can be done within 1 to 3 h, the preparation and extraction of samples and the detection of the amplified products is tedious, allows the throughput of only a few samples and can take two days.
We describe a method for the detection of hepatitis B virus DNA in serum with a homogeneous sample preparation and an ELISA like detection reaction on Boehringer Mannheim ES Systems.

The automated step comprises:
1) Hybridization of denatured DNA labelled with digoxigenin with a biotinylated capture probe.
2) Bound/free separation on a streptavidin solid phase.
3) Binding of peroxidase labelled anti-digoxigenin-antibody.
4) Quantitative detection via ES-substrate colour development.

Turn-round time is 3 h at 37 °C incubation temperature. By using an ES 300 instrument a maximum of 300 samples can be processed per day within 2 instrument runs.

Differentiation of the five serovar groups of the *Mycobacterium avium/intracellulare* complex by hybridization with oligonucleotides specific for subspecies

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In addition to our well adapted method of differentiation of mycobacteria, we introduced a molecular biological protocol via PCR and selective dot blot hybridization in our laboratory. To establish the method, we started analysing amplified 16S rDNA by hybridization with specific oligonucleotides of 7 strains from different serovar groups of the *Mycobacterium avium/intracellulare* complex. We chose a washing buffer that allowed hybridization selective down to one bp difference without elaborative analysis of T_m values. The procedure consists of five steps.

1) Amplification by PCR with a universal 5′ primer and a genus specific 3′ primer yielding a PCR product exclusively from mycobacteria.
2) Dot blot transfer of PCR products on nylon membranes.
3) Hybridization of Dig-11-ddUTP labelled oligonucleotides specific for the mycobacterial species, allowing differentiation down to the subspecies level in some cases.
4) Stringent washing with buffer containing tetramethylammonium chloride, which eliminates the dependence of the T_m values on the base composition of the probe. Additionally, by a given hybridization temperature appropriate for the length of the oligonucleotide, non-specific adsorption of the probes to solid supports is suppressed, resulting in lower non-specific backgrounds.
5) Non-radioactive chemoluminescent detection of hybridized probes using a commercially available detection kit (Boehringer, Mannheim, Germany).

For each of the strains examined, we found a unique hybridization pattern with the specific oligonucleotides used. The corresponding PCR product of a number of strains belonging to a total of 23 other mycobacterial species did not hybridize under the assay conditions outlined above.

Highly sensitive diagnosis of *Bordetella pertussis* DNA

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The 'gold standard' for the detection of *Bordetella pertussis* is the culture of bacteria. Usually a Bordet-Gengou or a Regan-Loewe medium is used, requiring immediate inoculation and incubation. Isolation and identification of *B. pertussis* with this method takes many days and often yields false negative results. Reliable detection of the causal agent is generally possible in the catarrhal stage. We describe a highly sensitive and quick method for detection of *B. pertussis*. With the help of the polymerase chain reaction a specific 183 bp DNA fragment was identified. The area of DNA is a repetitive sequence with 50–100 copies per cell and has a total length of 1.1 kbp. We compared three different methods of detection after PCR amplification: Enzymun-Test (Boehringer, Mannheim, Germany), PAGE with silver staining, and agarose gel with UV detection. For determination of the detection limit a positive control [target sequence cloned in plasmid KS+, (Stratagene)] was diluted from 1 ng to 10^{-8} ng and amplified in a PCR using a reaction mixture containing digoxigenin labelled primer (dig-Per1) and unlabelled primer (Per3rev). After the first amplification, detection was made with the Enzymun-Test. A biotinylated capture probe for hybridization of the denatured DNA frag-

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