Test report on the killing effect of VIRSHA (potassium hydrogen monopersulphate) on African swine fever virus

1. Quality requirements comply with ISO17025 quality management system specifications

2. Experimental location: Animal Biosafety Level 3 Laboratory of National Exotic Animal Disease Diagnostic Center;**Testing Lab:** National African Swine Fever Reference Laboratory, China Center for Animal Health and Epidemiology

3. Sample information

Product Name	Active ingredients and concentration	Traits	Requestor	Production Date
VIRSHA potassium hydrogen monopersulphate powder	potassium hydrogen monopersulphate (Available chlorine not be less than 10%)	light red granular powder	Hebei Erao Biotech Co., Ltd.	2021.06.20

4.Test objectives

Tests the effectiveness of VIRSHA products in inactivating African swine fever virus in cell cultures under specified working conditions.

5.Materials and methods

5.1 Materials

The epidemic strain of African swine fever virus was isolated and identified by the National African Swine Fever Specialist Laboratory. The virus titer was determined to be $10^{72}HAD_{50}$/mL.

Primary porcine alveolar macrophages, porcine red blood cells and serum were prepared and provided by the National African Swine Fever Reference Laboratory. Healthy pig lungs were collected aseptically, primary porcine alveolar macrophages were prepared by lavage, and red blood cells and serum were collected and prepared at the same time.

5.2 Method

5.2.1 Test concentration

Refer to the working concentration range of the VIRSHA instructions, reasonably select a low, medium, and high working concentration, and prepare the VIRSHA to 10 times the working concentration for later use.

5.2.2 Action program

For each working concentration group, add 1 volume of 10 times working concentration VIRSHA and 1 volume of virus liquid into 8 volumes of PBS containing 1% pig serum and mix thoroughly. The mixture was incubated at 4°C for 30 min and 20°C for 30 min.

5.2.3 Cell seeding

After the effect, the above mixture was continuously diluted 10 times to 10% with PBS containing 1% porcine serum, and the primary porcine alveolar macrophage culture was inoculated, and porcine red blood cells were added at the same time.

5.2.4 Cultivation and observation

Place the cell plate at 37°C and 5% CO_2 and culture for 6 days. Check each cell culture well every day to see if there is a red blood cell adsorption reaction (HAD). If there is no HAD phenomenon in the serially diluted cell culture wells, it is judged that the VIRSHA can effectively inactivate African swine fever virus under this condition; if there is HAD phenomenon in the serially diluted cell culture wells, it is judged that the VIRSHA cannot effectively inactivate the African swine fever virus under this condition. Effectively inactivates African swine fever virus. The test results cannot be judged. Passage once and judge the results again.

6.Test results

After each working concentration group interacts with virus liquid, primary porcine alveolar macrophages are inoculated and cultured for observation. The results of inactivation efficacy determination are detailed in the table below.

Product name	Conditions of action		
	Working concentration	20°C for 30 minutes	4°C for 30 minutes
VIRSHA	1:100	v	v
	1:200	v	v

	1:400	v	v

Note: " v " means that according to the test method, no HAD phenomenon occurs in the cell culture wells of serial dilution; "x" means that according to the test method, HAD phenomenon exists in the cell culture wells of serial dilution.

7.Conclusion

According to this test method, the VIRSHA products provided by Hebei Erao Biotech Co., Ltd are in the following working concentration range under the conditions of 4°C and 20°C for 30 minutes, including: dilution ratio of VIRSHA is ≤400 times, which is confirmed to be effective in killing African swine fever virus in cell culture.

Test by: 孔明霞 Review by: 高友龙 Approval by:

Tested party: China Center for Animal Health and Epidemiology

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