Genomics

Isolation of Bluetongue Virus (BTV) RNA on the MICROLAB® STAR

The Bluetongue virus, a double stranded RNA virus of the genus Orbivirus is a worldwide increasing threat for wild and domestic ruminants. BTV is transmitted by the bite of Culicoides spp. gnats and causes serious diseases in many livestocks. In 2006 BTV was initially detected in the Northern European region.

Commonly used, selective PCR tests have proven to be very sensitive and specific for BTV RNA. For a successful PCR reaction the viral RNA has to be purified from primary samples like whole blood. To handle the continuously growing number of samples for BTV surveillance testing, veterinary laboratories need fast and reliable solutions.

Equipment and Material

Equipment

- MICROLAB® STAR, Autoload, 8 channels, CO-RE Gripper
- 2x MICROLAB® BVS (Basic Vacuum Systems), incl. ME 4C Vario Membrane Pump and CVC 2000 Controller (Vacuubrand GmbH & Co. KG)
- 2x CAT SH10 shaker (CAT Ingenieurbüro, M. Zipper GmbH)

Labware

- NucleoSpin® 8/96 Virus Kit (MACHEREY-NAGEL GmbH & Co.KG) for Isolation of viral RNA and DNA
- SBS footprint 96 deepwell plates
- 120ml reagent reservoirs

Figure 1: *A MICROLAB® STAR, equipped with 8 individual 1000µl channels was used in this study.*

Decklayout

The deck is automatically loaded with reagents, disposable tips, elution plates and samples in deepwell plates on appropriate carriers, using the Autoload function of the robotic system. The MICROLAB® BVS and CAT SH10 shaker modules are mounted on two carriers which are fixed to the deck. The desired Deck Layout configuration enables a sample throughput of up to 192 samples/run.

Either 96-well NucleoSpin® Virus Binding plates or 8-well NucleoSpin® Virus Binding strips, mounted on special adapters (MACHEREY-NAGEL Column Holder A) can be used with the MICROLAB® BVS.

Application Software

The validated method was programmed using the MICROLAB® STAR Vector Software 4.1.

Protocol

Animal whole blood samples in deepwell plates are loaded to the deck. 75µl/well sample are transferred to the empty wells of a new deepwell plate and diluted 1:2 with PBS. Optional an additional usage of Proteinase K can be chosen. After adding lysis buffer RAV1 the samples are incubated, while mixing on a shaker which is programmed for 15 minutes at 70°C.

96% Ethanol is added to adjust the binding conditions and the samples are transferred to the NucleoSpin® Virus Binding Plate/Strips. A vacuum of -200mbar is applied by the MICROLAB® BVS for 5 minutes during the binding step and for 2 minutes during all subsequent washing steps with the 3 different washing solutions RAW, RAV3 and 96% Ethanol. The MN Wash Plate is used to eliminate cross-contamination during the binding and washing steps. After an efficient drying procedure for 10 minutes at -600mbar a variable volume of 75-200µl/well, which can be chosen by the user is added to the wells of the filter plate.

Purified viral RNA is efficiently eluted within a 2 minutes vacuum at -400mbar, ready to use in appropriate downstream applications.

Results

Reproducibility test

Viral RNA was purified from ruminant whole blood in 4 consecutive runs with 48 samples in each run, using

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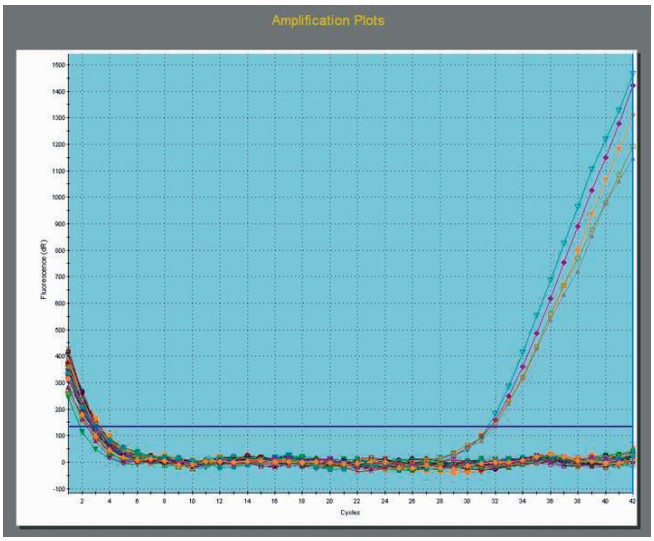

