Bioiberica
Taking life science further

INSTITUT PASTEUR

# Tested and certified by the Pasteur Institute

In **Bioiberica**, two thermic steps are applied:

1. After **Enzyneer**: 90°C2°C for 3 hours.
2. In Heparin purification process: 100°C5°C for 1 hour, 85°C5°C for 30 minutes.

In November 2018, a technical report from the **Institut Pasteur Texcell**, which studied these processes in the worst case and under attenuated conditions to obtain an inactivation kinetics and explore their robustness, certifies that both processes cause the inactivation of different viruses, including ASFV.

## Virus model

Due to the difficulties of working directly with VPPA, model viruses were used to test **Bioiberica's** viral inactivation process. ASFv is a very particular virus, that is not commonly found in laboratories involved in processes of viral validation. In line with European recommendations, Poxviridae like Vaccinia virus (dsDNA, enveloped) shares common characteristics with ASFv and thus it is an excellent virus model for a viral validation.

| VIRUS | ABBREVIATION | FAMILY | SIZE (nm) | PHYSICOCHEMICAL RESISTANCE |
|---|---|---|---|---|
| RNA Enveloped | | | | |
| Transmissible gastroenteritis virus | TGEV | Corobaviridae | 80-120 | Medium |
| RNA Non-Enveloped | | | | |
| Encephalomyocarditis virus | EMCV | Picornaviridae | 25-30 | Medium |
| DNA Enveloped | | | | |
| Pseudorabies virus | PRV | Herpesviridae | 120-200 | Medium |
| Vaccinia virus | Vaccinia | Poxviridae | 170-260 | Medium |
| African Swine Fever virus | ASFV | Asfarviridae | 175-215 | Medium |
| DNA Non-Enveloped | | | | |
| Porcine parvovirus | PPV | Parvoviridae | 18-24 | High |
| Canine parvovirus | CPV | Parvoviridae | 18-24 | High |

## After Enzyneer: 90°C2°C for 3 hours

Vaccinia virus, Encephalomyocarditis virus (EMCV; ssRNA, non-enveloped) and Canine Parvovirus (CPV; ssDNA, non-enveloped) were used to validate this step using two types of starting material such as an intermediate of production (intestinal porcine mucosa) and a surrogate (medium alternative media).

Attenuated conditions of 55°C 1 for Vaccinia virus and 65°C 1 for CPV and EMCV, showed the total inactivation of Vaccinia virus within 3 hours of treatment and EMCV in 10 minutes. CPV was partially inactivated as it was expected, because CPV has a high physicochemical resistance compared to Vaccinia virus and EMCV with medium resistance.

## In Heparin purification process: 100°C5°C for 1 hour, 85°C5°C for 30 minutes.

Four viruses were tested: Pseudorabies virus (PRV), Transmissible Gastroenteritis virus (TGEV), Encephalomyocarditis virus (EMCV) and Porcine parvovirus (PPV) which are representative of porcine adventitious viruses covering a large range of resistance to physicochemical treatments.

Scaling down of the thermal concentration step was performed in worst case conditions, with the following temperature parameters 93°C for 1h, 78°C for 30 minutes.

For the sensitive enveloped viruses PRV and TGEV, attenuated conditions were used: PRV was submitted to 45°C for 1h and then 30°C for 30 minutes, and TGEV was submitted to 60°C for 1h and then 45°C for 30 minutes.

***All four viruses were completely inactivated once the temperature reached 91º C.***

***The results of these experiments indicated the total inactivation of all tested viruses when the temperature of 87º C was reached.***

## EFFECTIVELY INACTIVATE THE ASF VIRUS

According to Texcell studies, these results can be safely extrapolated to other medium-strength wrapped viruses, such as VPPA, as the robustness of this inactivation has been fully validated in terms of temperature and duration of treatment.

**Using the worst-case approach, Texcell certifies that Bioibérica can extrapolate the result obtained with the most resistant virus used (PPV) to VPPA belonging to a less resistant type of wrapped virus.**

The results obtained with more sensitive viruses such as PRV and TGEV using attenuated conditions show a **very large safety margin**, both in terms of temperature and duration of treatment.