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

G. Schares[a], S. Kumar[a] and S. Steuber[b]

[a]Institut for Parasitology and Tropical Veterinary Medicine, Free University, Königsweg 67, D-14163 Berlin (Germany)
[b]Federal Institute for Health Protection of Consumers and Veterinary Medicine, Diedersdorfer Weg 1, D-12277 Berlin (Germany)

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
5×10^4	1/100	+++[2]	n.d.[3]	n.d.
10^4	0/100	++	++++	n.d.
5×10^3	n.d.	1	+++	n.d.
10^3	n.d.	1	+++	n.d.
5×10^2	n.d.	0	++	positive
10^2	n.d.	0	+	positive
5×10^1	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.