Diagnostic Techniques of Infection with equid herpesvirus-1(EHV-1)（rhinopneumonitis）

王科珂　乌鲁木齐海关技术中心

Technology center of Urumqi Custom
Keke Wang

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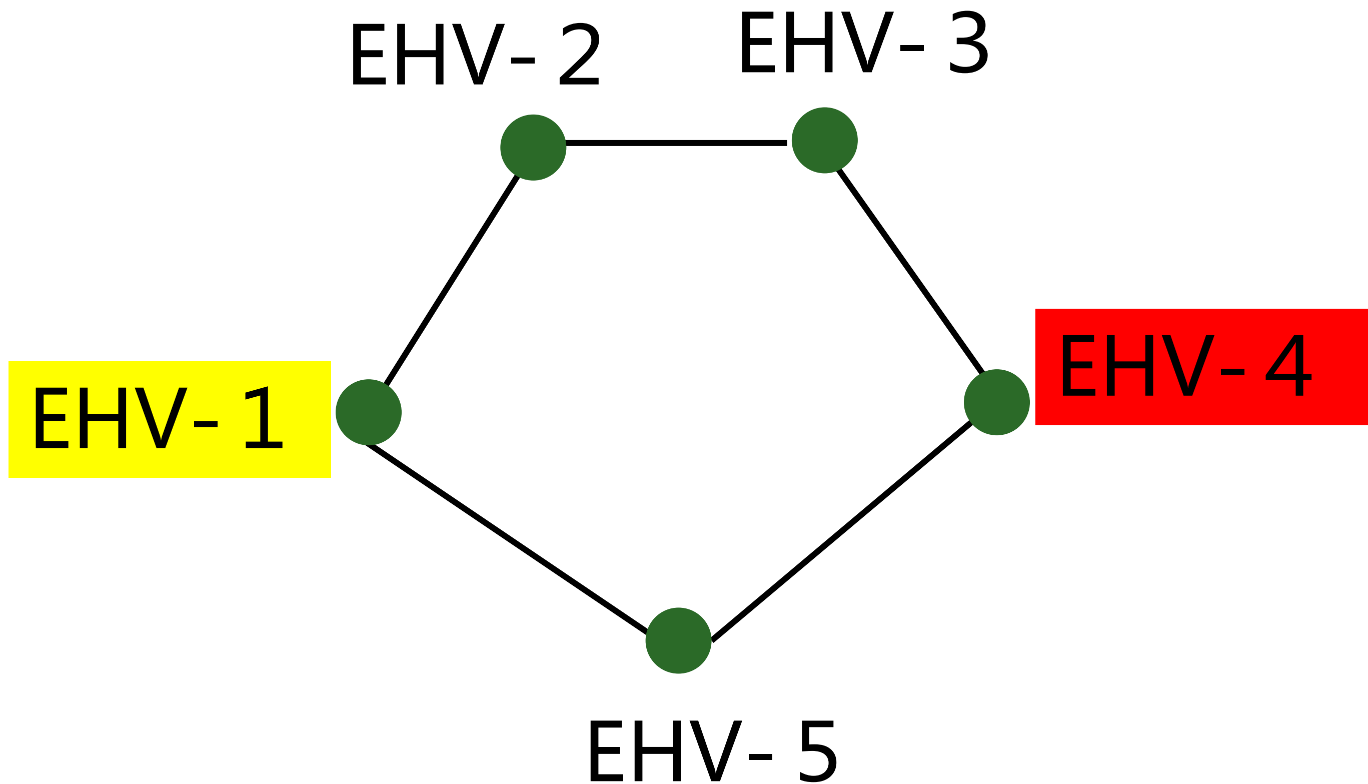
Infection with equid herpesvirus-1(EHV-1)（rhinopneumonitis）

Equine Rhinopneumonia (ER) is a highly contagious disease of equine animals caused by equine herpesvirus type 1 (EHV-1) and type 4 (EHV-4). Horses infected with the disease have abortion, respiratory and neurological diseases, and even death. They often show different clinical symptoms due to different immune time or age of horses.

Equine Rhinopneumonia is prevalent in horses all over the world, regardless of age and population, which brings serious harm to the development of horse industry. As one of the important diseases of horses, OIE classified it as class B animal disease. In China, it is listed as a class II animal infectious disease and one of the diseases that must be inspected in the entry and exit of equine animals.

病原 Antigen



Herpes virus

（equine herpesvirus， EHV）

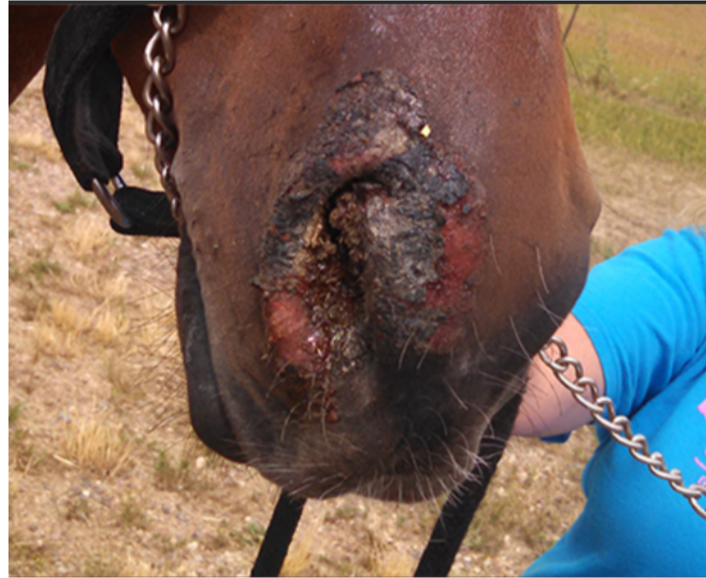
It belongs to the genus varicella virus of herpesvirus family. There are many types of EHV in classification, but there are five types that can infect horses.

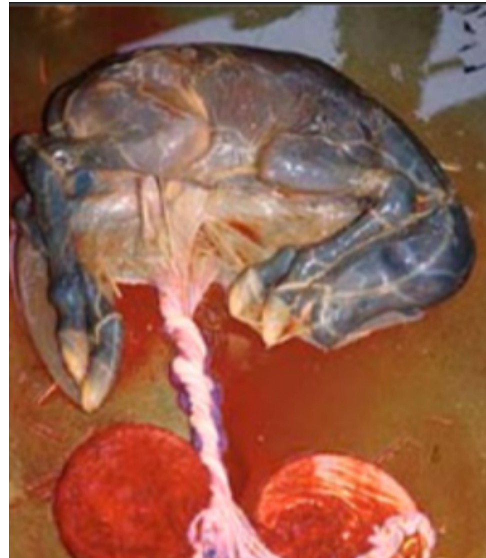
EHV-1 enters the horse's blood after entering the horse, causing toxemia, and then extends to various organs of the horse, which can cause respiratory symptoms, neurological symptoms and abortion.

