

**Spores or not spores, 'tis the question –  
Considerations for biological verification and  
technical feasibility of room fumigations**

Birmingham, 20.11.2019

## Overview

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### Background

- Who am I?

- IVI

- H<sub>2</sub>O<sub>2</sub> fumigation

### Inactivating FMDV

- VHP vs. aerosolised H<sub>2</sub>O<sub>2</sub> vs. formaldehyde

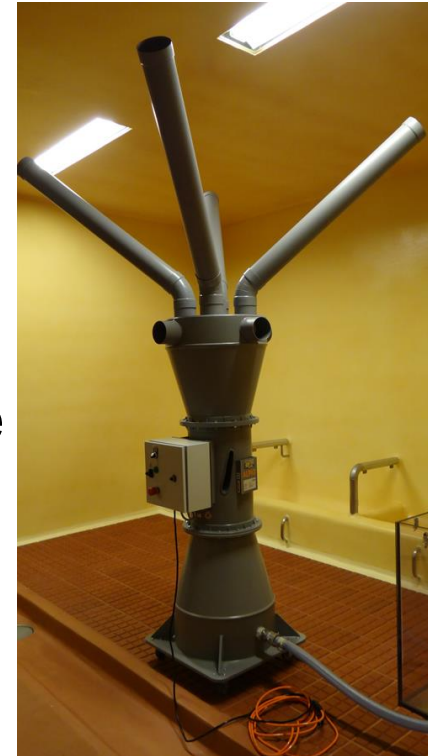
- Set-up

- Results

- FMDV vs. bacterial spore indicators

### Summary

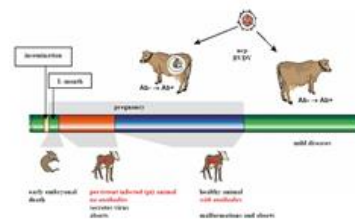
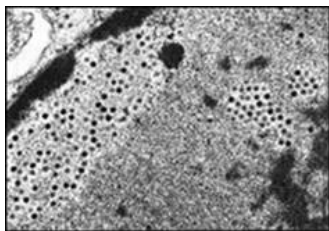
### Why bother?



# Background

## Who am I?

- M. Sc. in Biotechnology (ESBS) – master's thesis at AAHL
- PhD in Molecular Biology / Virology (Develogen AG / Humboldt Universität zu Berlin)
- Post-Doc (Veterinary Hospital University of Bern)
- Head of Biosafety LABOR SPIEZ (BSL3/4)
- Biosafety & Engineering IVI (BSL3/BSL3ag/BSL4)
- Biosafety Consultant (B&H)



