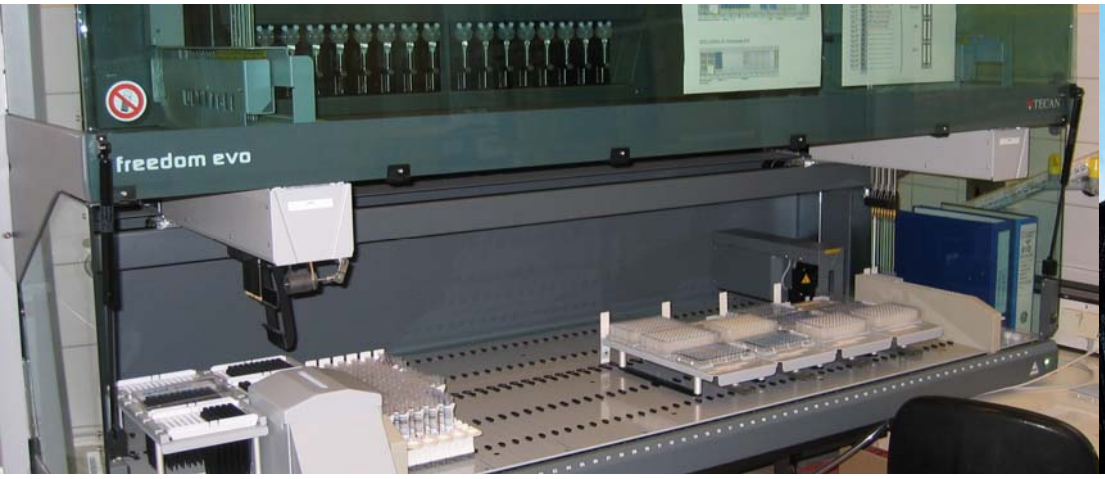
Sample preparation and archiving for veterinary diagnostics

Process automation simplifies workflows and enhances throughput



Introduction

The Landesuntersuchungsamt Rheinland-Pfalz (State Veterinary Laboratory of Rhineland-Palatinate) in Koblenz, Germany, is dedicated to ensuring human as well as animal health by executing measures to prevent and fight infectious diseases of man and animals.

In 1999, the Landesuntersuchungsamt (LUA) has established an extensive Bovine Herpesvirus 1 (BHV-1) eradication program. The program combines vaccination with test and slaughter strategies.

Recently, the eradication of Mucosal Disease (MD), caused by Bovine Viral Diarrhea Virus (BVDV), and the monitoring of Bluetongue disease have also become the focus of the LUA, due to the economic impact of these diseases.

Abstract

In order to increase reliability and throughput of its diagnostic services, the LUA has automated sample preparation for ELISA and PCR testing, including the preparation of archive plates in the same step, on a Tecan Freedom EVO® 200 workstation in 2008.

With this solution, the LUA can now easily process 3,000 bovine samples per day in peak season with multiple diagnostic assays according to its diagnostic programs. An added bonus is the reduced sample storage space and simplified hit-picking e.g. for re-tests, thanks to the creation of sample archive plates. The automated solution ensures full sample traceability from the beginning of the process.

Sample preparation for BHV-1, MD and Bluetongue diagnostics

The LUA performs a variety of diagnostic tests for each bovine sample received.

For example, the BHV-1 program consists of two different ELISA tests to discriminate between vaccinated and actively infected animals. Two different ELISA tests can also be performed for MD testing – the detection of antibodies to the virus as well as the detection of the virus itself. In contrast, the Bluetongue testing necessitates sample preparation for molecular diagnostics to detect the virus, in addition to ELISA testing for antibodies. Therefore, samples intended for Bluetongue testing require disposable pipette tips (DiTis) for sample preparation, while samples intended only for BHV-1 and/or BVDV tests can be prepared with fixed tips, combined with tip washing steps.

Furthermore, not all samples are tested with each of the available tests, and assay plates may be created over multiple sample loading runs, resulting in complex pipetting schemes that would be very difficult to track manually. The position of each sample on the microtiter or deep-well plates is administered in an Oracle® database. Automation of the sample preparation process on the Tecan Freedom EVO platform ensures process safety and the required sample throughput.

ELISA preparation, sample splitting and sample archiving on one platform

The Freedom EVO 200 is equipped with two independent liquid handling arms. The right arm is equipped with eight fixed tips and the left liquid handling arm is equipped with eight DiTis. While the right arm is used for processing samples that only need to be processed by ELISA testing, the left arm is used for samples that may need to be processed by PCR testing.