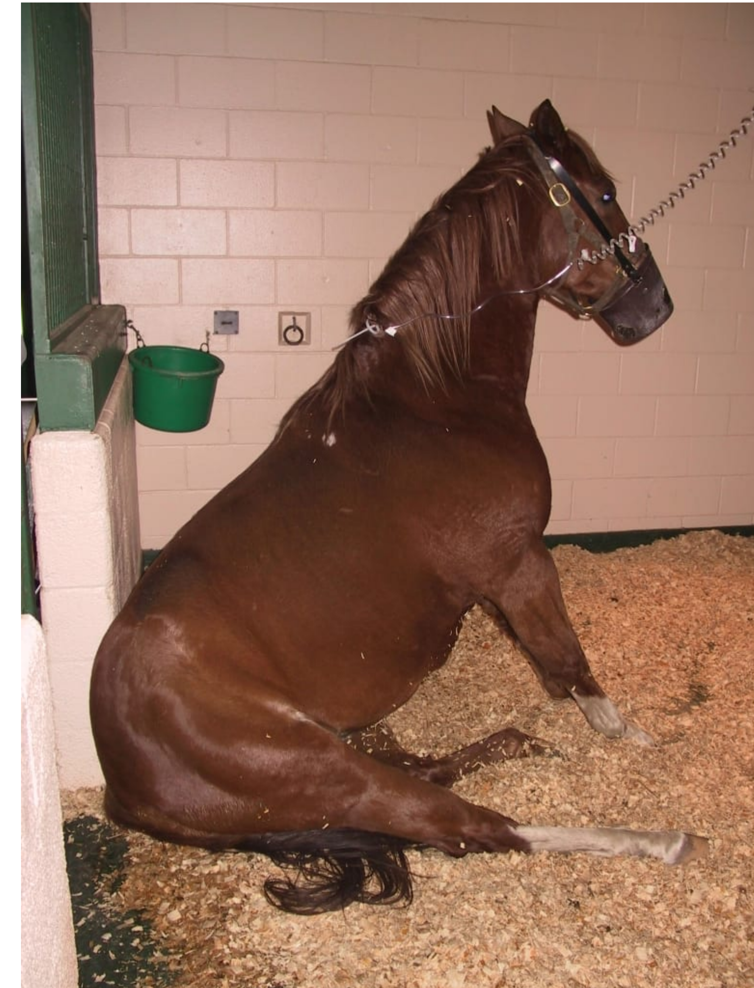
After EHV-4 infects horses, the virus only proliferates in the respiratory tract of horses, resulting in respiratory symptoms in horses. After EHV-1 and EHV-4 infect horses together, the same antigen can often be detected.

Clinical symptom

The clinical symptoms of diseased horses are different, generally including fever, depression, loss of appetite, runny nose, tears, conjunctivitis, eyelid edema, swelling of submandibular lymph nodes, leucopenia, edema of limbs, especially the lower end of hind limbs, swelling of scrotum and foreskin of male horses, and abortion of pregnant horses. The clinical symptoms of old mares and horses with poor nutritional status are more serious, and the symptoms of pregnant mares are more obvious than those of empty mares. If EAV is prevalent in pregnant horses, the abortion rate can be as high as 40% ~ 59%.







病原学检测

Collection and preparation of specimens

PCR
Polymerase chain reaction

Virus isolation

Direct immunofluorescent detection

Immunoperoxidase staining

Histopathology

Virus isolation

Sample collection

Sample delivery

Cell preparation

Sample processing

Culture of monolayer cells

5% CO_2 37℃

Virus attached

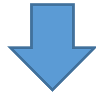
cytopathic effect (CPE)

blind-passaged

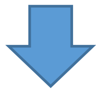
positive or negative

PCR聚合酶链式反应

Sample collection
1、 placenta and fetal tissues from suspect cases of EHV abortion， such as placenta, liver, lung, thymus and spleen
2、Nasal/nasopharyngeal swabs



DNA extraction： virus extraction kit or automatic nucleic acid extraction equipment



EHV 1 Forward: GGG-GTT-CTT-AAT-TGC-ATT-CAG-ACC

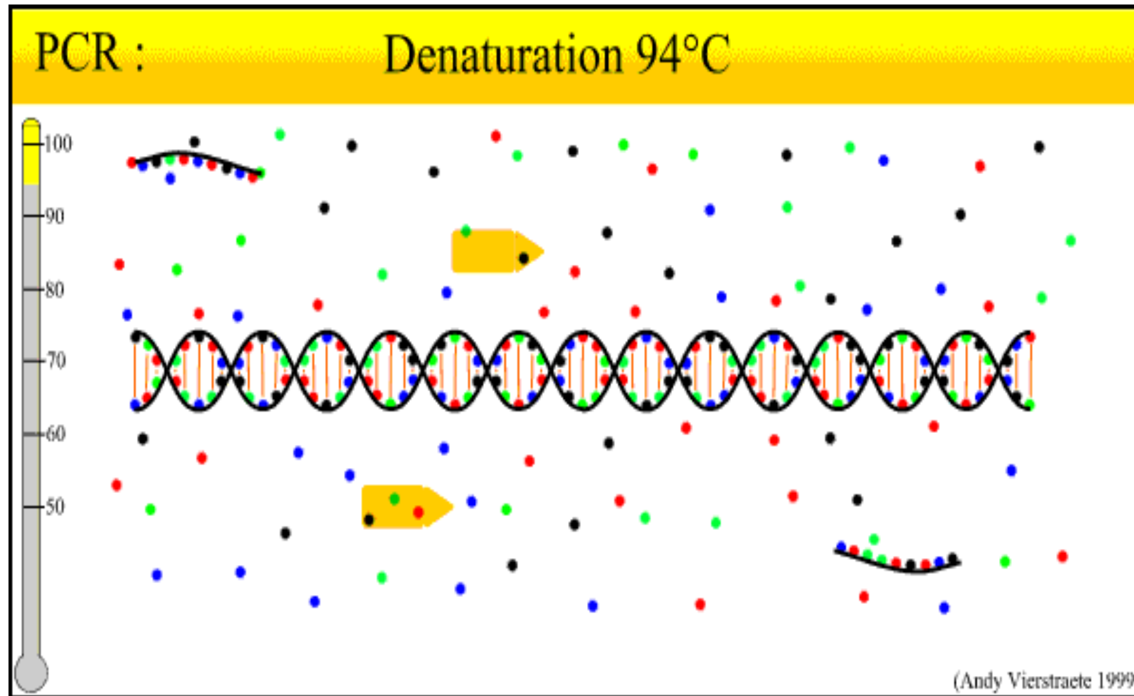
EHV 1 Reverse: GTA-GGT-GCG-GTT-AGA-TCT-CAC-AAG

EHV 4 Forward: TAG-CAA-ACA-CCC-ACT-AAT-AAT-AGC-AAG

EHV 4 Reverse: GCT-CAA-ATC-TCT-TTA-TTT-TAT-GTC-ATA-TGC

EHV1gB/probe: {FAM}TCT-CCA-ACG-AAC-TCG-CCA-GGC-TGT-ACC{BHQ1}

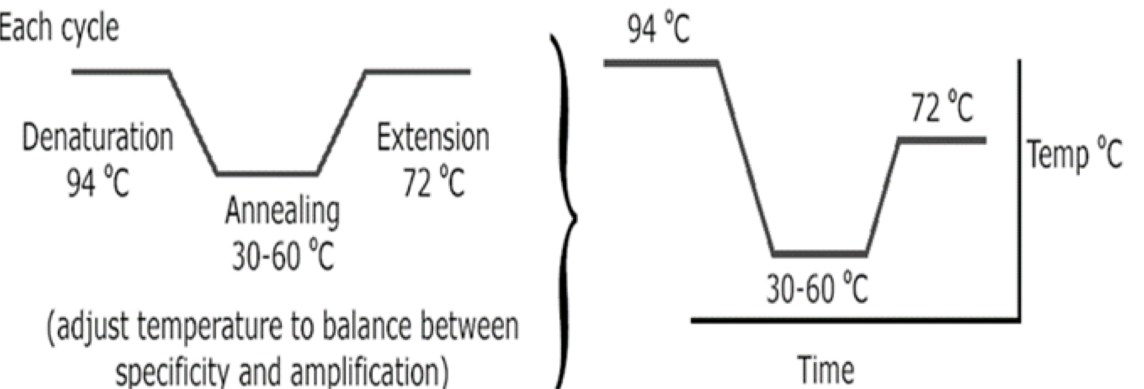
EHV4ORF17/probe: {JOE}CGG-AAC-AGG-AAC-TCA-CTT-CAG-AGC-CAG-C{BHQ1}



PCR reaction contains

- Target DNA (example: environmental DNA)
- 2 primers (20-30 nts long)
- Thermostable DNA polymerase
- Nucleotides (dNTPs)
- Mg^{2+} (cofactor for DNA polymerase)

Mix is subjected to temperature cycling



直接免疫荧光法

Rapid preliminary diagnosis of EHV antigen in tissue samples.The prepared effective EHV-1 polyclonal antiserum was combined with FITC.

