



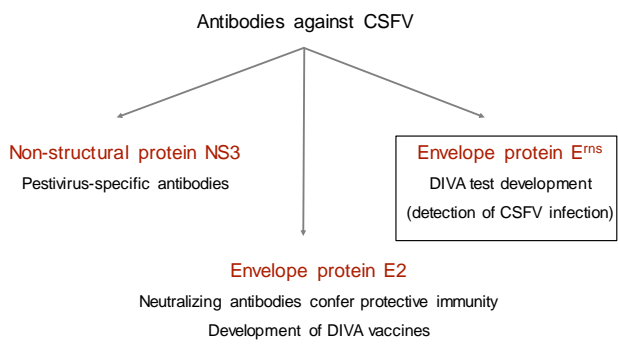
## Epitope mapping of the structural protein E<sup>ns</sup> of Classical swine fever virus

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## Antibodies against CSFV

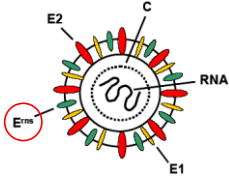


Antibodies against CSFV

- Non-structural protein NS3  
Pestivirus-specific antibodies
- Envelope protein E<sup>ns</sup>  
DIVA test development (detection of CSFV infection)
- Envelope protein E2  
Neutralizing antibodies confer protective immunity  
Development of DIVA vaccines

## Structural protein E<sup>ns</sup>

- Envelope protein
- Monomeric and homodimeric form
- Four intramolecular disulfide bridges
- Highly glycosylated:  
6 to 9 putative glycosylation sites
- RNase activity
- Antibody induction during viral infection



## Monoclonal antibodies

Institute of Virology, University of Veterinary Medicine Hannover:

13 monoclonal antibodies (mAbs)

- 5 mAbs directed against the BVDV E<sup>ns</sup>:**  
Antigenic domain
- 8 mAbs directed against the CSFV E<sup>ns</sup>:**  
Cross reactivity  
Epitope structure  
Antigenic domain  
Site-directed mutagenesis

## Cross reactivity

Infection of susceptible cells with a panel of pestiviruses  
↓ 48h  
Heat fixation for 4h at 80°C  
↓  
mAb titration in twofold serial dilutions (1:10 - 1:20,480; 12h at 4°C)  
↓ washing  
Incubation with a biotinylated anti-mouse antibody (1h at RT)  
↓ washing  
Incubation with a streptavidin-biotinylated peroxidase complex (1h at RT)  
↓ washing  
Incubation with substrate (30-45 min)  
↓  
Calculation of activity factor (AF = protein (mg/ml) / mAb titer)

## Cross reactivity

	CSFV-AMC/1107	CSFV-Berocia	CSFV-Guaterania	CSFV-South Africa	CSFV-Panamias	CSFV-Diunghai	CSFV-Kaibangwa	BVDV-Cong-Kemur	BVDV-AMBL-USA	BVDV-Silmer	BVDV-Cat	BVDV-382-1	BVDV-281	BVDV-0231	BVDV-Rainbow-Y189	BVDV-0108m	BVDV-Resco	Giraffe-virus
HC/TC128	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
HC/TC56	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
HC/TC57	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
HC/TC58	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
HC/TC60	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
HC/TC61	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
HC/TC66	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
HC/TC69	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
BVD/CT16	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
BVD/CT14	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

■ = high activity factor    □ = low activity factor    □ = no reactivity



## Conclusion



- The CSFV mAbs are directed against discontinuous epitopes.
- Two of the eight anti-E<sup>rns</sup> mAbs are CSFV-specific.
- One antigenic domain was identified for the CSFV E<sup>rns</sup> (aa 55-110) and one for the BVDV E<sup>rns</sup> (aa 111-167).
- The amino acids Ala<sub>102</sub> and Ala<sub>107</sub> are important for the binding of the CSFV mAbs.

**Optionally, the mAbs HC/TC56 and HC/TC66 could be used for the development of the DIVA-ELISA.**

## Outlook



- Analysis of the mAb reactivity of the CSFV-specific mAbs with an extended panel of BVDV and BDV isolates
- Site-directed mutagenesis to get more information about the epitopes in the antigenic domain of the CSFV E<sup>rns</sup> protein

## Acknowledgment



[Institute of Virology, University of Veterinary Medicine Hannover](#)

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