dye-terminator reactions). Sequence analysis of the p83/100 internal part showed an 18 bp insertion typical for *B. burgdorferi* sensu stricto strains and a 90 bp deletion typical for all investigated *B. afzelii* strains. Due to the insertions/deletions, the size of these PCR-amplified internal p83/100 fragments varies between the three species. The only exception was the internal fragment of *B. burgdorferi* sensu stricto, strain *B. pacificus*, which did not show the 18 bp insertion. Restriction analysis of PCR fragments with *Dra*I revealed three patterns, correlating to the species grouping.

**Conclusions.** As in OspA-serotyping experiments[5], the *B garinii* group (OspA-sterotype 3–7) showed highest diversity within this internal fragment of p83/100, whereas the *B. afzelii* group (OspA-type 2) and the *B. burgdorferi* sensu stricto group (OspA-type 1) were nearly identical. Determination of the size of the PCR products as well as restriction fragment length polymorphism analysis (*Dra*I) can be used for classification into the three species of *B. burgdorferi* sensu lato. Since p83/100 is chromosomally encoded, this protein might be a more stable marker for classification than the plasmid-encoded OspA. In contrast to the flagellin gene a subclassification of the *B. garinii* group is possible due to the diversity of the p83/100 internal fragment.

1 Eiffert, H., Schlott, T., Hoppert, M., Lotter, H., and Thomssen, R., J. med. Microbiol. *36* (1992) 209.
2 Luft, B. J., Mudri, S., Jiang, W., Dattwyler, R. J., Gorevic, P. D., Fischer, T., Munoz, P., Dunn, J. J., and Schuhbach, W. H., Infect. Immun. *60* (1992) 4309.
3 Perng, G. C., LeFebvre, R. B., and Johnson, R. C., Infect. Immun. *59* (1991) 1070.
4 Wilske, B., Preac-Mursic, V., Fuchs, R., Bruckbauer, H., Hofmann, A., Zumstein, G., Jauris, S., Soutschek, E., and Motz, M., in: New Antibacterial Strategies, pp. 47–63. Ed. H. C. Neu. Churchill Livingstone, London – Edinburgh 1990.
5 Wilske, B., Preac-Mursic, V., Göbel, U. B., Graf, B., Jauris, S., Soutschek, E., Schwab, E., and Zumstein, G., J. clin. Microbiol. *31* (1993) 340.

## Highly sensitive semi-quantitative method to detect amplified viral DNA in the microtitre format

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In many clinical situations it is important to know the virus titre of people chronically infected with HBV, HCV or other viruses, for example to monitor the success of an interferon treatment[2]. Nested PCR is useful to detect nucleic acids with sufficient sensitivity, but is hard to quantitate. We have developed a simple chemiluminescent method that allows the detection of HBV and HCV nucleic acids as sensitively as nested PCR. This method is performed in the microtitre format which makes working with many samples much easier.

**Extraction.** We compared different published or our own methods to extract HBV DNA (proteinase K, NaOH-/KOH-lysis, microwave or heat treatment, acetone) or HCV RNA (proteinase K, GITC, heat or QiaAMP Blood Kit, Qiagen)[1]. In our hands a modified NaOH-lysis method (from the Boehringer ES300 protocol) worked best for extraction of HBV DNA from serum (10 µl serum + 20 µl 0.2 N NaOH: 1 h 37 °C, + 30 µl 0.2 M TrisCl, pH 7.5; 20 µl of this mixture were used for PCR). Standard GITC extraction worked best for HCV.

**Chemiluminescent detection of amplified nucleic acids.** During PCR one biotinylated primer is integrated into the amplified PCR fragment. This amplicon is immobilized on white microtitre plates that are coated with covalently linked streptavidin (Dr. J. Diment, Kodak clinical diagnostics). After denaturation this template is hybridized to a DNA probe that is labelled with peroxidase. Target-bound peroxidase is detected by chemiluminescence in an Amerlite Analyzer.

As detection probe we tested single stranded or double stranded DNA of different lengths, or a labelled oligo. Ds and ss probes were labelled with ECL direct nucleic acid labelling system, Amersham; the oligo was 5'-labelled with ECL 5'thiol oligolabelling system, Amersham. The best probe in our hands was the single stranded probe (HBV or HCV probe not overlapping with the used primers, cloned into pBluescriptII SK$^+$, Stratagene, made single stranded with the helper phage R408, Stratagene). The detection limits were: ss-probe 8 pg, ds-probe 32 pg, and oligo 500 pg.

Hybridization was performed for 4 h in urea-containing hybridization buffer (ECL system) at 42 °C on a heated microtitre plate shaker (AIP4, Pasteur Diagnostics). Prehybridization decreased the sensitivity.

After the nucleic acid detection system had been optimized we compared nested PCR, the Boehringer ES 300 system and our inhouse method using a panel of sera obtained from a trial of Eurohep to standardize PCR for the detection of HBV DNA. The following titres were obtained for one serum by endpoint dilution and calculation according to ref. 3: nested PCR $2.21 \times 10^9$/ml, Boehringer ES 300 $2.3 \times 10^9$/ml, and inhouse method $3.43 \times 10^9$/ml. The titres that were determined by nested PCR and both semiquantitative methods were identical for this serum and others.