Advantages: the diagnostic reliability of the technology is close to that of virus isolation

Disadvantages: it is not suitable for serotyping and needs to be identified by PCR

Direct immunofluorescence

1. Samples of freshly dissected fetal tissues (lung, liver, thymus and spleen) (5 × 5mm pieces) frozen and sliced on a low temperature thermostat at - 20 ° C

2. Fixed on microscope slides with 100% acetone.

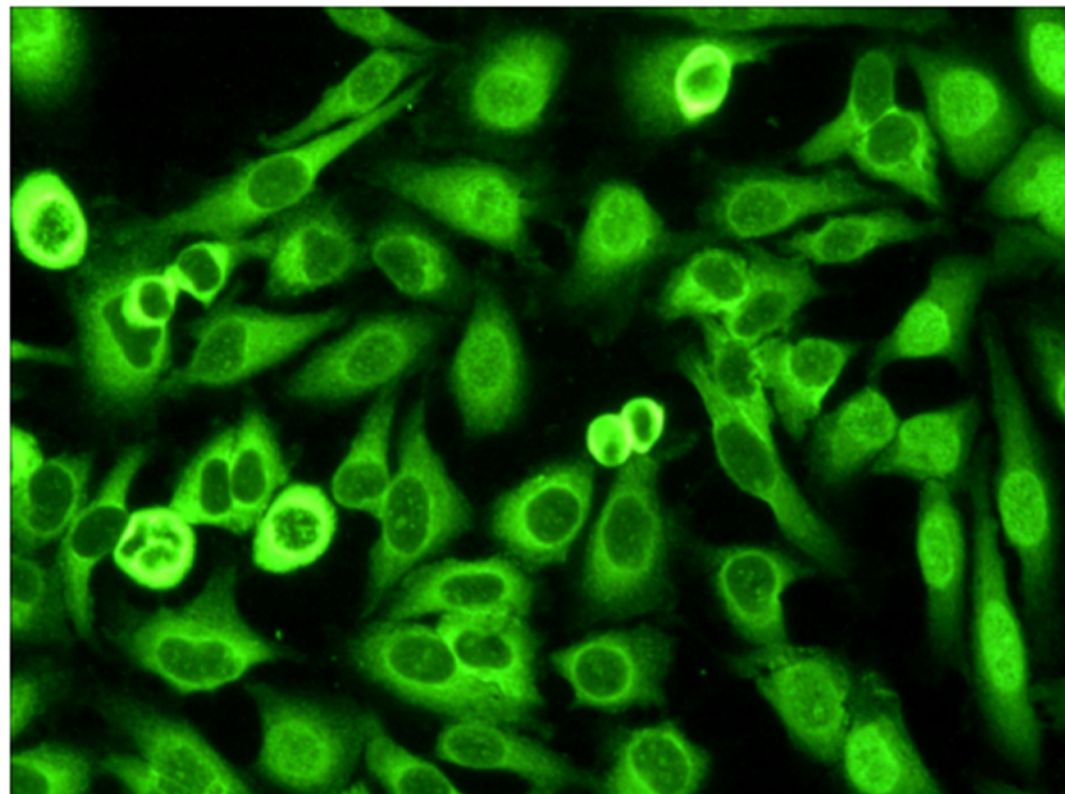
3. After drying, add appropriate EHV-1 binding antibody and culture the slices in a humid environment at 37 ° C for 30 minutes.

4. Wash twice with PBS to remove unreacted antibodies,

5. Cover the tissue with aqueous medium and solution, cover the slide,

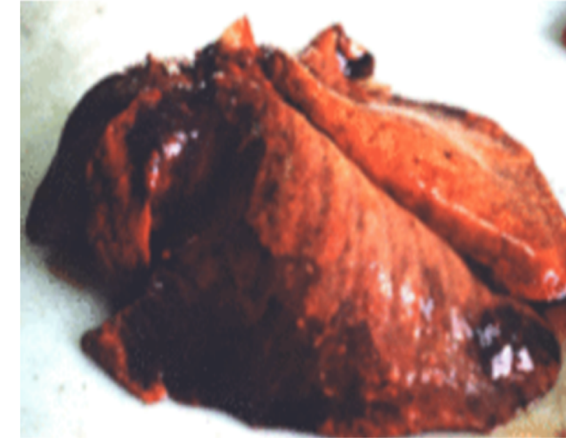
6. Observe whether there are fluorescent cells indicating EHV antigen.

Note: each test should include positive and negative controls, such as known EHV-1 infected and uninfected fetal tissues.

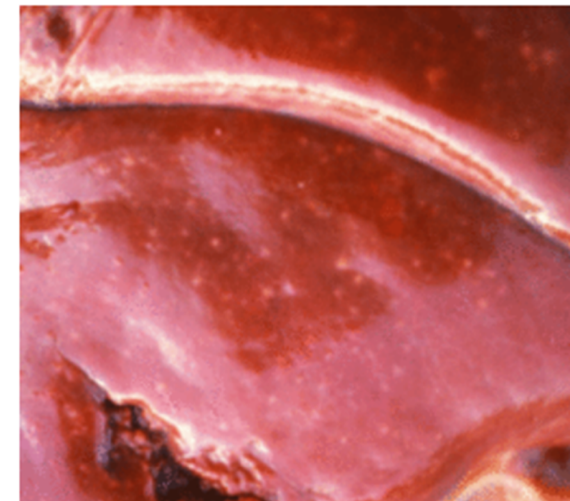


组织病理检测

The tissues of placenta, lung, liver, spleen, adrenal gland and thymus were taken from aborted fetuses and animals with damaged brain and spinal cord nervous system.



The presence of eosinophilic nuclear inclusions in the bronchiole epithelium or cells around the liver necrosis area is consistent with the diagnosis of herpesvirus infection. The characteristic microscopic lesions associated with EHV-1 neuropathy are degenerative cerebral or spinal small vessel thromboangiitis (perivascular cuff and inflammatory cell infiltration, endothelial cell proliferation and necrosis, and thrombosis).



