Cellular and Molecular Biology

Journal homepage: www.cellmolbiol.org

Different Detection Methods of Blood Nucleic Acid and Enzyme-linked Immunosorbent Assay for Blood Virus Screening

Qingchuan Ma[1], Junfang Xue[2], Xiqin Xin[3*], Ni Mu[4], Xi Wu[1], Bing Yang[1], Na Xu[1], Juan Du[1]

[1] *Department of Clinical Laboratory, Laboratory First People's Hospital of Tianshui, Tianshui, 741000, China*

[2] *Operation Room, People's Hospital of Gaomi, Gaomi, 261500, China*

[3] *Department of Clinical Laboratory, Tianshui maternal and Child Health Hospital, Tianshui, 741000, China*

[4] *Department of Pathology, Laboratory First People's Hospital of TianShui, Tianshui, 741000, China*

ARTICLE INFO

Original paper

Article history:
Received: November 02, 2021
Accepted: March 16, 2022
Published: March 31, 2022

Keywords:
blood virus detection, nucleic acid detection, enzyme-linked immunosorbent assay, blood virus screening

ABSTRACT

Through the detection of antigens or antibodies of related viruses in blood, the incidence of transfusion-transmitted diseases can be reduced, the comprehensive performance indicators of the human body can be judged, and the disease can be effectively treated and prevented. The purpose of this study is to analyze the screening results of blood viruses by different detection methods of blood nucleic acid and enzyme-linked immunosorbent assay. In this study, the comparison and data analysis of the two detection methods in the study were carried out through the comparison method and data analysis method, and the functional analysis is combined with the detection principle. The positive rate of anti-HCV was 1.67% (10 / 600) by ELISA and 0.34% (2 / 590) by nucleic acid. Conclusion enzyme immunoassay is not sensitive to the antigen-antibody reaction in the window stage, and there is a mistake in the detection. However, nucleic acid detection has high sensitivity because of its PCR principle, but it is also prone to false-positive.

DOI: http://dx.doi.org/10.14715/cmb/2022.68.3.45

Introduction

In recent years, nucleic acid detection has been gradually accepted by hospitals and physical examination personnel due to the shortening of window time, which also confirms the necessity of nucleic acid detection technology, but because of the complexity and technicality of its operation, it has not been fully popularized in basic detection institutions, so it can not completely replace enzyme-free detection (1, 2). This method can not only avoid the false leakage caused by the difference between reagents and different sensitivity but also detect the problem caused by the small amount of virus antibody in the window period, at the same time; the cost of nucleic acid detection is relatively high. The first use of enzyme-free detection for screening can also save the cost of reagents to a certain extent. It should also be noted that when detecting viral nucleic acids in the blood, it is necessary to keep the second enzyme immunoassay of the blood to confirm the test results. On the other hand, the detection personnel should pay more attention to enzyme-free detection, strengthen the study and training of detection technology and knowledge, and use the two detection technologies reasonably through rich detection experience to continuously improve the accuracy of blood detection results and ensure the safety of blood supply (3,4).

Ling f has established a stable, specific and affinity mouse hybridoma cell line, and he has also established indirect enzyme-linked immunosorbent assay and side flow Immunochromatography for the detection of geovirus in human blood (1). His haploid protein is coupled with bovine serum albumin or chicken ovalbumin by sodium propionic acid oxidation (2). The median inhibitory concentration of the highest sensitivity and specific antibody (IC < sub > 50 < / sub >) was 0.45ng/ml, the linear detection range was 0.293-0.7ng/ml, and the cross-reactivity with several dig analogues was low. The cut-off value of side flow immunochromatography was 5ng / ml. Immunochromatography side flow strip test provides a fast and simple method for the determination of plasma geovirus, which can directly observe the existence of geovirus in 5 minutes, and promote the

*Corresponding author. E-mail: xxq18093894995@163.com

rational use of drugs (3).

Zhu l believes that Bacillus cereus is increasingly recognized as one of the main causes of food poisoning in industrialized countries. He introduced a sensitive double antibody sandwich enzyme-linked immunosorbent assay (ELISA) for rapid detection of the blood-brain barrier (4,5). Cereals were prepared from rabbit antiserum and mouse ascites by caprylic acid / saturated ammonium sulfate precipitation and protein A-Agarose column, respectively. IgG homotype monoclonal antibody is a new peripheral multipoint immunity developed for the rapid acquisition of hybridoma. It can eliminate the cross-reaction with *Bacillus thuringiensis*, *Bacillus subtilis*, *Bacillus licheniformis* and other related species by subtraction screening. The linear detection range of this method is about 1 × 104-2.8 × 106cells / ml, and the detection limit (LOD) is 0.9 × 103cells / ml (6).

