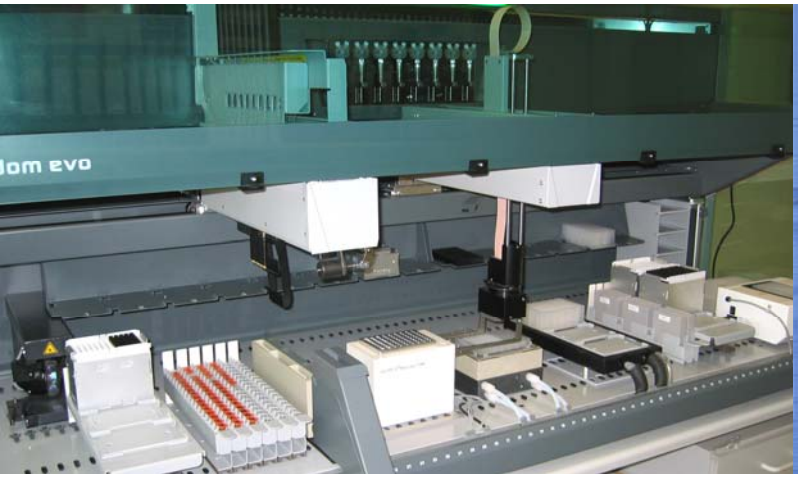
Avian influenza

Automated NAT for veterinarian diagnostics



Abstract

Avian influenza is a bird disease caused by the highly pathogenic influenza virus. Influenza viruses are inherently unstable and, as they lack a genetic proof-reading mechanism, small errors that occur when the virus copies itself go undetected and uncorrected. Specific mutations and evolution in influenza viruses cannot be predicted, making it difficult if not impossible to know if or when a virus such as H5N1 might acquire the properties needed to spread easily and sustainably among humans. This difficulty is increased by the present lack of understanding which specific mutations would lead to increased transmissibility of the virus among humans (www.who.int). Here we describe an automated method for nucleic acid extraction and avian influenza diagnosis.

Introduction

The Friedrich-Loeffler-Institut / *Federal Research Institute for Animal Health*, the national reference center in Germany, announced in February 2006 that the avian influenza (highly pathogenic influenza A/H5N1 type) was found in several wild birds on the German island Rügen. The number of positively tested wild birds on February 22 was 103, and other cases were detected in single individuals in Italy, Greece, Slovenia and Austria in early February (www.rki.de). The major economic risk is infection of domestic poultry, however transmission of the virus to humans cannot be excluded. Human infections were reported so far outside South-East Asia from Turkey, Iraq and Africa.

Diagnostics

Safe and proper diagnosis of avian influenza requires virus isolation and identification, including determination of the virus pathogenicity. This diagnosis is done in the national reference laboratories and, in Germany, this is the Federal Research Institute for Animal Health (Island Riems). The directive 92/40/EEC defines the diagnostic procedure for avian influenza as follows:
The sample material is organic material from brain and lung, as well as cloacal and pharyngeal swabs.
Identification and characterization of viral RNA is done by specific real-time RT-PCR assays for Influenza A Virus and the subtypes H5, H7 and N1. The high pathogenic subtypes H5 and H7 are confirmed by nucleic acid sequencing of a defined region of the hemagglutinin gene.

Sample preparation

Lysis of the samples is done with the standard lysis buffer of the "NucleoSpin® Viral RNA" kit (Macherey-Nagel) in the L4 security area of the laboratory. Samples are stored in 1.5ml Eppendorf tubes.

Automated nucleic acid extraction

Nucleic acids are extracted from samples using a Tecan Freedom EVO® 200 Workstation. Its layout and configuration are designed to minimize the risk of cross contamination and to maximize the yield of nucleic acid to meet the assay's performance requirements, ie that dilutions of 10^{-6} to 10^{-7} are still detectable.

The Freedom EVO 200 liquid handling workstation is equipped with an 8 channel liquid handling arm with 8 disposable tip adapters, a barcode reader for positive identification (PosID™) and a robotic manipulator arm.

