

ment was used. A second amplification with Per1 and a sequence-internal primer Per2rev was performed for electrophoretically detected samples.

Both a one-time PCR with Enzymun testing as well as a nested PCR with either of the electrophoresis methods was highly sensitive. Only 30 bacterial cells were needed for the Enzymun-Test starting after a one-time PCR.

Nasopharyngeal swabs from 53 children with whooping cough and from 50 children without infections were analyzed, using these methods. We found that 51 patients with whooping cough had positive results, a total of 2 of the sick patients and all the control children had negative results.

Evaluation of sample preparation methods for the detection of DNA from *Borrelia burgdorferi* and *Legionella* sp. in urine

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Testing of urine samples from patients is an attractive alternative compared to tests on other materials that can be difficult to obtain. *Borrelia burgdorferi* as well as *Legionellae* are pathogens that often pose problems for direct detection or cultivation. There have been several reports on the detection of *B. burgdorferi*-DNA in human urine samples using the polymerase chain reaction (PCR). However, it is currently unknown which components of the *Borreliae* appear in the urine. In the case of legionellosis, soluble antigens are excreted in the urine, which can be detected by immunoassays. The detection of *Legionella*-DNA in urine samples has not been reported. A common problem encountered with urine is the inhibitory activity to the PCR by some samples, and the need to concentrate the DNA prior to analysis. This prompted us to evaluate several methods for sample preparation using artificially contaminated urine samples and subsequently untreated urine samples from patients and from infected guinea pigs. Principles of the methods were filtration, centrifugation or adsorption of DNA to binding matrices. With artificially contaminated samples the various methods showed comparable sensitivities. Untreated urine samples from infected guinea pigs and from patients showed a variable degree of inhibition of the PCR reaction when treated with different methods. Adsorption of DNA to a binding matrix (GeneClean) proved to be the most reliable of the tested methods for untreated urine samples. Altogether, 114 urine samples of patients were tested using PCR for *B. burgdorferi*, and 37 guinea pig

samples as well as 34 samples from patients with pneumonia were tested using PCR for *Legionella* sp. The results indicate, that with both, borreliosis and legionellosis, soluble DNA or antigen-associated DNA is excreted in the urine rather than whole bacteria.

Reverse transcription and polymerase chain reaction – application to diagnosis of viral diseases in poultry

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Different extraction procedures are used for amplification of viral RNA or DNA from cell cultures and their supernatants, allantoic fluids of infected embryonating eggs, blood samples and organ specimens. Specific oligonucleotides have been used for reverse transcription and PCR to amplify the viral genome sequences of birna-, retro-, myxo- and paramyxoviruses. The reactions were specific and did not amplify extracts from uninfected controls.

Nonradioactive labelled probes complementary to sequences within the PCR amplification product have been used in hybridization assays to verify the PCR product and to increase the sensitivity.

Myxo- and paramyxoviruses have been detected in the same allantoic fluid by applying specific primers. Amplification products of virulence determining domains were directly used for cloning and sequencing. An evaluation of the degree of virulence was possible by comparing these with published sequences.

Ligase chain reaction for the differentiation of cowpox virus from other orthopoxvirus species based on the deletion of two adjacent nucleotides

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A ligase chain reaction (LCR) assay based on a double-base pair deletion in the A-type inclusion body gene