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nucleocapsid (N) gene of BCoV with published primers that could amplify all BCoV strains.

**Conclusion and Discussion:** This report is the first detection of BCoV in Iran. This study shows that bovine coronavirus is a significant virus in the fecal specimens of calves with diarrhea from farms in west of Iran and thus may be an important pathogen of calves.

**P48 Quality control assessment for the PCR diagnosis of TBEV infections**

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**Background:** RT-PCR is an efficient method for an early detection of tick-borne encephalitis virus (TBEV) RNA in blood and serum samples taken prior to the appearance of antibodies. Improving diagnostics is the most important step in detecting and handling this pathogen. Quality control measures are therefore essential tools.

**Aims:** To assess the diagnostic quality of laboratories we performed an external quality assurance (EQA) programme for the molecular detection of TBE infections.

**Methods:** A panel of twelve prepared human plasma samples were sent out to be tested for the presence of TBEV-specific RNA. This panel comprised 8 samples spiked with different TBEV strains of the European, Siberian as well as the Far Eastern subtype, including a 10-fold dilution series. Two further samples were prepared as specificity controls containing Louping ill virus and a pool of different other flaviviruses, while two other samples were used as negative controls.

**Results:** 23 laboratories from 16 European and two non-European countries participated in this EQA. Only two participants have correctly analysed all samples. Nine laboratories are ranging between 91.7% and 75.0% of correct test results, 7 laboratories between 66.7% and 58.3%, and 5 laboratories have less than 50% of correct results with increasing need for improvement of their methodology regarding sensitivity and/or specificity.

**Conclusions:** The EQA gives a feedback of the quality of the RT-PCR methods used by the participants and indicates a clear need for improvement.

**P49 Evaluation of the new NucliSens easyMAG® nucleic acid extraction system**

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**Aim:** The new automated NucliSens easyMAG® system was tested for its ability to extract DNA and RNA from blood plasma, stool, cerebrospinal fluid and throat swab specimens in virus transport medium. The manual QiaGen QiAamp® nucleic extraction systems served as reference systems for comparison.

**Methods:** For direct comparison, dilution series of virus reference strains or clinical samples were extracted in parallel and amplified in the same run of (RT-)PCR. Clinical materials stored at −80°C were retested by extraction with the NucliSens system and separate amplification.

**Results:** The NucliSens nucleic acid extraction reagent proved to have an excellent extraction efficiency for DNA and RNA from all the clinical materials tested. The simultaneous extraction of DNA and RNA eliminated the need for separate extraction which is a considerable advantage in routine PCR analysis. Using the NucliSens easyMAG® system no problems with extraction of any of the clinical materials tested were observed. The system handled even “dirty materials” such as stool specimens with a high degree of reproducibility and reliability. No carry-over was observed. No significant discrepancies with respect to the nucleic acid extraction efficiency between the QiaGen and the NucliSens extraction systems were detected for blood plasma, stool and swab specimens in virus transport media. Concentration of nucleic acids by lowering the volume of elution buffer may be indicated for cerebrospinal fluid.

**Conclusions:** The easyMAG® system proved to be extremely user-friendly. Hands-on time is short; once started the system operates fully automatically which is an important advantage in routine PCR diagnostics.

**P50 Diagnosis of Marek’s disease virus in broiler chickens by histopathology and nested-PCR in Iran**

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Marek’s disease (MD) continues to be a serious threat to poultry production, despite widespread use of vaccination programmers. Rapid and reliable diagnosis of MD remains an important issue. In this study, Marek’s disease virus in broiler chickens is diagnosed by histopathology and molecular methods. A polymerase chain reaction (PCR) and nested-PCR test based on genetic differences between pathogenic and non-pathogenic MDV-1 was utilized. PCR was carried out based on primers from the meq gene. PCR of the DNA extracted from an attenuated strain, amplified a 1200 bp fragment while the DNA from a pathogenic MDV-1 produced a 1062 bp amplicon. In the nested-PCR, the non-pathogenic strain produced a 500 bp DNA fragment and a 300 bp band was amplified from the DNA sample from tissue of infected broiler chicken with pathogenic strain. The nested-PCR procedure was found to be a simple and sensitive test for differentiation of pathogenic and non-pathogenic MDV-1 strains and can be used as a rapid diagnostic test.

**P51 Simultaneous detection of HSV-1, HSV-2 and VZV in clinical samples by multiplex polymerase chain reaction (MPCR)**

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**Introduction:** Human herpes virus such as Herpes simplex type 1 (HSV-1), type-2 (HSV-2) and Varicella Zoster virus (VZV) causing a wide range of acute infections in human which occasionally associated with significant morbidity and mortality. Encephalitis and blindness are the examples of such an occasion.

**Aim:** The aim of the study was to develop and use a multiplex PCR method for simultaneous detection of HSV-1, HSV-2 and VZV DNAs in different clinical sample. Furthermore, the mPCR results were compared with the results of virus.

**Materials and Methods:** A total of 93 clinical specimens including 63 skin lesions or vesicles, 28 corneal scraping or conjunctival swabs and 2 CSF samples were collected from patients admitted in Shiraz hospitals. All the specimens were cultured on Vero, HepII and MRC5 cell line. DNA was also purified from specimens by boiling method. Using a specific pair of primer for thymidine kinase gene, both HSV-1 and HSV-2 DNAs were amplified. A set of primer flanking a 208 bp of the DNA-polymerase gene was also used to amplify VZV DNA.

