

## Preparation of viral RNA for veterinary diagnostics

A versatile platform for automating sample preparation for testing virus-based diseases



### Abstract

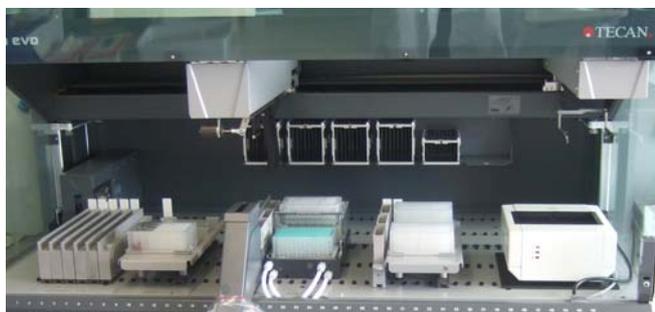
Bluetongue is an insect-borne disease caused by an orbivirus. It occurs primarily in sheep and cattle, and there is currently no effective treatment for the disease. The infection is caused by bites from midges of the genus *Culicoides*. The disease is normally found in Mediterranean regions, because this insect prefers the warmer parts of Europe. However, bluetongue was discovered in the Netherlands for the first time in August 2006 and this outbreak spread to the North Rhine-Westphalia region. The bluetongue virus belongs to serotype 8, which normally occurs in the sub-Saharan and in Central and South America. The disease is characterized by changes to the mucous linings of the mouth and nose, and the coronary band of the foot. The disease is potentially devastating to farmers and a country's economy, so it is critical that it can be strictly monitored using rapid, sensitive and reliable detection methods.

### Introduction

Laboratory workers at the Chemical and Veterinary Institute (Chemisches und Veterinäruntersuchungsamt Rhein-Ruhr-Wupper, CVUA-RRW) in Krefeld, Germany, provide a number of veterinary diagnostic tests on animal samples. This government institution is based in the administrative district of North Rhine-Westphalia, and has departments for pathology, serology, bacteriology, virology and molecular biology for the diagnosis of animal diseases. It also investigates food and feed quality, and performs pathogen detection in foodstuff. Dr Claudia Bunzenthel from the molecular biology department and her co-workers are also involved in diagnosing other viruses, such as avian influenza virus and classical swine fever virus, in animal samples from a variety of sources.

## Diagnostics prerequisites

One of the challenges faced by the Institute is the unpredictable number and type of samples that arrive each day, at different times. It is essential that the laboratory is able to respond rapidly to sudden outbreaks of diseases and continues to deliver test results on time, regardless of the sample load. The testing methods must also be consistent and reliable. The automated sample preparation platform Freedom EVO<sup>®</sup> has recently been installed at the molecular biology department in order to meet these demands (Fig 1).



**Fig 1** Freedom EVO platform configuration

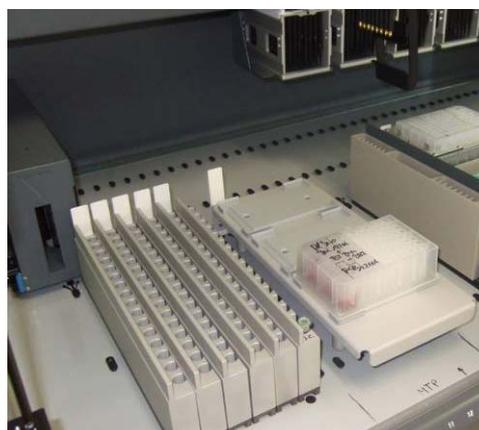
## Automated platform for preparing viral RNA

The Freedom EVO 150 workstation (Fig 1) is equipped with a liquid handling (LiHa) arm for eight disposable tips with a lower DiTi eject option (see Fig 3 and Fig 4) and robotic manipulator (RoMa) arm, all controlled by the Freedom EVOware<sup>®</sup> software. Additional hardware includes an integrated Te-VacS<sup>™</sup> vacuum separation module and a heated incubator for microplates [Biostep GmbH, Jahnsdorf, Germany] (see Fig 5). The workstation automates extraction of viral RNA using the NucleoSpin<sup>®</sup> 96 Virus kit from Macherey-Nagel GmbH & Co. KG, Düren, Germany.

