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. *Borrellia burgdorferi* as well as *Legionallae* 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

(ATI-gene) was developed to distinguish cowpox virus from other orthopoxvirus species. For this purpose, two primer pairs were designed, with one primer of each pair radioactively labelled. The discriminating bases are located at the 3'-end of the upper upstream primer (TT), at the 5'-end of the lower downstream primer (A), and at the 3'-end of the lower upstream primer (A), forming a one base overhang at the ligation site. Two mismatching bases (GG) were added at the 3'-ends of the upstream primers to prevent ligation at this end. Detection of the cowpox ligation product was achieved by autoradiography after denaturing polyacrylamide gel electrophoresis. To increase the sensitivity, the corresponding region of the ATI-gene was initially amplified by consensus primer detected polymerase chain reaction (PCR) prior to LCR.

All 9 cowpox viruses examined could be clearly discriminated from reference strains of camelpox virus, mousepox virus and monkeypox virus, as well as seven vaccinia virus isolates. The PCR-coupled LCR was shown to be a reliable tool for the screening of cowpox virus infections, which is of particular importance with respect to the increasing number of unusual hosts.

To our knowledge, this is the first report of a LCR that uses a double nucleotide deletion. This LCR does not require any fill-in reaction (substitution of bases) and is not limited to the detection of base pair changes from A-T/T-A to G-C/C-G or vice versa.

## Identification of Epstein-Barr virus transactivator BZLF1 mRNA in uncultured peripheral blood lymphocytes by RT-polymerase chain reaction

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The cascade of gene expression during the lytic replication of *Epstein-Barr* virus (EBV) is initiated by three immediate early regulatory proteins. Based on a nucleic acid detection method for one of these factors, the proof of active EBV replication is possible even before the onset of serological markers and will therefore be of great value for diagnosis and treatment. For early detection of lytic replication in the case of EBV associated diseases, we developed a reverse transcription polymerase chain reaction (RT-PCR), followed by a second 'nested' PCR with primers overlapping the first and second exon coding for BZLF1.

We report detection of mRNA for the immediate early transactivator BZLF1 in peripheral blood lymphocytes (PBL) of donors with reactivation of EBV and chronic infection (CAEBV). No transcripts were detected in PBL of normally latently infected individuals of EBV-negative BJAB cells.

The significance of this observation for pathobiology, diagnosis and treatment of clinical forms of EBV infections like infectious mononucleocleosis (IM), CAEBV, chronic fatigue syndrome (CFS) or reactivation of latent EBV and rejection of organ transplants needs further evaluation and will be discussed.

## Dependence on the taxon composition of clone libraries for PCR amplified, naturally occurring 16S rDNA, on the primer pair and the cloning system used

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Recent advances in molecular techniques have allowed the determination of the genetic variety of microbial inhabitants in the environment. Encouraging progress has been made in the elucidation of as yet undetectable biodiversity in different natural samples, such as marine environments, forest soil, acidothermal soil, hot spring environments and the environment of a continuous bioleach bioreactor. Despite the novelty of the results, certain problems associated with the methods applied to quantitatively describe the microbial populations have been recognized. Unsolved problems in the cloning strategy include: 1) the recovery of cells and/or nucleic acids from the environment, 2) the conditions under which PCR amplification of the target genes is performed, including selection and specificity of primers, the dependence of PCR amplification on the quality of DNA and the possibility of capture of *Taq* polymerase by abundantly occurring DNA, 3) the influence of the cloning strategy and cloning vectors, such as shotgun cloning (minute percentage of DNA are rrn operons), sticky end cloning (presence of restriction sites within the rrn operons may prevent cloning of the amplified product), blunt end cloning (low transformation efficiency), and clonability of heterologous rDNA, 4) the detection of clones by sequence analysis