

2nd
PAN-AMERICAN
Conference for Alternative Methods

August 23-24, 2018
Rio de Janeiro

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

***Beatriz L C Moreira,**

Ana P L G Sbalqueiro, Jorge M F Inagaki, Sonia M Raboni

*Masters student, Post-Graduation Program on Microbiology, Parasitology and Pathology, UFPR

Bioindustrial Analyst, Immunobiological Development and Production Center, TEPCAR



VIRUS TRANSMISSION



Saliva of infected animals



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.



HOW TO PREVENT RABIES TRANSMISSION FROM DOGS?



Raise public awareness

Learn dog body language



NO DOG BITE = NO RABIES

FATALITIES

Rabies affects **poor rural communities** mostly in Asia and Africa



About **One death** every



40% of the victims are children younger than 15

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



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.



HOW TO PREVENT RABIES TRANSMISSION FROM DOGS?



Raise public awareness

Learn dog body language



NO DOG BITE = NO RABIES

FATALITIES

Rabies affects **poor rural communities** mostly in Asia and Africa



About **One death every**



40% of the victims are children younger than 15

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



VIRUS TRANSMISSION



Saliva of infected animals



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.



HOW TO PREVENT RABIES TRANSMISSION FROM DOGS?



Raise public awareness

Learn dog body language



NO DOG BITE = NO RABIES

FATALITIES

Rabies affects **poor rural communities** mostly in Asia and Africa



About **One death every**



40% of the victims are children younger than 15

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



VIRUS TRANSMISSION



Saliva of infected animals



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.



HOW TO PREVENT RABIES TRANSMISSION FROM DOGS?



Raise public awareness

Learn dog body language



NO DOG BITE = NO RABIES

FATALITIES

Rabies affects **poor rural communities** mostly in Asia and Africa



About **One death every**



40% of the victims are children younger than 15

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



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



40% of the victims are children younger than 15

HOW TO PREVENT RABIES TRANSMISSION FROM DOGS?



Raise public awareness

Learn dog body language



NO DOG BITE = NO RABIES

VACCINATING DOGS SAVES HUMAN LIVES

Rabies is 100% preventable

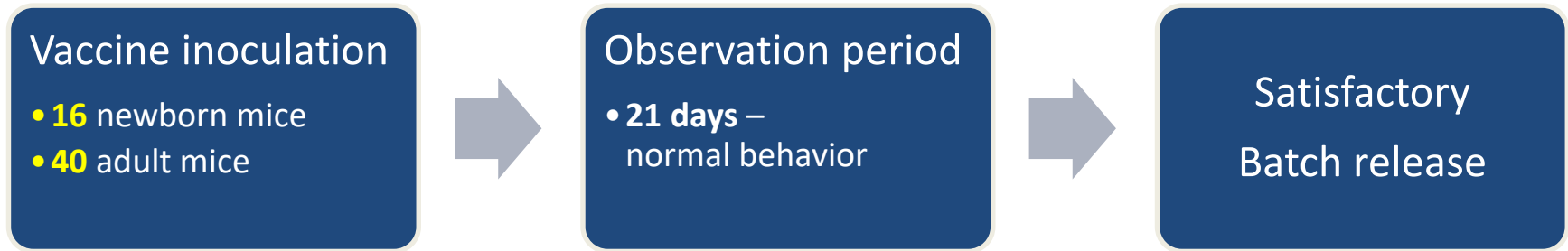


Vaccinating **70%** of dogs **breaks rabies transmission cycle** in an area at risk