Samples are positioned in the Eppendorf sample carrier (Fig 1); the red tubes contain material from the L4 safety area of the lab.

Figure 1: Sample area of the nucleic acid extraction workstation.

Sample pipetting is done with disposable tips placed on a standard microplate carrier close to the samples. The disposable tip racks are positioned in transportable Diti boxes and can be reloaded with the robotic manipulator arm during the process; a lower Diti eject option reduces contamination. Disposable tips are used to pipette from sample tubes into the deep well plates on the cooled carrier (see Fig 3).

The Macherey-Nagel NucleoSpin Viral RNA Kit is used for nucleic acid extraction, with minor modifications and an additional ethanol washing step.

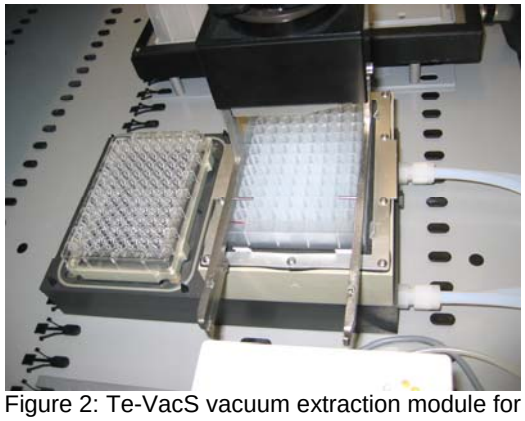


Figure 2: Te-VacS vacuum extraction module for use of M-N NucleoSpin Viral RNA Kit.

The Te-VacS™ vacuum extraction module with vacuum block C and the spacers 3 and 6 is placed on the worktable (Fig 2). The Te-VacS is used with a special solid phase extraction protection plate that prevents cross contamination during extraction, when the vacuum is applied to the M-N extraction block. A cooled carrier (water circulator under the instrument) is used for temperature control of the deep well plates. On the right hand side of the cooled carrier are additional disposable tips, which are used only for transfer steps from deep well plates to the vacuum station and for the reagent addition and washing steps. With this disposable tip layout, it is assured that the liquid handling arm will not move across clean areas with used tips, risking cross contamination.

Additional modules on the system include two shelves and a hotel for storage of consumables (disposable tips and deep well plates), and two trough racks with three 100 ml reagent troughs each. A magnetic bead separation module and a heating shaker are available on the worktable, providing the flexibility for magnetic bead separation kits to also be used on this system.

Conclusion

Tecan's nucleic acid extraction workstation allows the lab to react flexibly to changing sample entries. This set-up allows 96 samples to be extracted in parallel in 1.5 hours and four to six disposable tip racks (96 tips each) are necessary for one batch. A wide range of vacuum extraction and magnetic bead separation kits can also be used with this set-up.

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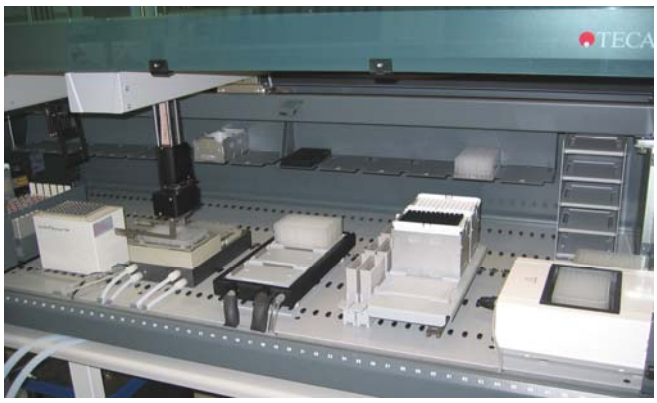

Figure 3: Worktable layout:
Sample area, magnetic bead separator, Te-VacS vacuum station, cooled carrier, reagent area, disposable tip area, heating block (from left to right).

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