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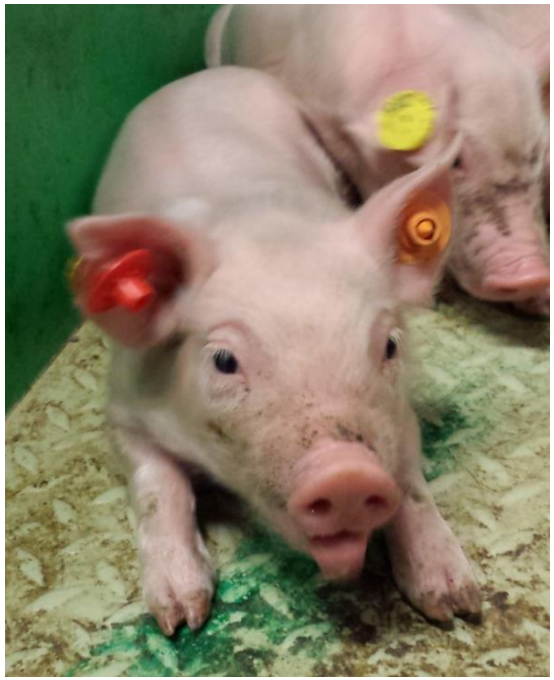
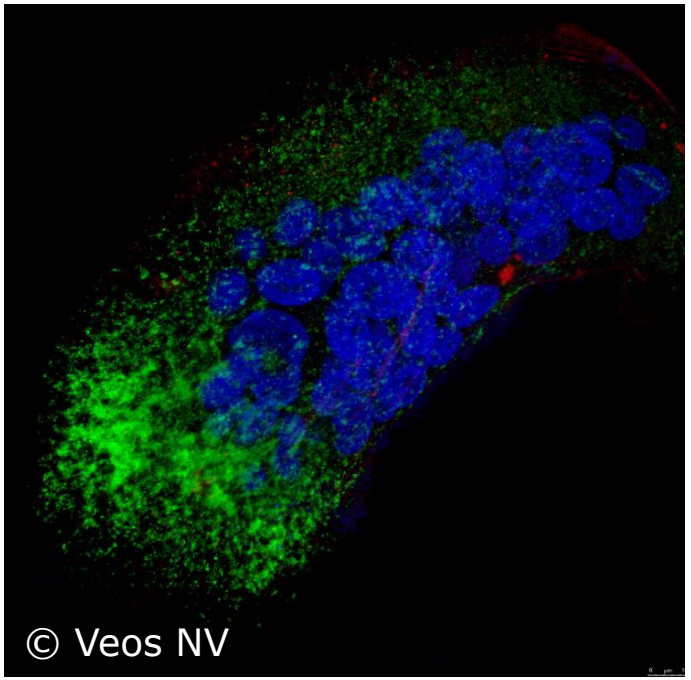
Warshaw, PL

piglet feed

Natural ingredients

Inactivation of porcine epidemic diarrhea virus (PEDV) by heat-alkalinity-time (HAT) pasteurization





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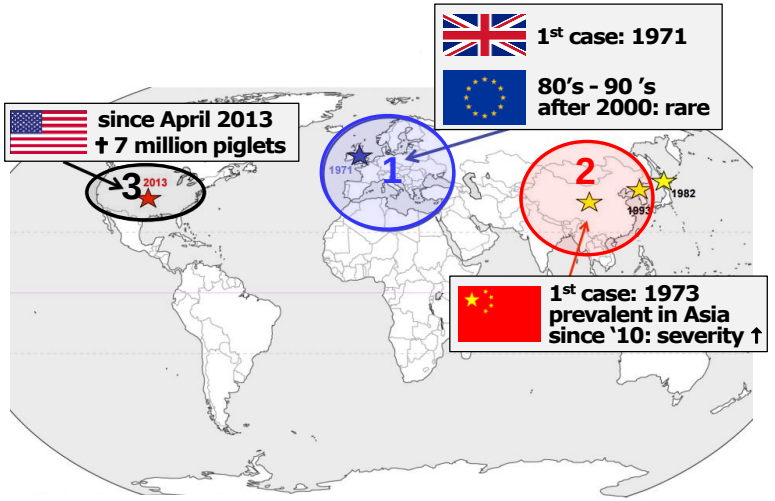
Porcine Epidemic Diarrhea Virus (PEDV)

- swine alfa-corona virus
- faecal-oral transmission
- highly enteropathogenic
  - → villus atrophy
  - → acute, watery diarrhea



After Opriessnig, 2014

- Clinical outcome depends on age at infection
  (virus strain, lactogenic immunity, co-infections, ...)
  - ❑ neonatal & suckling : up to 100% mortality
  - ❑ weaners to adults : (usually mild) self-limiting
  - ❑ gestating sows : reproductive performance ↓

Transmission of PEDV by feed

- US Field cases with **entirely vegetal diets** (Dee et al., 2014)
  - Feed-borne (corn, SBM and Vit & Min diets)

- Ontario cases (USA → Eastern Canada)
  - Epidemiology: feed-borne transmission
  - Infectious PEDV in SDPP sampled at a feedmill

❶ (Pasick et al., 2014)

  - Non-infectious PEDV in SDPP at the production plant

(US FDA, 2014)