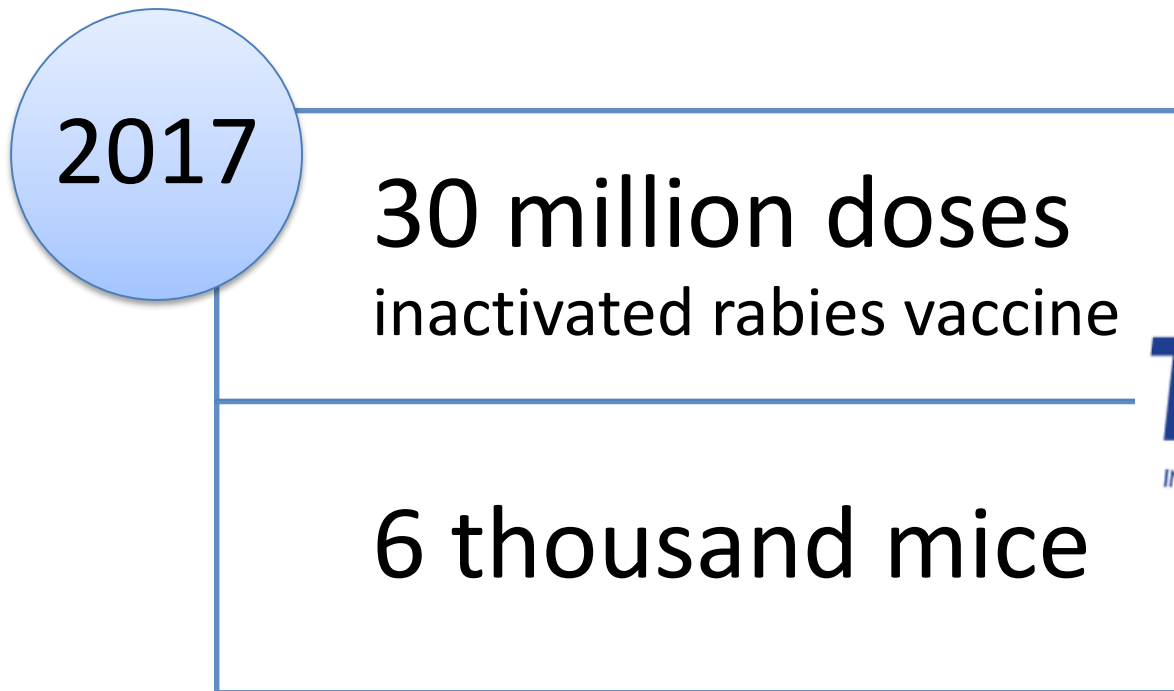
Every dog owner is concerned



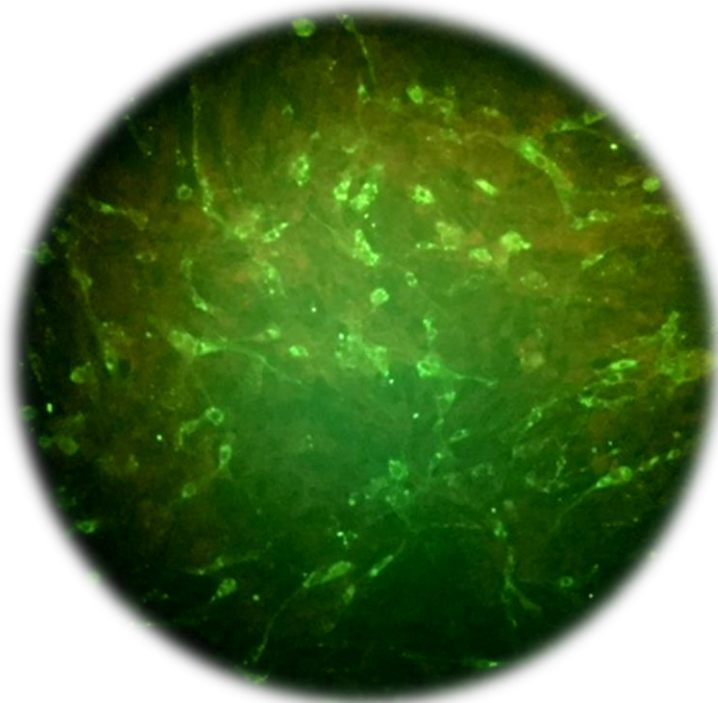
Residual Live Virus Assay



Residual Live Virus Assay



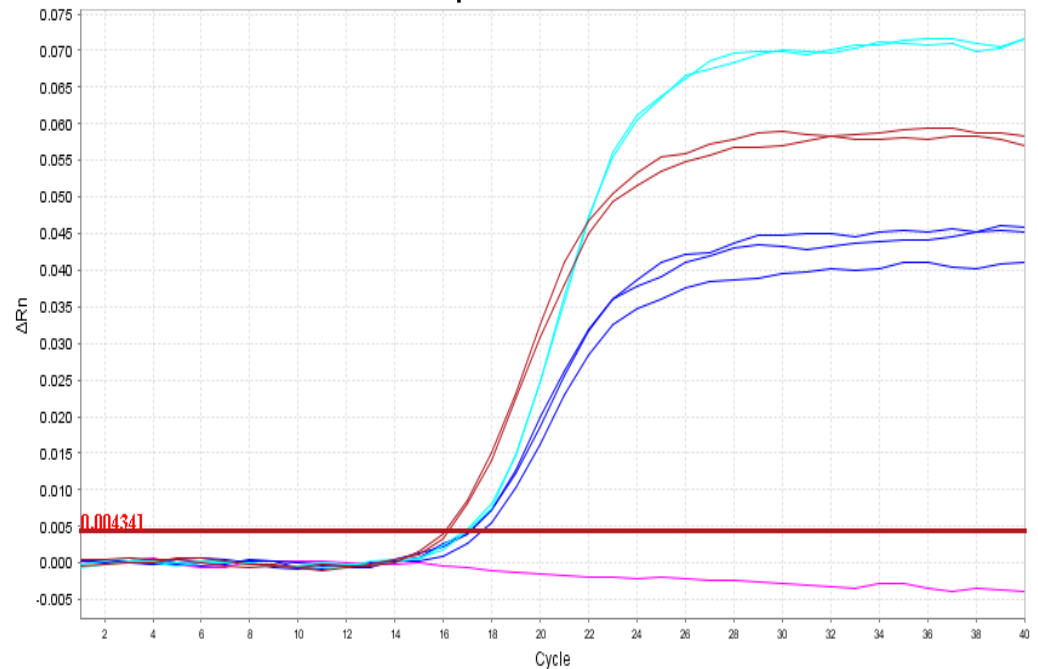
In Vitro Methodology



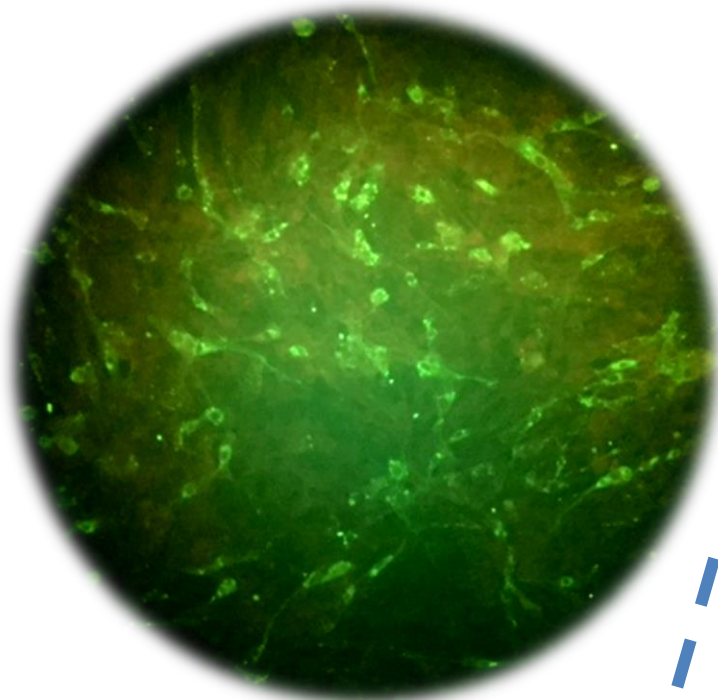
BHK-21 cells

VIRAL CULTURE + DIFA & RT-qPCR

Amplification Plot

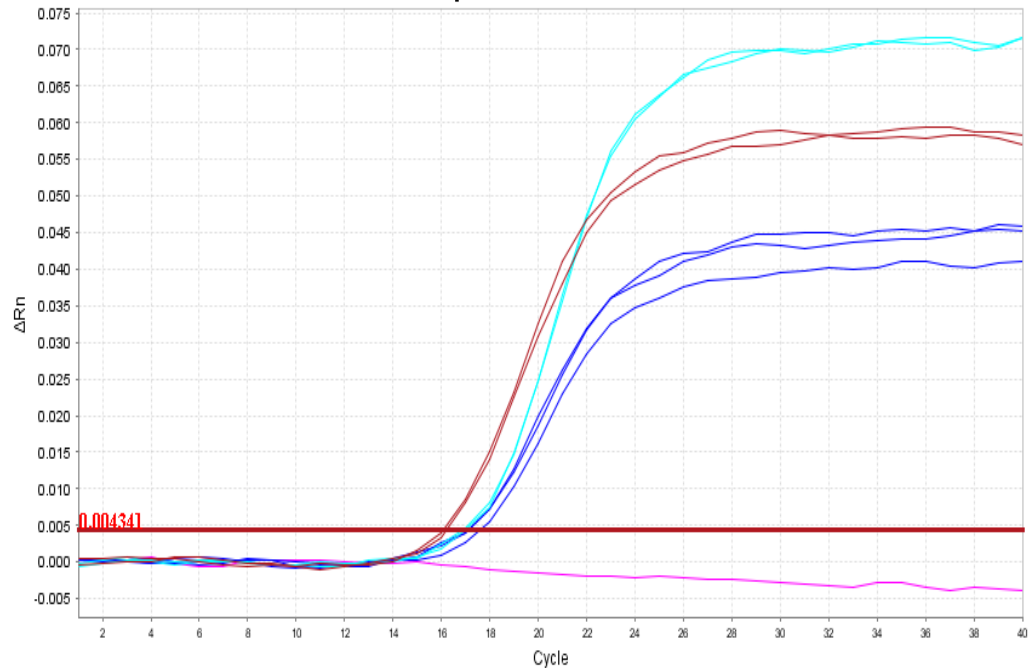


In Vitro Methodology



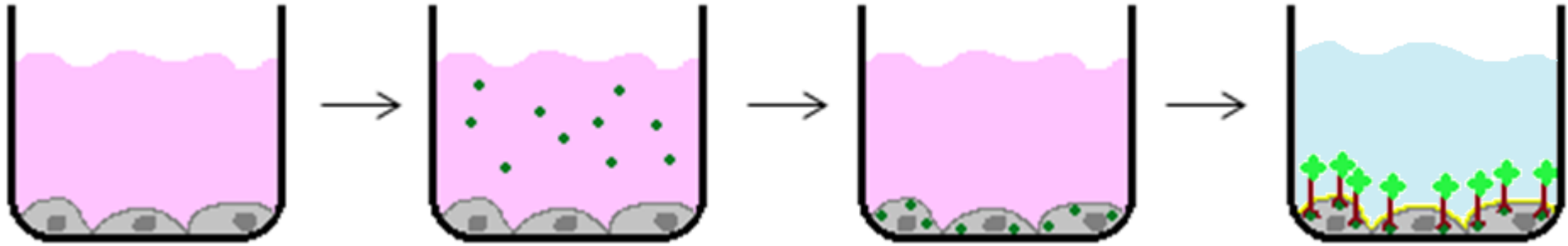
