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**Section** **Fields (of activity)**

**Style sheet (Bitte die Vorlage direkt mit Ihrem Text überschreiben)**

# Development of a procedure for the diagnosis of outbreaks of emerging, mutated or novel viral pathogens

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**Key words**
Outbreak diagnostics, emerging viruses, virome analysis, next generation sequencing, hepatitis E virus

**Aim of the study**
The overall aims of the project were: (I) to develop easy to follow protocols for the preparation of different sample materials and species, and (II) provide the optimal setup for the sequencing process and data analysis. Using next generation sequencing (NGS) technology, identification and discovery of viruses is possible without the need to target a specific infectious agent – all viruses present in a sample are sequenced. The established procedure should be tailored to the veterinary diagnostic field regarding flexibility of sample material and animal species with the aim to be applicable in cases of disease outbreaks where standard routine diagnostics such as ELISA or PCR fail to identify the infective agent.

**Material and methods**
In order to establish a NGS protocol for virus detection in host samples, several points have to be attained. Parameters such as filter pore size, nuclease treatment, extraction method and amplification cycle numbers were tested and compared using three types of samples from pigs (lung tissue, feces and nasal swabs) that were spiked with several known RNA and DNA viruses. Effectiveness of each method regarding removal of host nucleic acid and preservation of viral genomes (=virus enrichment) was measured by specific real-time (RT)-PCR and DNA and RNA concentration measurements on the Qubit Fluorometer. Finally, the most promising setups for sample preparation were compared in a first NGS run. The spiked pig samples were divided into four groups depending on the preparation method, DNA sequencing libraries prepared and subsequently sequenced in NextSeq Series Desktop Sequencing System at the Functional Genomics Center Zurich (FGCZ). Based on the results of the initial comparative sequencing run, the most promising enrichment protocol was tested, using different “real’’ sample materials collected at different clinics of the Tierspital (UZH) from several species (pig, horse, cow, sheep, bison, dog) with or without known virus infection. In addition, a human stool sample and raw pork sausage containing pig liver that were shown to be Hepatitis E virus (HEV) positive by a commercial RT-qPCR were essayed. To test the application of the protocol on collective samples for disease monitoring in pigs, individual faecal and respiratory samples of a group of animals as well as pooled faeces from floor and saliva from chewing rope were compared.

**Results and significance**
Analysis of the sequencing data showed that enrichment for virus particles such as filtration, centrifugation and nuclease treatment as well as amplification have a significant influence on the number of viral genomic sequences detected and has to be applied. Sequencing data from samples collected at different clinics showed that the virome protocol worked well with different types of sample material such as faeces, nasal swabs, different organs and blood from various species and NGS data reflected nicely the results of previously performed specific (RT)-PCR results. In samples with high viral load it was easily possible to obtain the full-length genome sequences, such as for swine influenza virus (H1N1) from a nasal swab and porcine circovirus 2 (PCV-2) and

porcine parvovirus (PPV) from porcine tissue. In addition, the full-length sequence of Hepatitis E virus from human stool and pork sausage was possible [1]. In other cases, where specific testing was negative, new or unexpected viruses where detected such as Torque-Teno sus virus in the brain of a pig with neurological signs. Comparison of NGS data from individual animals and collective samples demonstrated that the combination of pooled faeces from the pen floor and saliva from the chewing rope represented the faecal and respiratory viral spectrum best and might hence be promising for virus surveillance in pig herds.

**Publications, posters and presentations**

Jakub Kubacki, Münchenwiler meeting for Virology students, Münchenwiler (BE) 27.-28.10.2016 and 02.-03.11.2017 Oral presentation

Jakub Kubacki, Virology–colloquium, Institute of Virology, Vetsuisse faculty, University of Zurich 12.05.2017 Oral presentation

Kubacki J, Fraefel C, Jermini M, Giannini P, Martinetti G, Ripellino P, Bernasconi E, Sidler X, Stephan R, Bachofen C. 2017. Complete genome sequences of two Swiss hepatitis E virus isolates from human stool and raw pork sausage. Genome Announc 5:e00888-17. https://doi.org/10.1128/genomeA.00888-17

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