**Results:** See the table.

**Comparison between mPCR results and virus isolation on 93 clinical specimens**

| Disease | mPCR(+) | mPCR(−) | Culture(+) | Culture(−) |
|---------|---------|---------|------------|------------|
| Cutaneous | 83 | 49(77.6) | 14(22.2) | 32(50.8) |
| Ocular | 29 | 17(58.6) | 11(38.2) | 17(60.7) |
| CNS | 2 | 1(50) | 1(50) | 0(0.0) |
| Total | 93 | 67(72) | 26(28) | 43(46.2) |

In general the sensitivity of the MPCR for detection of HSV and VZV in clinical samples were 80.9% and 95% respectively. Whereas the sensitivity of cell culture for isolation of HSV and VZV was 62.9% and 72.7% respectively. Interestingly, both HSV and VZV DNAs were detected in 3 out of 93(3.2%) specimens exclusively by MPCR.
Conclusion: Using statistical analyzing of the results a significant difference between virus isolation and mPCR detection for HSV-1, HSV-2 and VZV in clinical samples was noticed (p < 0.005). Results show that mPCR is amore reliable method in rapid diagnosis of herpes viruses DNA in clinical samples than virus isolation. However, more studies for application of the mPCR in clinical diagnosis of viral infection are recommended.

P52 A novel screening assay reveals high prevalence of variant erythroviruses

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Background: Recent data from European populations has demonstrated the existence of novel human Erythroviruses as distinct from Parvovirus B19. An initial study failed to detect such variants in a North American population sample.

Methods: In order to further examine the prevalence of variant Erythroviruses in the North American pediatric and maternal population, we have developed a novel molecular genovar screening assay which can specifically highlight specimens containing sequences related to but genetically distinct from a type sequence. We applied this assay in the retrospective analysis of approximately 100 pediatric and maternal specimens which had been submitted to our clinical hospital laboratory for B19 testing. Results of this analysis were compared with pre-existing sample parvovirus serology and classical B19-specific molecular diagnostics results where available.

Results: We detected genotypic variants of B19 with 4–19% sequence divergence from the B19-Au type strain in 20% of this clinically selected specimen population. In addition, we present clinical and diagnostic data suggestive of serological infection of a patient by two distinct Erythrovirus genovars, indicating that prior infection with one genovar may not afford cross-protective immunity to others.

Discussion: The novel assay method presented here is useful for screening a population specifically for variants of a target sequence. Using it, we have demonstrated evidence for greater diversity in human Erythrovirus sequences than has been previously reported. Our data suggests these may be of clinical relevance and may be missed by current serological and molecular methods aimed at known Erythrovirus genovars.

P53 Evaluation of an automated and highly sensitive real-time PCR assay for CMV DNA in whole blood samples

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Introduction: Highly sensitive diagnostic assays are crucial for obtaining information on early onset of viral replication of CMV after organ transplantation. In our study, we evaluated the sensitivity and accuracy of a commercially available CMV PCR kit when used in conjunction with a fully automated nucleic acid extraction system.

Methods: A total of 147 whole blood patient samples were extracted in parallel with the Roche MagNA Pure and Abbott m2000sp extraction kit. Both plasma and whole blood was collected from each patient and EBV utilising affigel | EBV trender. Three of the patients were on immune suppression.

Results: The HPeV tagman assay has an analytical sensitivity of 300 copies of viral cDNA per reaction. All 4 HPeV serotypes could be detected while EVs, rhinoviruses and HAV. To establish diagnostic relevance 522 cerebral spinal fluid (CSF) from children <5 years negative for enterovirus were tested.

Discussion: HPeV infections in young children may lead to serious conditions as neonatal sepsis and meningitis. Therefore, HPeV should be included in viral diagnostics of neonatal sepsis and meningitis in young children.

P54 Rapid detection of human parechoviruses in clinical samples by real-time PCR

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Introduction: Human parechoviruses (HPeVs) have been associated with mild respiratory and gastrointestinal symptoms in predominantly young children, but more severe conditions such as transient paralysis, neonatal sepsis on meningitis have also been reported. Therefore, a rapid detection of HPeVs in clinical samples from young children is essential in viral diagnostics.

Materials and Methods: We have developed a 5‘UTR real time tagman PCR assay specific for HPeVs. Serial dilutions of HPeV wild type RNA were tested in a background of HPeV/EV RNA-negative cerebrospinal fluid to determine the dynamic range and lower limit of detection of the assay. The specificity and efficiency of the assay were tested by using high positive and low positive cell cultures of three previously described HPeV serotypes, the new HPeV serotype we found (HPeV4), enterovirus serotypes (EVs), rhinoviruses and hepatitis A (HAV).

Discussion: HPeV infections in young children may lead to serious conditions as neonatal sepsis and meningitis. Therefore, HPeV should be included in viral diagnostics of neonatal sepsis and meningitis in young children.

P55 Epstein–Barr virus in plasma and whole blood

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Introduction: Highly sensitive diagnostic assays are crucial for obtaining information on early onset of viral replication of EBV after organ transplantation. In our study, we evaluated the sensitivity and accuracy of a commercially available CMV PCR kit when used in conjunction with a fully automated nucleic acid extraction system.

Methods: Two plasma and whole blood samples were collected from each patient. Both plasma and whole blood was collected from each patient and EBV utilising affigel | EBV trender. Three of the patients were on immune suppression.

Results: The HPeV tagman assay has an analytical sensitivity of 300 copies of viral cDNA per reaction. All 4 HPeV serotypes could be detected while EVs, rhinoviruses and HAV. To establish diagnostic relevance 522 cerebral spinal fluid (CSF) from children <5 years negative for enterovirus were tested.

Discussion: HPeV infections in young children may lead to serious conditions as neonatal sepsis and meningitis. Therefore, HPeV should be included in viral diagnostics of neonatal sepsis and meningitis in young children.