Gao believes that enzyme-linked immunosorbent assay (ELISA) is a technique for the detection and quantification of biomolecules in liquid samples. It is a powerful tool for clinical diagnosis, food safety and environmental monitoring. However, the main limitation of conventional ELISA is its low sensitivity, which cannot meet the requirements of complex (biological) sample analysis (7-8). In addition to therapeutic applications of metal and metal oxide NPs (3-5), these nanomaterials can be used as novel carriers to load enzymes and antibodies for signal amplification, as analog enzymes to replace natural enzyme tags, and as a signal, sensors to provide fluorescent signals as alternative outputs (9-10).

In this study, we compared the effect of the nucleic acid test (NAT) and enzyme-linked immunosorbent assay (ELISA) on virus detection. Nat is widely used in the clinic because of its high sensitivity and specificity. However, NAT technology requires a high level of operation and blood sample of the testing personnel, and the proportion of false positives is large, while ELISA The detection of an antibody requires a certain concentration. After the virus enters the human body, the immune response of the body will take a period of virus replication and antibody production to be detected. Therefore, the detection "window period" of this method is relatively long.

Materials and methods

Principle of experimental detection

Biochemical detection principle

The pyruvate content was determined by measuring the absorbance of dinitrobenzene at the wavelength of 480-530nm, and the contents of GPT and GST were determined (11-12).

The detection principle of enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (EIA) is an enzyme marker formed by the enzyme labeling of antigen and antibody, which interacts with the corresponding antigen or antibody in the sample to be tested to form an enzyme-labeled antigen-antibody complex (13). The enzyme-labeled on the complex is used to catalyze the substrate color development, so as to determine the content of the substance to be tested, including the double antigen sandwich method, indirect method, competition method, etc(14-15).

Principle of nucleic acid detection

Three basic reaction steps are composed of denaturation, annealing and extension (16).

At 72 °C, deoxy ribonucleoside triphosphate was used, DNTP) is the raw material, and the target sequence is the replication template. According to the principle of complementary base pairing and the principle of half reserved replication, a new chain complementary to the template DNA chain is generated (17). The three processes of denaturing annealing extension are repeated continuously and more "replication chains" are obtained gradually, and the newly generated replication chain can be used as the replication template for the next cycle (18). It takes about 3 minutes for each cycle to be completed, and 2 to 3 hours to amplify the target gene to be expanded by an N-power multiple of 2 ($n \geq 30$). In amplification technology, a pair of primers is added together with a special fluorescent probe. The probe is an oligonucleotide, a report fluorescent group and a quenched fluorescent group are labeled on both sides of the fluorescent probe (19-20). When the probe is intact, the quenching group absorbs the fluorescence signal emitted by the reporting group, and the fluorescence signal cannot be detected; at the beginning of amplification, the probe binds to a corresponding single strand of DNA; during PCR amplification, Taq has used The 5 '- 3' end

exonuclease of the enzyme cleaves and degrades the probe, separating the reported fluorescence group and the quenched fluorescence group on the probe, so that the fluorescence monitoring system can receive the fluorescence signal, that is, when amplifying a DNA chain, a new fluorescence molecular signal is detected, realizing the synchronous formation process of PCR product and the accumulation of fluorescence signal(21-22).

The testing process included a Biochemical test, Treponema pallidum test and Secondary enzyme-linked immunosorbent assay

Detection of HIV antigen and antibody

The index is higher than the corresponding blood sample of positive control (23-24). The biochemical test and enzyme-free test flow of the blood sample are shown in Figure 1. The flow chart of biochemical and enzyme immunoassay of blood samples is shown in the figure. The blood samples of the donors with negative blood samples and positive blood samples from the secondary enzyme-linked immunoscreening were tested for NAT respectively (25).