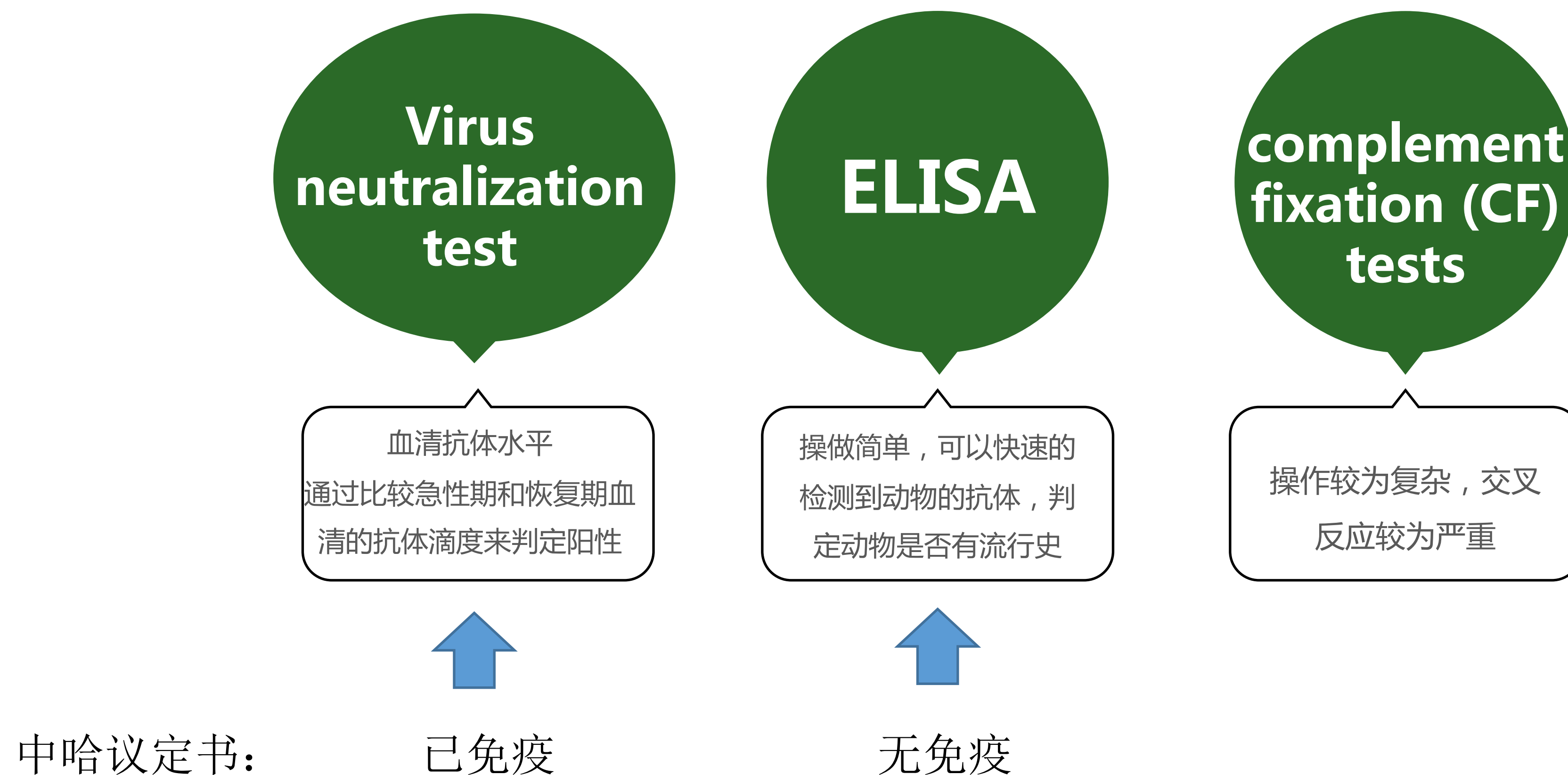
血清学检测 Serological tests

Serological tests

The positive diagnosis is based on a significant increase (four times or more) in antibody titers in the serum of the acute and convalescent phases

- Virus neutralization test
- ELISA
- complement fixation (CF) tests

血清学方法比较



中哈议定书：　已免疫　无免疫

Virus neutralization test

Test reagent consumables requirements:

1. Known titer standard virus strain
2. E-derm or cell
3. Antibody positive and negative control horse serum
4. Flat bottom 96 well microtiter plate (tissue culture grade)

Virus neutralization test

Test environment and equipment requirements:

1. Biosafety level 2 or above Laboratory

2. CO_2 incubator

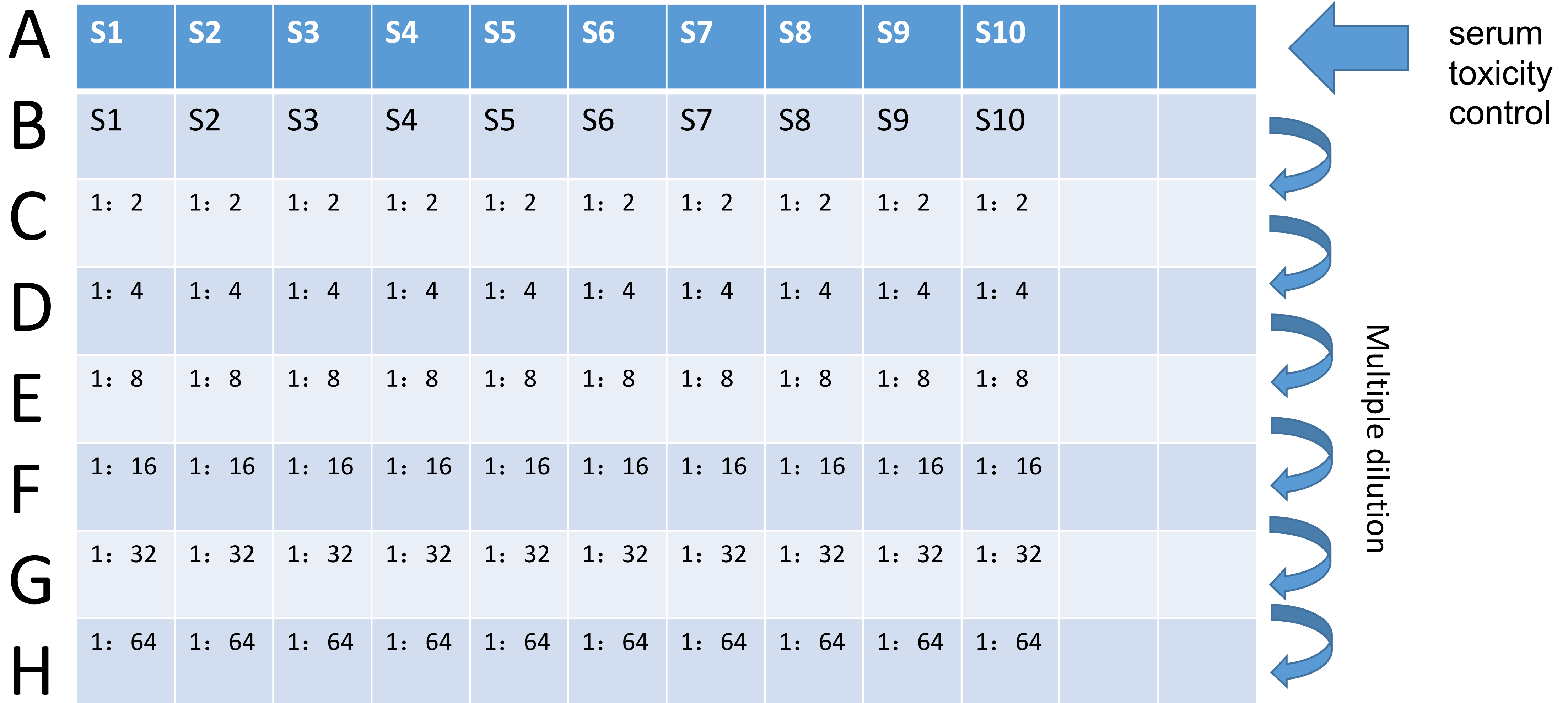
3. Biosafety cabinet

Professional and technical personnel must be trained in corresponding standards and laboratory standard operating procedures, pass the examination and obtain authorization; They should have solid technical skills, be able to correctly use reagents according to standards and reagent instructions, regulations on chemical and waste treatment in numerical laboratory, relevant biosafety regulations, laboratory emergency disposal, results confidentiality regulations and positive results reporting procedures, etc.

Personnel entering the laboratory work area must wear personal protective equipment. The most basic protection includes long sleeved experimental clothes, disposable gloves, disposable medical masks, etc; Appropriate personal protective equipment shall be selected according to the biological factor risk assessment report.

Test procedure

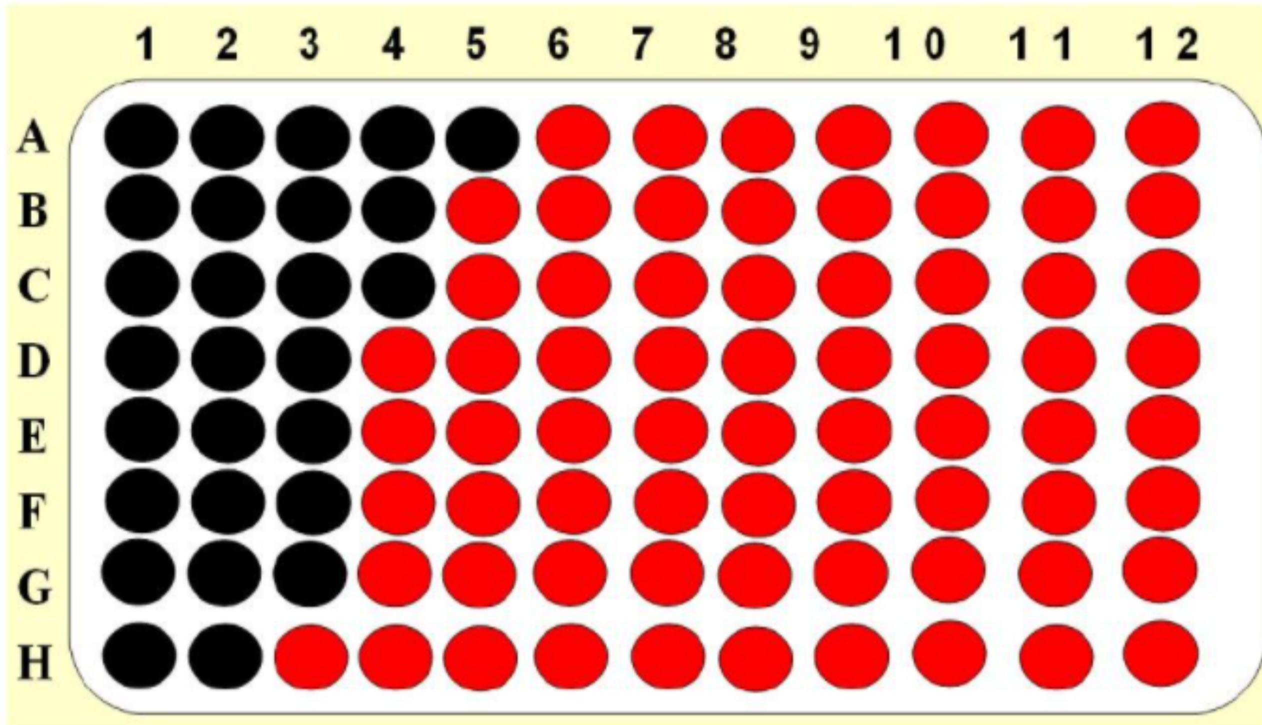
ii) The test serum and control serum were inactivated in water bath at 56 ℃ for 30 min.

