

## TEST RESULT OF ANTI- ASFV ABILITY OF STERIPLANT

### 1. Cell preparation

PAM cells ( $2 \times 10^6$  cells/ml/well) isolated from healthy 2 months old pig were prepared on 24-well plate.

### 2. Preparation of SteriPlant for safety test:

The safety of SteriPlant was tested on PAM cells. In detail, two-fold serial dilutions (0, 1/2, 1/4 and 1/8) of SteriPlant were prepared in cell culture medium (RPMI medium) and then inoculated into prepared PAM cells. PAM cells were then cultured for 96h. SteriPlant was considered as safety to cells if the cells grow normally, not died.

**Table 1.** *Designed experiment for safety test of SteriPlant on PAM cells using a 24-well plate. SteriPlant was prepared as two-fold serial dilutions*

	1	2	3	4	5	6
A	0	1/2	1/4	1/8	Cell only	Cell only
B	0	1/2	1/4	1/8	Cell only	Cell only
C	0	1/2	1/4	1/8	Cell only	Cell only
D	0	1/2	1/4	1/8	Cell only	Cell only

### 3. Preparation of SteriPlant and ASFV

Mixing 500 $\mu$ l of ASFV solution ( $10^5$  HAD<sub>50</sub>) with 500 $\mu$ l SteriPlant (using optimal concentration of SteriPlant that did not kill the PAM cells) and then incubated at room temperature for 3 different time intervals: 1 min, 5 min and 20 min. The mixture incubated at 3 different time intervals was inoculated into prepared PAM cells. PAM cells were then cultured for 96h and check the CPE daily.

**Table 2.** *Designed experiment for testing Anti-ASFV ability of SteriPlant on PAM cells using a 24-well plate. Mixture of SteriPlant (origin) and ASFV was incubated at 3 different time intervals*

0	1	2	3	4	5	6
A	1 min	5 min	20 min	ASFV only	SteriPlant	Cell only
B	1 min	5 min	20 min	ASFV only	SteriPlant	Cell only
C	1 min	5 min	20 min	ASFV only	SteriPlant	Cell only
D	1 min	5 min	20 min	ASFV only	SteriPlant	Cell only

**Table 3.** *Designed experiment for testing Anti-ASFV ability of SteriPlant on PAM cells using a 24-well plate. Mixture of SteriPlant (2 times dilution) and ASFV was incubated at 3 different time intervals*

<b>1/2</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>
<b>A</b>	1 min	5 min	20 min	ASFV only	SteriPlant	Cell only
<b>B</b>	1 min	5 min	20 min	ASFV only	SteriPlant	Cell only
<b>C</b>	1 min	5 min	20 min	ASFV only	SteriPlant	Cell only
<b>D</b>	1 min	5 min	20 min	ASFV only	SteriPlant	Cell only

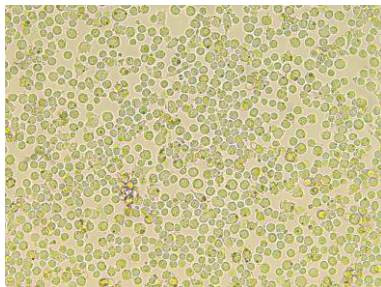
#### 4. Result interpretation

- The SteriPlant HAS anti- ASFV activity if PAM cells DON'T SHOW the CPE (cytopathogenic effects) because the virus was inactivated by SteriPlant.
- The SteriPlant HAS NOT anti- ASFV activity if PAM cells SHOW the CPE (cytopathogenic effects) because the virus was not inactivated by SteriPlant.

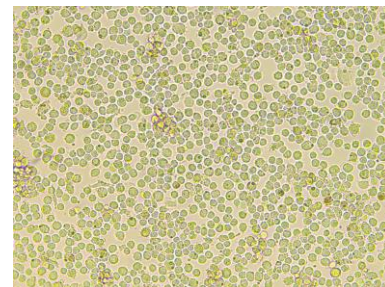
#### 5. Results

##### 5.1. Safety test results of SteriPlant on PAM cells:

- SteriPlant (origin concentration) was safe and not harmful to PAM cells, all the PAM cells grow normal when treated with SteriPlant.



*PAM cells were treated with SteriPlant*

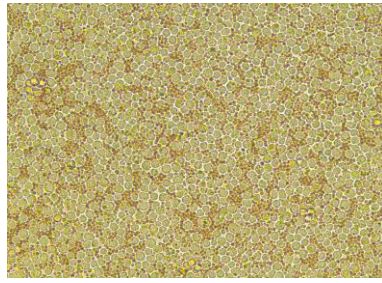


*Control PAM cells were not treated with SteriPlant*

##### 5.2. Results of testing anti- ASFV ability of SteriPlant

- All PAM cells treated with mixture of SteriPlant and ASFV at 3 different time incubation intervals SHOWED the CPE, however, the percentage of CPE of PAM cells treated with mixture of SteriPlant and ASFV was LOWER than ASFV- infected PAM cells (positive control).

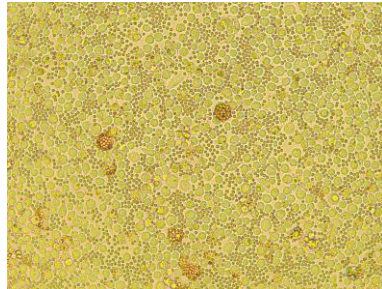
September 16, 2019



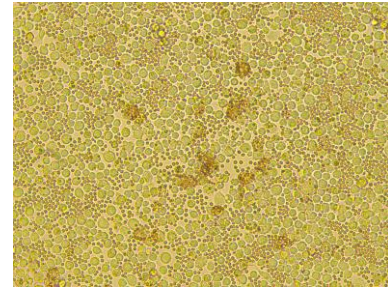
Uninfected PAM cells



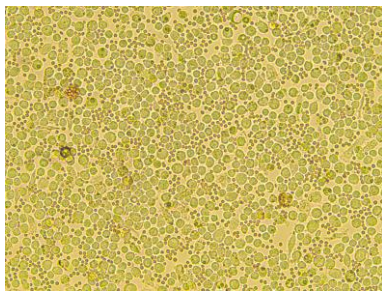
Control SteriPlant



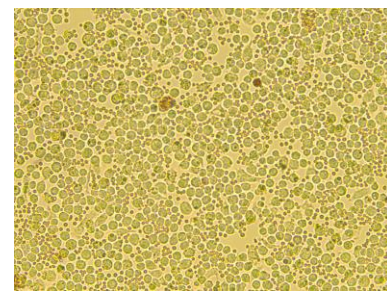
ASFV-infected PAM cells



PAM cells treated with mixture of SteriPlant and ASFV at 1 min incubation



PAM cells treated with mixture of SteriPlant and ASFV at 5 min incubation



PAM cells treated with mixture of SteriPlant and ASFV at 20 min incubation

## 7. Conclusion

- SteriPlant (origin concentration) was safe and not harmful to PAM cells
- SteriPlant seems to have signal of viral activity against ASF virus with titter of  $10^5$  HAD<sub>50</sub>.

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