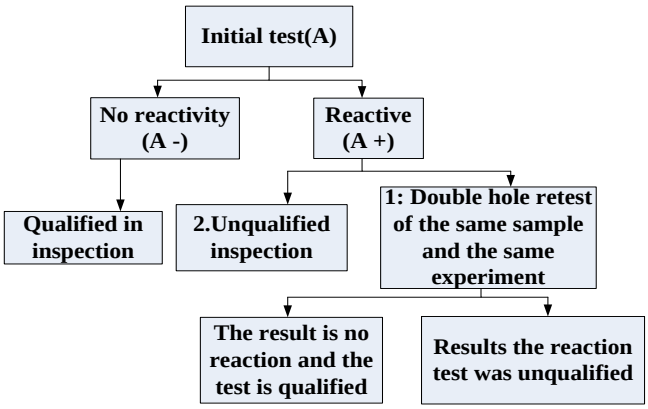

Figure 1. Biochemical test and enzyme-free test flow of blood sample.

Nat detection

1) Sample preparation and collection for nucleic acid screening

2) Virus nucleic acid extraction

3) Nucleic acid amplification

4) Interpretation of results

Detection of parvovirus in plasma and blood products

The extraction method of parvovirus nucleic acid

Take 200 μ l blood sample from each plasma sample, use the high-purity nucleic acid extraction kit, extract the nucleic acid from each raw plasma sample according to the method instructions, and finally obtain 50 μ L / share of nucleic acid, and store it at -20 °C; after diluting the blood products, extract the nucleic acid according to the same method, 50 μ L / tube, and store it at - 20 ℃. Blood product dilution method: according to the blood product manual provided by the manufacturer, dilute with 0.9% sodium chloride injection: thrombin / bottle + 2ml NaCl solution; coagulation factor Ⅷ / bottle + 10ml NaCl solution; prothrombin complex (PCC) / bottle + 20ml NaCl solution; fibrinogen / bottle + 25ml NaCl solution. Take 200 μ L from each sample to extract nucleic acid.

Extraction steps of parvovirus nucleic acid

Using Roche high purity nucleic acid extraction kit (centrifugal column method) to extract nucleic acid from mixed plasma and blood products, the virus-cell diagram in the blood is shown in Figure 2. the main steps are as follows:

(a) In 1.5ml EP tube (autoclave), add 200ul serum, plasma or whole blood, add 200ul binding buffer containing Polya, add 50ul protease K, mix immediately, and incubate at 72 °C for 10 minutes.

(b) Add 100ul binding buffer and mix. Put the high purity filter column into the collection tube, and then add the sample into the filter column.

(c) Centrifuge 8000g for 1min.

(d) After centrifugation, discard the waste liquid and collection pipe, and replace them with new ones.

(e) Add 500ul inhibitor removal buffer into the filter column, and centrifugate 8000g for 1min.

(f) After centrifugation, discard the waste liquid and collection pipe and replace them with new ones.

(g) Add 450ul wash buffer into the filter column and centrifuge 8000g for 1min.

(h) After centrifugation, discard the waste liquid and collection pipe and replace them with new ones. Add 450ul wash buffer into the filter column and centrifuge 8000g for 1min. Waste liquid after centrifugation. Centrifuge for 10 s at maximum speed (about 13000 g) to remove the remaining wash buffer.

(i) Discard the waste liquid and collection pipe, and insert the filter column into a new sterile EP pipe.

(g) Add 50uelution buffer into the filter column and centrifuge 8000g for 1min. Take the DNA solution and store it at - 20 °C.

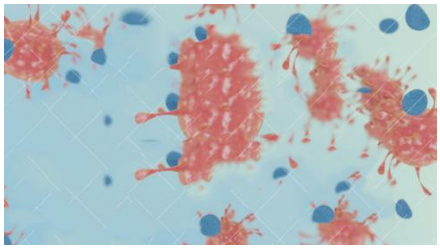

Figure 2. Virus cell diagram in the blood (25)

Experimental design of blood virus detection

Experimental instrument

Hitachi automatic biochemical analyzer 7600; Haier hr40 - Ⅱ A2 biosafety cabinet; Xanthus automatic sampling instrument; fame automatic enzyme immunoassay analyzer; uranusae200 automatic enzyme immunoassay analyzer; Haier hr40 - Ⅱ A2 biosafety cabinet; Hamilton star sample collector; ezbeam system-32 magnetic bead extractor; abi7500 fluorescence quantitative PCR instrument;