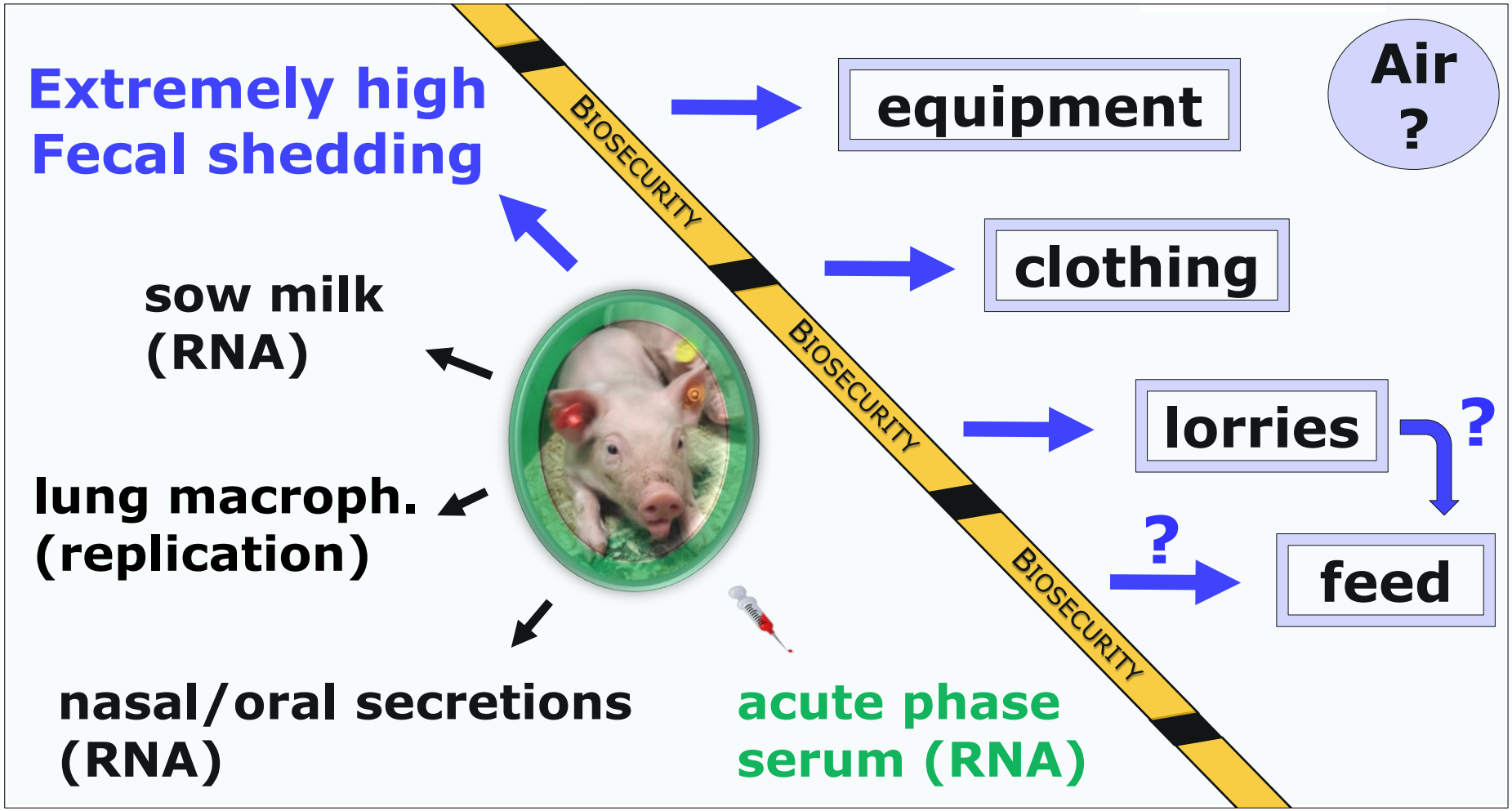
❸ ❷

  - PEDV is sensitive to spray-drying and dessication
  - SDPP was produced 10 wks prior to Ontario cases

Ingredient specific sensitivity (Dee et al., 2015)
outdoor storage (-25 to +20 °C): $10^{4.2}$ TIC_{50}/g
inactivation: SDPP < 7 d vs SBM < 210 d (>180 d)

Matrix Effect

Porcine Epidemic Diarrhea Virus (PEDV)



Adequate biosafety measures should <u>also</u> be in place for feed and its ingredients

Present Trial

Sensitivity of PEDV to HAT-pasteurisation			
Heat	**Alkalinity**	**Time**	**Matrix**
Product temp	**Product pH**	**Holding time**	**product**
[T_{IN} ; T_{OUT}]	**[pH_{IN} ; pH_{OUT}]**	**[flow]**	**[plasma]**

