Results: IDS iSYs VCA IgM: Sens. during PrI = 97.5% (95%CI:92.6–99.4). Spe. during AI and PastI = 97,4% (95%CI:92.6–99). Spec with interfering sera from other viral primary infections =88.1% (95%CI:79.2–93.5).

IDS iSYS VCA IgG: Sens. during PrI and PastI = 71.2% (95%CI:62.3–78.7) and 98.2 (95%IC 95.4–99.3) respectively. Spe. during AI = 94.4% (95% CI:92.2–97.6)

IDS iSYS EBNA IgG: Sens. during PastI = 97.3 (95%CI 93.6–98.4). Spe. during AI and PrI = 98.9 (95%CI:93.9–99.8) and 90.1% (95%CI: 83.4–94.4) respectively.

Agreement for the determination of EBV status between IDS iSYS system and reference test = 84. 4 (95%CI:80.6–87.6).

Conclusion: The IDS- iSYS is a real "Walk-Away System", easy to use, fast and secure and appears to offer new reliable commercial immunoassays for the determination of EBV serological status.

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Evaluation of process control "Mengo Virus" using 3 RNA extraction kits and 2 different types of methods of one-step real-time RT-PCR in Donax sp (Palabritas)

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There are many problems to extract viral genetic material that is contaminating bivalve molluscs, this is due to bivalve molluscs, specifically the hepatopancreas, have many inhibitors to PCR, for that reason extraction methods should consider a virus process control used to measure the efficiency of extraction. In the market there are many commercial kits for extracting the nucleic acid of the virus and to perform One-step real-time RT-PCR, but most are not tested on bivalve mollusks, for this reason, the aim to evaluate the efficiency of extraction process control (Mengo virus) using 3 different RNA extraction kits and 2 treatments of One-Step real time RT-PCR. They were used to study 30 samples of hepatopancreas of *Donax sp.* (Palabritas) to which was added 10 μl of Mengo virus at a concentration of 1.6×10^4 particles/μl, and processing of the sample according to the ISO/TS 15216-2:2013, then RNA was extracted of each sample with the kits: 1. BioMerieux NucliSENS® system (bioMerieux SA, France), 2. PureLink™ RNA Mini Kit (Ambion-Life Technologies™, USA) and 3. Hugh Pure RNA Tissue Kit (Roche SA, Germany). Once extracted RNA was performed one-step real-time RT-PCR using the following treatments: 1. the UltraSense One-step qRT-PCR (Invitrogen, USA) kit according to the ISO/TS 15216-2:2013 was used and 2. Kit Mengovirus@ceeramTools™ (CEERAM, France) according to the manufacturer's specifications was used. It turned out that the measuring efficiency of the extraction process control (Mengo virus) the best method of extraction was BioMerieux NucliSENS® system with an efficiency 10 times greater than the second; and with respect to matters related to Kits One-step real-time RT-PCR it can be concluded that treatment 1 kit UltraSense One-step qRT-PCR has an efficiency of 32% over the Mengovirus@ceeramTools™ Kit.

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Ion Torrent next generation sequencing for accurate genotyping and detection of resistance associated variants in HCV and HIV

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Background: Detection of resistance-associated mutations is well established in HIV ART (as DRMs) and is increasingly used in HCV patients selected for treatment (as RAVs) with direct acting antiviral agents (DAAs). Both for DAA treatment and conventional interferon-based therapy accurate determination of HCV genotypes (GTs) is essential. Sanger sequencing has recognized limitations in sensitivity and turn around time. NGS provides excellent accuracy, speed and sensitivity enabling detection of rare mutants, HCV subtypes as well as mixed infections.

Objectives: To develop improved detection of clinically relevant viral mutations using ion torrent based NGS in an automated workflow.

Materials and methods: We have used NGS in combination with workflow automation on a newly developed platform based on the emotion 5075 system (Eppendorf, Germany) consisting of a continuous robotic process starting with sample extraction and RT-PCR followed by automated library preparation, Ion Torrent deep sequencing and direct online data analysis to determine HCV genotypes and RAVs as well as DRMs in HIV. We have employed target sequences from the HCV NS3, NS5A and NS5B regions. For HIV sequences in reverse transcriptase, protease and integrase were selected for NGS.

Results: We are reporting results from an evaluation study conducted on >200 HCV sera comparing HCV genotyping with line probe assay. Two cases of mixed GT infections were detected. Confirmation of discrepant results between NGS and line probing by Sanger sequencing indicated 100% accurate GTs by NGS whereas in several cases line probe results would have led to selection of suboptimal therapy regimens. In an HIV pilot study ($n = 112$ patients), comparing NGS results to TruGene sequencing the *Sentosa* SQ HIV Genotyping Assay detected 100% (199/199) of all mutations in the protease gene and more that 98% mutations (427/435) in the reverse transcriptase gene.

Conclusions: Given the crucial role of accurate sequencing analysis in HCV and HIV treatment management, workflow automated NGS appears as a highly reliable tool for differentiating HCV GTs and RAVs, which can help to prevent diagnostic errors potentially leading to suboptimal treatment.