VIRAL CULTURE + DIFA & RT-qPCR

Amplification Plot

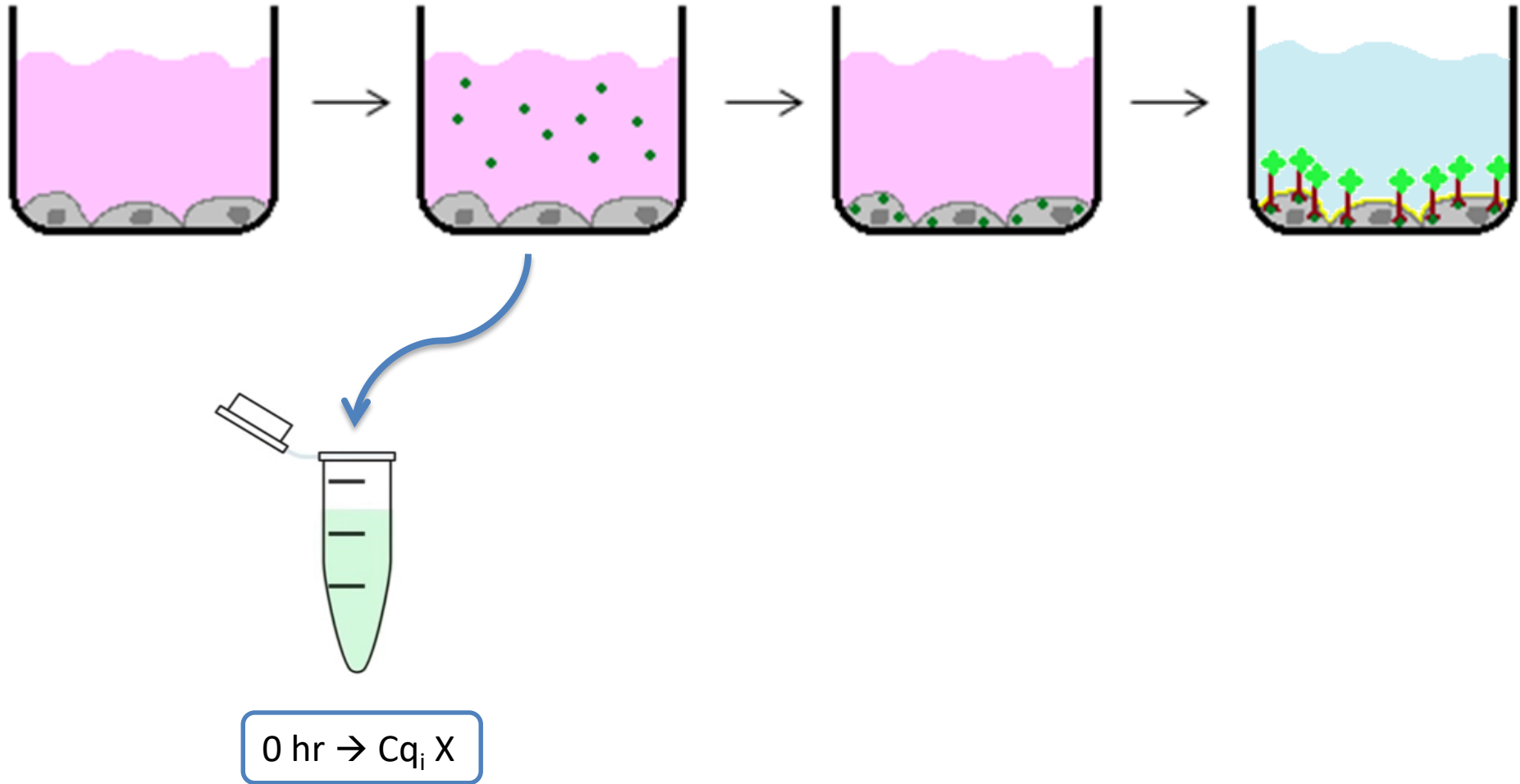


**REDUCE 2/3 MICE –
INTERNAL QUALITY
CONTROL**

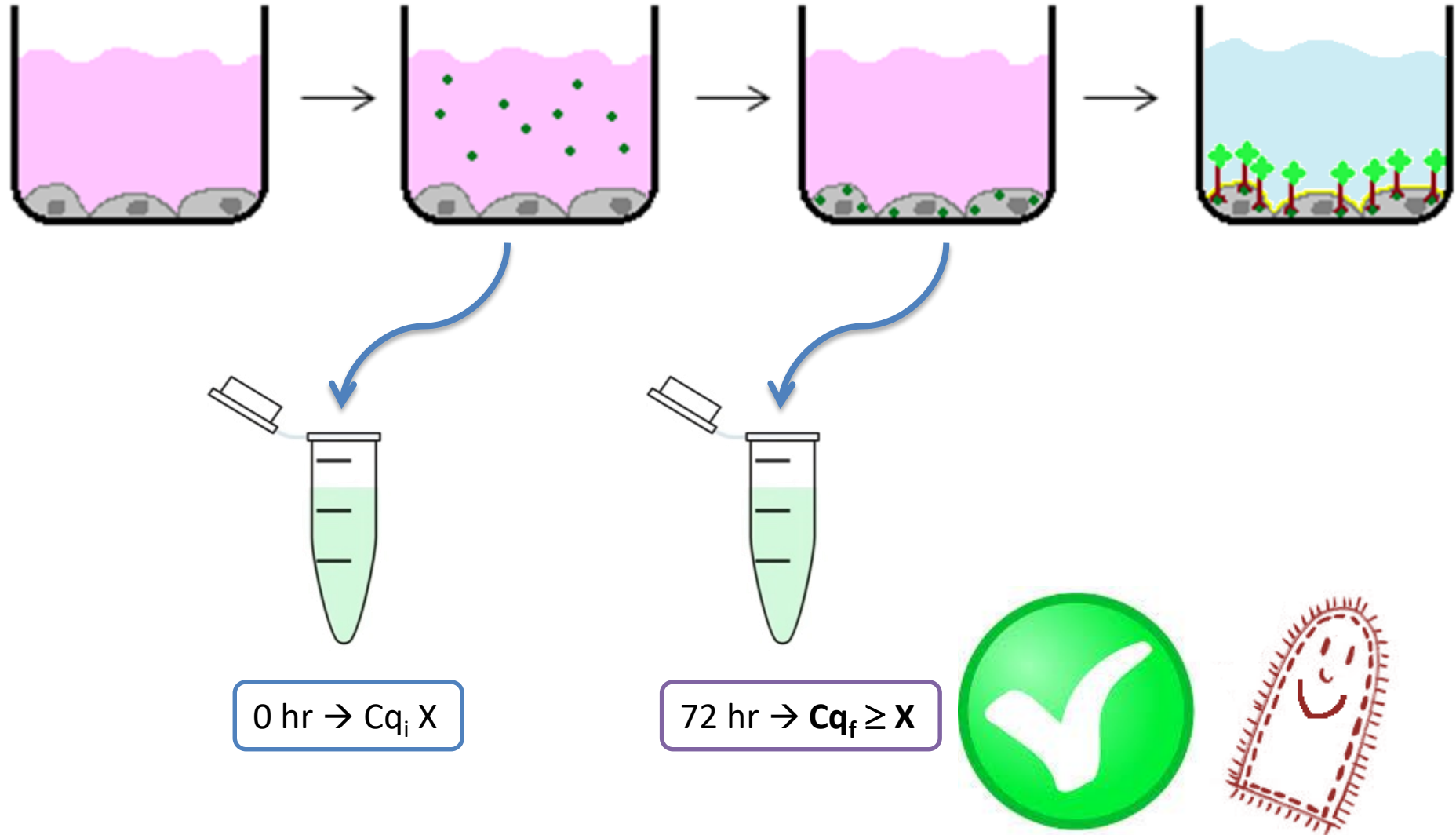
Detection of residual virus



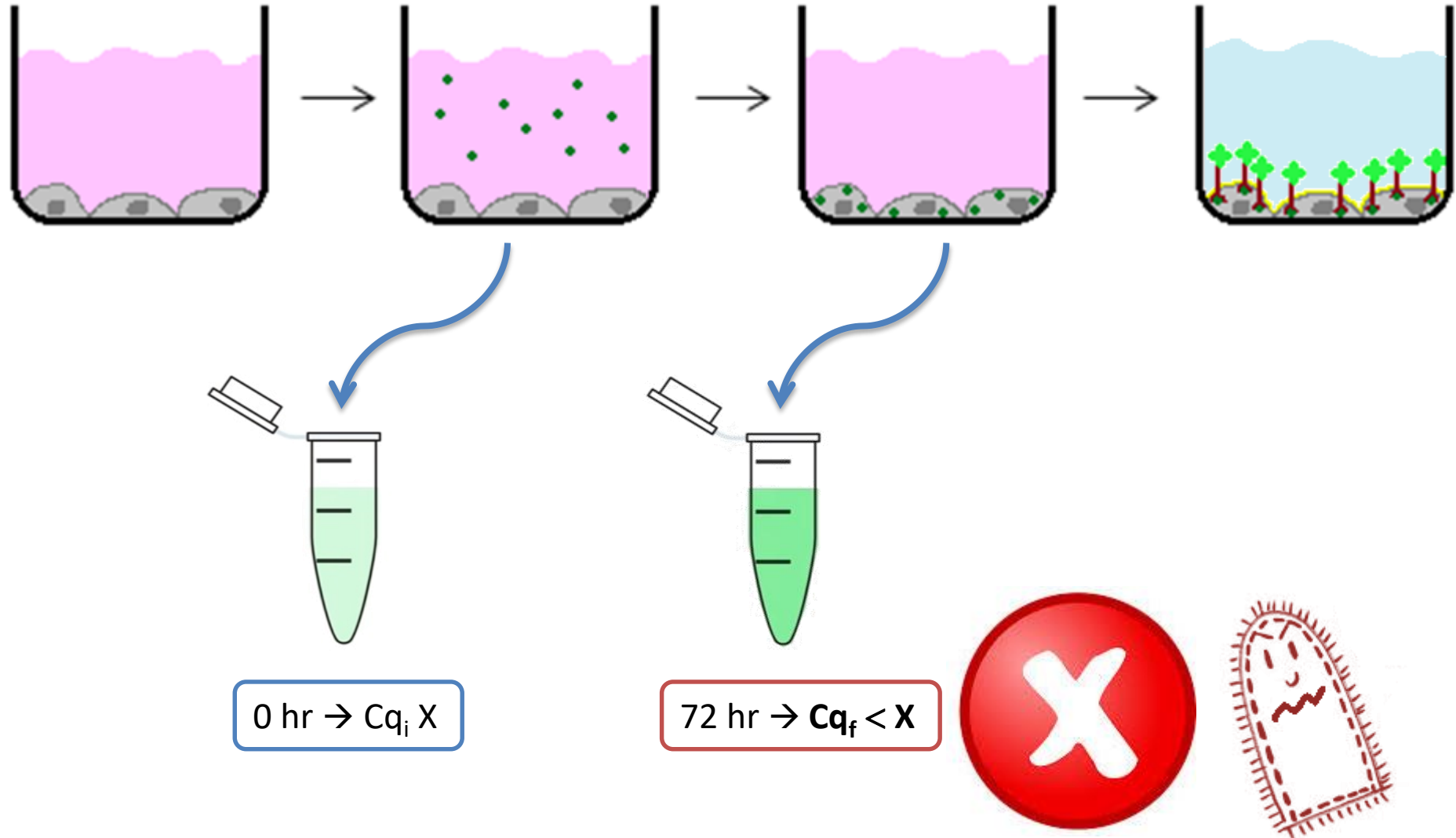
Detection of residual virus



Detection of residual virus



Detection of residual virus



RT-qPCR

Table 1: Oligonucleotides sequences of primers and probes used in this study.

Name	Sequence 5'-3'	Gene	Position ^a	Product size (nt)
RABV-FN1	5'- GAAGAGATCGCACATACGGAGAT -3'	Rabies Virus Nucleo-protein	1260-1282	
RABV-RN1	5'- TGTTTAGAAACTCGGCGAATGA -3'		1342-1321	82
RABV-P1	5'- 6FAM-AGTCAGTTCCAATCATCAAGCTCGTCCAAA-BBQ -3'		1290-1319	
ACTB-F*	5'- CAGCACCATGAAGATCAAGATCATT -3'	BHK-21 cells β-actin	1083-1107	
ACTB-R*	5'- CGGACTCATCGTACTCCTGCTT -3'		1213-1192	131
ACTB-P*	5'- VIC-TCACTGTCCACCTTCCAGCAGATGT-BBQ -3'		1159-1183	

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).