Experimental materials

5ml EDTA vacuum blood collection test tube; 40 hole test tube rack; disposable sampling gun suction head; washing bottle; 7mledta vacuum blood collection test tube; 50 hole test tube rack; disposable PCR sampling gun filter cartridge suction head; PCR amplification reaction disk, eight link reaction tube; soaking barrel; alcohol spray pot, 84 Keywords liquid spout; sterile gauze and absorbent cotton; disposable sterile gloves, shoe covers, masks and caps; medical garbage bag;

General information

600 samples were from volunteers who came to the blood station from June 2016 to June 2018 to voluntarily donate blood. They informed the volunteers of the specific process and content of the study. After informed consent of the volunteers, they were included in the study. Finally, 600 volunteers were selected to participate in the study. Blood samples were collected from volunteers. The ratio of males and females was 347:253. The average age was (38.63 ± 4.29) years. All volunteers who provided blood samples were voluntary donors and supported the study.

Methods

HBV, HCV and HIV-1 / 2 were detected. All the blood samples involved in the study were collected and stored in 5ml vacuum blood collection anticoagulant tube containing separation gel and 7ml vacuum negative pressure EDTA anticoagulant tube respectively. The samples were labeled and stored in a 4 °C refrigerator temporarily after collection for centralized separation and detection. Enzyme-linked immunosorbent assay (ELISA) was performed on blood samples in 7 ml vacuum tube within 24 hours after collection. The enzyme immunoassay system adopts the fame 24 / 20 full-automatic detection system produced after adding samples using the full-automatic sample processing system, it enters the enzyme immunoassay board automatic analysis system for detection and issues the result report. Anti HIV-1 / 2, anti HCV and HBsAg of blood samples were tested with enzyme immunoassay kits made by two different manufacturers The test results of the two kits are positive when they are both positive, and negative when both results are negative. When the two results are inconsistent, the second test is carried out. When both the secondary test results are negative, it is determined as negative. If there is a single hole or double hole positive situation, it is determined as positive.

Observation index

Record the results of the two methods, sort out the positive and negative data of HBV, HCV and HIV-1 / 2, compare and analyze the test results, and discuss the differences between the two methods.

Statistical treatment

The test data were input into SPSS 19.0 software, and statistical analysis was carried out after the test. The measurement data was expressed in (x ± s), and the count data was expressed in rate (%). Using x2 test, $P < 0.05$ was statistically significant. A comparison between HIV nucleic acid test and enzyme immunoassay is shown in Table 1.

Table 1. Comparison between HIV nucleic acid test and enzyme immunoassay

Enzyme immunoassay	Nucleic acid detection		Total
	Positive	Negative	
Positive	15	26	41
Negative	0	29811	29811
Total	15	29837	29852

χ^2_{HIV}= 24.04, $\chi^2_{HIV} > \chi^2_{0.05(1)}$= 3.84 P < 0.05, according to the level of α = 0.05, the difference is statistically significant. It can be considered that the detection rate of the two detection methods in HIV blood samples is different, the detection rate of the HIV nucleic acid method is 15 / 29852 = 0.50 ‰, and the detection rate of HIV enzyme immunoassay is 41 / 29852 = 1.37 ‰, and the detection rate of HIV nucleic acid is lower than that of HIV enzyme immunoassay. Interpretation of HIV results is shown in Figure 3.

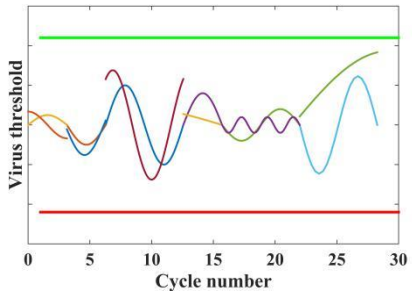

Figure 3. Interpretation of HIV results

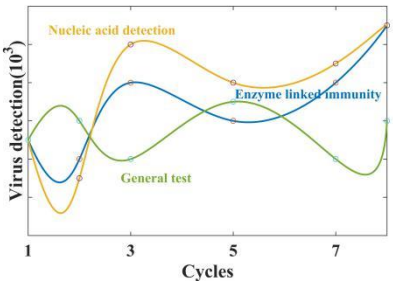

Figure 4. Renderings using different detection methods

Renderings using different detection methods are shown in Figure 4. Although nucleic acid detection has many advantages, its requirements for samples; detection environment and technical level of the personnel are more stringent than general experiments. If the quality of the sample can not be guaranteed before the test, the significance of nucleic acid detection will no longer exist, because the degradation of the viral nucleic acid will lead to the failure of the whole experiment, that is, missed detection. If the factors such as reagents, equipment and personnel of nucleic acid detection affect the sensitivity and quality of detection, it will also miss detection or even cause pollution.

