Alternatives for in vivo Foot-and-mouth disease vaccines potency tests: Serotype dependency of the in vitro vaccine potency models

[1] *Unit of Vesicular and Exotic Diseases, Department of Virology, CODA-CERVA-VAR, Veterinary and Agrochemical Research Centre, Groeselenberg 99, 1180 Brussel, Belgium (VAR)*
[2] *Biological and Technical Control Department, Federal Governmental Institution "Federal Centre for Animal Health", 600901, Yur'evets, Vladimir, Russia (ARRIAH)*
[3] *Research and Developments Department, Intervet International GmbH, Betriebsstätte Köln, Osterather Strasse 1A, 50739 Köln, Germany.*

AUTHORS: Willems T[1], Lefebvre DJ[1],De Vleeschauwer A[1], Diev VI[2], Borisov VV[2], Paul G[3],De Clercq K[1]

Introduction and objectives

Foot-and-mouth disease (FMD) vaccine potency testing is performed by experimentally infecting vaccinated cattle. The *in vivo* 50% Protective Dose (PD_{50}) test is the standard European procedure for the quality control of FMD vaccines. Due to ethical reasons, current research alliances focus on the replacement of the *in vivo* viral challenge by *in vitro* alternatives. Previously, Goris *et al*. (Vaccine, 2008) have validated an *in vitro* model for the FMDV reference strain O_1 Manisa. The present study aims to develop comparable models for serotype A and to test both models for serotype-dependency.

First step: replicates of the in vivo PD_{50} test

Five replicate *in vivo* PD_{50} tests are performed with a FMDV A Iran 96 vaccine. Twenty-one days post vaccinal (21DPV) serum samples are collected to perform the serological analyses on which the alternative *in vitro* vaccine potency models will be based.
Each PD_{50} tests is performed with 17 naïve cattle that are randomly divided into 2 control animals and 3 groups of 5 animals, each group receiving a different vaccine dose. At 21 DPV all animals are challenged with homologous FMD A Iran 96 virus (A96). Eight days post challenge (8DPC) they are clinically inspected for generalisation of FMD and animals are classified as protected or unprotected against challenge. Then the Kärber (1931) formula is used to calculate the PD_{50} for the vaccine batch.

Day 0: Vaccination

Vaccine dose groups

2ml 0.5ml 0.125ml control

21 DPV: Virus challenge (10^4 BID_{50})

Vaccine dose groups

2ml 0.5ml 0.125ml control

8 DPC: Clinical inspection of cattle

PD_{50} result

Second step : *in vitro* vaccine potency model

All 21 DPV sera are analysed (serum neutralisation (SNT A96) & liquid phase blocking ELISA (LPBE A96)) and with the resulting serum titres (t_i) a logistic correlation model is built. This model correlates the serum titres with a probability of being protected against virus challenge. This results in a cut off titre (t_{co}) wich will be used to classify the animal sera. Then the Kärber formula is used to calculate the EPD_{50} for the vaccine batch.

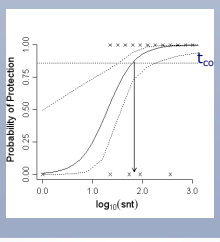
Day 0: Vaccination

Vaccine dose groups

2ml 0.5ml 0.125ml control

21 DPV:

Serum sampling



Serum titre Classification with Logistic regression model

$t_i \geq t_{co}$ p : protected
$t_i < t_{co}$ p : unprotected

Estimated EPD_{50} result

Third step : comparison PD_{50} vs. EPD_{50}

In vitro vaccine potency models are built for 2 different serological assays and 2 different laboratories. The EPD_{50} results are estimated using the cut off from the A96 model. Alternatively the EPD_{50} is also estimated against the cut off from an earlier developed O_1 Manisa model (Goris *et al*., 2008). The table below shows the mean EPD_{50} estimates for the 5 trials for each serological test (Test) and model cut off (Model). There is a difference between the mean *in vivo* PD_{50} (20.5 with 95%CI between 14.9 and 27.4) and the estimated values, except for the LPBE A96 test from VAR which confidence intervals have a good overlap of the *in vivo* results.

Laboratory	Test	Model (t_{co})	Mean EPD_{50}	95% CI
ARRIAH	SNT A96	SNT A96 (1.34)	11.1	9.2 - 19.1
VAR	SNT A96	SNT A96 (1.53)	10.2	8.4 - 19.1
ARRIAH	SNT A96	SNT O_1 (1.51)	5.8	5.0 – 14.0
VAR	SNT A96	SNT O_1 (1.68)	8.7	6.9 – 14.6
ARRIAH	LPBE A96	LPBE A96 (1.34)	6.0	5.0 – 11.2
VAR	**LPBE A96**	**LPBE A96** (1.73)	**14.7**	**12.5 – 21.1**
ARRIAH	LPBE A96	LPBE O_1 (1.65)	2.1	1.7 – 3.1
VAR	**LPBE A96**	**LPBE** O_1 (1.95)	**13.1**	**11.2 – 20.5**

Conclusion

For each serological assay the corresponding alternative *in vitro* vaccine potency model has to be validated for every individual laboratory and its serotype dependency has to be investigated.

Acknowledgements: The research leading to these results has received funding from the European Community's Sixth Framework programme (EC-EPIZONE FOOD-CT-2006-016236), theEuropean Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement no 226556 (FMD-DISCONVAC), the Belgian Federal Public Service of Health, Food Chain Safety and Environment (grant RT-05/06-ALTANDI-2) and the RPPJ of the VAR institute. Special thanks to Rim Dhambri for her technical assistance during the laboratory analysis of the samples.

Reference

European Pharmacopoeia, 2006. Foot-and-mouth disease (ruminants) vaccine (inactivated) 04/2005:0063. 5th ed., Version 5.5.
Goris, N., Willems, T., Diev, V.I., Merkelbach-Peters, P., Vanbinst, T., Van der Stede, Y., Kraft, H.P., Zakharov, V.M., Borisov, V.V., Nauwynck, H.J., Haas, B., De Clercq, K., 2008. Indirect foot-and-mouth disease vaccine potency testing based on a serological alternative. Vaccine, 26, 3870-3879.
Kärber G., 1931. Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Arch Exp Pathol Pharmakol, 162,480–483.