✓ **Completely characterised proces**

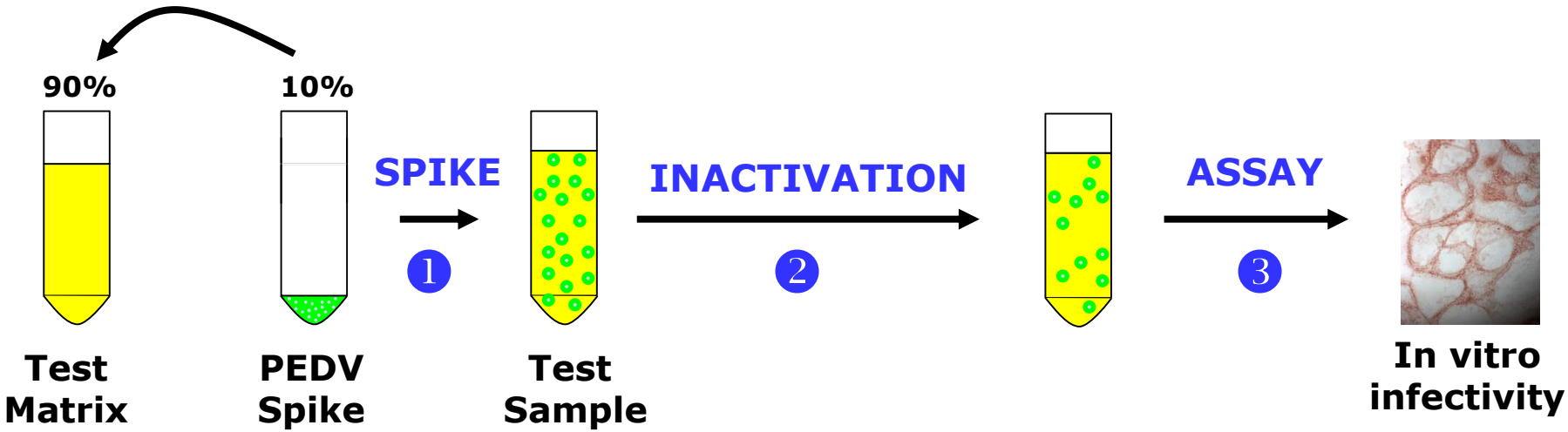
⇩ **Enables**

Laboratory replication of industrial conditions

Determination of sensitivity of PEDV

D-value = time needed to inactivate 90% of initial infectivity (1 log)

Materials & Methods: Spike Inactivation Assays



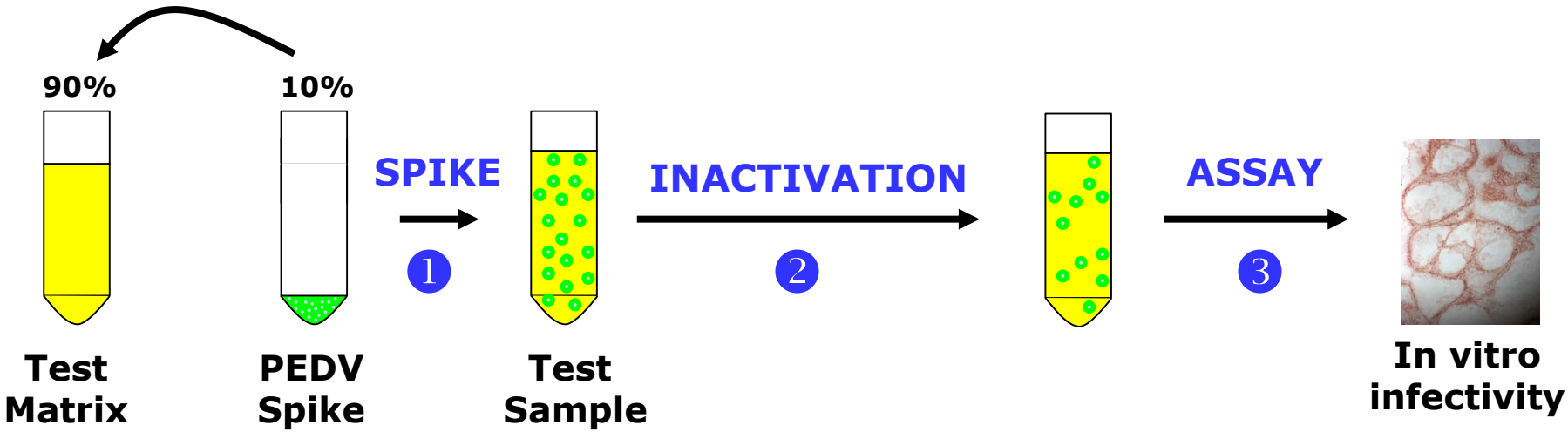
❶ **Test-samples: matrix + virus (9:1-ratio)**

Matrix: • **Minimum Essential Medium (MEM)**

• **porcine plasma**

sterile filtered
heat inactivated } **LDL⬆**
seronegative for PEDV

Materials & Methods: Spike Inactivation Assays

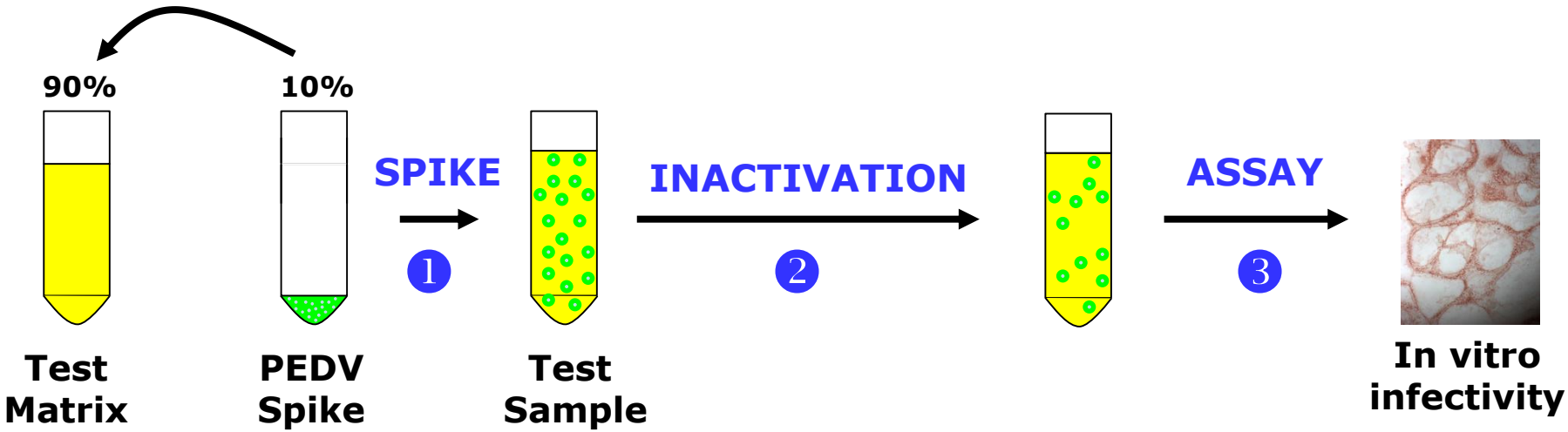


❷ Inactivation (treatment)

