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-