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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 efficacy 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 efficacy 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 programmes. 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 p 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 Zoaster (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	No.	MPCR(+)	MPCR(-)	Culture(+)	Culture(-)
Cutaneous	63	49(77.8)	14(22.2)	32(50.8)	31(49.2)
Ocular	28	17(60.7)	11(39.2)	11(39.2)	17(60.7)
CNS	2	1(50)	1(50)	0(0.00)	2(100)
Total	93	67(72)	26(28)	43(46.2)	50(53.8)

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 were 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.