Triplicate

Condition	pH 7.2, 9.2 or 10.2 Temperature 4, 40, 44 or 48°C
Duration	8 time-points up to 120 min (0.25, 1, 3, 5, 10, 30, 60 or 120 min)
Matrix	MEM or porcine plasma

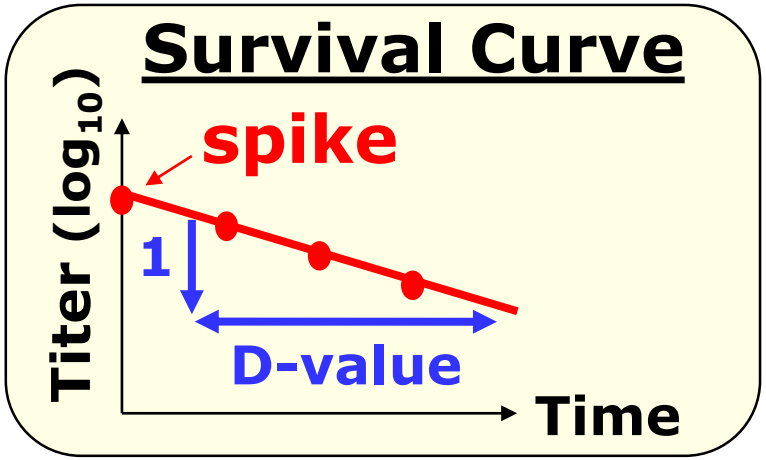
Materials & Methods: Spike Inactivation Assays



❸ Virus titration

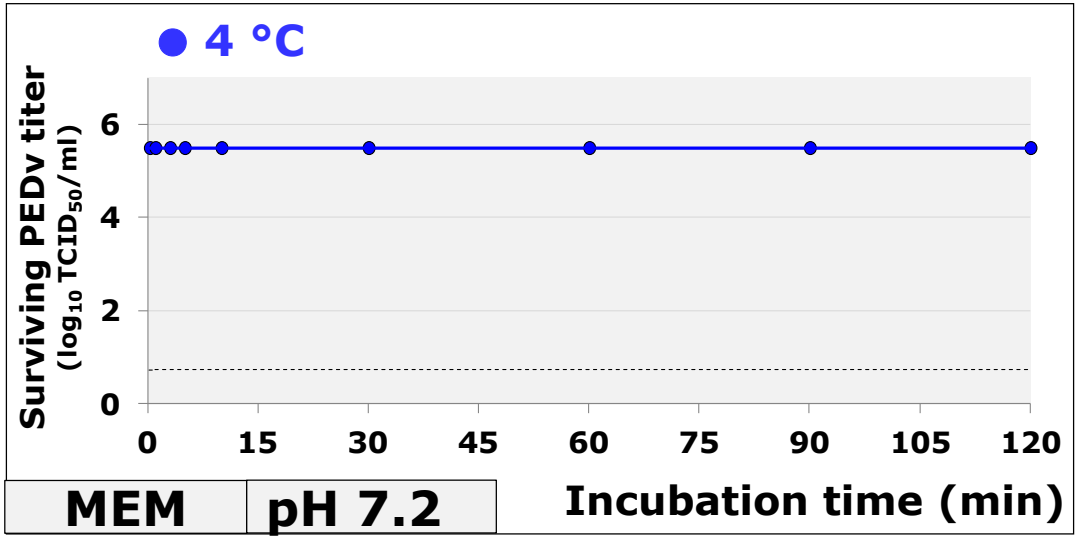
- residual infectivity
- whole test-sample
- end-point dilution assay
- 96-well plates

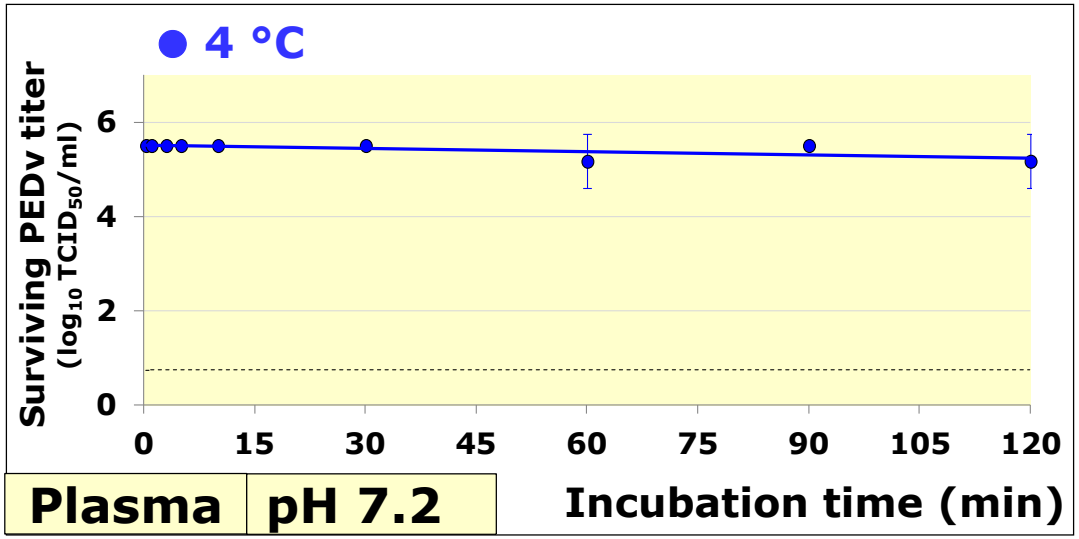
Confirmation assays
➡ **D-value, PEDV sterility**



