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CUTTING EDGE TECHNOLOGY: DIRECT FIELD TESTING USING A PORTABLE INSTRUMENT WITHOUT NUCLEIC ACID EXTRACTION

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Introduction

Worldwide, Central Reference Laboratory systems are being overwhelmed by the volume of African Swine Fever (ASF) samples being submitted. The surge volume is causing substantial delays in the reporting which confounds real-time decision making for control efforts. Here we demonstrate results of cutting edge technological developments that facilitate on site testing for Food and Mouth Disease (FMD) and African Swine Fever (ASF) viruses.

The NAHLN laboratories in the US utilize an ASFV real-time PCR based on a publication from Zsak, et al., 2005 which was designed and developed by Tetracore in 2000. The 16 year old design was evaluated in-silico in 2016 and modernized with an additional primer probe set to increase the potential for detection of contemporary strains of ASFV. The modernized test was evaluated in the field in collaboration with the National Veterinary Institute (SVA) in Uppsala, Sweden, an OIE Collaborating Centre for Biotechnology-based Diagnosis of Infectious Diseases in Veterinary Medicine and the National Animal Disease Diagnosis and Epidemiology Center (NADDEC) in Entebbe, Uganda.

The World Organization for Animal Heath (OIE) Terrestrial Manual Chapter on FMD (3.1.8) recognizes two independent real-time RT-PCR assays, one targeting the 5'UTR and the other targeting the 3D region of the viral genome. The FMDV 3D assay was designed and developed in 2000 and published by Tetracore (Callahan et al, 2002). As viruses evolve over time it is prudent to periodically review the assay design against contemporary FMD sequences. In 2016 the 3D assay was updated with an additional primer / probe set that was added to the original assay design. The modernized test was then validated in collaboration with the Pirbright Institute, an OIE reference laboratory for FMD.

Materials and Methods

For ASF detection, this study was conducted in three stages over three years (2015-2017) as part of a project under the OIE in partnership with the SVA and NADDEC. Stage one focused on the adaptation of the magnetic beads-based protocol for nucleic acids extraction from 64 blood samples. In stage two, two sample testing strategies were tested in parallel: (1) direct testing of samples diluted in PBS were tested by the dried-down ASFV PCR kit with internal control (IC) (Tetracore Inc., Rockville, Maryland) on the portable real-time PCR thermocycler T-COR 8™ (Tetracore Inc.), and (2) samples underwent nucleic acid extraction and were tested by the OIE recommended Universal Probe Library (UPL) assay (Fernández-Pinero et al. 2013) on a Stratagene Mx3000P at NADDEC.

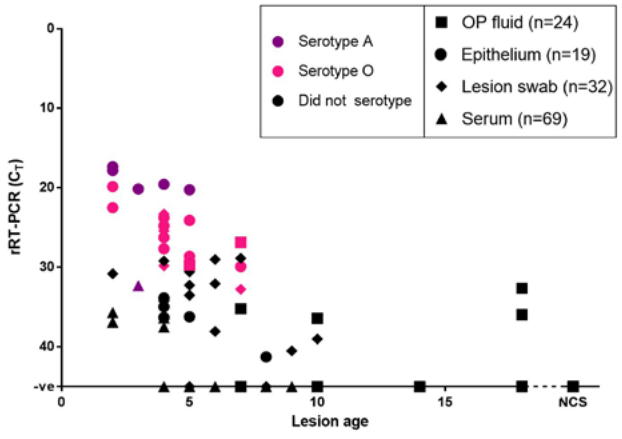

Figure 1: T-CORTM 8 field evaluation results for the 144 samples tested in East Africa. Shapes represents sample type. Colours represent T-CORTM 8 serotyping results. Samples were tested directly (no extraction) in duplicate