## Testing procedures

Samples to be tested for bluetongue virus usually arrive at the CVUA-RRW in the form of blood samples. The samples undergo two different testing procedures to confirm the results: ELISAs are performed in the serology department and PCR-based assays are carried out in the molecular biology department. The molecular biology department's Freedom EVO 150 automated platform from Tecan isolates and purifies RNA from the samples (Figs 1-5).

Subsequent detection of the specific RNA segment of the virus is tested by reverse transcription and real-time PCR. The platform can be easily adapted for RNA preparations of other viruses and is fully compatible with a variety of labware, including Eppendorf<sup>®</sup> tubes, deep-well plates and blood tubes (see Fig 2); it is currently used to detect avian influenza, bluetongue and classical swine fever viruses.



**Fig 2** Flexibility of the Freedom EVO platform: sample loading section. Depending on the sample preparation required, serum can be pre-diluted in a deep-well plate prepared in the serology department. As shown on the image, starting samples might be prepared in Eppendorf tubes. Alternatively, regular blood tubes can also be loaded on the worktable using the dedicated rack (not shown on the image).



**Fig 3** Safety: the lower DiTi eject option enables smooth dropping of used disposable tips directly into the waste. The risk of splashing remaining liquid droplets while dropping the tips from the tip adapters is avoided.

## Sample preparation

The RNA preparation procedure described below is the protocol used for testing bluetongue disease at the CVUA-RRW; the protocol comes from the Friedrich-Loeffler Institute in Insel Riems, the National Reference Laboratory, which is also equipped with a Tecan instrument.

Whole blood samples (with added EDTA) arrive at the CVUA-RRW's serology department where they are labeled with barcodes and entered into the laboratory information system (LIS). The serology department carries out ELISAs on all the samples to detect viral antibodies, using automated ELISA processor from Tecan. In addition, each sample is distributed into a deep-well plate and diluted (1:1) with PBS. These samples are delivered to the molecular biology department for RNA preparation using the Freedom EVO 150 workstation followed by qualitative PCR assays.



**Fig 4** Additional storage places for consumables save space on the worktable. The flexible number and size of shelves allow initial loading of necessary consumables such as DiTi box carriers, microplates and deep-well plates to reduce operator actions during processing.

## Workflow

The automated viral RNA preparation is performed in 96-well plates, allowing 86 samples to be tested together with negative and positive controls. Every eleventh sample serves as an internal negative control to check for cross-contamination and, as long as these remain negative, the process is considered to be sufficiently free of contamination. Processing of the entire 96-well plate takes 2 hours and 15 minutes.

The viral RNA extraction procedure is carried out using the NucleoSpin® 96 Virus kit, according to the manufacturer's manual [Macherey-Nagel] and the Friedrich-Loeffler Institute's protocol, with minor modifications as follows: the sample lysis is performed directly on the Freedom EVO's worktable (see Fig 5), an extra ethanol wash step followed by drying for 20 minutes allows better recovery and quality of RNA. Additionally, the final elution of RNA is done by centrifugation in order to obtain higher concentrated viral RNA. RNA preparations are used immediately for qualitative real-time PCR, according to the protocol provided by the Friedrich-Loeffler Institute.



**Fig 5** Complete process automation. The diluted serum is pipetted into the deep-well plate and lysis buffer is added. The lysis can be carried out on the worktable at room temperature or in a heated incubator. Here a third party microplate incubator from Biostep, GmbH (Jahnsdorf, Germany) was integrated to reduce manual sample handling and to increase the workflow efficiency.

## Conclusions

The automated Freedom EVO workstation for viral RNA preparation for real-time PCR at the CVUA-RRW molecular biology laboratory provides a reliable, consistent and fast platform to monitor the spread of potentially devastating diseases in livestock. Its flexibility makes it easy to switch between different disease tests, allowing the laboratory to respond rapidly to changing sample loads on any given day. The workstation is suitable for integrating with a variety of additional devices, including from third parties, making it easy to adapt for future application requirements.

## Acknowledgements

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