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(JCVVP1), BK polyomavirus VP1 (BKVVP1), glycoprotein D of herpes simplex virus type 1 (HSV-1gD) and varicella zoster virus glycoprotein E (VZVgE).

Methods: A plasmid based expression system (InsectDirect; Novagen, USA) was utilised. Recombinant plasmids were generated using ligation independent cloning. Sf9 insect cells were then transfected. Expressed proteins were characterised using immunofluorescence, SDS-PAGE, Dot Blot and western blot.

Results: Using the InsectDirect expression system, JCVVP1, BKVVP1, HSV-1gD and VZVgE were successfully expressed in insect cells 48 h p.i. JCV and BKV VP1 and the HSV-1gD and VZVgE were detected in cytoplasm and cell membranes using immunofluorescence analysis. The InsectDirect approach took 5 days from initial cloning to the production of protein.

Conclusion: The InsectDirect system employs an optimal expression vector and produces high efficiency transfection. It is ideal for rapid expression of protein in insect cells. Although, the system does not provide large scale protein expression, it provides a rapid and simple method to construct and screen recombinants.

P.036

GeneXpert and TaqMan-PCR assay for the detection of enterovirus/parechovirus in cerebrospinal fluid

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A prospective comparative study was performed to evaluate the efficacy of the Cepheid GeneXpert (GX) enterovirus (EV) assay, a fully automated and rapid real-time PCR assay for the diagnosis of viral meningitis.

Methods: From May 2007 till September 2008, cerebrospinal fluid samples from 210 patients with a clinical suspicion of meningitis were tested for the presence of EV RNA using the GX and an EV and parechovirus (HPEV) specific Real-Time PCR (TqM) assay, 107 samples had enough material left to perform viral culture.

Results: From the 107 samples tested by virus culture, 6 (5.6%) were positive compared to 18 (16.8%) and 22 (20.6%) using the GX and TqM assay, respectively. One sample tested positive with virus culture, but was negative in both molecular assays. In total 33 (15.7%) of the 210 samples were positive for EV using the TqM assay. 26 of these samples were also positive in the GX assay, whereas 6 were negative and 1 gave an invalid result. Finally, 7 (3.4%) samples were positive (HPEV) RNA using the HPEV-specific TqM assay. As expected, these samples were negative in the GX assay. **CONCLUSIONS:** Viral culture has a lower sensitivity than both molecular assays. The TqM PCR seems to be more sensitive than the GX assay for the detection of EV RNA from CSF samples and is suitable for the detection of HPEV, in contrast to the GX assay. Therefore, the TqM assay is preferred over the GX assay for the diagnosis of picornavirus meningitis in a clinical setting.

P.037

PCR on Dried Salivary Swab (DSS-test): a useful method for diagnosis of congenital cytomegalovirus infection?

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Purpose: An early laboratory diagnosis (first 2 weeks of life) is essential for the identification and treatment of congenital Cytomegalovirus (cCMV) infected babies especially because 85–90% are asymptomatic and 10–15% of these will develop sequelae. The gold standard is viral isolation from urine and/or saliva but it is very expensive and laborious. CMV-DBS test has demonstrated to be a valid method to overcome these problems. Our aim is to identify an alternative method that can be useful for cCMV diagnosis, as the detection of CMV-DNA on Dried Salivary Swab (DSS).

Methods: We tested series of DSS loaded with 10-fold dilution (107–101 copies/ml) of cell grown CMV by means of a nested-PCR (gB region). We tested urine, saliva and DSS samples of 39 asymptomatic newborns (≤14 days of life), group A, and 12 cCMV infected babies in follow-up (>14 days), group B.

The results of DSS-test were compared with rapid isolation assay (RI-p72) on urine and saliva.