In conclusion, NAT and ELISA are complementary. The implementation of NAT requires sufficient preparation, careful evaluation and verification, and continuous improvement of quality to achieve good results.

Results and discussion

29852 unpaid blood samples were negative for HBsAg, HCVAb and having AB and normal for ALT in the secondary immune screening. After NAT screening, 15 blood samples were positive for HBV DNA, and the missed rate was 0.50 ‰. No HCV RNA and HIV RNA positive blood samples were found.

The results of two methods for detecting HBV, HCV and HIV-1 / 2 were analyzed. The positive rate of HBsAg was 2.33% (14 / 600) in blood samples detected by enzyme immunoassay and 0.51% (3 / 586) in negative samples detected by the nucleic acid qualitative method. The positive rate of anti HCV was 1.67% (10 / 600) by ELISA, and 0.34% (2 / 590) by nucleic acid. The positive rate of anti HIV-1 / 2 was 2.17% (13 / 600) by ELISA, and 0.68% (4 / 587) by nucleic acid test. There was no significant difference in the positive rate of enzyme immunoassay between the two groups (P > 0.05).

Studies have shown that more than 90% of the risk of HIV and HBV and more than 75% of the risk of HCV in transfusion-transmitted infections in the United States come from blood donation by "window period" blood donors. It has been reported that the positive rate of HBV-DNA can reach 0.4% ~ 0.92%; the positive rate of HCV-RNA can reach 0.01% ~ 0.19%; in 70% ~ 90% area of HBV exposure, 7% ~ 19% of blood donors in this area are HBsAg negative HBV carriers or infected persons due to the high exposure rate, while HBsAg negative HBV-DNA 18% of the positive blood can lead to hepatitis B infection after blood transfusion. It can be seen that the negative results of enzyme-linked immunosorbent assay for HBsAg, anti-HCV and anti-HIV can not completely exclude the risk of HBV, HCV and HIV infection, and even become the main cause of threat to blood safety.

The two detection methods have their advantages and disadvantages. Because of the poor sensitivity to the antigen-antibody reaction in the window stage, the

enzyme immunoassay has missed the detection errors, while the nucleic acid detection is due to its PCR The principle has high sensitivity, but it is also prone to false-positive, and the price of nucleic acid detection reagents is relatively high. Therefore, in the actual blood station sample detection, it is reasonable to implement the sample detection mode of enzyme-free detection first, then nucleic acid detection for qualified samples. At the same time, it is necessary to improve the technical level and theoretical knowledge of operators and improve the detection accuracy In the practical application of virus detection, the two methods can be selected flexibly according to their respective advantages.

Acknowledgments

Not applicable.

Interest conflict

The authors declare that they have no conflict of interest.