RT-qPCR – Duplex x Singleplex

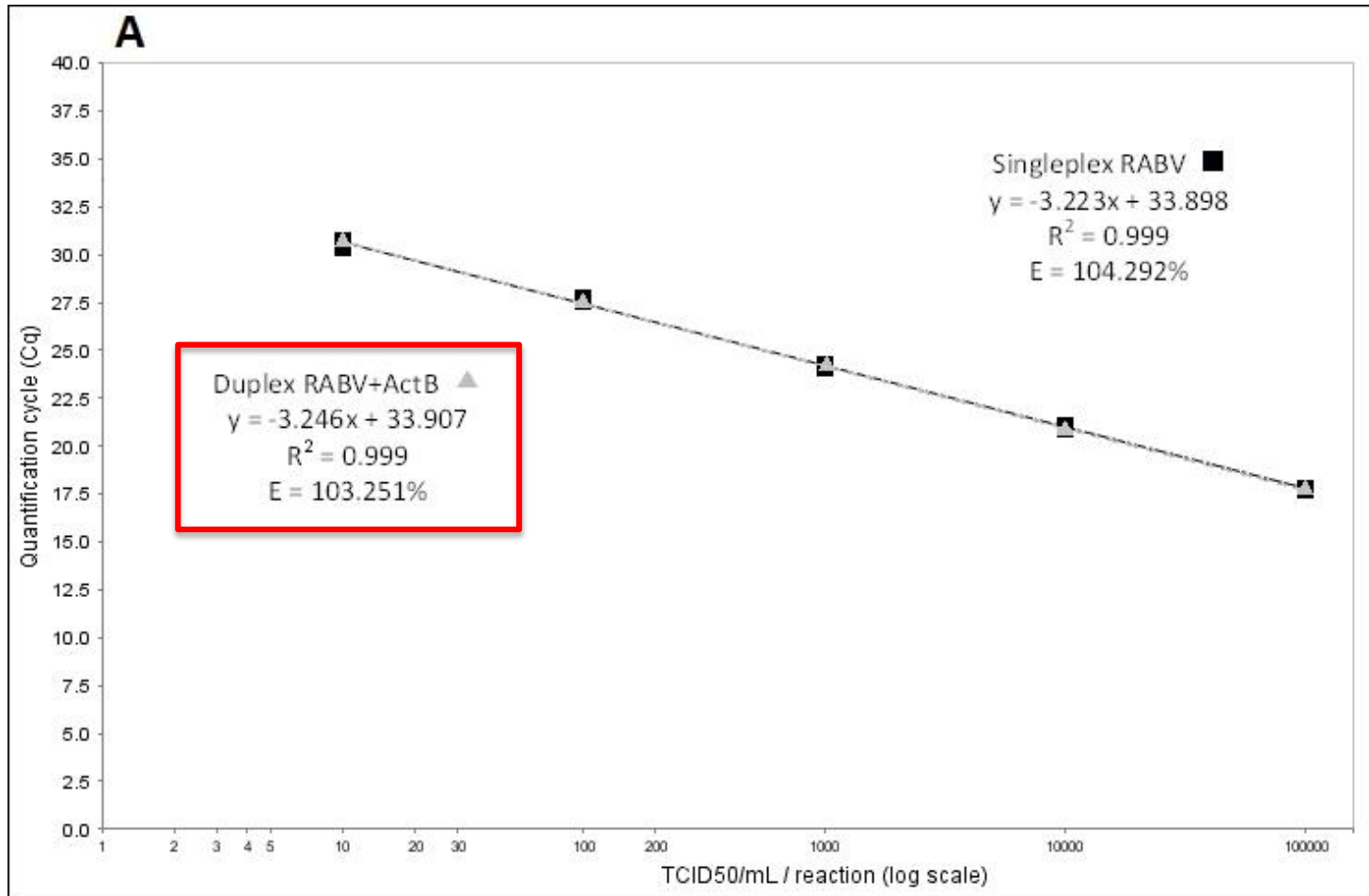


Fig1: Comparison between singleplex assay targeting only Rabies nucleoprotein (RABVgp1) and duplex assay targeting RABVgp1 and BHK-21 b-actin mRNA.

RT-qPCR – LDR & LOQ

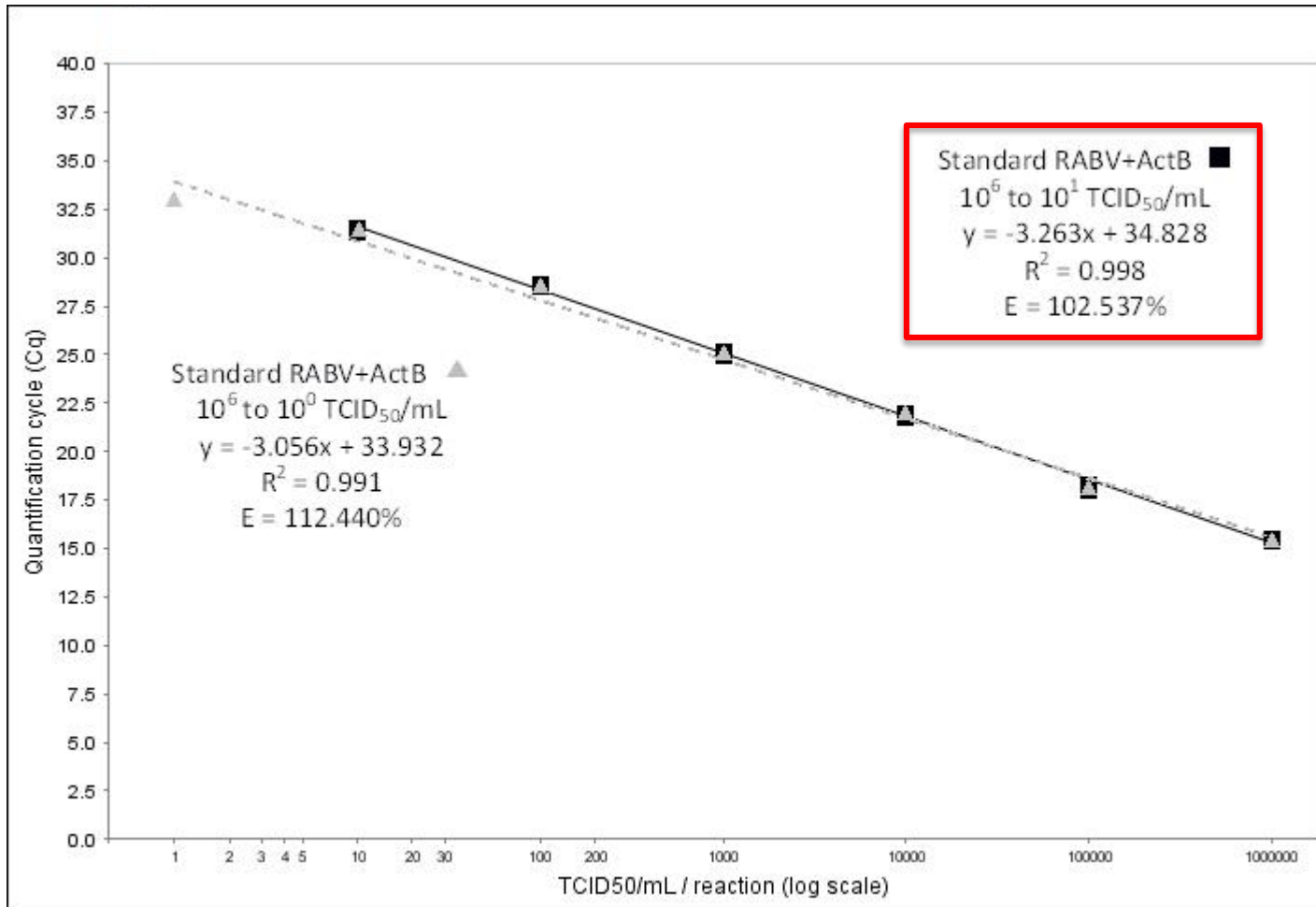


Fig2: Validation of the duplex assay targeting RABVgp1 and BHK-21 b-actin mRNA. Linear dynamic range (LNR) for the assay targeting RABVgp1, determines 10^1 TCID₅₀/mL as the Limit of Quantification (LOQ).

RT-qPCR – LOD & Specificity

Repeatability

- 10^0 TCID₅₀/mL Cq(m) 31,88 (9,2%CV)
- $10^{-0.5}$ TCID₅₀/mL Cq(m) 32,65 (2,9%CV)
- 10^{-1} TCID₅₀/mL Cq(m) **33,41** (2,5%CV) → **LOD**