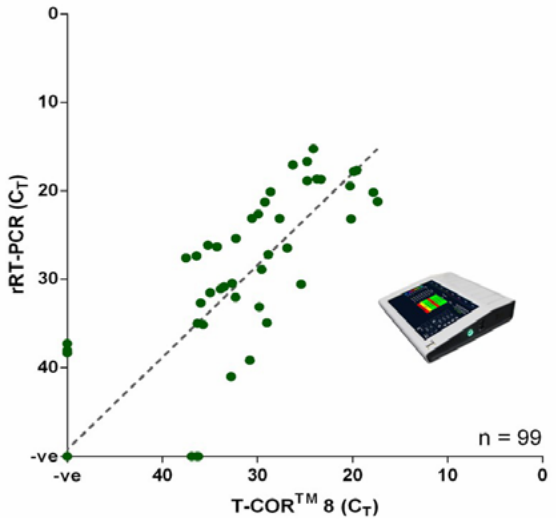

Figure 2: Comparison between laboratory based and field-based rRT-PCR

This parallel testing approach was also evaluated with selected samples in two villages in Northern Uganda during a 3-day outbreak investigation in 2016. In the third and last stage, further comparison of two diluents was performed by testing 46 blood samples in an austere lab setting in affected villages.
In another field study in Africa (Kenya, Tanzania, and Ethiopia), Tetracore's field deployable FMD detection assay was validated by utilizing epithelial tissue suspensions, serum, esophageal–pharyngeal (OP) fluid and oral swabs. The positive FMD samples from the study were then tested with a serotype specific field deployable Real-Time PCR assay, which covers the following serotypes O, A, Southern African Territories [SAT] 1 and 2. The positive results were confirmed by sequencing at the Pirbright Institute. Additionally, a robust sample preparation method for serum, esophageal-pharyngeal fluid and epithelial suspensions was developed to negate the need for RNA extraction prior to rRT-PCR.

Results

ASF - Pigs from two of the five suspected outbreak sites investigated were positive for ASFV using the ASF kit on the T-COR 8™. For blood diluted in PBS, inhibition was prevalent in 20-fold diluted and present in some 40-fold diluted samples. Archived samples were also tested and in total samples for twenty-two pigs were positive for ASFV out of sixty-nine tested.
These results matched those of the reference method in the lab at NADDEC with 100% correlation. Overall, the portable platform performed on par with the reference method.
FMD - The final rRT-PCR protocol and associated lyophilized reagents were field evaluated in three endemic settings (Kenya, Tanzania and Ethiopia), consistently detecting both clinical and subclinical FMD infections. Results of 145 samples tested in three test sites combined showed a 100% correlation between lab-based and field-based results.
The field studies in Africa showed that the reagents can be successfully lyophilized and stored under extreme conditions.

Discussion and Conclusion

Current delays in reporting from Centralized Reference Laboratories confound real-time decision making for animal control and disease containment efforts. These studies showed that confirmation of an outbreak can be performed on-site within 1.5-2 hrs, which would allow for real-time decisions to be made on animal control measures and containment efforts. The experience of performing the PCR assays in remote areas highlighted several factors that need to be carefully considered before deployment of portable technology: including biosafety issues, simplicity and effectiveness of sample preparation and turn-around time. Technical advances demonstrated are: dried assays stored at ambient temperatures; new chemistries that allow direct testing; and mobile, fieldable PCR instruments. Decentralized, on-site testing methods are undergoing validation efforts with OIE Reference Laboratories to enable the adoption of the technology by National Animal Disease Control authorities. The study demonstrated that the results of testing of samples at the point of care in remote field situations correlate very well with data generated from the same samples tested in OIE laboratories using reference methods.

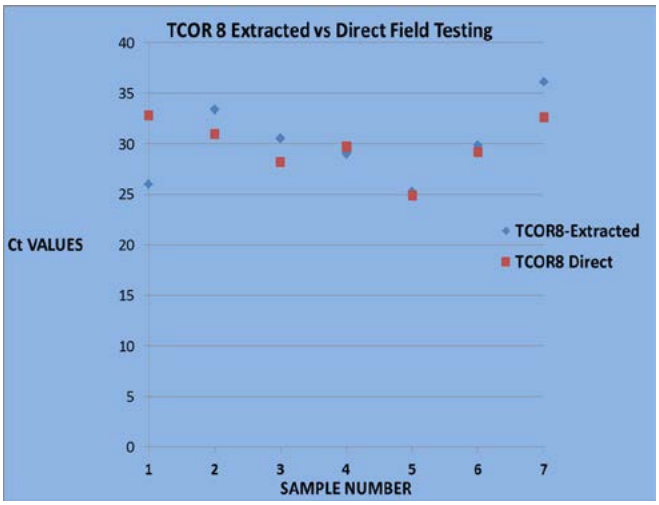

Figure 3: Comparison of direct and extracted whole blood samples