REF 428

New approaches for standardization and validation of quantitative qRT PCR assays for quantitation of yellow fever on clinical samples with high quality parameters

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The development and production of viral vaccines involves several steps that need the monitoring of viral load throughout the process (antigen production, purification, inactivation). Currently, these steps are monitored by plaque lysis titration assay, whose results take 7 10 days to come out. With the advent of real time RT PCR, we have a faster approach available to this issue. In this context, the development, standardization and validation of a technique to quickly and efficiently quantify the yellow fever (YF) virus in the aforementioned stages is extremely important. To accomplish that, we constructed a plasmidial standard curve and validation parameters were evaluated. Furthermore, we defined the limits of detection and quantification of the test. To ensure high quality, internal controls were established in order to avoid false negative results. The statistical analysis revealed an excellent correlation between the results obtained in RNA copies/mL quantified by qRT PCR and the viral titer calculated by lysis plaques tests (R=0.96). In addition, a correlation factor for conversion of the real time PCR data to plaque assays was generated. The results analysis showed that the validation experiments sufficed all parameters defined by the quality control sector. The technique herein standardized proved to be effective for determining YF viral load both in vivo and in vitro, thus becoming a very important tool in all projects developed in LATEV, and may eventually be adopted as the gold standard laboratory analysis and quality control for vaccine production.

REF 429

Comparison of the EZ1 XL advanced and the Magna Pure instruments for the extraction of whole blood before DNA quantification of CMV, EBV, HHV 6 and Adenovirus

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Background: the extraction is a key step for real time PCR and can be facilited by ease to use instrument like EZ1 XL advanced (Qiagen). Here we have compared the EZ1 and the Magna Pure LC (MP) (Roche) before an amplification with CMV, EBV, HHV 6 and Adenovirus (AdV) R gene kits (Argène Biomerieux). **Methods:** whole blood samples (200 µl) were extracted (CMV n=156, EBV n=75, HHV 6 n=49, AdV n=32) with MP and EZ1 before amplification. Viral loads (VL) below the threshold were considered as "detected". The concordance of the results was verified. The agreement was evaluated by the intra class correlation coefficient (ICC) (acceptable if ICC>90%). **Results:** no known negative samples was positive above the threshold. CMV: 45 samples positive in the 2 assays, 1 positive EZ1 (784 copies/ml) detected with MP, 1 positive MP (519 copies/ml) detected with EZ1. EZ1 VL were on average 0.03 Log lower than MP VL, ICC=95%. EBV: 46 samples positive in the 2 assays, 2 positive MP (520 and 731 copies/ml) detected with EZ1. EZ1 VL were on average 0.19 Log lower than MP VL, ICC=96%. HHV 6: 25 samples positive in the 2 assays, 1 positive MP (1315 copies/ml) detected with EZ1. EZ1 VL were on average 0.12 Log lower than MP VL, ICC=97%. AdV: 9 samples positive in the 2 assays. EZ1 VL were on average 0.13 Log lower than MP VL, ICC=97%. **Conclusion:** The extracts obtained with EZ1 or MP give similar results when amplified with CMV, EBV, HHV 6 or AdV R gene. With a throughput of up to 14 samples per run in 1 hour, EZ1 has the same capacity than MP and is easier to use but it is also more expensive.

REF 430

Development of A Sensitive and Specific Epitope Blocking ELISA for Universal Detection of Antibodies to Human Enterovirus 71 Strains