## Background

**IVI** - National Reference Centre for highly infectious animal diseases:

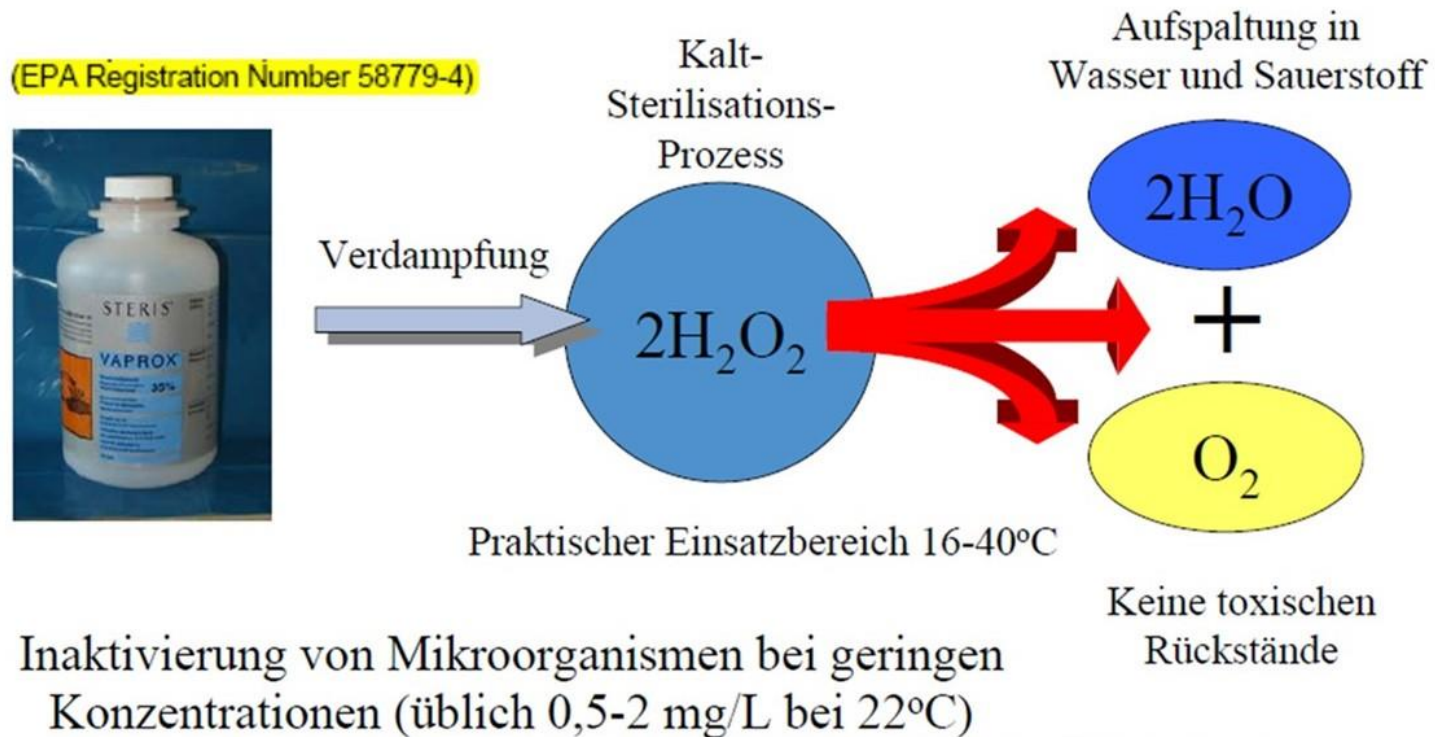
- \_ Diagnosis, surveillance and control of highly contagious animal diseases
- \_ Approval authority for vaccines and sera for animals
- \_ Research and development
  - \_ Foot and mouth disease
  - \_ Classical swine fever
  - \_ Avian influenza
  - \_ Swine vesicular disease
  - \_ Japanese encephalitis
  - \_ West Nile
  - \_ SARS / MERS