Results and Discussion: Survival curves

Spike-Inactivation Assay



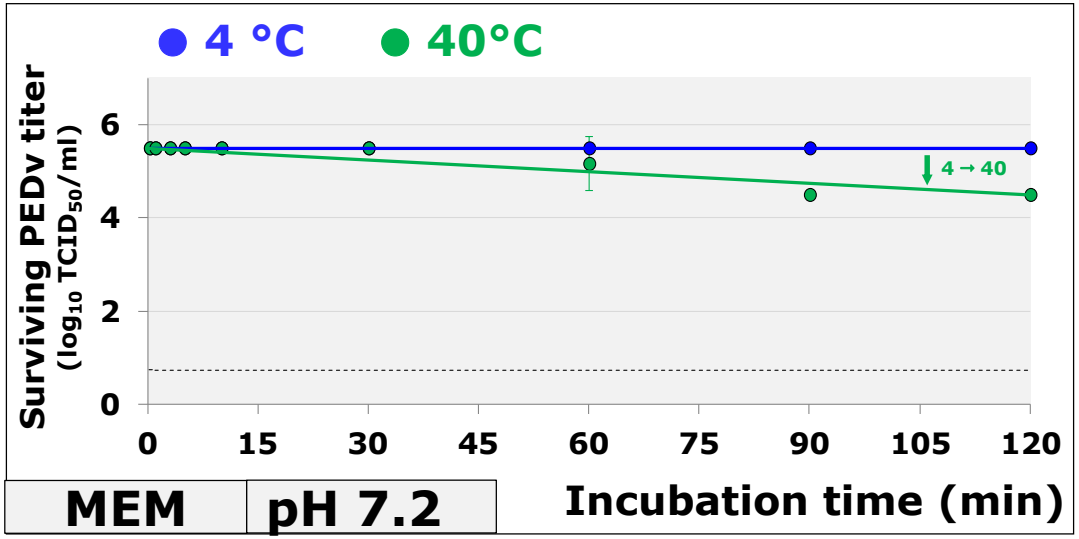


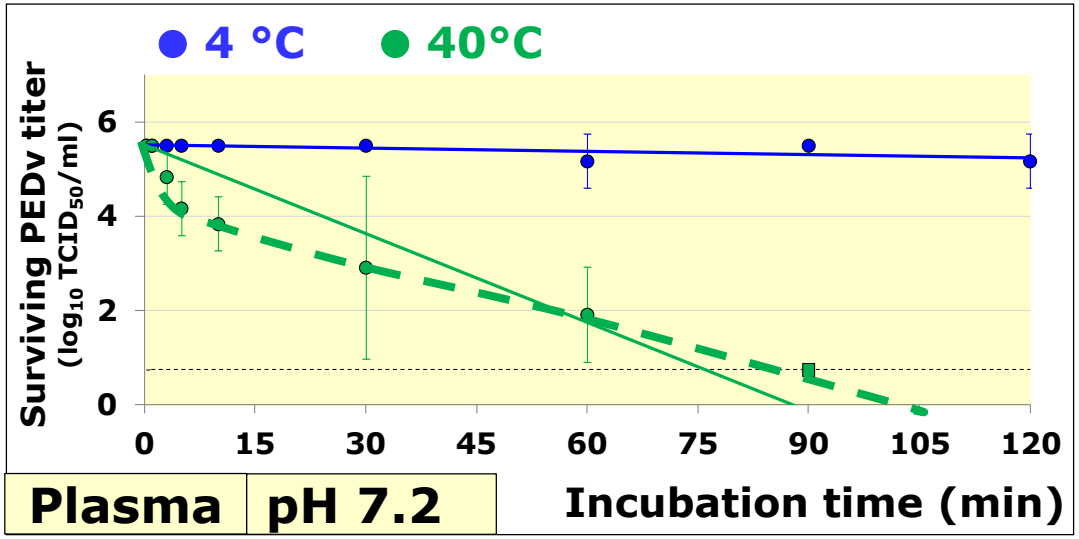
Sensitivity of PEDV

Stable in MEM at 4°C
Stable in plasma at 4°C

Results and Discussion: Survival curves

Spike-Inactivation Assay





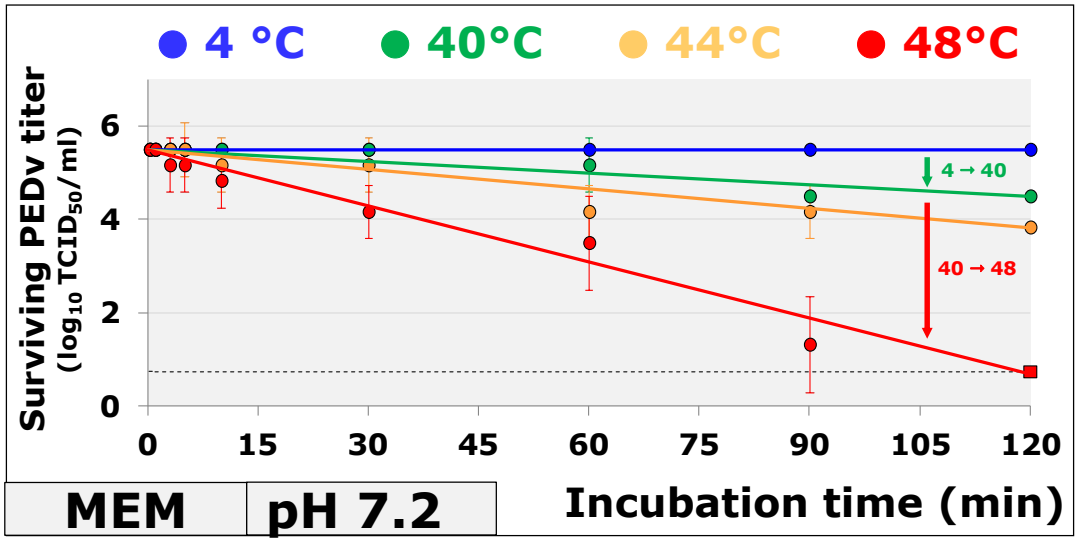
Sensitivity of PEDV

Stable in MEM at 4°C
Stable in plasma at 4°C