Reproducibility

- 10^0 TCID₅₀/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

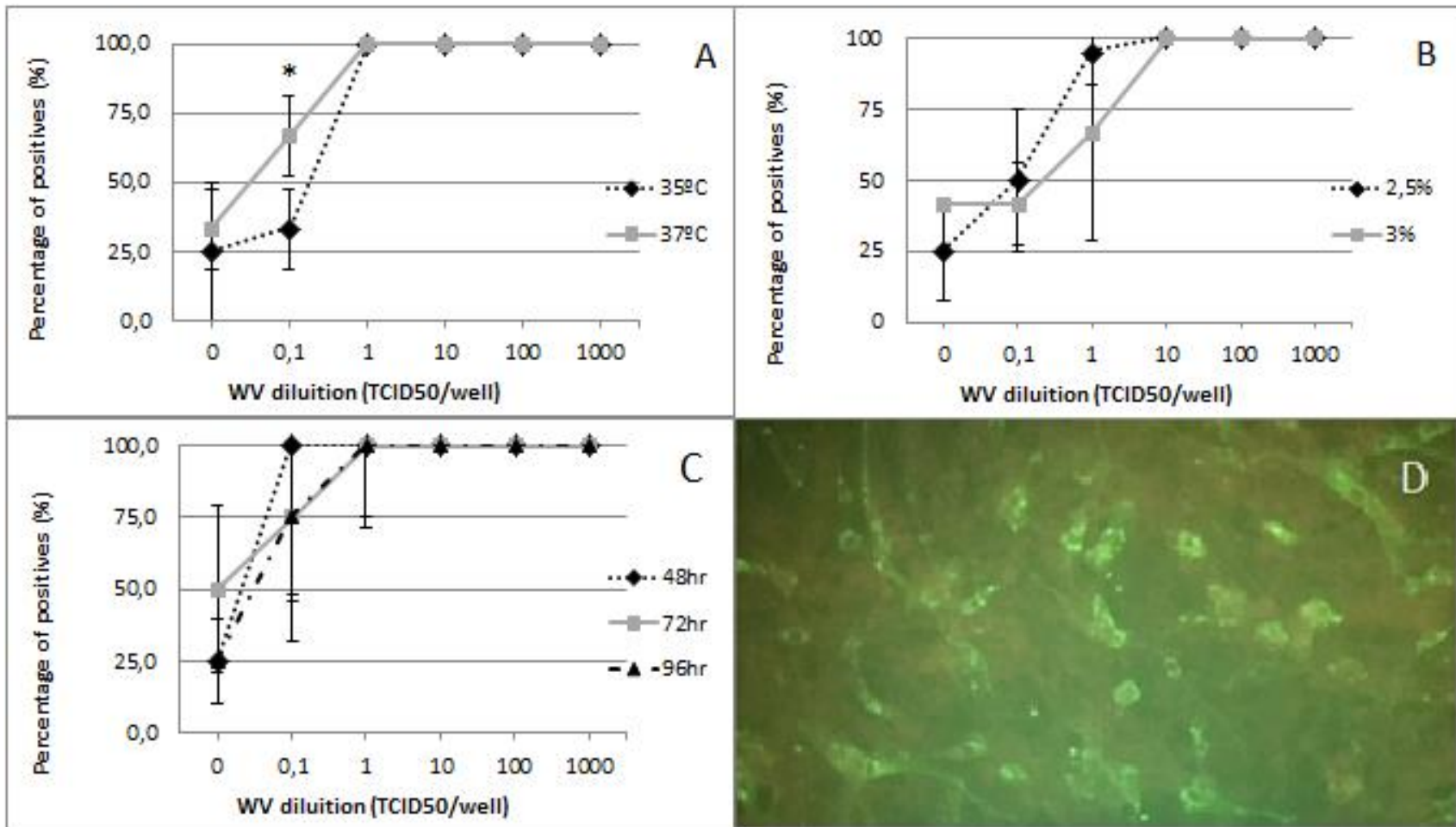


Fig3: Analysis of optimal culture conditions. WV = working virus, DIFA = Direct Immunofluorescence Assay

Viral Culture – Temperature

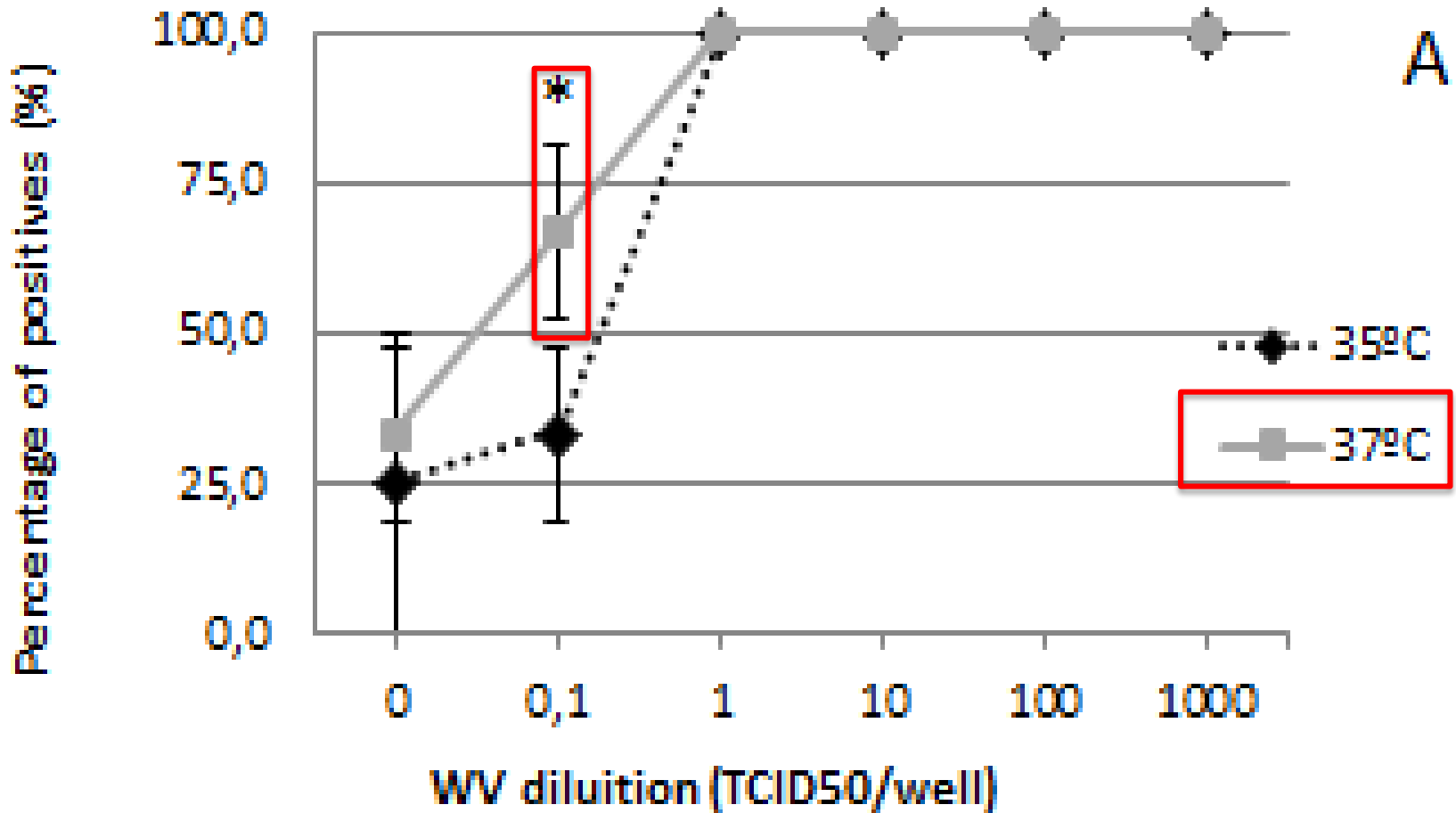


Fig3: Analysis of optimal culture conditions. WV = working virus, DIFA = Direct Immunofluorescence Assay