We have used the same method to detect HCV RNA after amplification. So far we can detect 32 pg of amplified HCV product. Using our method it appears possible to quantitate the titre of HCV RNA in serum of infected people as sensitively as with a nested PCR.

1 Athanassiou, N., Vereinfachte Methoden zum Nachweis von Hepatitis B Virus DNA in Serum mittels Polymerase Ketten Reaktion. MD Thesis (1994), University Giessen, Germany.
2 Perrillo, R. P., Semin. Liver Dis. *9* (1989) 240.
3 Reed, L. J., and Muench, H., Am. J. Hyg. *27* (1938) 493.

## Comparison of DNA isolation methods from blood for use in trypanosome specific polymerase chain reaction

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Over the last few years, oligonucleotide primers have been designed from repetitive DNA sequences which can be used for the sensitive detection of trypanosome DNA in blood samples[2,3,4]. An important aspect in the use of PCR for the detection of trypanosome DNA is sample preparation. The optimal procedure should generate template DNA in sufficient concentration and purity, it should be fast and inexpensive, and it should comprise only a few steps, in order to prevent cross contamination. The aim of the present study was to evaluate three different methods for DNA isolation employing a PCR method for the specific detection of *Trypanosoma congolense* (Type savannah) as an example[4].

**Methods and results.** In comparison to several standard methods (table 1) PCR is extremely sensitive for the detection of *T. conglense* (Type savannah); 0.008 pg of isolated trypanosome DNA, which amounted to 10% of the total DNA content of a single cell, still leads to a specific amplification product of 326 bp. However, sample preparation for the PCR can be costly and time consuming. Therefore, the following three methods of sample preparation have been evaluated: (i) isolation of cell nuclei and subsequent proteinase K digestion[1], (ii) DNA extraction employing $SiO_2$ [5] and (iii) DNA isolation after phenol extraction[4].

Table 1. Sensitivity of different parasitological standard techniques (blood smear, haematocrit centrifugation technique [HCT], mini-anion exchange centrifugation technique [m-AECT] and polymerase chain reaction [PCR]).

| Concentration (parasites/ml) | Blood smear (parasites/field)[1] | HCT (parasites) | m-AECT (parasites) | PCR |
|---|---|---|---|---|
| $5 \times 10^4$ | 1/100 | +++[2] | n.d.[3] | n.d. |
| $10^4$ | 0/100 | ++ | ++++ | n.d. |
| $5 \times 10^3$ | n.d. | 1 | +++ | n.d. |
| $10^3$ | n.d. | 1 | +++ | n.d. |
| $5 \times 10^2$ | n.d. | 0 | ++ | positive |
| $10^2$ | n.d. | 0 | + | positive |
| $5 \times 10^1$ | n.d. | n.d. | + | positive |
| $10^1$ | n.d. | n.d. | 2 | postive |
| 5 | n.d. | n.d. | 0 | positive |
| 1 | n.d. | n.d. | n.d. | positive |
| 0 | n.d. | n.d. | n.d. | negative |

[1] Magnification: ×250.
[2] Number of detected parasites: ++++ > 100, +++ = 51–100, ++ = 11–50, + = 5–10.
[3] n.d. = not done.

If the complete parasites are present in blood samples, all the DNA extraction methods tested are suitable for the detection of a single cell by PCR. If the blood samples only contain free parasite DNA, the $SiO_2$ and the phenol extraction method lead to the best results. By limiting dilution, 0.08 pg of the parasite DNA is detectable by PCR employing these two techniques. The more simple and rapid sample preparation method based on the isolation of cell nuclei is less sensitive. Using this method a detection limit of 0.8 pg of trypanosome DNA could be achieved.

**Conclusions.** The three methods tested are convenient for the preparation of samples to be analyzed by PCR for the repetitive satellite DNA sequences of trypanosomes. Despite a slightly reduced sensitivity of detection of free trypanosome DNA, the preparation method based on the isolation of cell nuclei seems to be the most suitable and rapid technique for the routine analysis of a large number of blood samples.

1 Erlich, H. A., PCR-Technology: Principles and Applications for DNA Amplification. Stockton Press, New York 1989.
2 Majiwa, P. A. O., Maina, M., Waitumbi, J. N., Mihok, S., and Zweygarth, E., Parasitology *106* (1993) 151.
3 Masiga, D. K., Smyth, A. J., Hayes, P., Bromidge, T. J., and Gibson, W. C., Int. J. Parasitol, *22* (1992) 909.
4 Moser, D. R., Cook, G. A., Ochs, D. E., Bailey, C. P., McKane, M. R., and Donelson, J. E., Parasitol. *99* (1989) 57.
5 Zeilinger, R., Schneeberger, C., Speiser, P., and Kury, F., Biotechniques *14* (1993) 202.