Report number: XF-2022006

Testing report of the effectiveness of VIRSHA (potassium monopersulphate compound) in killing porcine epidemic diarrhea virus

Requester: Hebei Erao Biotech Co.,Ltd

Sample name: VIRSHA (potassium monopersulphate compound)

Test item: Detection of the effectiveness of VIRSHA in killing porcine epidemic diarrhea virus

Testing time: November 2022 to December 2022

Testing lab: Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences

December 26, 2022

Testing Report

Sample Name	VIRSHA (potassium monopersulphate compound)	**Sample acceptance number**	XF-2022006
Sample specifications	1000g	**Number of samples**	1 bag
Requester	Hebei Erao Biotech Co.,Ltd	**Sample status**	pink powder
Test items	VIRSHA in killing porcine epidemic diarrhea virus	**Date of submission**	November 19, 2022
Test results	The samples submitted for inspection are diluted in a certain proportion and mixed with the porcine epidemic diarrhea virus in equal volumes. After the cell test level is diluted at a ratio of 1:150 or less, VIRSHA can completely kill the porcine epidemic diarrhea virus for 5 minutes or more. Kill viruses.		
Inspector		**Date**	2022年12月26日
Reviewer		**Date**	2022年12月26日
Signer		**Date**	2022年12月26日
Remark	Please see the attachment for the test report		
Test lab	Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences		

APPENDIX

VIRSHA in killing porcine epidemic diarrhea virus at the cellular level

With reference to the "Technical Specifications for Disinfection" (2002 Edition), "Technical Specifications for the Identification of Veterinary Disinfectants" (1992 Edition), "European Standard for Testing the Virus Killing Efficacy of Veterinary Disinfectants" (EN14675) and other methods, our laboratory conducted the Hebei Erao Biotech Co., Ltd. sent for testing VIRSHA for qualitative evaluation of killing porcine epidemic diarrhea virus (PEDV) at the cellular level. The test results are reported below.

1 Materials and methods

1.1 Test materials

Cells used for testing: African green monkey kidney cells (Vero cells).
Porcine epidemic diarrhea virus (PEDV) strain: PEDV CH/HNLB/2017 was provided by the State Key Laboratory of Pathogen Biology of Livestock Diseases, Lanzhou Institute of Veterinary Medicine, Chinese Academy of Agricultural Sciences, with a virus titer of $10^{-6.125}TCID_{50}$ /ml.
Sample information: VIRSHA (potassium monopersulphate compound)
Test consumables: DMEM culture medium and fetal calf serum were purchased from Gibco; $75cm^2$ cell culture flask; 96-hole cell culture plate; 10ml pipette; 200μl, 1000μl gun tip; 1.5 ml, 2 ml Eppendorf tube, centrifuge tube and other experimental consumables were purchased from Corning Company.

1.2 Test methods

1.2.1 Material preparation

Prepare pH 7.6 DMEM 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 maintenance solution, mix thoroughly, and prepare 10x working fluid.

1.2.2 Toxicity measurement of Vero cells by samples submitted for inspection

Reaction mode: sample submitted for inspection + maintenance solution + cells-cell control group
Aspirate 10× working solution and dilute it 2 times. According to 10×, 20×, 40×, 80×••10240×, add to the 96-hole plate in sequence, 100μl per holel, one column for each titer, repeat 8 holes. At the same time, set up a column to add the negative control group of cells. Add 100μl of Vero cell suspension to each hole that has grown into a monolayer within 24 hours. Place the 96-hole plate in an incubator at 37°C, 5% CO_2 and constant temperature CO_2. Observe whether cytotoxicity occurs in each cell culture hole. The final result will be judged after 96 hours.

1.2.3 Determination of the effective concentration of inactivated porcine epidemic diarrhea virus in samples submitted for inspection

Reaction mode (1): different concentrations of submitted samples + virus + cells as one experimental group
Take an appropriate amount of the sample working solution for inspection and dilute it with maintenance solution so that the final concentration is 100×, 150×, 200× and 300×. Mix the samples submitted for testing with different concentrations with an equal volume of $10^{-6.125}TCID_{50}$ /ml virus liquid, and let it react for 10 minutes. Each concentration experimental group was diluted 10 times with maintenance solution, and then added to the 96-hole plate in the order of supernatant stock solution, 10^{-1}, 10^{-2}, 10^{-3}, ••10^{-9}", 100μl for each hole, one column for each titer, repeat 8 holes, and add 100μl of Vero cell suspension that has grown into a monolayer within 24 hours to each hole. Also set up negative controls and blank controls. The 96-hole plate was placed at 37°C, 5% CO_2 cultivate in a constant-temperature CO_2 incubator, observe whether cytopathic effect (CPE) appears in each cell culture hole, and finally judge and calculate the virus titer ($TCID_{50}$) after 96 hours.
Reaction mode (2): virus+maintenance solution+cells-virus control group
Absorb an appropriate amount of $10^{-6.125}TCID_{50}$ /ml virus liquid, mix it with an equal volume of maintenance solution, and incubate at room temperature for 10 minutes. For example, in reaction mode (1), dilute 10 times with maintenance solution according to 10^{-1}, 10^{-2}, Add 10^{-3} and •• 10^{-9} in sequence to the 96-hole plate, 100μl per hole, one column for each titer, repeat for 8 holes, and add 100μl to each hole of the Vero cell suspension that has grown into a monolayer within 24 hours. Also set up negative controls and blank controls. The 96-hole plate was cultured in an incubator at 37°C, 5% CO_2 and constant temperature CO_2. Regularly observe whether there was a cytopathic effect (CPE) in each cell culture hole. The virus titer ($TCID_{50)}$ was finally judged and calculated after 96 hours.).

1.2.4 Determination of the effective action time of inactivated porcine epidemic diarrhea virus in samples submitted for inspection

Take 150x of the sample to be tested and mix it thoroughly with an equal volume of $10^{-6.125}TCID_{50}$ /ml virus liquid, and separate at room temperature.
Act for 5 minutes and 10 minutes respectively, and measure the virus titers respectively as in (1) in 1.2.3.

2 Results

2.1 Determination of Vero cell toxicity of submitted samples

The cells in the supernatant experimental group diluted from 10× to 2560× were all lethal, and the cells in the supernatant experimental group diluted from 5120× to 10240× were normal. Negative control cells and blank control group were normal.

2.2 Determination of the effectiveness of inactivating porcine epidemic diarrhea virus in samples submitted for inspection

The 100×, 150×, 200×, and 300× diluted suspensions of the samples to be tested were mixed with equal volumes of porcine epidemic diarrhea virus respectively. After 10 minutes of action, all the viruses in the 100× and 150× supernatants were killed, and

the 200× supernatant was The virus titer of 300× supernatant dropped from $10^{-6.125}$ $TCID_{50}$ /ml to $10^{-4.25}TCID_{50}$ /ml, and the virus titer of 300× supernatant dropped from $10^{-6.125}TCID_{50}$ /ml to $10^{-4.375}TCID_{50}$/ml; The samples submitted for inspection were diluted 150×, and all the viruses in the supernatant were killed after 5 minutes of action. Negative control cells and blank control group were normal.

3 Conclusion

After the sample to be tested is diluted in a certain proportion, it is mixed with the porcine epidemic diarrhea virus in equal volumes. At the cell test level, VIRSHA (potassium monopersulphate compound) is diluted in a ratio of 1:150 or less, and then reacted with the porcine epidemic diarrhea virus for 5 minutes, and above the time can completely kill the virus.

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