Viral Culture – % FBS

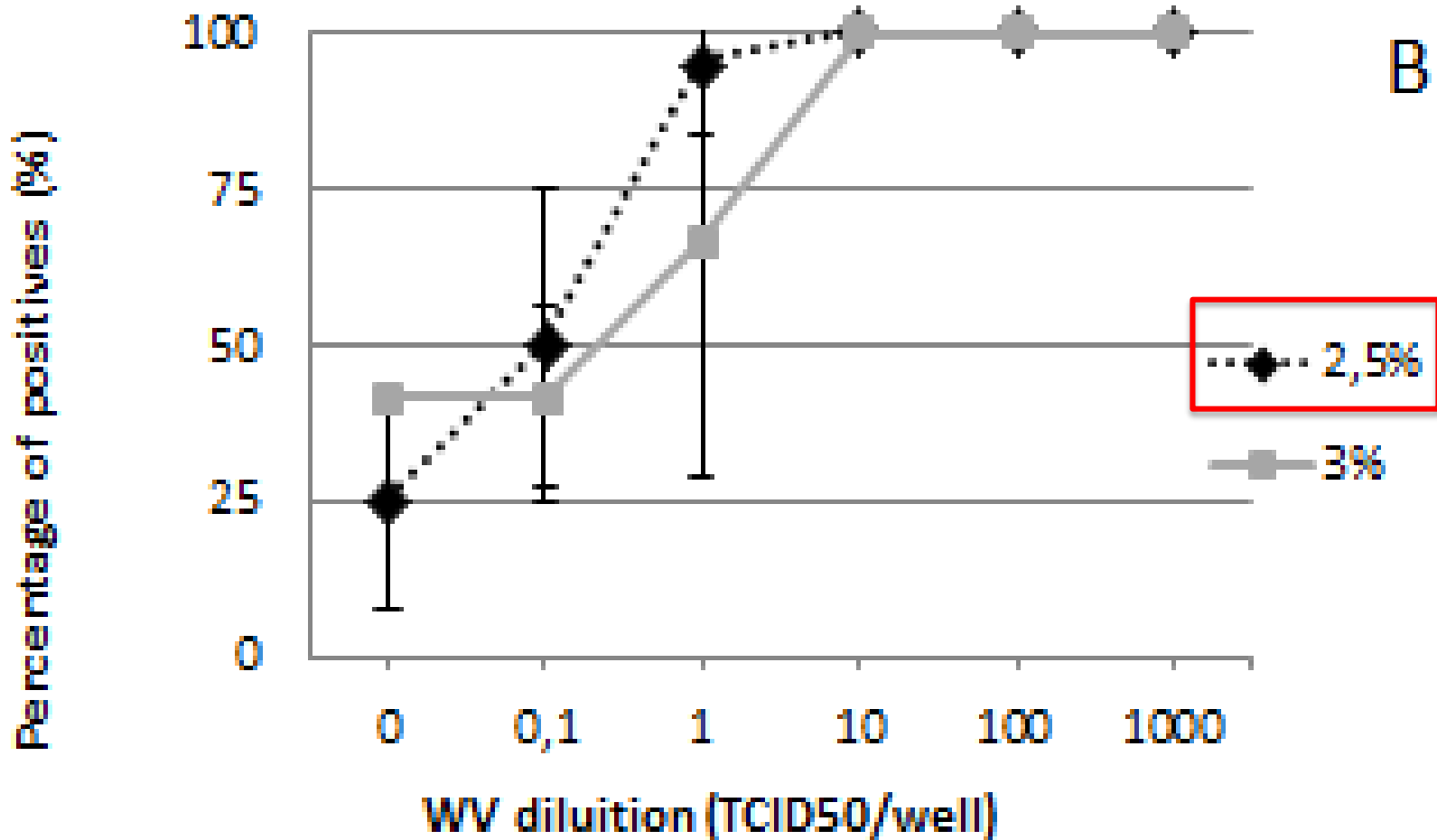
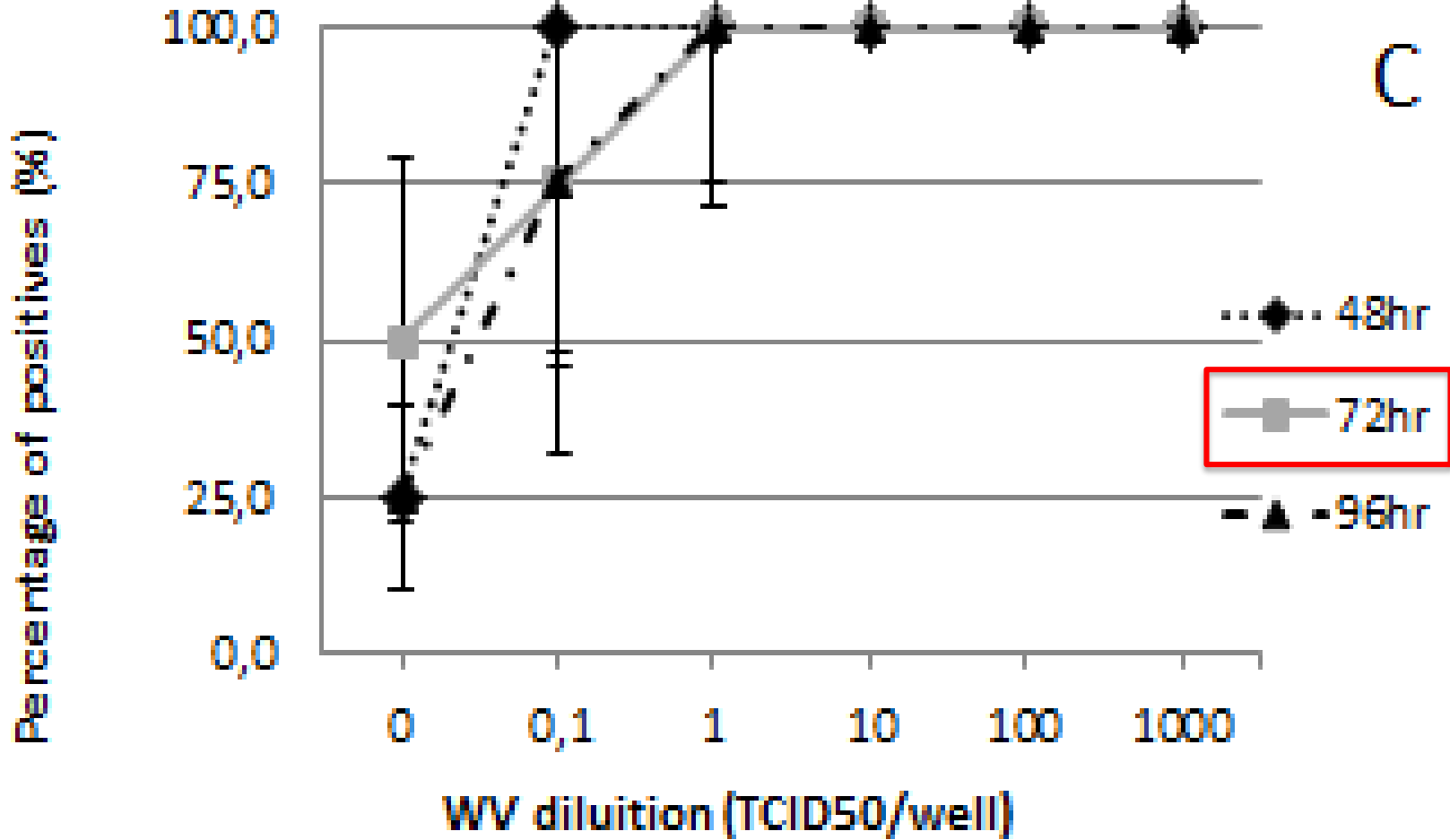


Fig3: Analysis of optimal culture conditions. WV = working virus, DIFA = Direct Immunofluorescence Assay

Viral Culture – Incubation



C

Fig3: Analysis of optimal culture conditions. WV = working virus, DIFA = Direct Immunofluorescence Assay