# Background

## H<sub>2</sub>O<sub>2</sub> fumigation

### Vaporized Hydrogen Peroxide “VHP®”



## Inactivating FMDV

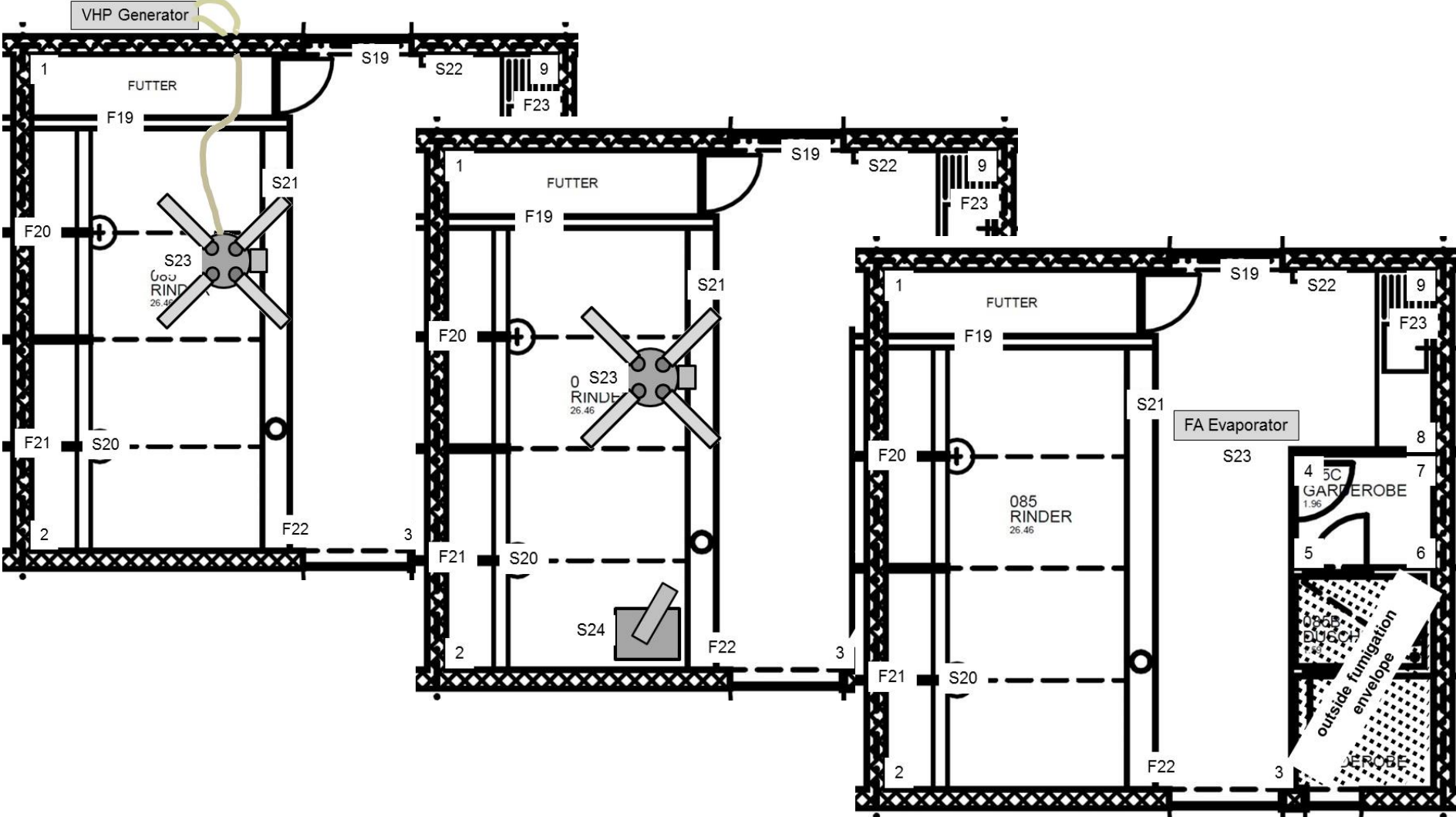
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### Goal

- Replace formaldehyde as the standard fumigation method at IVI
  - As an FMDV reference lab, IVI adheres to the EU FMDV standard\*
  - Formaldehyde still the fumigation method of choice according to the EU FMDV standard\*
- Replace ethylene oxide as a fumigation method
- Validate the fumigation / inactivation of FMDV with H<sub>2</sub>O<sub>2</sub>

\* EUFMD. Minimum Biorisk Management Standards for Laboratories Working with Foot-and-Mouth Disease Virus. Version GS40/4.2bis as Adopted by the 40th General Session of the EUFMD Commission. Rome, Italy: EUFMD; 2013.