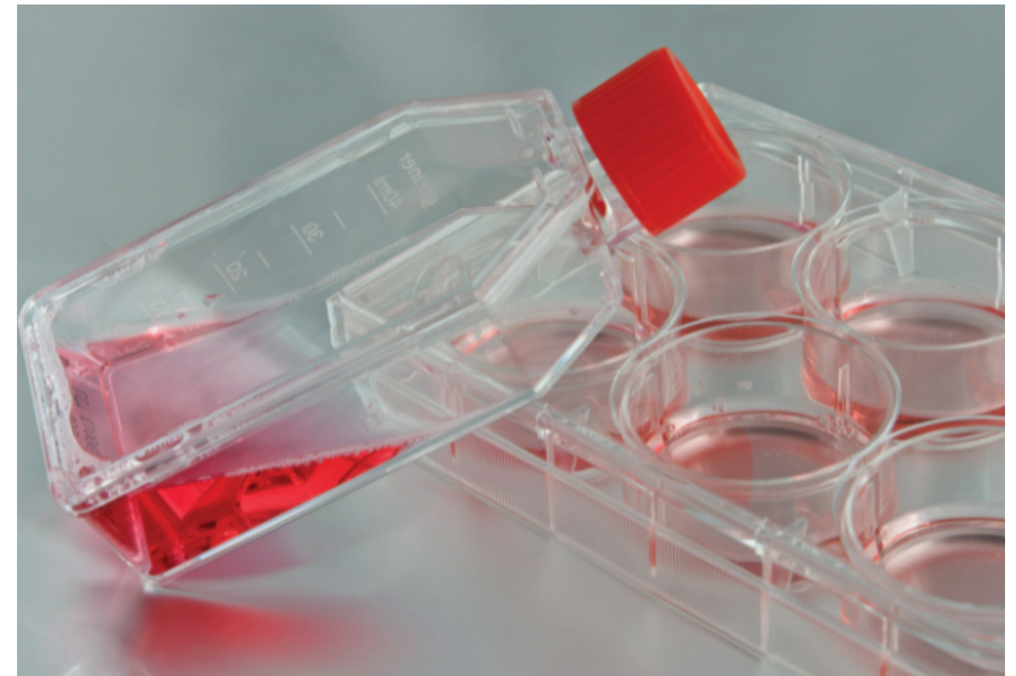


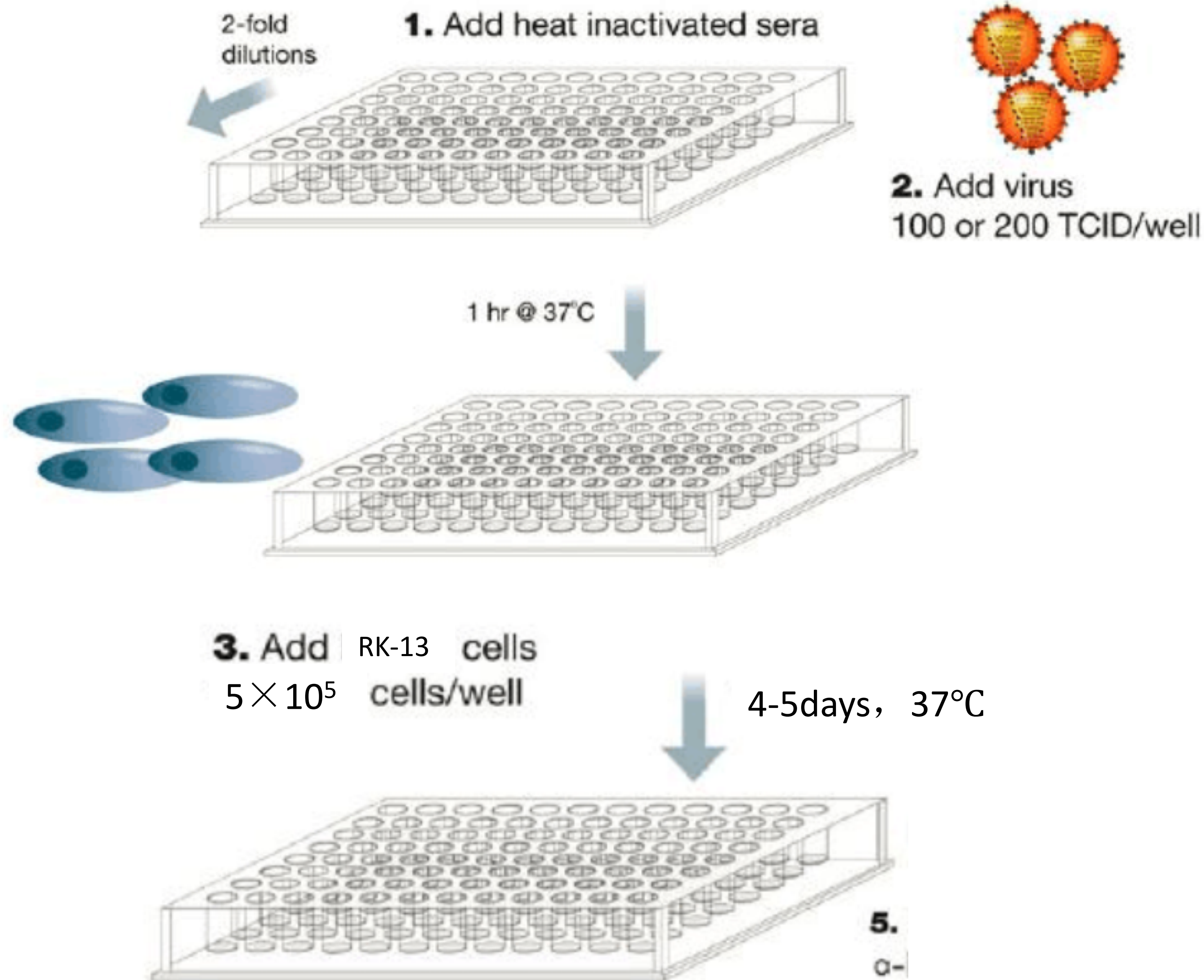
A	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**		
B	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10		
C	1: 2	1: 2	1: 2	1: 2	1: 2	1: 2	1: 2	1: 2	1: 2	1: 2		
D	1: 4	1: 4	1: 4	1: 4	1: 4	1: 4	1: 4	1: 4	1: 4	1: 4		
E	1: 8	1: 8	1: 8	1: 8	1: 8	1: 8	1: 8	1: 8	1: 8	1: 8		
F	1: 16	1: 16	1: 16	1: 16	1: 16	1: 16	1: 16	1: 16	1: 16	1: 16		
G	1: 32	1: 32	1: 32	1: 32	1: 32	1: 32	1: 32	1: 32	1: 32	1: 32		
H	1: 64	1: 64	1: 64	1: 64	1: 64	1: 64	1: 64	1: 64	1: 64	1: 64		

II) add 25 μ l MEM to all holes of the microplate.
III) pipette 25 μ l of test serum into rows a and B of the microplate.Line a was used as the serum toxicity control, and line B was used as the first dilution test of serum toxicity. Gradient dilution from row B to the last row.