DIFA x RT-qPCR

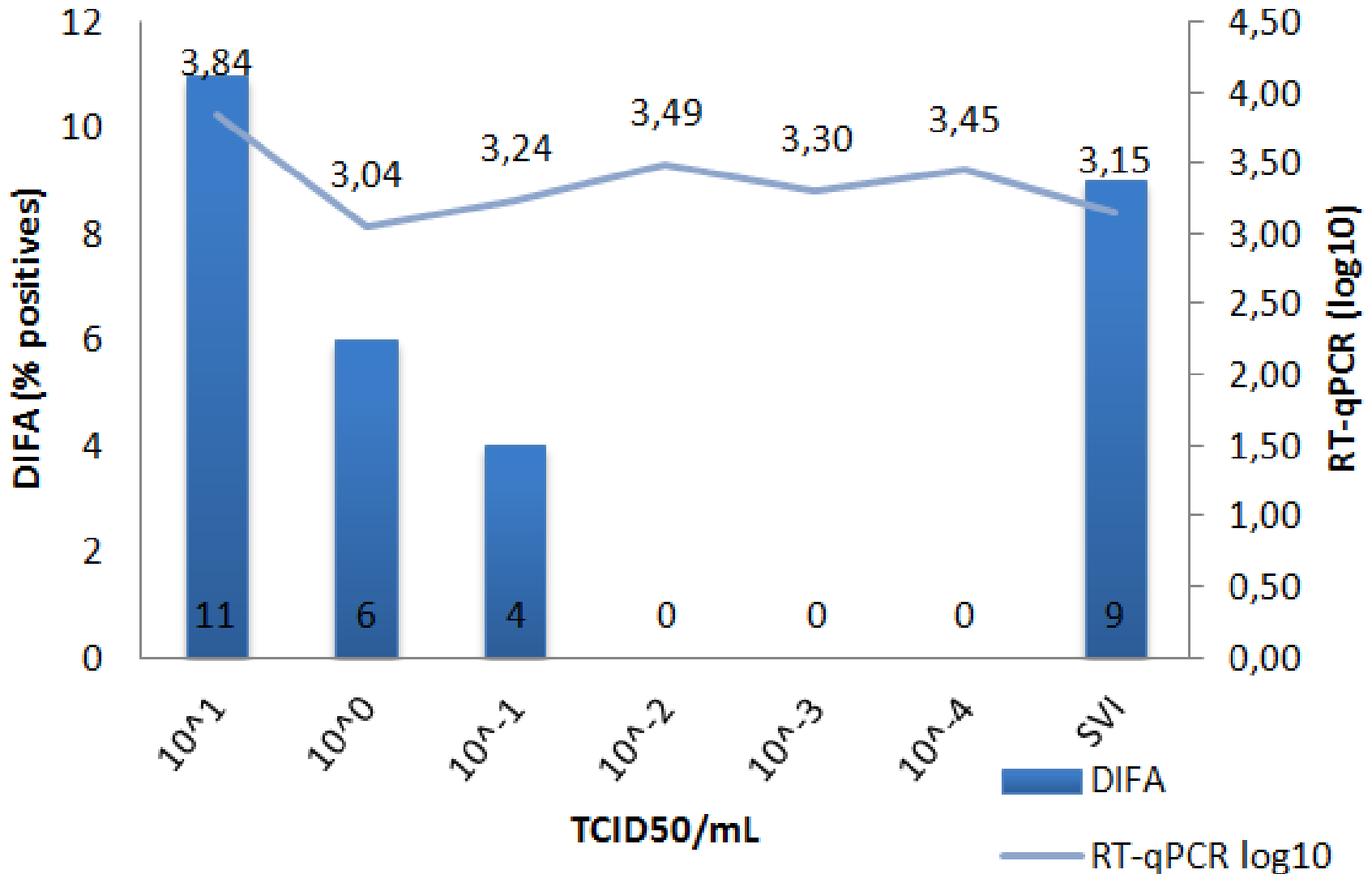


Fig4: Evaluation of RT-qPCR in combination with *in vitro* method.

Perspectives

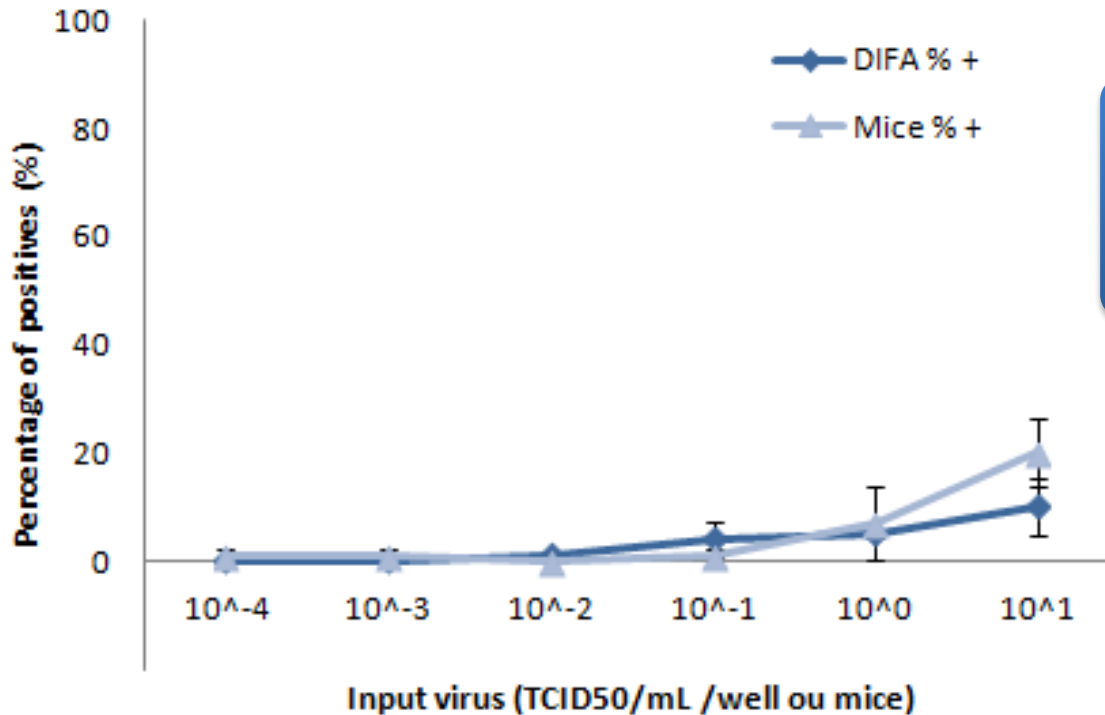


Fig5: Comparative study between *in vitro* and *in vivo* assays.

Next

Sensitivity test

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

References

- BALARAM, D. *et al.* World Rabies Day – a decade of raising awareness. *Tropical Diseases, Travel Medicine and Vaccines*, v. 2, p. 19, 2016.
- BRUCKNER, L. *et al.* Three Rs Approaches in the Quality Control of Inactivated Rabies Vaccines: The Report and Recommendations of ECVAM Workshop 48. *ATLA* v. 31, p. 429-454, 2003.
- HILL, R. E. Alternative Methods to Reduce, Refine, and Replace the Use of Animals In the Development and Testing of Veterinary Biologics in The United States; a Strategic Priority. *Procedia in Vaccinology*, v. 5, p. 141–145, 2011.
- MAPA. Portaria nº 228, 25/out/1988 – Production and comercialization control of veterinary antirabies vaccine and serum, 1988.
- Stokes, W. *et al.* Report on the international workshop on alternative methods for human and veterinary rabies vaccine testing: State of the science and planning the way forward. *Biologicals*, v. 40, p. 369-381, 2012.
- TAKAYAMA-ITO, M. *et al.* A sensitive invitro assay for the detection of residual viable rabies virus in inactivated rabies vaccines. *Biologicals*, v. 42, n. 1, p. 42–47, 2014.
- WHO. Rabies. Fact Sheet Update September 2017. Available in: <http://www.who.int/mediacentre/factsheets/fs099/en/>
- Zhang, Y., et al. Preparation and characterization of a stable BHK-21 cell line constitutively expressing the Schmallerberg virus nucleocapsid protein. *Mol. Cell. Probes*, v. 29 (4), p. 244-253, 2015.

2nd
PAN-AMERICAN
Conference for Alternative Methods

August 23-24, 2018
Rio de Janeiro

Thank you!



Beatriz L. C. Moreira

blcorreia@tecpar.br

(41) 3316-3229

TEC PAR
INSTITUTO DE TECNOLOGIA DO PARANÁ