Fig. 1. Freedom EVO configuration. Two liquid handling arms for alternative use of fixed or disposable tips. The arm for DiTis is equipped with the lower DiTi eject option to minimize the risk of cross-contamination.

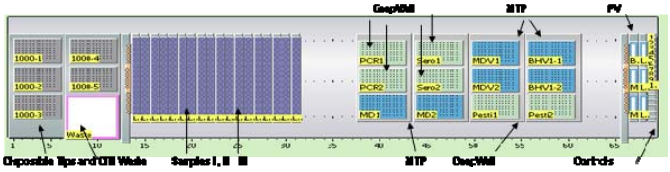


Fig. 2. Worktable layout. The worktable contains a DiTi loading and waste station on the left. Next to it are 18 grids dedicated to sample loading, allowing for three sets of 96 samples to be loaded in one step.

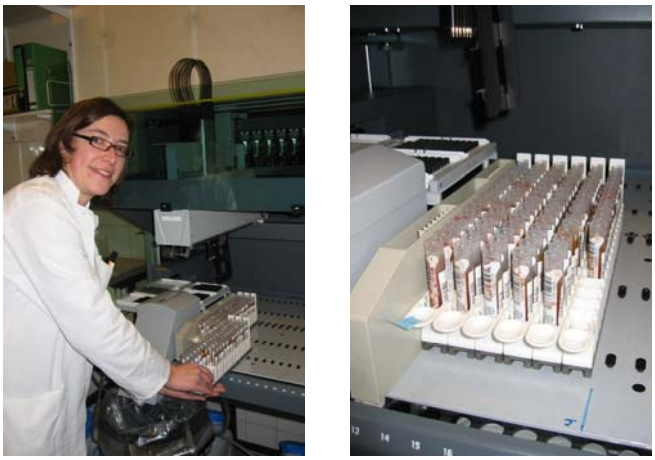


Fig. 3. Loading area. Liquid level and clot detection ensures safe sample transfer.

Fig. 4. Sample distribution. The destination area has space for 12 microtiter or deep-well plates, allowing the pipetting of all of the required range of diagnostics assays as well as preparing the sample archive plates.

The required amount of sample is transferred directly from the individual tube to the required number of assay and archive plates. Capacitive liquid level detection and error handling for detected clots ensure safe processing of the samples. Reagent troughs and racks contain the required ELISA buffers for predilution and ELISA controls.

Intensive washing of fixed tips using CARRY STOP 'low ionic' (LvL 'System Clean', Laborbedarf von Lueder, www.lvl-laborbedarf.com) as system liquid has been validated by the LUA, effectively preventing carryover and allowing the use of fixed pipette tips for all ELISA tests.

Process security – enhanced reliability with sample tracking

Sample identification is performed with a PosID™ unit via individual barcodes on the blood tubes and plate barcodes on the destination plates. The worklist for the individual samples is received from the LIMS (LADIA, BALVI GmbH) and the sample information created during the automated process is returned to the LIMS, so that full sample traceability is ensured. Tailor-made software add-ons allow ELISA plates to be filled over multiple sample loading runs and still track the samples reliably. User management and audit trails add to the process security.

Therefore, samples can be automatically processed according to the diagnostic programs of the LUA, following even complex diagnostic schemes.

Validation of the automated set-up

The full automated process has been validated by the LUA, both for the diagnostic performance of the ELISA tests in comparison to manual processing, as well as the reliability of the sample tracking.

Conclusions

Tecan's sample preparation and archiving solution allows flexible sample preparation and sample archiving in a high throughput scenario, preparing 3,000 samples per day for multiple diagnostic assays corresponding to 30-35 assay plates per diagnostic assay.

The flexibility of the configuration makes it easy to switch between different testing schemes and sample loads, allowing the laboratory to respond rapidly to changing needs.

Sample archiving

The LUA archives samples for a period of four weeks, to enable re-testing or additional testing. Hence, in the automated process, each sample is additionally archived on a deep well plate (450 µl per sample) during the assay preparation step. Consequently, storage space for sample archiving is significantly reduced compared to the archiving of individual blood tubes.

Fig. 5. Sample archiving. Deep-well plates can be stacked for archiving, significantly reducing the required storage space.

Hit-picking is also simplified, as deep-well plates can be loaded directly onto the ELISA processor, a Tecan Genesis Freedom® 200 platform.

Acknowledgements

We thank Cornelia Arft at the Landesuntersuchungsamt Rheinland-Pfalz, Institut für Tierseuchendiagnostik (Institute for Animal Disease Diagnostics), in Koblenz, Germany, for generously sharing her expertise in routine diagnostics using the Tecan Freedom EVO platform.

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396 028 V1.0, 07-2009