IV) add 25 µ l of properly diluted EHV-1 or EHV-4 virus stock solution (100 TCID50 / well) to each well. Row a is excluded, and row a is used to monitor the serum toxicity of serum control well indicating cells.







v) The actual number of viruses used in the test was calculated using a separate control plate, including titration of serum with known titers of negative and positive horses, cell control (virus-free), virus control (serum-free) and virus titration.

VI) incubate the dish at 37 ° C in 5% CO_2 atmosphere for 1 hour.

VII) add 50 μ l of e-derm or rk-13 cell suspension (5) per well × 105 cells / ml)

VIII) incubate at 37 ° C with 5% carbon dioxide in the air for 4-5 days.IX) check the CPE of each hole with a microscope and record the results on the worksheet.

XI) calculate the neutralization titer of each test serum and compare the increase of serum titer in acute and convalescent stages

5.3.2 or vaccinated equidae: two serum neutralization tests of paired samples at 14 days interval, with the negative result of second blood serum with no less than 4-fold increase in titre.

ELISA 检测

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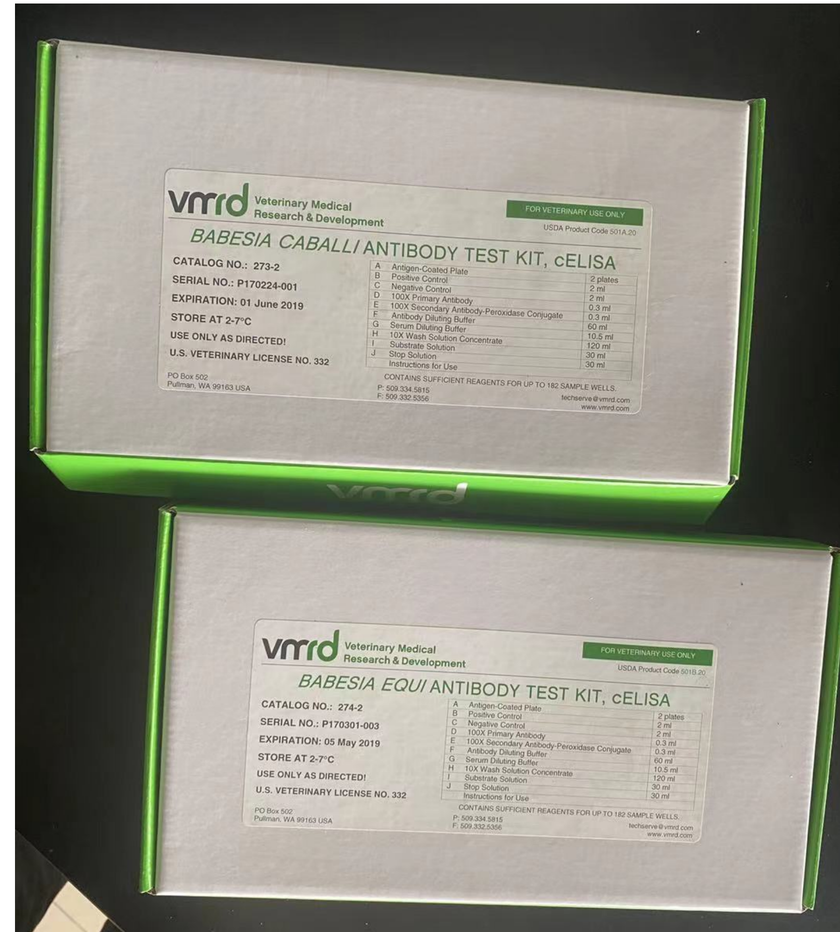
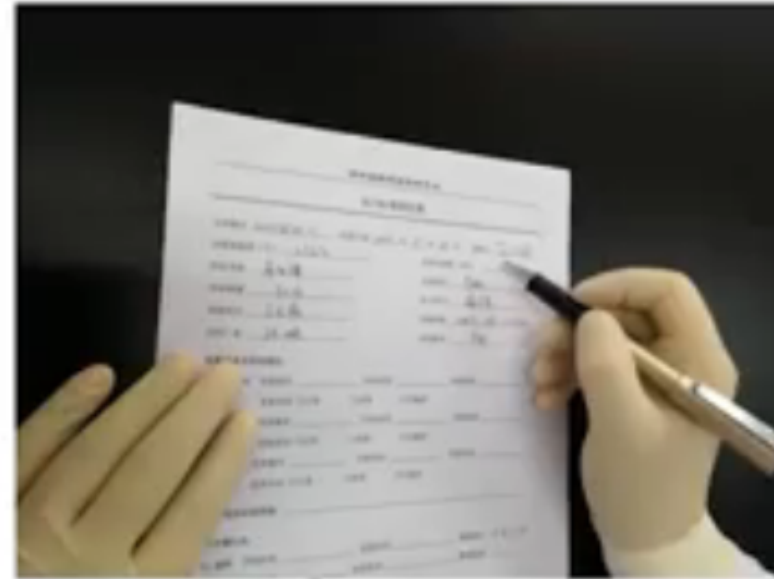


Personnel requirements

Professional and technical personnel must be trained in corresponding standards and laboratory standard operating procedures, pass the examination and obtain authorization; They should have solid technical skills, be able to correctly use reagents according to standards and reagent instructions, regulations on chemical and waste treatment in numerical laboratory, relevant biosafety regulations, laboratory emergency disposal, results confidentiality regulations and positive results reporting procedures, etc.

Personnel entering the laboratory work area must wear personal protective equipment. The most basic protection includes long sleeved experimental clothes, disposable gloves, disposable medical masks, etc; Appropriate personal protective equipment shall be selected according to the biological factor risk assessment report.

The ambient temperature and humidity shall be controlled according to the requirements of testing equipment and specific ELISA test, and relevant records shall be made.

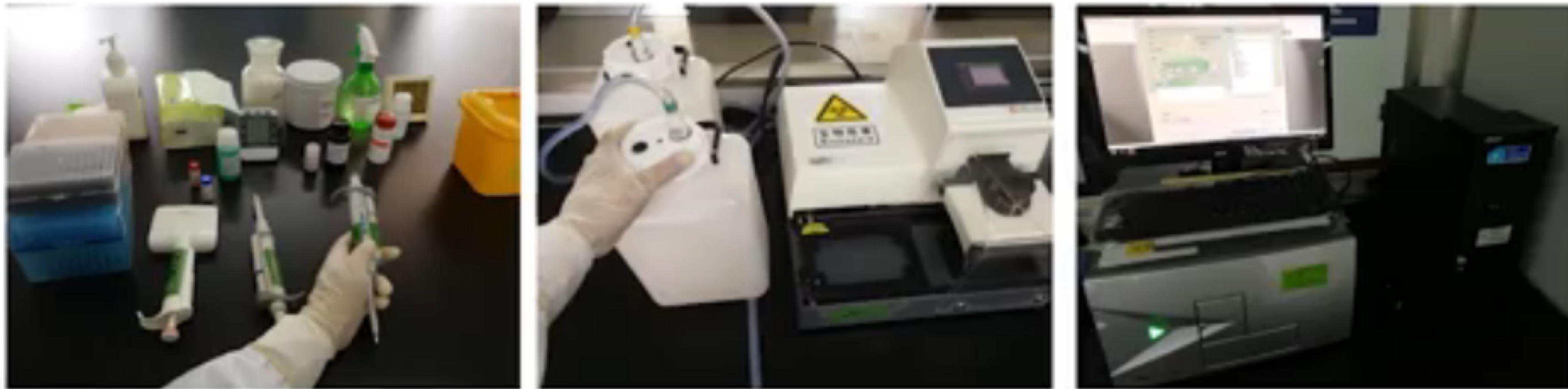


If necessary, the laboratory can be equipped with uninterruptible power supply (UPS) or dual power supply to ensure the normal operation of key equipment (such as plate washer, enzyme labeling instrument, incubator, refrigerator, etc.).