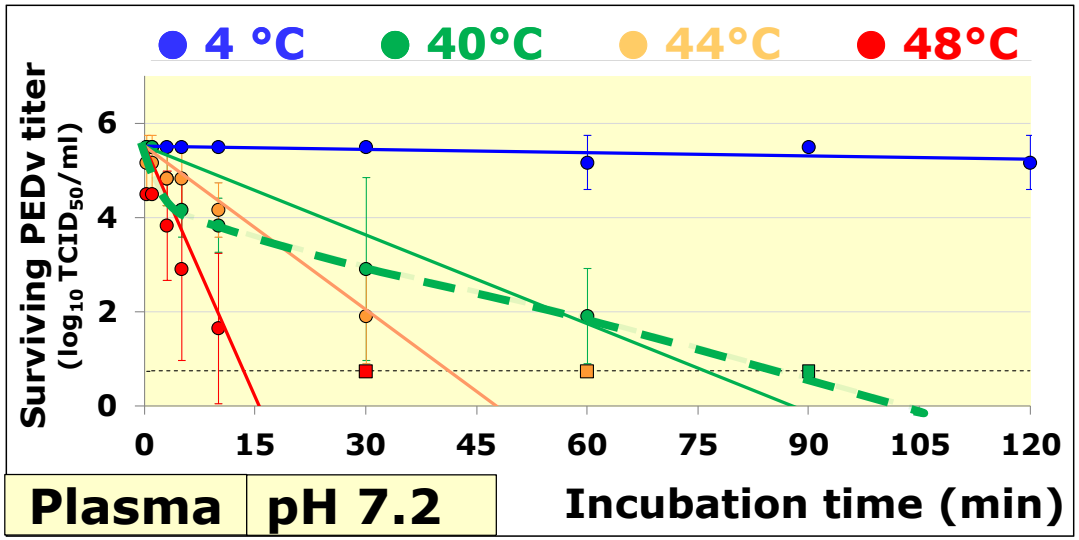
Stable in MEM at 40°C
Sensitive to 40°C in plasma (tailing effect in plasma)

Results and Discussion: Survival curves

Spike-Inactivation Assay



MEM | pH 7.2



Plasma | pH 7.2

Sensitivity of PEDV

Stable in MEM at 4°C
Stable in plasma at 4°C

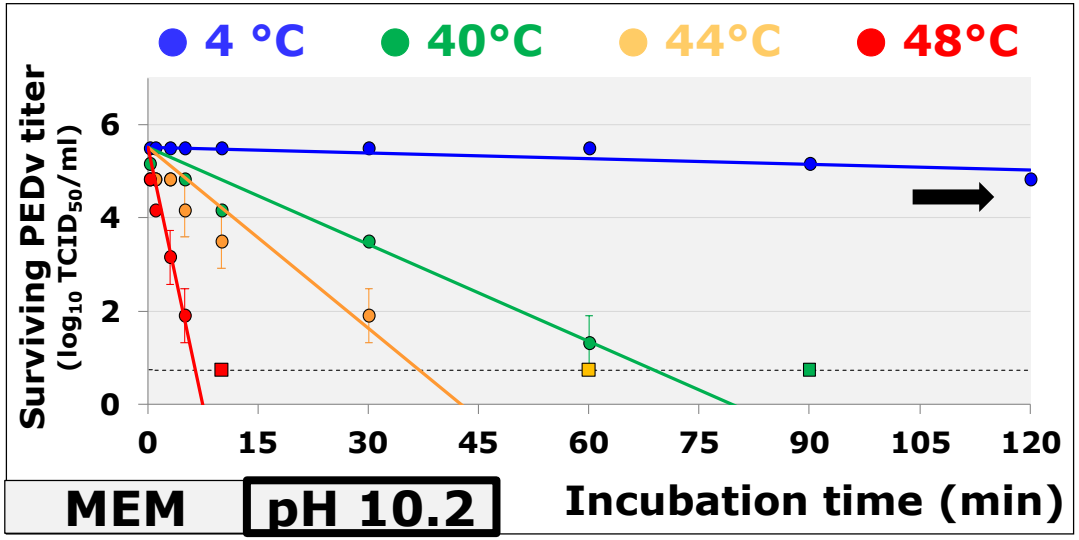
Stable in MEM at 40°C
Sensitive to 40°C in plasma
(tailing effect in plasma)