Results: In the validation session we could detect CMV-DNA up to 102 copies/ml. In the group A (1/39 cCMV infected), the comparison of the DSS-test both urine and saliva RI-p72 showed a concordance of 97.4%, a sensitivity of ~100% and a specificity of 97.4%. For the other group, we

obtained a higher concordance with urine RI-p72 and a perfect one with PCR on no-dried salivary swab.

Conclusion: The detection of CMV-DNA in DSS seems to be a favourable method to diagnose cCMV. The confirmation of these results will permit to apply advantageously DSS-test also for screening programmes.

P.038

Virological-molecular study of aetiological agents associated to Influenza-Like Illness (ILI)

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Purpose: During 2006/2007 winter season, within the Italian Influenza Surveillance Network, a virological-molecular study was carried out to identify the aetiological agents responsible of Influenza-Like Illness (ILI): Influenzavirus (A/H1, A/H3, B), Respiratory Syncytial Virus (RSV), human Metapneumovirus (hMPV), human Parainfluenzavirus (hPIV) and human Coronavirus (hCoV).

Methods: 277 respiratory samples were collected from outpatients with a clinical presentation of ILI during the 2006/2007 season (weeks 46/06–16/07). The respiratory viruses were detected through specific PCR assays.

Results: 39% (109/277) of samples were positive to influenzaviruses (78% A/H3, 15.6% A/H1, 6.4% B). 21% (58/277) of samples were positive to at least one of the other viruses considered. Particularly, RSV was identified in 9%, hMPV in 6.5%, hCoV in 4% and hPIV in 1.4% of ILI cases.

RSV epidemic wave almost overlapped the influenzaviruses one, with a peak in weeks 05/07–07/07. RSV infection was mainly detected in subjects aged <4 years. No epidemic trends were observed for hMPV, hPIV and hCoV. HMPV infections were more frequently observed among subjects aged 0–4 years and those aged 15–64 years.

Conclusion: Although an influenzavirus infection was identified in most subjects who presented ILI, the other detected viruses were a major cause of disease as well. Overall, the proportion of ILI attributable to respiratory viruses infection during 2006/2007 winter season was about 60%.

This study showed that about 20% of all ILI cases was due to an RSV, hMPV, hPIV or hCoV infection.

Further molecular investigations for Rhinovirus, Enterovirus, Adenovirus and Bocavirus are currently in progress.

P.039

Is replacement of tAN cells in routine viral culture possible?

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Purpose: At our laboratory viral cultures of faeces are routinely performed on tAN, RD-C and HFL cells and cultures of respiratory materials on tAN, HFL and R-HELA cells. The production of tAN is uncertain and the quality of these cells is unstable. Therefore the performance of 2 other cell lines for culture of faeces (CaCo2 and HT-29) and 3 other cell lines for culture of respiratory samples (MDCK, LLC-MK2 and HT-29) was compared with the performance of tAN in the routine setting.

Methods: All 1251 faeces samples and 413 respiratory samples sent to our laboratory for viral culture during 3 seasons were analyzed. A rapid (IF detection) and a conventional (CPE) culture method was used. Performance of the cell lines was compared for isolation of adenovirus, parechovirus, coxsackie B, coxsackie A/echovirus, Influenza virus and Parainfluenza virus.

Results: In 862 faecal samples (69%) no virus was isolated. 107 (9%) adenoviruses (96% in CaCo2/HT29 and 55% in tAN), 136 echo/coxsackie A viruses (92% and 71% respectively), 41 coxsackie B viruses (95% and 90% respectively) and 38 parecho viruses (82% and 55% respectively) were isolated.

In 279 respiratory samples (68%) no virus was isolated. Adenovirus was isolated in 30 (7%) samples (57% on tAN versus 80% on MDCK/LLC-MK2). Influenza virus was isolated in 9 (2%) samples (89% on tAN and 100% on MDCK). Parainfluenza virus was isolated in 16 (4%) samples (63% on tAN and 88% on R-HELA/HFL).

Conclusion: Replacement of tAN cells in routine viral culture is possible.