Figure 2: *All positive samples show a CT value of 30-32, while all negative samples do not show a signal even after 42 PCR cylces.*

NucleoSpin® Virus Binding 8-well strips. All samples were analyzed by a PAN-BTV-S5 RT-PCR[1]. An internal control system[2] was added to the mastermix and measured in parallel during the PCR. The CT-values of all detected BTV-positive samples show a low variety and thus the high reproducibility of results.

Sensitivity test

To check the sensitivity of the application a bovine, BTV-positive control was diluted 1:1, 1:10, 1:100, 1:1000 and 1:10000. Three replicates of each dilution, with negative samples in between were purified with the above mentioned method on the MICROLAB® Star. The same samples where purified following a validated, manual procedure.

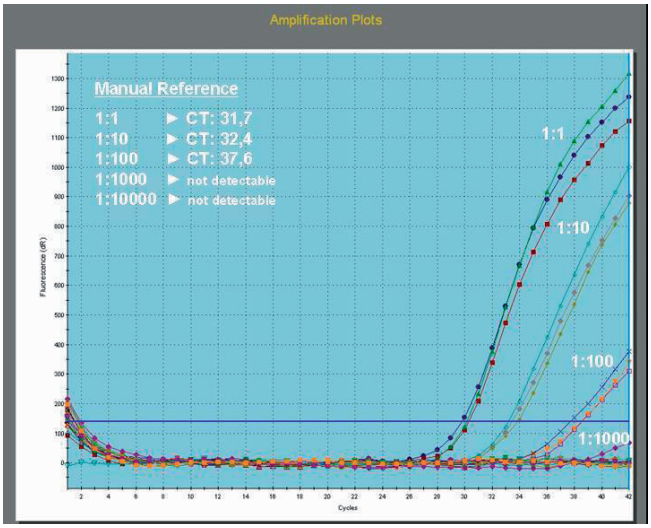

Figure 3: *All replicates of each dilution of the BTV-positive control sample are highly reproducible. The manual references show very similar CT-Values and the detection limit for the desired dilutions.*

Discussion

The fully automated NucleoSpin® 8/96 Virus Kit (MACHEREY-NAGEL) on the MICROLAB® Star offers a reliable solution for cross-contamination free high-throughput purification of a broad range of viral nucleic acids.

With the application variant for BTV-RNA purification, which is shown in this application note HAMILTON and MACHEREY-NAGEL have developed a proven system for surveillance testing in veterinary laboratories. The highly flexible MICROLAB® Star system can easily be adapted to further applications and kits from MACHEREY-NAGEL.

Features and Benefits

- Fully-automated walkaway purification of viral nucleic acids
- High sample throughput of up to 192 samples/run on two MICROLAB® BVS modules
- On deck heat lysis incubation without user intervention
- Complete tracking of sample information through the whole process
- Automation of additional applications like pooling, reaction setups, numerous purification methods

Acknowledgements

We would like to thank Dr. Irene Klingelhöfer and Dr. Astrid König, Landesuntersuchungsamt Rheinland-Pfalz, and Dr. Thomas Zinn of Macherey Nagel GmbH & Co. Kg for codeveloping this method and kindly providing the data used in this Application Note.

[1] **PAN-BTV-S5 RT-PCR** Developed by Dr. B. Hoffmann, Friedrich-Löffler-Institute-Insel-Riems based on Toussiant et al. (2007, J. Virol Methods 140, S. 115-123).

[2] Hoffmann et al. (2006, J. Virol Methods 136, S. 200-209).

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Lit. No. MR-0810-02/00 ©HAMILTON Bonaduz AG 08/12 Printed in Germany

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