Report number: XF-2022003

Testing report of anti-foot-and-mouth disease virus effect of VIRSHA (potassium monopersulphate compound)

Requestor: Hebei Erao Biotech Co.,Ltd
Sample name: VIRSHA (potassium monopersulphate compound)
Test item: Detection of anti-foot-and-mouth disease virus effect of VIRSHA (potassium monopersulphate compound)
Testing time: May 2022 to July 2022
Testing lab: Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences
July 20,2022

Report number: XF-2022003

Testing Report

Sample Name	VIRSHA(potassium monopersulphate compound)	**Sample acceptance number**	XF-2022003
Sample specifications	500g	**Number of samples**	1 bag
Requester	Hebei Erao Biotech Co.,Ltd	**Sample status**	pink powder
Test items	Detection of anti-foot-and-mouth disease virus effect of VIRSHA (potassium monopersulphate compound)	**Date of submission**	April 06, 2022
Test Results	After the samples submitted for inspection are diluted in a certain proportion, they are mixed with an equal volume of foot-and-mouth disease virus solution. At the cell test level, VIRSHA can completely kill the foot-and-mouth disease virus when it is diluted at a ratio of 1:150 or less and interacts with the foot-and-mouth disease virus for 10 minutes or more.		
Inspector	吴卿敖、侯俊玉	**Date**	2022.7.20
Reviewer		**Date**	2022.7.20
Signer		**Date**	2022.7.20
Remark	Please see the attachment for the test report		
Test Lab	Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences		

APPENDIX

Detection of anti-foot-and-mouth disease virus effect of VIRSHA (potassium monopersulphate compound)

Refer to the "Technical Specifications for Disinfection" (2002 Edition), "Technical Specifications for the Identification of Veterinary Disinfectants" (1992 Edition), "European Veterinary Disinfectant Virus Killing Efficacy Testing Standards" (EN14675) and "Foot-and-mouth Disease Diagnostic Technology" (GB/T18935 -2018) and other methods, this laboratory has used VIRSHA manufactured by Hebei Erao Biotech Co.,Ltd, which was entrusted with the inspection, conducts qualitative evaluation of anti-foot-and-mouth disease virus (FMDV) at the cellular level.The test results are reported below.

1 Materials and methods

1.1 Test materials

Cells used for testing: suckling hamster kidney cells (BHK-21).
Foot-and-mouth disease virus strain: FMDV/O/Mya98/2010 was provided by the National Foot-and-mouth Disease Reference Laboratory.
The poison titer is $10^{-5.8}TCID_{50}/50\mu l$.
Sample information: VIRSHA (sample submitted for inspection)
Test consumables: MEM medium and fetal bovine serum were purchased from Gibco; 25cm² cell culture flask; 96-hole cell culture plate; 10ml pipette; 200μl, 1000μl gun tip; 1.5ml Eppendorf tube and other experimental consumables.The materials were all purchased from Corning Company.

1.2 Test methods

1.2.1 Material preparation

Prepare pH7.6 MEM culture medium (referred to as maintenance medium, without adding double antibodies and serum) as sample diluent and negative control. Weigh 1g of the sample to be submitted for inspection, dissolve it in 10ml of maintenance solution, mix thoroughly, and prepare a 10× working solution.

1.2.2 Determination of toxicity of submitted samples to BHK-21 cells

Reaction mode: test sample+maintenance solution+cells→cell control group
Aspirate and dilute 10× the supernatant of the sample working solution to be submitted for inspection, and perform a 2-fold gradient dilution. Add 10×, 20×, 40×, 80×...10240× to the 96-hole plate in sequence, 100μl per hole, one column for each titer, repeat 8 holes; at the same time, set up a negative control column. Add 100 μl of BHK-21 cell suspension that has grown into a monolayer within 24 hours to each hole. Place the 96-hole plate in a 37°C, 5% CO_2 constant-temperature CO_2 incubator. Observe whether there is cytotoxicity in each cell culture hole every 24 hours. The final result will be judged after 72 hours.

