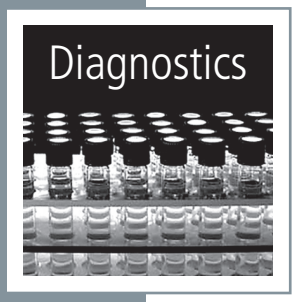

Avian influenza and BVD diagnostics on the MICROLAB® STAR

Surveillance testing for avian influenza, bovine virus diarrhoea is becoming an increasing challenge for veterinarian laboratories. Routine surveillance already reaches 3 million tests every year in European countries but reaches seasonal peaks that are particular challenging.

In addition to serological testing, nucleic acid based testing that investigates presence of viral RNA or DNA in samples is becoming a powerful, economical and fast alternative.

Since viral RNA is present long before viral protein can be detected, this method also closes the diagnostic gap between infection and outbreak of the disease.

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Equipment and Materials

Equipment

- MICROLAB® Star, Autoload, 8 pipetting channels
- BVS Vacuum Filtration System

Figure 1: *HAMILTON STAR used in this Application Note. The flexible workstation is equipped with an additional AGOWA box that is used for plasmid isolation in a different application.*

Software

All methods were programmed using the MICROLAB® Vector Software.

Labware

- SBS footprint 96 plates
- 1.5ml Eppendorf tubes
- 40ml reservoirs

Reagents

- MACHEREY-NAGEL NucleoSpin® 96 Virus kit for isolation of viral RNA or viral DNA

Protocol

Method for purification of viral RNA

In order to assess process safety, every second of 96 BVDV negative serum samples was spiked with a positive sample to obtain a checker board pattern of positive and negative samples.

After 10 minutes incubation with MACHEREY-NAGEL buffer RAV1 (lysis) at room temperature, Ethanol (96%) is added, mixed and transferred to the NucleoSpin® virus binding plate. Vacuum (-200mbar) is applied for 5 minutes and the plate is successively washed 4 times with different wash solutions according to the MACHEREY-NAGEL protocol.

After extensive drying (10 minutes) of the plates with vacuum (-600mbar) 100µl Elution buffer is added to the wells. The plate is then removed and RNA is collected by a 5 minute centrifugation. Using this protocol, 96 samples can be purified in less than 90 minutes.

Figure 2 shows the amplification plots of the 96 samples.

Results

To test for the presence of viral RNA in the samples, 1 µl of the eluate were used as template in a Real Time PCR. All 48 samples show a positive signal after 16 cycles of amplification, whereas the negative samples do not give a positive signal even after extensive amplification of 35 cycles.

HAMILTON

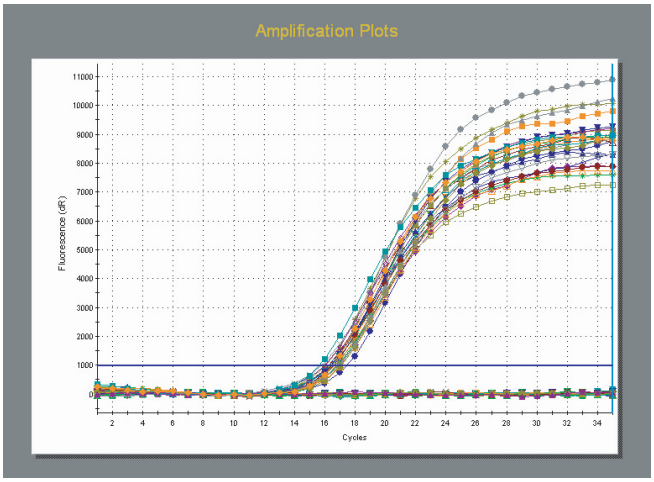

Figure 2: *Amplification plots of the checker board experiment. No cross contamination can be observed. The 48 positive samples show a signal starting at 17 PCR cycles, while the interspearsed negative samples do not give a signal even after 35 cylces of amplification.*

Dilution test for sensitivity

To assess sensitivity of the described test, plasma of BVDV positive specimen was diluted in BVDV negative plasma. Viral RNA was successively isolated using the above described protocol and the final eluate was tested in a Real Time PCR system. Figure 3 shows that BVDV RNA can be reliably detected in dilutions up to 1:3125 and are traceable in dilutions up to 1:15625. This result impressively emphasizes the diagnostic power of this automated BVDV test.

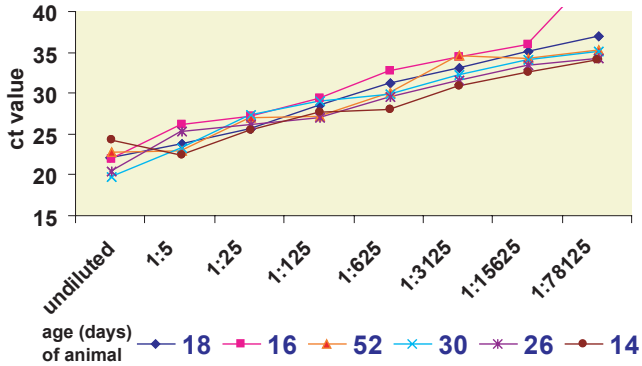

Figure 3: *CT-values for the tested dilutions. 6 differently aged samples were diluted from 1:5 to 1:78125. The graphic shows the CT-value (Threshold Cycle) for the different samples. Cut off was set to 35 cycles, allowing safe identification of positive samples in 1:3125 dilutions.*

Discussion

By using MACHEREY-NAGEL's NucleoSpin® 96 Virus kit for isolation of viral RNA or viral DNA on the MICROLAB® STAR, users get a fully automated RNA preparation system that offers ultimate process safety. This application note shows, that Monitored Air Displacement in combination with HAMILTON's CO-RE technology offers contamination free, fully automated BVD testing.

The NucleoSpin® 96 Virus kit has also been successfully used to isolate RNA from different samples, for example avian influenza virus RNA from infected birds or blue tongue virus from cattle blood and hence is a complete solution for the veterinarian laboratory.

Features and Benefits

- Flexibility of the software and hardware (automation of different applications on the same platform)
- Serological testing on the same system
- High level of precision and accuracy using the CORE pipetting technology
- Reliability of the obtained results

Acknowledgements

We would like to thank Dr. Wolfgang Gaede of the "Landesamt für Verbraucherschutz Sachsen-Anhalt" and Dr. Thomas Zinn from MACHEREY-NAGEL GmbH & Co. KG for performing the described experiments in the laboratories of the "Landesamt für Verbraucherschutz Sachsen-Anhalt".

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Lit. No. MR-0703-02/00 ©HAMILTON Bonaduz AG 07/03 Printed in Germany

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