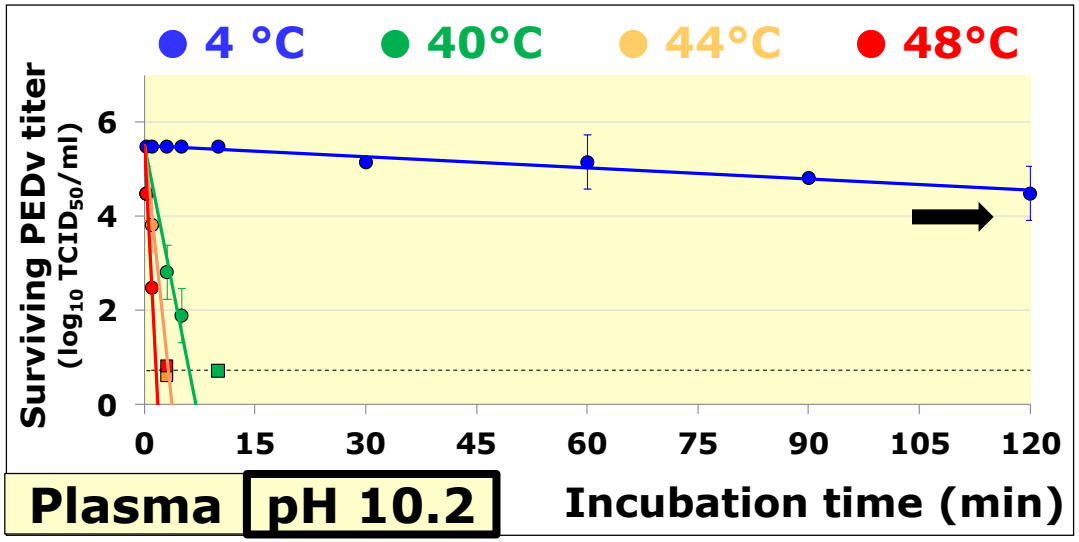
Temp ⬆ ⇨ Sensitivity ⬆

⇨ $Sensitivity_{plasma}$ ⬆⬆
⇨ $Tailing_{plasma}$ ⬇

Results and Discussion: Survival curves

Spike-Inactivation Assay





Sensitivity of PEDV

Stable in MEM at 4°C
Stable in plasma at 4°C

Stable in MEM at 40°C
Sensitive to 40°C in plasma
(tailing effect in plasma)

Temp ⬆ ⇨ Sensitivity ⬆

⇨ $Sensitivity_{plasma}$ ⬆⬆
⇨ $Tailing_{plasma}$ ⬇

pH 10.2 ⇨ Not sensitive
pH ⬆ ⇨ $Sensitivity_{Temp}$ ⬆⬆

$D_{48°C,\ pH\ 10.2}$ = 35 s in plasma
(UCL_{95}) 114 s in MEM

Results and Discussion: Confirmation assays

Spike-inactivation assay in tissue culture flasks

HAT determinants: H = 48°C; A = pH 10.2; T = 2.5 min and T = 5 min

✓ Confirmation of D value

Matrix	Spike (log_{10} $TCID_{50}$)	Vol	pH	Temp (°C)	Time (min)	Surviving PEDV #	Measured D value (sec or min)	Expected D value mean	Expected D value [UCL95]
Plasma	7.37	10 ml	10.2	48	2.5	4	23 sec	20	[35] sec
Plasma	7.65	1 ml	10.2	48	2.5	25	25 sec	20	[35] sec

- Measured D value (23-25 sec) < UCL_{95} of expected D value (35 sec)
- D value is not dependent on test volume or magnitude of virus spike
- Similar results in confirmation assays of other HAT determinants

In other words

start (spike) over 31 million infectious particles

is reduced to 25 infectious particles in 2.5 min HAT-pasteurisation

Results and Discussion: Confirmation assays

Spike-inactivation assay in tissue culture flasks

HAT determinants: H = 48°C; A = pH 10.2; T = 2.5 min and T = 5 min

✓ Confirmation of obtained PEDV sterility

Matrix	Spike (log_{10} $TCID_{50}$)	Vol	pH	Temp (°C)	Time (min)	Surviving PEDV # PFU	Sterility obtained ? (yes/no)	Expected time to sterility mean	Expected time to sterility [UCL95]
Plasma	7.37	10 ml	10.2	48	5	0	YES	2.4	[4.2] min
Plasma	7.65	1 ml	10.2	48	5	0	YES	2.5	[4.4] min

HAT-treatment at 48°C and pH 10.2 during 5 min
resulted in PEDV sterility of plasma spiked to 7.65 log_{10} $TCID_{50}$ per ml

In other words

start (spike) over 31 million infectious particles
is reduced to 25 infectious particles in 2.5 min HAT-pasteurisation
and is reduced to 0 infectious particles in 5 min HAT-pasteurisation

