Validation of PCR test for BoHV-1

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Objectives

★ Increased use of glykoprotein E (gE) deleted BoHV-1 marker vaccine strains:
   protection + Differentiation of Infected from Vaccinated Animals (DIVA)

★ reactivation of live marker vaccine virus possible (stress, disease, immunosuppression)
   ★ potential time coincidence of vaccination and respiratory disease (weanlings)

Need for a discriminatory test system
Verifying BoHV-1 field virus introduction (notifiable to the authorities!) ↔
monitoring spread of vaccine virus

- Indirect diagnostics: gE seroconversion 14->21days
- Direct diagnostics: virus isolation in cell culture

😊 Gain of time by molecular diagnostics
Biosecurity measures, emergency vaccination
• Triplex real time BoHV-1 PCR published in 2011
qPCR: low risk of cross contamination by absence of post-amplification handling, semi-quantitative analysis and simultaneous amplification of 3 target sequences

- **Glykoprotein D** (gD): highly conserved for BoHV-1, lesser conserved among ruminant non BoHV-1 viruses than gB

- **Glykoprotein E** (gE): lacking in marker vaccine virus strains

Conventional PCRs for gE (Schynts et al., 1999, Fuchs et al., 1999): high detection limits: 1000-3000 viral genomes per rctn

- β-actin: housekeeping gene → control of DNA extraction and PCR inhibition
Validation

▶ Virus strains

**Inclusivity:** 15 international BoHV-1.1 and 1.2 reference strains + 25 BoHV-1 field strains

**Exclusivity:** all commercial gE-deleted BoHV-1 vaccine virus strains (Boehringer/Pfizer/Zoetis; Intervet/MSD; Hipra)

BoHV-2, BoHV-4, BoHV-5, and BoHV-6

Ruminant herpesviruses: CvHV-1, CvHV-2, BuHV-1, CapHV-1; (not ElkHV-1)

▶ Matrices

BoHV-1 positive nasal swabs, serum, trigeminal ganglia, tissue samples and spiked semen samples

▶ Reference methods

- Virus isolation in cell culture according to the OIE manual
- OIE validated BoHV-1 gB qPCR  

▶ ASe

BoHV-1.2 virus DNA, gE / gD expression plasmids ≤10 copies per rcttn;

▶ ASp

100% for gD ↔ Wang gB PCR: BoHV-5, BuHV-1, CpHV-1, CvHV-1, CvHV-2

X-reactions only for gE: BuHV-1, CvHV-1, CvHV-2
► Repeatability and reproducibility

gD and gE: intra and inter assay repeatability within accepted range of coefficient of variation (CV) limits

β-actin: 4 replicates of cattle DNA from blood cells extracted on 4 separate days: mean cq 33.08 / CV 1.72%

Fig. 2. Reproducibility and repeatability of the simplex BoHV-1 qPCR. The Cq values (y-axis) of 16 replicates of the BoHV-1 gD-assay (a) and of the BoHV-1 gE-assay (b) using a 10-fold dilution series of the BoHV-1 "Schroederboeck" standard DNA (x-axis: log10 initial quantity) are shown. Mean values are indicated alongside each boxplot, standard deviations are depicted in parentheses and coefficients of variation in square brackets. Boxplots were designed by the R software (R Development Core Team, 2009).
Repeatability and reproducibility (2)
Second phase:
3 collaborating labs: comparison of blinded panel of samples
Ring trial (2009); update 2014

Implementation
In 2009 established in the first German official regional diagnostic labs

Revalidation
Finalised protocol in 2010; no further relevant modifications necessary
Virus dilutions: comparable to v.i. and Wang gB qPCR
Semen: comparable to v.i., lower detection limit than Wang

Experimentally infected reference animals +
field samples with defined status
comparable to Wang gB qPCR
Both PCRs: nasal shedding: 1-3 d longer positive than v.i.
DSp
41 nasal swabs, 24 serum, 10 semen, and 17 tissue samples with known negative status:
92 samples - 100% negative PCR results
Cut-off determination cq 40 (no false positive results)

DSp FIELD VALIDATION
Mandatory BoHV-1 eradication programme in Germany → defined status for each holding and each adult dairy and breeding cattle

Official regional vet labs:
Since 2009 LAV Stendal, Saxony-Anhalt (0.34 mil. cattle)
Since 2012 LVI Oldenburg, Lower-Saxony (2.58 mil. cattle)

Hundreds of samples tested in comparison to v.i.
- no naturally occurring false positive reactions reported (x-reactions, primer-probe incompatibilities)
- numerous outbreaks confirmed
### Field samples

<table>
<thead>
<tr>
<th>Test results</th>
<th>Known positive 212</th>
<th>Known negative 92</th>
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<tbody>
<tr>
<td>Positive</td>
<td>gE 210 gD 204</td>
<td>gE and gD 0</td>
</tr>
<tr>
<td>Negative</td>
<td>2 8</td>
<td>92</td>
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<table>
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<tr>
<th>Diagnostic sensitivity*</th>
<th>Diagnostic specificity*</th>
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<tr>
<td>gE 99.5%</td>
<td>100±2%</td>
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<tr>
<td>gD 96.2%</td>
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SUMMARY

Triplex qPCR is a **sensitive and specific** method for confirming the presence of BoHV-1 genomes

Published by Wernike et al., 2011
FLI homepage: collection of officially prescribed methods

**Differentiation** between wild type virus and gE deleted marker vaccine strains in a single PCR reaction possible

**DSe** comparable to OIE validated gB PCR (Wang et al.) and virus isolation as gold standard

Since years applied by German official vet labs:
Excellent **DSp**

“**FIT FOR PURPOSE**” robust, sensitive, specific
Thank you for your attention