Validation of an *in vitro* assay for the detection of residual viable rabies virus in inactivated rabies vaccines

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**Virus Transmission**
- Saliva of infected animals
- 99% of human cases are caused by dog bites

The virus attacks the brain. Rabies is fatal once symptoms appear.

**Treatment**
- Thorough washing of the wound with soap, and vaccine injections can avoid symptoms and save lives. Seek immediate medical care if bitten.

**Fatalities**
- Rabies affects poor rural communities mostly in Asia and Africa.

About one death every 15 minutes.

- 40% of the victims are children younger than 15.

**How to prevent Rabies transmission from dogs?**
- Learn dog body language
- Raise public awareness

**No dog bite = No rabies**

**Vaccinating dogs saves human lives**
- Rabies is 100% preventable

Vaccinating 70% of dogs breaks rabies transmission cycle in an area at risk.
- Every dog owner is concerned.
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**Rabies: The Facts**

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Residual Live Virus Assay

Vaccine inoculation
- 16 newborn mice
- 40 adult mice

Observation period
- 21 days – normal behavior

Satisfactory Batch release

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2017

30 million doses inactivated rabies vaccine

6 thousand mice

In Vitro Methodology

VIRAL CULTURE + DIFA & RT-qPCR

BHK-21 cells
In Vitro Methodology

VIRAL CULTURE + DIFA & RT-qPCR

REDUCE 2/3 MICE - INTERNAL QUALITY CONTROL
Detection of residual virus
Detection of residual virus

\[0 \text{ hr} \rightarrow C_{q_i} X\]

\(C_q =\) quantification cycle
Detection of residual virus

\[ \text{Cq} = \text{quantification cycle} \]
Detection of residual virus

0 hr $\rightarrow$ C$q_i$ $\neq$ X

72 hr $\rightarrow$ C$q_f$ $< X$

C$q$ = quantification cycle
Table 1: Oligonucleotides sequences of primers and probes used in this study.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence 5’-3’</th>
<th>Gene</th>
<th>Positiona</th>
<th>Product size (nt)</th>
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</thead>
<tbody>
<tr>
<td>RABV-FN1</td>
<td>5’-GAAGAGATCGCACAATACGGAGAT-3’</td>
<td>Rabies Virus Nucleoprotein</td>
<td>1260-1282</td>
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<tr>
<td>RABV-RN1</td>
<td>5’-TGTGGAGAAACCTCGGCGATGA-3’</td>
<td>BHK-21 cells β-actin</td>
<td>1342-1321</td>
<td>82</td>
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<tr>
<td>RABV-P1</td>
<td>5’-6FAM-AGTCAGTTCCAATCATAAGCTCGTCCAA-BBQ-3’</td>
<td>ACTB- F*</td>
<td>1390-1319</td>
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<tr>
<td>ACTB-F*</td>
<td>5’-CAGCACCATGAAGATCAAGATCATT-3’</td>
<td>BHK-21 cells β-actin</td>
<td>1083-1107</td>
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<tr>
<td>ACTB-R*</td>
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<td>ACTB- F*</td>
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<tr>
<td>ACTB-P*</td>
<td>5’-VIC-TCACTGTCCACCTTCAGCAGATGT-BBQ-3’</td>
<td>ACTB- F*</td>
<td>1159-1183</td>
<td></td>
</tr>
</tbody>
</table>

6FAM, 6-carboxyfluorescein; VIC, 2’-chloro-7’phenyl-1,4-dichloro-6-carboxy-fluorescein; BBQ, blackberry quencher; nt, nucleotides.

a Corresponding nucleotide positions of RABVgp1 (GenBank Ac. No. 001542.1), and of Mesocricetus auratus b-actin mRNA (GenBank Ac. No. AJ312092).

Fig1: Comparison between singleplex assay targeting only Rabies nucleoprotein (RABVgp1) and duplex assay targeting RABVgp1 and BHK-21 b-actin mRNA.
Fig2: Validation of the duplex assay targeting RABVgp1 and BHK-21 b-actin mRNA. Linear dynamic range (LNR) for the assay targeting RABVgp1, determines $10^1$ TCID50/mL as the Limit of Quantification (LOQ).
RT-qPCR – LOD & Specificity

**Repeatability**
- $10^0$ TCID$_{50}$/mL Cq(m) 31.88 (9.2%CV)
- $10^{-0.5}$ TCID$_{50}$/mL Cq(m) 32.65 (2.9%CV)
- $10^{-1}$ TCID$_{50}$/mL Cq(m) 33.41 (2.5%CV) → **LOD**

**Reproducibility**
- $10^0$ TCID$_{50}$/mL Cq(m) 30.49 (6.4%CV)

**Specificity**
- EV // CMV // HSV2/VZV // HSV1 // ErithroB19 // HHV6 // EBV
- All Cq higher than LOD → **negative**

*Cq* = quantification cycle; *LOD* = Limit of Detection

EV = Enterovirus non-polio; CMV = Cytomegalovirus; HSV2/VZV = Herpesvirus 2/Varicella Zoster virus; HSV1 = Herpesvirus 1; ErithroB19 = Erithrovirus B19; HHV6 = Human Herpexvirus 6; EBV = Epstein-Barr virus
Viral Culture + DIFA

Fig 3: Analysis of optimal culture conditions. WV = working virus, DIFA = Direct Immunofluorescence Assay
Viral Culture – Temperature

Fig3: Analysis of optimal culture conditions. WV = working virus, DIFA = Direct Immunofluorescence Assay
Viral Culture – % FBS

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Fig3: Analysis of optimal culture conditions. WV = working virus, DIFA = Direct Immunofluorescence Assay
Fig 4: Evaluation of RT-qPCR in combination with *in vitro* method.
Perspectives

Fig 5: Comparative study between *in vitro* and *in vivo* assays.

Next

Sensitivity test

Analysis of previously released batches to validate the *in vitro* method.
References


Thank you!

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