References

1. Ling F, Liu L, Kuang H, et al. Development of Indirect Competitive Enzyme-Linked Immunosorbent Assay and Lateral-Flow Immunochromatographic Strip for the Detection of Digoxin in Human Blood. ACS Omega 2020; 5(3):1371-1376.
2. Zhu L, He J, Cao X, et al. Development of a double-antibody sandwich ELISA for rapid detection of Bacillus Cereus in food. Sci Rep 2016; 6(1):16092.
3. Gao Y, Zhou Y, Chandrawati R . Metal and Metal Oxide Nanoparticles to Enhance the Performance of Enzyme-Linked Immunosorbent Assay (ELISA). ACS Appl Nano Materials 2020; 3(1):1-21.
4. Vos A S D, Janssen M P, Zaaijer H L, et al. Cost-effectiveness of the screening of blood donations for hepatitis E virus in the Netherlands: Cost-Effectiveness Hev Screening in the Netherlands. Transfusion 2017; 57(2):258.
5. Sirakov I, Peshev R, Koburova K, et al. Development of blocking enzyme-linked immunosorbent assay for detection of Caprine herpesvirus 1 antibodies in Bulgaria. Comptes rendus de l'Acadeìmie bulgare des sciences: Sciences Matheìmatiques Et Naturelles 2016; 69(9):1159-1166.
6. Levashova A I, Artiushkova O Y, Morozova V S, et al. Pressure algometry and enzyme-linked immunosorbent assay of natural antibodies in the evaluation of pain syndrome. Doklady Biochem Biophy 2016; 466L43-46.
7. Neal G. Satterly, Matthew A. Voorhees, Abbe D. Ames. Comparison of MagPix Assays and Enzyme-Linked Immunosorbent Assay for Detection of Hemorrhagic Fever Viruses. J Clin Microbiol 2017; 55(1):68.
8. Sathe M, Srivastava S, Agrawal S K, et al. Effect of Spacer and the Enzyme-linked Immunosorbent Assay. Defense J 2016; 66(5):471-478.
9. Yuan L, Liu B, Yin K, et al. Development of an enzyme-linked immunosorbent assay for quantification of estriol in milk. Food Agric Immunol 2019; 30(1):817-828.
10. Nhari R M H R, Hanish I, Mokhtar N F K, et al. Authentication approach using enzyme-linked immunosorbent assay for detection of porcine substances. Quality Assurance Safety Crops Foods 2019;11(5):1-10.
11. Rajeswari, Shome, Natesan, Krithiga, Padmashree, B Shankaranarayana. Genotyping of Indian antigenic, vaccine, and field Brucella spp. using multilocus sequence typing. J Infect Dev Countries 2016; 10(3):237.
12. Singh R, Singh G, Urhekar A D . Enzyme-Linked Immunosorbent Assay for Detection of Aspergillus fumigatus Antibodies. International J Current Microbiol Appl Sci 2016; 5(10):240-244.
13. Ayanur A, Singh K P, Cherian S, et al. Sero-epidemiology and molecular detection of Bluetongue virus in Indian ruminants. Veter Italia 2016; 52(3-4):305-311.
14. Hause B M, Duff J W, Scheidt A, et al. Virus detection using metagenomic sequencing of swine nasal and rectal swabs. J Swine Health Prod 2016; 24(6):304-308.
15. Visser M, Bester R, Burger J T, et al. Next-generation sequencing for virus detection: covering all the base. Virol J 2016; 13(1):85.
16. Muhammad I, Sale P, Salisu M, Muhammad T, Abubakar B, Maidala A, Nuwanyada E. Molecular analysis of Bio-makers of Chloroquine resistance in Plasmodium falciparum Isolate from Gombe Local Government Area, Gombe State, Nigeria. Cell Mol Biomed Rep 2022; 2(1): 42-55. doi: 10.55705/cmbr.2022.335753.1033
17. Shehata D M, Hadi S . Applications of gold nanoparticles in virus detection. Theranostics 2018;8(7):1985-2017.
18. Zhang J, Yue L, Li Y L . Clinical Significance of Peripheral blood EB Virus Detection in NK/T Cell Lymphoma Patients. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2017; 25(2):460-464.
19. Jaewook L, Kenshin T, Enoch P . Plasmonic Nanomaterial-Based Optical Biosensing Platforms for Virus Detection. Sensors 2017; 17(10):2332-.
20. Sung G, Ahn C, Kulkarni A, et al. Highly efficient in-line wet cyclone air sampler for airborne virus detection. J Mechanic Sci Technol 2017; 31(9):4363-4369.
21. Suresh N, Rahin A S, Rohit C, et al. Recent Advances in Biosensor Development for Foodborne Virus Detection. Nanotheranostics 2017; 1(3):272-295.
22. Yanai H, Iizasa H, Chihara D, et al. Epstein-Barr virus detection using gastric biopsy specimens after rapid

urease test for Helicobacter pylori. Endoscopy International Open 2019; 07(04):E431-E432.
23. Yi S Y, Yoon K, Kwon J, et al. Expression and Purification of Recombinant Mayaro Virus Envelope Glycoproteins E1 and E2 to Develop a Mayaro Virus Detection System. J Bacteriol Virol 2020; 50(1):25.
24. Janjic A . The Need for Including Virus Detection Methods in Future Mars Missions. Astrobiol 2018; 18(12):1611-1614.
25. Ozcelik D, Jain A, Stambaugh A, et al. Scalable Spatial-Spectral Multiplexing of Single-Virus Detection Using Multimode Interference Waveguides. Sci Rep 2017; 7(1):12199.