# Inactivating FMDV



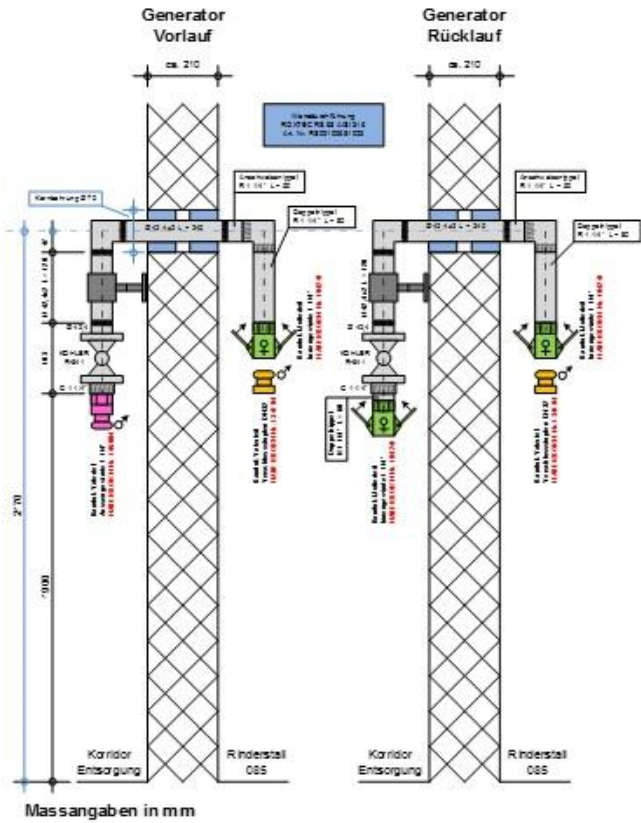
# Inactivating FMDV - VHP


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7.4.2017 *Marin Kandy*

Wanddurchführung für H2O2-Generator





## Inactivating FMDV - VHP



## Inactivating FMDV – Aerosolised H<sub>2</sub>O<sub>2</sub>



# Inactivating FMDV – Formaldehyde

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## Inactivating FMDV – Summary of Results

**Table 2.** FMDV Titers Before and After Exposure to Fumigants.

Fumigation Method	Validation Run	Negative/Total FMDV BIs	Cytotoxicity Control	Titer <sup>a,b</sup> of Climate Controls <sup>c</sup>	Titer <sup>a,b</sup> of Positive Controls <sup>d</sup>	Titer <sup>a</sup> of Virus Suspension <sup>e</sup>	Reduction in FMDV Titer <sup>f</sup>
VHP	1	23/23	Negative	4.91 ± 0.16	4.91 ± 0.63	6.83	4.17 ± 0.53
	2	23/23	Negative	3.91 ± 0.16	4.35 ± 0.16	6.83	
	3	23/23	Negative	3.69 ± 0.16	4.35 ± 0.32	6.83	
Aerosolized H <sub>2</sub> O <sub>2</sub>	1	23/23	Negative	3.91 ± 0.42	4.35 ± 0.16	6.83	3.72 ± 0.14
	2	23/23	Negative	3.57 ± 0.16	3.8 ± 0.00	6.83	
	3	23/23	Negative	3.69 ± 0.16	3.91 ± 0.16	6.83	
FA	1	23/23	Negative <sup>g</sup>	4.32 ± 0.16	4.32 ± 0.31	6.83	4.25 ± 0.05
	2	23/23	Negative <sup>g</sup>	4.21 ± 0.16	4.54 ± 0.31	6.83	
	3	23/23	Negative <sup>g</sup>	4.21 ± 0.16	4.54 ± 0.42	6.83	

BI, biological indicator; FA, formaldehyde FMDV, foot-and-mouth disease virus; VHP, vaporized hydrogen peroxide.

<sup>a</sup>Titer expressed as log<sub>10</sub> TCID<sub>50</sub>.

<sup>b</sup>Mean value of three BIs.

<sup>c</sup>In sealed box in animal room during fumigation (no exposure to fumigant).

<sup>d</sup>In sealed box in laboratory (no exposure to fumigant).

<sup>e</sup>Original virus titer (24 hours at 4°C following thawing).

<sup>f</sup>Mean value and standard deviation of three validation runs expressed as log<sub>10</sub> TCID<sub>50</sub> reduction based on the climate controls which reflect all losses due to drying onto the discs and exposure to the environmental conditions.

<sup>g</sup>Following purification over Sephadex columns.

## Inactivating FMDV – BIs vs. FMDV - VHP

Step	Duration	Flow	rel. hum.	Injection rate
Dehumidification	20 min	34 m3/h	10 mg/l	-
Conditioning	15 min	32 m3/h	-	10 g/min
Decontamination	150 min	32 m3/h	-	9.5 g/min
Aeration	60 min	34 m3/h	-	-

Step	Duration	Flow	rel. hum.	Injection rate
Dehumidification	20 min	34 m3/h	10 mg/l	-
Conditioning	30 min	32 m3/h	-	12 g/min
Decontamination	300 min	32 m3/h	-	11 g/min
Aeration	60 min	34 m3/h	-	-

Bacterial spore indicators  
Total volume H<sub>2</sub>O<sub>2</sub>: **1575 ml**

