China University
Laboratory of Animal Microbiology & Immunology
Report on the evaluation of
Jorenku A/S Staldren® hygiene powder

1. MATERIALS

1.1 Biological Materials

Vero cells, Porcine Epidemic Diarrhea Virus (PEDV), and African Swine Fever Virus (ASFV) nucleic acids were obtained from the university's Microbiology and Immunity Laboratory for preservation.

1.2 Disinfectants

Staldren®, the main ingredients are Chloramine-T, iron oxide, sodium chloride, pine oil, calcium carbonate, and Rollovit calcium.

Production date: 2023.07.27.

1.3 Main Reagents

GoScript Reverse Transcription Mix. Random primers were purchased from Promega (Beijing) Biotechnology Co.

Animal Detection U+ Probe qPCR Super PreMix was purchased from Nanjing Vazyme Biotechnology Co.

Fetal bovine serum was purchased from Aibi Biotechnology Co.

Fetal bovine serum was purchased from Ebixon (Shanghai) Biotechnology Co.

Probes. The probes and primers were synthesised by Sangon Bioengineering (Shanghai) Co.

1.4 Instruments

LEGEND MICRO21R centrifuge, pipettes, Sorvall MTX150 ultracentrifuge were purchased from Thermo Fisher Scientific.Co

AIRTECH ultra-clean bench was purchased from Suzhou Airtech Air Technology Co.

BIO-RAD C1000 Dx Thermal Cycler with CFX DX ORM Real-Time PCR System was purchased from Bio-Rad Life Medical Products (Shanghai) Co.

The BIO-RAD C1000 Dx Thermal Cycler with CFX DX ORM Real-Time PCR System was purchased from BIO-RAD Medical Products (Shanghai) Co.

The automatic nucleic acid extractor was supplied by Nanjing Vazyme Biotechnology Co.

2. Methods

2.1 Viral nucleic acid preparation

2.1.1 Cell culture

Take out the Vero cells frozen in liquid nitrogen, put them in a 37°C water bath for 2 min, wait for them to melt and then centrifuge them at 3000 g/min for 2 min.

Centrifuge the cells at 3000 g/min for 2 min, discard the upper layer of cryo-preservative, and resuspend the cells by gently blowing with cell culture

medium containing 10 % FBS.

The cells were spread in T25 cell flasks and incubated at 37°C in a 5 % CO_2 incubator.

2.1.2 Virus amplification

The well-grown Vero was washed with PBS for 3 times, and 100 μL of PEDV solution was diluted to 1 mL with DMEM and added to Vero.

Dilute 100 μL of PEDV solution with DMEM to 1 ml and add it to Vero and mix gently. Incubate at 37°C for 2 h, shaking every 20 min.

After incubation, add 4 ml of maintenance solution and incubate in 5 % CO_2 incubator for about 2 days to observe the effect of cell disease.

Observe the effect of cell disease. After more than 75 % of the cells in the field of view showed lesions, the virus liquid was collected by freezing and thawing several times at -70°C.

2.1.3 Viral nucleic acid extraction

The nucleic acids of the amplified PEDV were extracted using a fully automated nucleic acid extractor, and then determined by Quantitative real-time PCR （qPCR） of PEDV and ASFV.

The initial Ct values of PEDV and ASFV nucleic acids were determined by qPCR, and the ASFV nucleic acids were diluted 10 times to 10 times.

10-fold dilution of ASFV nucleic acid to 10.

ASFV nucleic acid was diluted 10-fold to 10^{-2}, i.e., 2 dilutions, and all nucleic

acids were set aside.

2.2 "Staldren®" treatment of PEDV solution, ASFV nucleic acid

Sprinkle 1 g of Staldren® into 200 uL of PEDV solution for 1h. Take 200 uL of PEDV solution and expose it to air for 1 hour.

PEDV solution was exposed to air for 1 hour as a control group. 200 uL of PEDV solution was exposed to air for 1 hour, and after the PEDV solution was exposed to Staldren® for 1 hour, the dry powder was sampled using a cotton swab.

After 1 hour of interaction of the PEDV solution with Staldren®, the dry powder was sampled using a cotton swab. Evaluate the effect of Staldren® on the PEDV solution.

Sample 200 uL of 10^{-1}, 10^{-2}concentration of Staldren®.

10^{-1},10^{-2} concentration of ASFV to 200 uL of ASFV nucleic acid with 1 g of Staldren® for 1 hour. Take 200 uL 10-1,10-2 concentration of ASFV nucleic acid.

ASFV nucleic acid at concentrations of 10^{-1} and 10^{-2} was exposed to air for 1 hour as a control group.

After 1 hour of interaction of ASFV nucleic acid with Staldren®, the dry powder was sampled using a cotton swab. The effect of Staldren® on different concentrations of ASFV nucleic acids was evaluated.

The effect of Staldren® on different concentrations of ASFV nucleic acid was evaluated.

3. Results

3.1 Effectiveness of Staldren® in treating **PEDV virus** liquid

The Ct value of PEDV virus liquid was 21.8, and 1g of Staldren® could completely degrade PEDV virus liquid after 1h of interaction with PEDV virus liquid.

After using 1 g of "Staldren®" with PEDV stock solution for 1 hour, PEDV virus liquid can be completely degraded, see Table 1.

Table 1 Results of "Staldren®" treatment of PEDV solution for 1 hour.

Groups	Ct value
Control subjects	23.3
Experimental group	40

Note: For the convenience of statistical data, the absence of values is indicated by a Ct value = 40.

3.2 Effect of Staldren® treatment on **ASFV nucleic acid**

ASFV nucleic acid was diluted twice at 10-fold, and the Ct values were 10^{-1}18.7 and 10^{-2}=22.4, respectively.

The Ct values of ASFV nucleic acid after two 10-fold dilutions were 10^{-1}=18.7 and 10^{-2}=22.4.

The Ct values were 10^{-1}=18.7 and 10^{-2}=22.4. 1 g of Staldren® was used with 10^{-1} and 10^{-2} concentrations of ASFV.

After using 1 g of Staldren® with 10^{-1} and 10^{-2} concentrations of ASFV nucleic acid for 1 hour, the 10^{-1} concentration of ASFV nucleus and the 10^{-2} concentration of ASFV nucleic acid were the same as the 10^{-1} concentration.

After 1 hour of interaction of 1 g of Staldren® with 10^{-1} and 10^{-2}concentrations of ASFV nucleic acid, the 10^{-1} concentration of ASFV nucleic acid and the 10^{-2} concentration of ASFV nucleic acid were completely degraded as shown in Table 2.

Table 2 Results of "Staldren®" treatment of ASFV nucleic acid for 1 hour.

Groups	ASFV nucleic acid concentration	Ct value
Control groups	10^{-1}	20.6
	10^{-2}	25.3
Experimental groups	10^{-1}	40
	10^{-2}	40

Note: For statistical convenience no values are expressed as Ct value = 40.

4. Conclusion

(1) "Staldren®" can completely degrade PEDV nucleic acid when interacted with PEDV virus liquid for 1 hour.

(2) "Staldren®" can completely degrade ASFV nucleic acid by interacting with 10^{-1}, 10^{-2} concentration of ASFV nucleic acid for 1 hour.