid testing effectively detects the HBsAg infection of blood samples with 1.0≤S/CO≤3.0 and 0.7≤S/CO<1.0. In this study, we believe that the reason is that the principle of ELISA to detect HBsAg is the specific reaction of antibodies and antigens, and now reports point out that it results in the endogenous infection factors such as complement, cross-reactive substances, etc. and the exogenous factors such as bacterial infection and other factors.

CONCLUSION

In conclusion, in the clinical test of blood-borne disease, the joint test of nucleic acid testing and ELISA reduces the occurrence of virus omission and plays a positive role in preventing from spreading diseases through blood transfusion.

REFERENCES

Ba S, Raugi DN, Smith RA, Sall F, Faye K, Hawes SE, Sow PS, Seydi M, Gottlieb GS; University of Washington–Dakar HIV-2 Study Group (2018). A trial of a single-tablet regimen of elvitegravir, cobicistat, emtricitabine, and tenofovir disoproxil fumarate for the initial treatment of human immunodeficiency virus type 2 infection in a resource-limited setting: 48-week results from Senegal, West Africa. *Clin Infect Dis,* **67**(10):1588-1594.

Bashover EM, Stefaniuk CM, Harding CV, Maitta RW (2019). Use of a whole-cell ELISA to detect additional antibodies in setting of suspected heparin-induced thrombocytopenia. *Eur. J. Haematol.*, **103**(2): 99-106.

Behnke G, Tiscione NB, Rakus J and Richards-Waugh L (2019). Validation of the neogen ELISA benzodiazepine kit using clonazepam as the target molecule for blood and urine. *J. Anal Toxicol.*, **43**(3): 399-405.

Belanger KM, Crislip GR, Gillis EE, Abdelbary M, Musall JB, Mohamed R and Sullivan JC (2020). Greater T regulatory cells in females attenuate DOCA-salt-induced increases in blood pressure versus males. *Hypertension*, **75**(6): 1615-1623.

Bhatia R, Stewart J, Moncur S, Cubie H, Kavanagh K, Pollock KGJ and Cuschieri K (2019). Development of an in-house ELISA to detect anti-HPV16-L1 antibodies in serum and dried blood spots. *J. Virol. Methods*, **264**: 55-60.

Bushman ET, Theilen LH, Monson M, Hammad I, Esplin I and Esplin MS (2020). Effect of blood contamination on amniotic fluid detection *in vitro* using immuno-assays. *J. Matern Fetal. Neonatal Med.*, **33**(17): 2909-2912.

Chileshe J, Goosen WJ, Buss PE, van Helden PD, Warren R, Parsons SDC and Miller MA (2019). A commercial ELISA for detection of interferon gamma in white rhinoceros. *J. Vet. Diagn Invest*, **31(4)**: 531-536.

Dalkan C, Uncu M, Duran S and Bahçeciler NN (2020). Association of cord blood bisphenol A (BPA) with cord blood adiponectin, leptin, fetal growth; Adiposity and neoantal complications in a newborn cohort. *J. Matern Fetal Neonatal Med.*, **33**(15): 2588-2593.

Damola AB, Adeniji JA, Bakarey AS (2019). Hepatitis C virus seropositivity and the risk factors for transmission among blood donors in some selected centers in Lagos State, Southwest Nigeria. *J. Immunoassay Immunochem.*, **40**(5): 528-539.

El-Hage CM, Bannai H, Wiethoelter AK, Firestone SM, Heislers CM, Allen JL and Gilkerson JR (2019). Serological responses of Australian horses using a commercial duplex indirect ELISA following vaccination against strangles. *Aust. Vet. J.*, **97**(7): 220-224.

Gadermaier E, Tesarz M, Wallwitz J, Berg G and Himmler G (2019). Characterization of a sandwich ELISA for quantification of total human soluble neuropilin-1. *J. Clin. Lab Anal.*, **33**(7): e22944.

Kumar A, Mhatre S and Dikshit R (2019). Utility of dried blood spots in detecting helicobacter pylori infection. *Indian J. Med. Microbiol.*, **37**(4): 514-520.

Oakes JM, Xu J, Morris TM, Fried ND, Pearson CS, Lobell TD and Yue X (2020). Effects of chronic nicotine inhalation on systemic and pulmonary blood pressure and right ventricular remodeling in mice. *Hypertension*, **75**(5): 1305-1314.

Saravanan N, Rajendiran P, Sankar S, Ramamurthy M, Sasimohan A, Vineeta V and Sridharan G (2020). Detection of *scrub typhus* by real-time polymerase chain reaction and immunoglobulin M ELISA among patients with acute febrile illness. *J. Nat. Sci. Biol. Mede*, **11**(3): 66.

Saushkin NY, Samsonova JV, Osipov AP, Kondakov SE (2019). Strip-dried blood sampling: Applicability for bovine leukemia virus detection with ELISA and real-time PCR. *J. Virol Methods*, **263**: 101-104.

Sisti G, Minis E, Di Tommaso M, Magro Malosso ER, Parenti A, Paccosi S and Witkin SS (2020). T-cell immunoglobulin mucin-3 and galectin-9 levels in peripheral blood mononuclear cells predict fetal acidemia in twin pregnancies. *J. Matern Fetal Neonatal Med*, **33**(10): 1683-1687.

Sun JL, Li S, Lu X, Feng JB, Cai TJ, Tian M and Liu QJ (2020). Identification of the differentially expressed protein biomarkers in rat blood plasma in response to gamma irradiation. *Int. J. Radiat Biol.*, **96**(6): 748-758.

Jehn U, Schütte-Nütgen K, Bautz J, Pavenstädt H, Suwelack B, Thölking G and Reuter S (2020). Clinical features of BK-polyomavirus and cytomegalovirus co-infection after kidney transplantation. *Sci Rep.,* **10**(1): 22406.

Yaşar O, Karatayli E, Cengiz G, Kızılpınar M, Yurdcu E, Albayrak R and Karatayli SC (2019). HEV seroprevalence in blood donors in Turkey by two commercial total anti-HEV Ab ELISA kits. *J. Med. Virol.*, **91**(12): 2174-2181.

Zhang X, Liu X, Hu G, Zhang G, Zhao G and Shi M (2020). Ginsenoside Rd attenuates blood-brain barrier damage by suppressing proteasome-mediated signaling after transient forebrain ischemia. *Neuroreport*, **31**(6): 466-472.