FMDV  
Total volume H<sub>2</sub>O<sub>2</sub>: **3660 ml**

## Inactivating FMDV – BIs vs. FMDV – Aerosolised H<sub>2</sub>O<sub>2</sub>

Step	Duration of injection	Injection rate	End concentration	Incubation time
1	30 min	33 ml/min	10 ml/m <sup>3</sup>	60 min
2	30 min	33 ml/min	10 ml/m <sup>3</sup>	60 min
3	30 min	33 ml/min	10 ml/m <sup>3</sup>	60 min

Bacterial spore indicators

Total volume H<sub>2</sub>O<sub>2</sub>: **2970 ml**

Step	Duration of injection	Injection rate	End concentration	Incubation time
1	35 min	33 ml/min	12 ml/m <sup>3</sup>	60 min
2	35 min	33 ml/min	12 ml/m <sup>3</sup>	120 min
3	-	-	-	-

FMDV

Total volume H<sub>2</sub>O<sub>2</sub>: **2310 ml**

# Inactivating FMDV – BIs vs. FMDV – Formaldehyde

Step	Formaldehyde	Water	End concentration	Incubation time
1	1090 ml	1040 ml	4 g/m <sup>3</sup>	-
2	-	50 ml	-	-
3	-	-	-	~15 h



# Inactivating FMDV – BIs vs. FMDV

**Table I.** Number of Spore Biological Indicators Inactivated Out of the Total Number of Indicators Placed in the Animal Room and Around the HEPA Filters.

BI Manufacturer	Spore Amount/BI	Fumigation Method		
		VHP	Aerosolized H <sub>2</sub> O <sub>2</sub>	FA
MesaLabs	10 <sup>4</sup>	ND	ND	0/30
	10 <sup>5</sup>	ND	ND	0/30
	10 <sup>6</sup>	25/27	24/28	0/30
3M	10 <sup>5</sup>	ND	ND	27/27

BI, biological indicator; FA, formaldehyde; ND, not done; VHP, vaporized hydrogen peroxide.



## Summary

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- \_ H<sub>2</sub>O<sub>2</sub>, both as a vapour or aerosol, may be used to inactivate FMDV => validated fumigation processes
- \_ All methods have their pros and cons
- \_ All methods have their limits
- \_ Bacterial spores are not viruses

## Summary

### Cost comparisons

	Quantity	VHP	Aerosol	FA
Generator	1	~ 85'000	~ 5000	~ 5000
Vaprox	6x950 ml	840 (540)*	-	-
Sanosil S015	10 l	-	125 (29)*	-
FA 36%	1 l	-	-	15 (16)*
chem. Indicators	200	380	20 / 380	257

Costs in CHF; \*: costs per run

## Why Bother?

Fumigation using distilled water

- \_ FMDV dried onto stainless steel discs and positioned together with BIs
- \_ Climate controls in plastic box (no H<sub>2</sub>O exposure)
- \_ Same cycle as previously



**=> Climate controls inactivated as efficiently as all other samples!!!**

**=> Heat room to above 40°C (at elevated rel. humidity) to inactivate FMDV ....**



**ABSA**  
**INTERNATIONAL**  
*The Association for Biosafety and Biosecurity*

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**Thank you!**

Basler & Hofmann Zürich

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## Literature Overview

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- Kümin D., Signer J., Portmann J. and Beuret C., Of a Storm in a Teacup and a Gutter Heater – Practical Aspects of VHP Room Fumigation, Applied Biosafety, 2015, 20: 146-154.
- Kümin D., Portmann J., Signer J., Beuret C. and Strasser M., The IndicatorSafe – A Simple Tool to Confirm Successful Fumigation of a HEPA Filter Housing, Applied Biosafety, 2015, 20:179-183.
- Kümin D., Gsell Albert M. and Summermatter K., Comparison and Validation of Three Fumigation Methods to Inactivate Foot-and-Mouth Disease Virus, Applied Biosafety, 2018, 23: 70-76.
- Kümin D., Gsell Albert M. and Summermatter K., Case Study – Room Fumigation Using Aerosolized Hydrogen Peroxide: a Versatile and Economic Fumigation Method, Manuscript in preparation.