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Human Enterovirus 71 (EV71) is a common cause of hand, foot and mouth disease (HFMD) in young children. It is often associated with severe neurological diseases and mortalities in recent outbreaks across the Asia Pacific region. Currently, there is no efficient universal antibody test available to detect EV71 infections.
Methodology/Principal Finding: In the present study, an epitope blocking ELISA was developed to detect specific antibodies to human EV71 viruses in human or animal sera. The assay relies on a novel monoclonal antibody (Mab 1C6) that specifically binds to capsid proteins in whole EV71 viruses without any cross reaction to any EV71 capsid protein expressed alone. The sensitivity and specificity of the epitope blocking ELISA for EV71 was evaluated and compared to microneutralization using immunized animal sera to multiple virus genotypes of EV71 and coxsackieviruses. Further, 200 serum sample from human individuals who were potentially infected with EV71 viruses were tested in both the blocking ELISA and microneutralization. Results indicated that antibodies to EV71 were readily detected in immunized animals or human sera by the epitope blocking ELISA whereas specimens with antibodies to other enteroviruses yielded negative results. This assay is not only simpler to perform but also shows higher sensitivity and specificity as compared to microneutralization. **Conclusion:** the epitope blocking ELISA based on a unique Mab 1C6 provided highly sensitive and 100% specific detection of antibodies to human EV71 viruses in human sera.

REF 431

Evaluation of a new rapid test for the detection of norovirus antigen in comparison with real time RT PCR

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Objectives: noroviruses (NoV) are recognized as the leading cause of gastroenteritis worldwide. Diagnosis of NoV infection mainly relies on molecular methods. A detection of viral antigens can also be performed by immunochromatographic assays. In outbreak settings, these rapid detection tests (RDT) may be useful. The aim of this study was to compare the performances of the new RDT ImmunoCardSTAT!® Norovirus (Meridian Bioscience®, Europe) with a real time RT PCR. **Methods:** on the basis of the symptoms, 205 samples from patients were selected. Their status was determined by real time RT PCR (Stals et al, J Virol Methods, 2009): 68 positive, and 137 negative of whose 16 samples were positive for other enteropathogens in order to evaluate the specificity of the RDT. Fifty of the 205 samples originated from nosocomial NoV outbreaks during which a genotyping of the strains was performed. **Results:** we observed an overall agreement between the RDT and the RT PCR of 81%. The specificity and the sensitivity of the RDT were respectively 96.3% and 50%. Regarding to

the 50 samples from the nosocomial NoV outbreaks (genotypes GII.4 and GII.16), the RDT showed a specificity of 100% and a sensitivity of 68.8% (95%CI). None interference was observed with enteropathogens tested. **Conclusion:** according to our evaluation study, this RDT is very specific but exhibits inadequate sensitivity to be used for the diagnosis of sporadic cases. However, in front of gastroenteritis outbreaks, this TDR is an effective method for the early detection of NoV and the fast implementation of prevention measures.

REF 432

No additional value of throat swabs in the diagnosis of enterovirus infections

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Introduction: it is currently unknown what the additional value is of sampling other sites than cerebrospinal fluid, such as throat or feces, for the diagnosis enterovirus (EV) meningitis. The presence of EV RNA in feces or throat is considered to be a strong indication of EV sepsis or meningitis. This study aims defining the additive value of testing throat and/or fecal samples for diagnosis of EV infections. **Methods:** we analyzed samples that were tested with an EV PCR between 1 January 2007 and 1 October 2012. In total, 566 patients had one or more samples tested for EV, 322 of them were below the age of one year. We used a composite reference standard with the following definition: patients that were positive in any of the PCR's for CSF, throat or feces were regarded as EV positive. **Results:** in the group of patients with both CSF and throat swab testes, a small group had a negative CSF PCR but a positive throat PCR. However, in all patients that had also their feces tested, the feces PCR was positive, indicating that a throat PCR does not provide extra information when a feces PCR is performed. In the group of patients with both CSF and feces PCR, we observed that a substantial number of patients had a positive feces PCR and negative CSF PCR. EV meningitis would not be missed when a PCR on feces and CSF is done.
Conclusions: our data show that including a throat swab in the diagnosis of EV infection has only limited additional value and can be omitted. This will lead to a reduction in costs without compromising sensitivity, which is important in the current economic situation.