Considering the pivotal role of DRMs in HIV patients under HAART the *Sentosa* SQ HIV Genotyping workflow appears as a valuable new tool for detecting clinically relevant HIV variants. Given its high sensitivity compared to Sanger based systems and the

comparatively short turnaround time of two days the workflow offers relevant improvements in HIV DRM detection.

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Mass spectrometry of influenza virus using clinically available MALDI-TOF platform

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Viral respiratory disease caused by influenza viruses has an important medical, epidemiological and economic impact on global population. Ideal screening assays for influenza viruses directly from clinical specimens must be not only of high sensitivity and specify, but also must have short turn-around times (less than two hours). In many laboratories, screening for influenza virus directly from clinical specimens is based on express Direct Fluorescent Antibody Assay, which is quite specific, but not very sensitive, when compared to RT-PCR (reverse transcriptase PCR). On the other hand, nucleic acid amplification assays, such as RT-PCR, are not characterized by quick turn-around times. Little is known if mass spectrometry technology may be used as an alternative screening approach for influenza virus identification directly from clinical samples.

The main objective of our study was to analyse mass spectra for influenza virus identification using a mass spectrometer, which is routinely used in our Clinical Microbiology Diagnostic Laboratory for bacterial or fungal identification. In this preliminary study, we used cell culture or egg-amplified influenza viruses as well as their corresponding recombinant neuraminidase (NA) and hemagglutinin (HA) proteins. Mass spectra were generated using clinically available Biomerieux Vitek® MS MALDI-TOF mass spectrometer. All proteins and whole viruses were pre-treated by lysis solution, sonication, boiling and microwaving. Influenza viruses and their corresponding HA and NA proteins were either directly applied on MALDI-TOF target or a minimal pre-treatment with either formic acid extraction and/or short trypsin digestion was used prior to target application of samples. Overall, the turn-around time for specimens was from less than an hour (without trypsin digestion) to three hours (with trypsin digestion). Mass spectra for H1N1, H3N2 and B influenza viruses as well as their corresponding HA and NA were analysed. We used VENN diagrams to manually analyze spectra of HA and NA and the corresponding whole virus in order to identify potential peak (m/z) candidates for the influenza virus identification by MS MALDI-TOF. On average, three to five peak candidates were identified for each influenza virus based on mass spectra analysis of HA, NA and entire influenza virus mass spectrometry.

Engineering and implementation costs for a mass spectrometer, which may be used in a Clinical Microbiology setting and yet possess a resolution capacity comparable to that of research use only platforms, is quite lengthy and financially demanding procedure. In this preliminary study we investigated whether already clinically available mass spectrometer may be used for influenza virus identification. We obtained a number of peak candidates, which may be used for a peak database creation in the future experiments. In the future, we are planning to identify influenza viruses directly from virus-spiked clinical specimens as well as from clinical specimens derived from symptomatic patients.

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Increasing blood safety by diagnosing Zika, Chikungunya and Dengue in times of massive outbreaks

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The Aedes trio (Dengue, Chikungunya and Zika) are arthropodborne viruses that are transmitted by mosquitos of different Aedes species (*Aedes aegypti, Aedes albopictus*). They have been reported in Africa, the Americas, Asia and the Pacific Islands. Dengue virus is a flavivirus, closely related to Zika whereas Chikungunya belongs to the alphaviruses. Dengue shares some clinical signs with Chikungunya and Zika and they can be misdiagnosed in areas where these arboviruses are common. As Dengue infection may cause a rash that could be confused with other diseases such as Chikungunya, Zika and measles, these other diseases do need to be ruled out. Diagnosis of Zika will first and foremost be by exclusion of other diseases such as Chikungunya and Dengue, based on symptoms and travel history. It is known that these diseases can also be transmitted by blood transfusion. Since a great proportion of infected persons are asymptomatic special care has to be taken in respect to blood safety.

Surveillance and testing algorithm for these three co-circulating arboviruses are needed since they show high impact on the socio economic burden in endemic countries. WHO proposed very recently a testing guidance for laboratory detection and diagnosis of these diseases. On 1 February 2016 the WHO declared a Public Health Emergency of International Concern (PHEIC) regarding a recent cluster of microcephaly cases and other neurological disorders and the possible association of these illnesses with Zika virus infections. The WHO recommended efforts towards improved surveillance of Zika virus which is only possible with an accurate diagnostic system for Dengue and Chikungunya as well.

Here we show how the BEP® III and BEP 2000 Advance® systems of Siemens Healthcare GmbH in combination with the Novagnost® ELISA assays can help in the management of outbreaks, proper diagnosis of individuals and surveillance of populations at risk.

The combination of highly sensitive and specific ELISA assays (Dengue IgG sensitivity >95%/specificity >95%; Dengue IgM sensitivity 82,3%/specificity >95%; Chikungunya IgG sensitivity >98.6%/specificity 100%; Chikungunya IgM sensitivity >98.8%/specificity 100%/Zika IgM sensitivity >100%/specificity 98.2%) fulfills the criteria of the WHO testing guidance for high throughput screening of these diseases and therefore seems to be an excellent tool for surveillance of blood products.

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