The laboratory shall be equipped with necessary protective facilities, such as emergency eye washer, emergency equipment, etc.

The laboratory identification is clear and standardized

Ensure that the performance and parameters of enzyme labeling instrument, plate washer, pipette and other equipment meet the test requirements; Key equipment shall have correct equipment status identification within the calibration validity period.

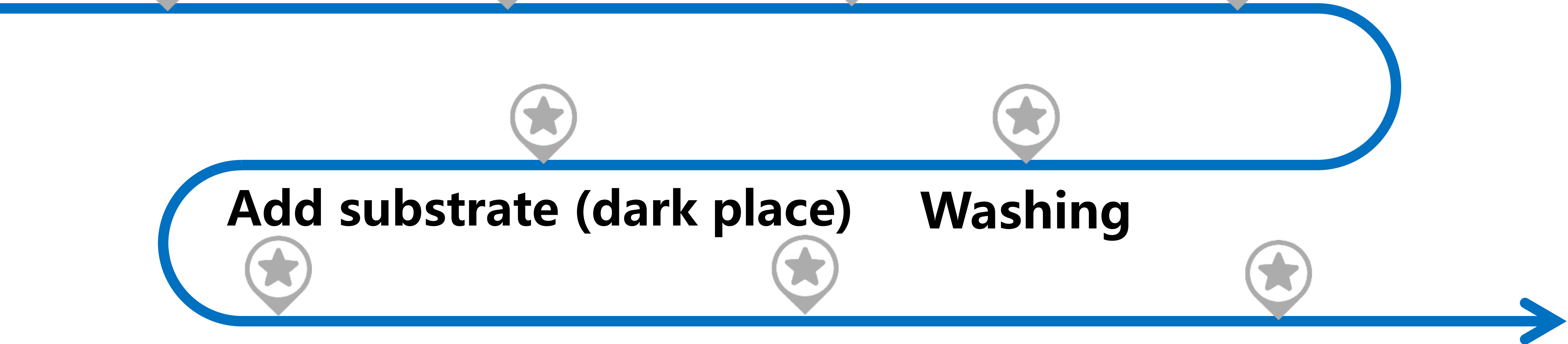


Biosafety cabinet and high-pressure steam sterilizer shall be verified regularly.

Periodic verification, performance monitoring and regular maintenance shall be carried out for key equipment.

Before each test, the equipment shall be started for inspection, and the test can be carried out only after it is confirmed to be normal.

LOGO 公司标志



Key points:
Read standards or SOPs and prepare original records carefully

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SN

中华人民共和国出入境检验检疫行业标准

SN/T 1142—2011
代替 SN/T 1142—2002,SN/T 1377—2004

马病毒性动脉炎检疫技术规范

Quarantine protocol for equine viral arteritis

2011-05-31 发布 2011-12-01 实施

中华人民共和国国家质量监督检验检疫总局 发布

CHAPTER 3.6.10.

EQUINE VIRAL ARTERITIS (INFECTION WITH EQUINE ARTERITIS VIRUS)

SUMMARY

Equine viral arteritis (EVA) is a contagious viral disease of equids caused by equine arteritis virus (EAV), an RNA virus classified in the genus, Arterivirus, *family* Arteriviridae. *Equine arteritis virus is found in horse populations in many countries world-wide. Although infrequently reported in the past, confirmed outbreaks of EVA appear to be on the increase.*

Description of the disease: *The majority of naturally acquired infections with EAV are subclinical. Where present, clinical signs of EVA can vary in range and severity. The disease is characterised principally by fever, depression, anorexia, dependent oedema, especially of the limbs, scrotum and prepuce in the stallion, conjunctivitis, an urticarial-type skin reaction, abortion and, rarely, a fulminating pneumonia, enteritis or pneumo-enteritis in young foals. Apart from mortality in young foals, the case-fatality rate in outbreaks of EVA is very low. Affected horses almost invariably make complete clinical recoveries. A long-term carrier state can occur in a variable percentage of infected stallions, but not in mares, geldings or sexually immature colts.*

Identification of the agent: *EVA cannot be differentiated clinically from a number of other respiratory and systemic equine diseases. Diagnosis of EAV infection is laboratory dependent and based on virus isolation, detection of nucleic acid or viral antigen, or demonstration of a specific antibody response. Detection and identification of EAV nucleic acid in suspect cases of the disease can be attempted using various reverse-transcription polymerase chain reaction (RT-PCR) assays. The identity of isolates of EAV should be confirmed by RT-PCR assay, neutralisation test, or by immunocytochemical methods, namely indirect immunofluorescence or avidin–biotin–peroxidase techniques.*

Where mortality is associated with a suspected outbreak of EVA, a wide range of tissues should be examined for histological evidence of panvasculitis that is especially pronounced in the small arteries throughout the body. The characteristic vascular lesions present in the mature animal are not a notable feature in EVA-related abortions, diagnosis of which is based on virus isolation, viral nucleic acid detection by RT-PCR or demonstration of EAV antigens by immunohistochemical examination of placental and various fetal tissues.

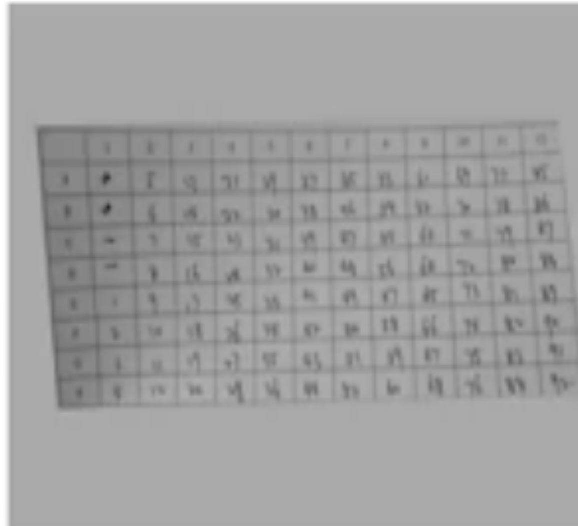
Serological tests: *A variety of serological tests, including virus neutralisation (VN), complement fixation (CF), indirect fluorescent antibody, agar gel immunodiffusion, the enzyme-linked immunosorbent assay (ELISA), and the fluorescent microsphere immunoassay assay (MIA) have been used for the detection of antibody to EAV. The tests currently in widest use are the complement-enhanced VN test and the ELISA. The VN test is a very sensitive and highly specific assay of proven value in diagnosing acute infection and in seroprevalence studies. Several ELISAs have been developed. Although none have been as extensively validated as the VN test, some offer comparable specificity and close to equivalent sensitivity. The CF test is less sensitive than either VN test or ELISA, but it can be used for diagnosing recent infection.*

Requirements for vaccines: *Two commercial tissue culture derived vaccines are currently available against EVA. One is a modified live virus (MLV) vaccine prepared from virus that has been attenuated for horses by multiple serial transfers in primary equine and rabbit kidney cells and in an equine dermal cell line. It has been confirmed to be safe and protective for stallions and nonpregnant mares. Vaccination of foals less than 6 weeks of age and of pregnant mares in the final 2 months of gestation is not recommended. There is no evidence of back reversion of the vaccine virus to virulence or of recombination with naturally occurring strains of EAV following its*

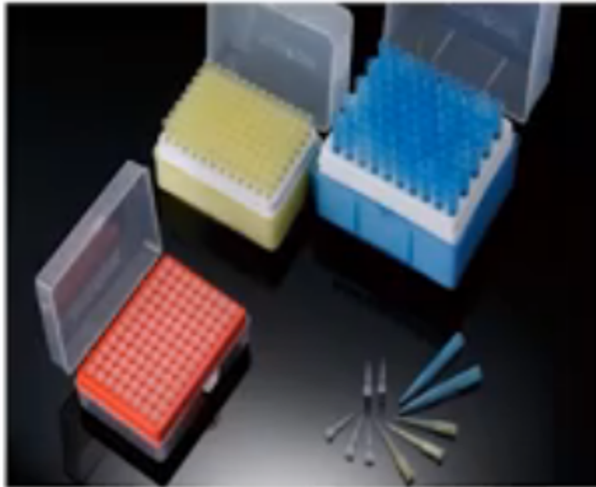
Sample preparation:
During sample acceptance, check whether the sample status, name, number, sample quantity and packaging meet the requirements, and make relevant records. There are many types of ELISA test samples, the most commonly used is serum.