REF 433

Fast and accurate RSV A/B detection by PCR on a BDmax platform

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Patients on a pediatric ward are screened for RSV infection to prevent spread of this highly contagious respiratory virus. Patients that are tested positive for RSV are nursed in isolation to prevent the spread of the virus. The decision whether a patient should be isolated upon admission is made on the outcome of fast diagnostic tests, such as antigen or molecular tests. Antigen tests are fast, but have a low positive predictive value (PPV), risking the spread of RSV by false negative results. The molecular tests that are based on reverse transcriptase PCR (rtPCR) however, are superior to the antigen tests with respect to sensitivity and specificity. In this report we describe an in house developed rtPCR for RSV A and B on a BDmax platform. The BDmax is a fully automated platform that combines RNA/DNA isolation with real time PCR. The test detects both RSV A and B in a single run and the time to result is only 2 hours. We compared for 60 samples the RSV BDmax test with the BinaxNow antigen test and found a PPV of 65% for the antigen test that missed 7 of the 20 BDmax positive samples. When comparing the BDmax test to a routine multiplex molecular test (Respifinder, Pathofinder, NL) we found a good concordance of both tests. Finally, we tested the QCMD 2012 proficiency panel for the in house test on the BDmax. All samples were correctly analyzed on the BDmax, again demonstrating the good performance of the test. We conclude that the BDmax is a flexible and reliable platform for the implementation of in house developed tests in a clinical molecular laboratory setting.

REF 434

Development of biplex real time RT PCR for detection and differentiation of human parainfluenza viruses

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Objectives: Development of biplex real time PCR for detection and differentiation of human parainfluenza viruses (HPIV) 1 to 4. Clinical evaluation and comparison with conventional methods: direct fluorescence assay (DFA) and viral isolation techniques (VIT). **Material and methods:** primers and probes used for real time PCR have been described and evaluated in original publications (Templeton et al., 2005, Garbino et al., 2009) and tested in silico. Each PCR was primarily set up as a monospecific assay and then combined in two biplex reactions and optimized further. Both assays have the same PCR protocol. Amplification, detection and data analysis were performed with SMARTCycler, Cepheid®. Retrospective study used 304 nasopharyngeal aspirates to evaluate the biplex PCR and compare obtained results with those of conventional techniques. **Results:** monospecific PCR parameters were maintained in biplex assays. Optimization focused on concentration of MgCl2, probes, and dNTP. PCR specificity and repeatability testing showed no non specific reaction and no variation exceeding ±1Ct. The biplex PCR positive specimens included 92 samples that were positive by DFA/VIT and 9 additional ones. Discrepant results were tested by a conventional PCR. Obtained results were supporting our findings except for one sample. **Conclusion:** biplex real time PCR was found to be a sensitive and specific alternative for classical DFA and VIT. Conventional methods remain the gold standard for detection of respiratory viruses. However, rapid laboratory diagnosis can be critical for clinical management of patients.

REF 435

Evaluation of the new Abbot Architect assays for the detection of EBV antibodies in routine diagnostics

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Objective: The aim of the study was to evaluate the performance of 3 new automated Abbot Architect EBV diagnostic test of IgG antibodies against Epstein Barr virus Nuclear Antigen 1 (EBNA 1) and Viral Capsid Antigen (VCA) and IgM antibodies against VCA. **Material and methods:**204 consecutive samples, and 59 selected samples were analyzed by Biotest (EIA) for IgG EBNA, VCA IgG and VCA IgM and Abbott Architect (CMIA) for EBNA IgG, VCA IgG and VCA IgM. Dissenting samples were also analyzed by bioMerieux Vidas (ELFA) EBNA IgG, VCA/EA IgG or VCA IgM. A cross reactivity panel of 61 samples were analyzed by Architect VCA IgM. **Results:** If Biotest results were used as gold standard the sensitivity for EBNA 1 IgG, VCA IgG and VCA IgM were 96,5%, 100% and 94,3% respectively and the specificity 97,8%, 84,6% and 92,8% respectively.If a combination of Biotest, Architect and Vidas results (2 out of 3) were used as gold stand the sensitivity for EBNA 1 IgG, VCA IgG and VCA IgM were 98,8%, 100% and 100% respectively and the specificity 98,9%, 95,6% and 98,4% respectively. The concordance was 89,1%. Crossreactity was shown for CMV, VZV, Mycoplasma and hepatitis A. **Conclusion:** The Architect assays show good performance