1.2.3 Determination of the effective concentration of inactivated foot-and-mouth disease virus in samples submitted for inspection

Reaction mode (1) Samples submitted for inspection at different concentrations + virus + cells → Experimental group
Take an appropriate amount of the working solution of the sample to be submitted for inspection, and dilute it with the maintenance solution to make the final concentration 100×, 150× and 200×. Mix the sample suspensions of different concentrations with an

equal volume of $10^{-5.8}$TCID$_{50}$/50μl virus liquid. After 10 minutes of incubation, centrifuge, aspirate the supernatant, and perform 10-fold gradient dilution with maintenance solution. Follow the instructions above. Add clear stock solution 10^{-1} , 10^{-2} , 10^{-3} ...10^{-9} in sequence to the 96-hole plate, 100μl/hole, repeat for 8 holes, add 100μl to each hole.The BHK-21 cell suspension grew into a monolayer within 24 hours. Also set up negative controls and blank controls. Place the 96-hole plate in a 37°C, 5% CO_2 constant temperature CO_2 incubator, and observe each cell culture hole every 24 hours.
Whether there is a cytopathic effect (CPE) in the virus, a final judgment will be made at 72 hours and the virus titer (TCID$_{50}$) will be calculated.
Reaction mode (2) virus + maintenance solution + cells → virus control group
Take an appropriate amount of $10^{-5.8}$TCID$_{50}$/50μl virus liquid, dilute it 10 times with maintenance solution, and add it to the 96-hole plate in the order of 10^{-1}, 10^{-2}, 10^{-3} ...10^{-9}. 100μl per hole, one column for each titer, repeat 8 holes, add 100μl to each hole. The BHK-21 cell suspension grew into a monolayer within 24 hours. Also set up negative controls and blank controls. The 96-hole plate was cultured in a constant temperature CO_2 incubator at 37°C and 5% CO_2. Every 24 hours, each cell culture holewas observed to see whether there was a cytopathic effect (CPE). The final judgment was made at 72 hours and the virus titer (TCID$_{50}$) was calculated.

1.2.4 Determination of the effective time of inactivating foot-and-mouth disease virus in samples submitted for inspection

Take 150× sample suspension and mix it thoroughly with an equal volume of $10^{-5.8}$ TCID$_{50}$/50μl virus liquid, incubate for 5min and 10min respectively at room temperature, centrifuge, and pipette the supernatant, as in 1.2.3 (1) to measure virus titers respectively.

2 Results

2.1 Test results of BHK-21 cytotoxicity of samples submitted for inspection

The cells in the supernatant experimental group diluted 3200×~10240× of the samples submitted for inspection were normal, while the cells in the supernatant experimental group diluted 10×~1600× were all lethal. Negative control cells and blank control group were normal.

2.2 Determination of the effectiveness of inactivating foot-and-mouth disease virus in samples submitted for inspection

The 100×, 150×, and 200× diluted suspensions of the samples to be inspected were mixed with equal volumes of foot-and-mouth disease virus respectively. After 10 min of action, all the viruses in the 100× and 150× supernatants were killed, and the 200× supernatant viruses were The titer dropped from $10^{-5.8}$TCID$_{50}$/50μl to $10^{-2.2}$TCID$_{50}$ /50μl. The sample submitted for inspection was diluted 150× and incubated for 5 minutes. The virus titer of the supernatant dropped from $10^{-5.8}$TCID$_{50}$ /50μl. $10^{-1.5}$ TCID$_{50}$/50μl. Negative control cells and blank.The control group was normal,

3 Conclusion

After diluting the sample in a certain proportion and mixing it with the same volume of foot-and-mouth disease virus solution, at the cellular testing level, VIRSHA can completely kill the foot-and-mouth disease virus after being diluted in a ratio of 1:150 or less and interacting with the virus for 10 minutes or more.