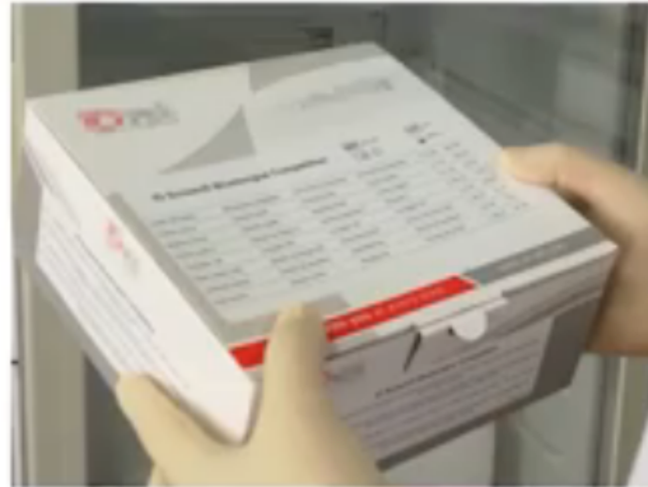
Key points of detection

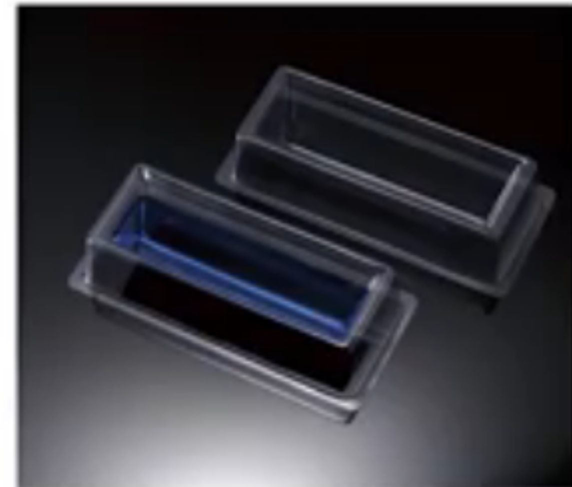
No.: number the sample plate to be tested and the enzyme label plate accordingly; Mark the positive and negative control, and register the samples to be tested in the micropores one by one.



Preparation of reagent consumables: prepare sufficient reagent consumables according to the sample quantity, and carefully check the reagent packaging, validity period, reagent composition and other information before the test.





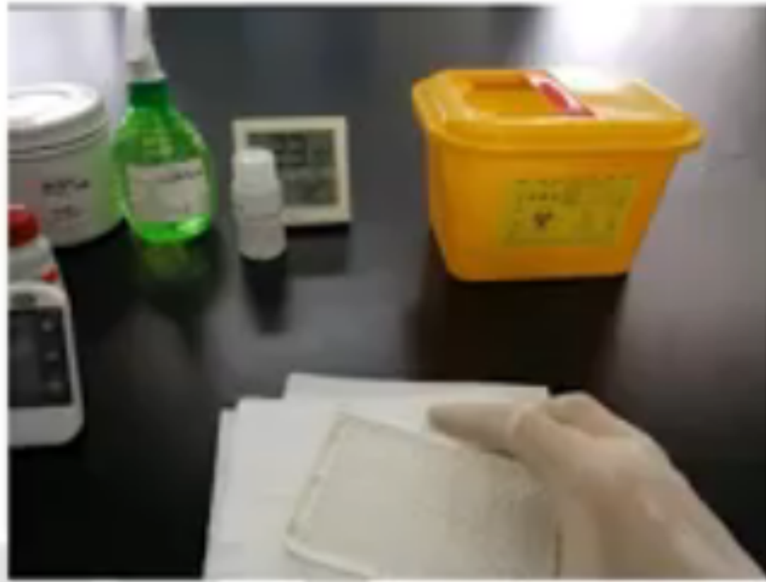


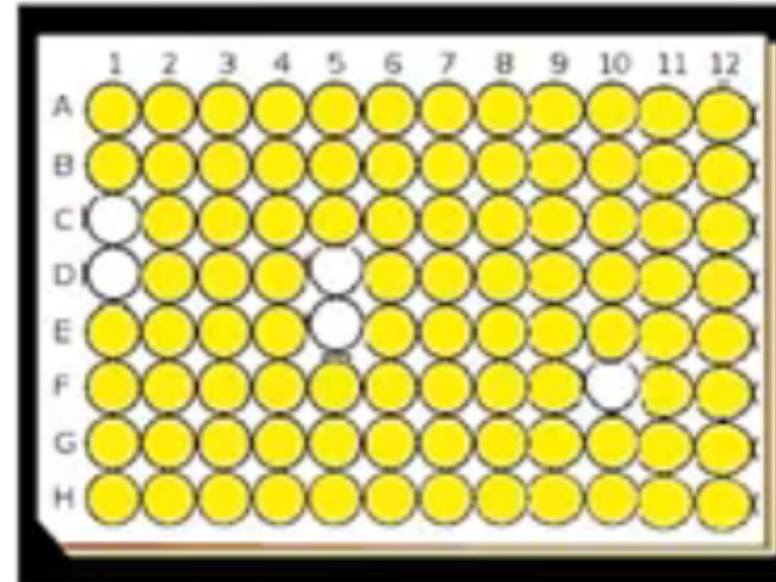
Solution preparation: the lotion, enzyme and tuberculosis in the kit shall be prepared in strict accordance with the reagent instructions, and shall be used and prepared as soon as possible.

Liquid addition: when adding samples, yin-yang control, enzyme conjugates, substrate solution and termination solution, ensure that the liquid addition amount is accurate, and mix well after each liquid addition to avoid pollution.

Accurate liquid transfer technique: after sucking, use the suction head to contact the side of the "container" to remove the excess liquid outside the suction head. When using the pipette, if the microplate is empty, please extend the suction head to the lower corner of each hole (without touching the bottom); If there is liquid in the microplate, please put the tip of the suction head above the liquid. Try to suck the liquid for 1 ~ 2 times, and observe whether the liquid level in the suction head is flush.

Washing: wash in strict accordance with the number of times the kit is washed and the amount of detergent in the kit. Cross contamination should be avoidedColor development: avoid light, accurately time and stop the reaction in time.



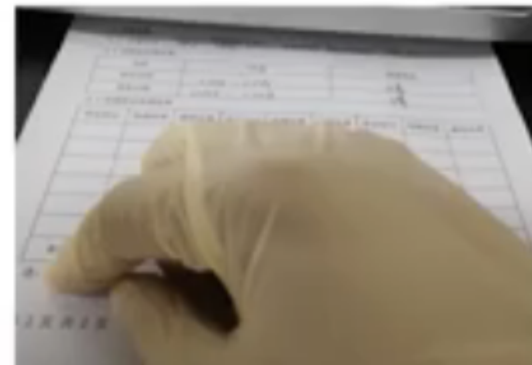


Incubation: it is necessary to confirm the incubation temperature and incubation time, and time them accurately

Reading: the program setting of the microplate reader meets the requirements of the kit. First observe the color of the microplate with the naked eye, confirm that the reading is accurate, and properly save the original data

Key points of detectionResult calculation: when the negative and positive control results were established, the calculation was accurate; If the negative and positive control results are abnormal, it is necessary to find out the cause and retestResult

Determination: it shall be determined in strict accordance with relevant standards or kit instructions. Positive samples and suspicious samples shall be retested for confirmation.



Waste treatment: after high-pressure sterilization, submit it to the medical waste treatment company and make relevant records.

Cleaning and disinfection: after the experiment, the laboratory environment, facilities and equipment shall be thoroughly disinfected, the ultraviolet lamp shall be turned on for disinfection for more than 30 minutes, and relevant records shall be made.

- Thanks