Results and Discussion: Confirmation assays

Spike-inactivation assay in tissue culture flasks

HAT determinants: H = 48°C; A = pH 10.2; T = 2.5 min and T = 5 min

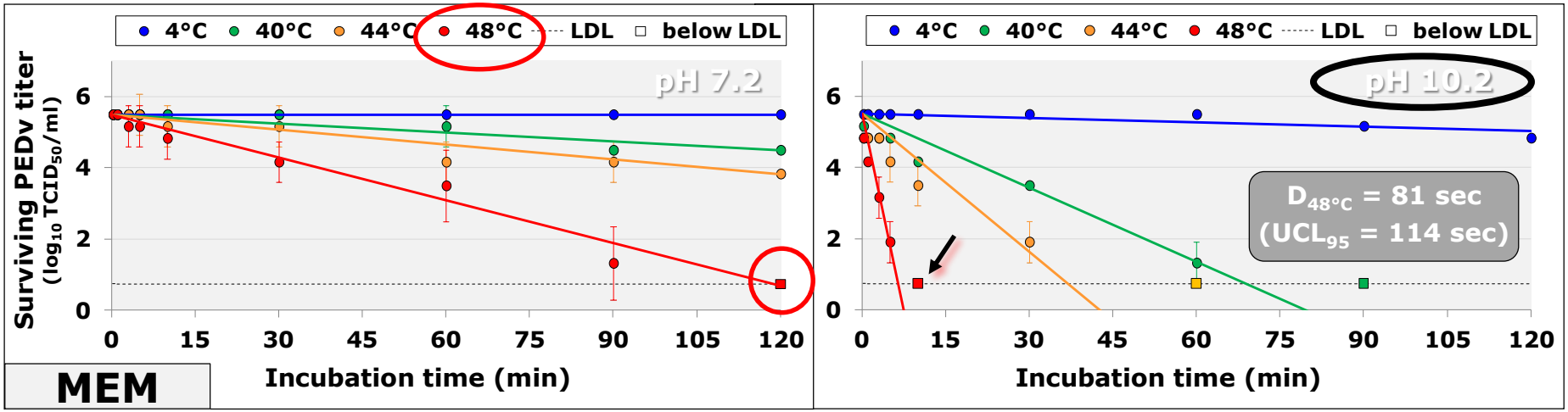
✓ Confirmation of obtained PEDV sterility

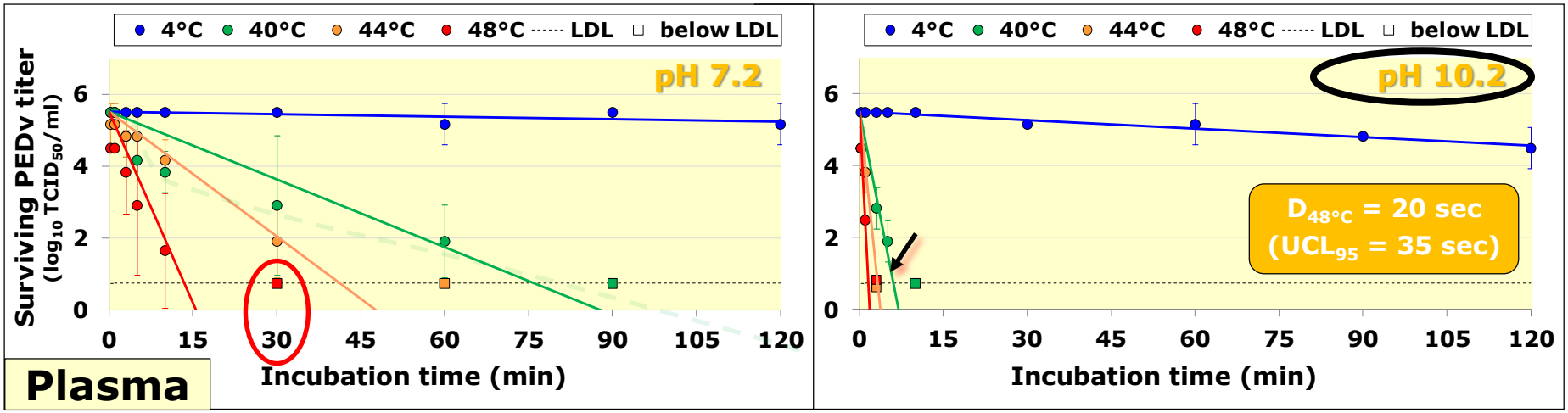
Matrix	Spike (log_{10} $TCID_{50}$)	Vol	pH	Temp (°C)	Time (min)	Surviving PEDV # PFU	Sterility obtained ? (yes/no)	Expected time to sterility mean	Expected time to sterility [UCL95]
Plasma	7.37	10 ml	10.2	48	5	0	YES	2.4	[4.2] min
Plasma	7.65	1 ml	10.2	48	5	0	YES	2.5	[4.4] min
Additional assay									
Plasma	5.80	1 ml	10.2	48	4	0	YES	1.9	[3.4] min
Plasma	5.80	1 ml	10.2	48	3	0	YES	1.9	[3.4] min

Time to sterility occured within the expected time

Summary

Spike-Inactivation Assay - survival curves





Conclusions

1. **Inactivation of PEDV is <u>facilitated in plasma</u>**

2. **Inactivation assays should take <u>matrix</u> into account**

3. **HAT-pasteurisation at $H_{48°C}A_{pH10.2}T_{10min}$**
   ⇨ Inactivates 17.4 log_{10} $TCID_{50}$ / ml plasma

(Quist-Rybachuk *et al.*, submitted 2015)

4. **PEDV is highly sensitive to HAT-pasteurisation, a redundant additional safety-step ?**

<u>Standard processing of SDPP</u>

- **Spray-Drying** (Gerber *et al.*, 2014; Pujols and segalés, 2014)
  ⇨ **Inactivates min 4.2 log_{10} $TCID_{50}$ / ml plasma**

- **Storage at low Aw** (Pujols and segalés, 2014)
  ⇨ **Inactivates min 2.8 log_{10} $TCID_{50}$/g SDBP in 3 w-4°C, 2 w at 12°C, 1 wk at 21°C**

Further Take Home Messages

1. **ALL ingredient types can be vectors of PEDV**
   (vegetal (Dee et al., 2014) , animal (Pasick et al., 2014) , micro-ingredients)
   Risks of feedborne transmission of PEDV
   are NOT limited to animal-based ingredients.

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2. **Inactivation of event. infectious agents is <u>anticipated</u>**
   in the processing of animal-based ingredients.
   Processing implies a safety-guarantee, not a safety-risk.

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3. **Securing feed-safety necessitates proper biosecurity**
   <u>at all points</u> of the distribution chain.

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4. **PCR-tests do not inform on virus infectivity,**
   they inform on standard necessity of processing.

Acknowledgments