

# Detection of all human *Plasmodium* species by a telomeric DNA fragment cloned from *Plasmodium berghei*

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*A telomeric DNA fragment that was cloned from Plasmodium berghei was used to detect the genomic DNA of P. falciparum, P. vivax, P. malariae, and P. ovale. The fragment hybridized to the DNA of all four of these human Plasmodium species and can be used as an interspecific probe to detect human malaria.*

Probes that specifically recognize plasmodial DNA are potential diagnostic tools in malaria research and primary health care (1). In addition to the available probes that recognize only a single *Plasmodium* species, it would be useful to find interspecific probes that could be used to detect the presence of malaria parasites in peripheral blood in instances where determination of the particular species is not required.

A possible candidate for interspecific recognition of plasmodial DNA is the telomeric probe that has recently been cloned from *P. berghei* DNA (2). This probe, which we have used to investigate the organization of the plasmodial genome, appears to cross-hybridize with DNA from *P. chabaudi* and *P. yoelii* as well as with DNA from the human malaria parasite *P. falciparum* (3).

Here, we report the results of an investigation to determine whether the telomeric sequences of the human plasmodia *P. vivax*, *P. malariae*, and *P. ovale* are conserved—a feature that would make the probe a useful interspecific tool for *Plasmodium* species.

## MATERIALS AND METHODS

### Parasites

Human plasmodia were obtained from aliquots of infected blood that had been frozen in liquid nitrogen according to standard procedures (4).

*P. malariae* parasites were isolated from samples of blood taken from an experimentally infected chimpanzee (ISIS-TAN-strain), while the other plasmodial species were obtained from samples of human peripheral blood from various geographical origins. After

the blood had been thawed and carefully washed in hypertonic salt solutions, the erythrocytes were washed twice in phosphate-buffered saline (PBS) and a smear was made to determine the parasitaemia level. Serial twofold or tenfold dilutions for blood spots were made using uninfected human blood to give parasitaemia levels that ranged from 4 to  $4 \times 10^5$  per spot. Plasmid twofold dilutions were performed in PBS.

### Preparation of blood spots

The procedure followed to prepare blood spots was that described by Mucenski et al. (5).

### Preparation of lysed blood spots

A sample of infected blood (100  $\mu$ l) was added to 100  $\mu$ l of TET buffer (100 mmol/l Tris-hydrochloride (pH 8.8), 10 mmol/l ethylenediaminetetraacetic acid, 0.4% Triton X-100, and 200  $\mu$ g/ml proteinase-K) and incubated for 90 minutes at 37 °C. To this solution was then added 200  $\mu$ l of 0.6 mol/l sodium hydroxide and the mixture was incubated at 0 °C for 5 minutes. Subsequently, 400  $\mu$ l of 2 mol/l ammonium acetate (pH 7.4) was added. The mixture was centrifuged in an Eppendorf microcentrifuge for 10 minutes, the supernatant spotted onto nitrocellulose paper using a Schleicher and Schull manifold apparatus, and the spots were washed with a 1-mol/l solution of ammonium acetate (pH 7.4). The procedure could be used for up to 200  $\mu$ l of infected blood.

### The DNA probe

The insert of the original telomeric clone (2) contains a stretch of highly repetitive species-specific *P. berghei* DNA along with about 500 base pairs (bp)

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of a tandemly repeated CCCT $\delta$ AA heptanucleotide, which is the telomeric sequence common to other *Plasmodium* species (3). The insert was reduced using *Bal31* nuclease and cloned in the vector pEMBL 9 (6). The new clone obtained (pBBal10) contains an insert of 430 bp of the heptanucleotide repeats (T. Pace et al., unpublished results).

#### Labelling procedure

A sample of the DNA fragment (0.1–0.5  $\mu$ g) containing the telomeric array of heptanucleotide repeats was labelled by conventional nick translation using  $3.7 \times 10^6$  Bq of [ $\alpha$ - $^{32}$ P]-dATP (approximately  $10^{15}$  Bq/mmol).<sup>a</sup> The specific activity of the labelled probe was  $1\text{--}3 \times 10^8$  counts per minute per  $\mu$ g of DNA.

#### Hybridization

Nitrocellulose filter-papers that had been spotted with samples of normal and pre-lysed blood were pre-hybridized and hybridized using the procedure described by Maniatis et al. (7). The last washing step was carried out under stringent conditions (0.1 $\times$  standard saline citrate (SSC), 0.1% sodium dodecyl sulfate (SDS) at 65 °C for 30 minutes).

Autoradiographs were recorded on commercially available X-ray films.<sup>b</sup> Exposure times ranged from 12 hours to 96 hours.

### RESULTS

The sensitivity of the telomeric probe was comparable for the four human *Plasmodium* species and ranged from  $4 \times 10^3$  to  $1.5 \times 10^4$  parasites per 10  $\mu$ l spot of whole blood (Fig. 1). Lower levels of parasites could not be clearly detected with the probe, the resultant spots being difficult to visualize against the background (see Fig. 1, row 2 (arrow)). Also, when 100  $\mu$ l of lysed human blood was spotted (Fig. 2a), the signal intensities were similar to those obtained with samples of 10  $\mu$ l whole blood containing the same number of parasites, and the spot corresponding to 5000 parasites was clearly positive (see Fig. 2a, second spot from the left in rows 2 and 3). If, however,  $10^4$  parasites are taken to be the minimum number that can be reliably detected using the method, a parasitaemia level of 0.02% in 10  $\mu$ l whole blood and 0.002% in 100  $\mu$ l of lysed blood could be detected (assuming an erythrocyte count of  $5 \times 10^9$  per ml blood). The rodent parasites *P. berghei* and

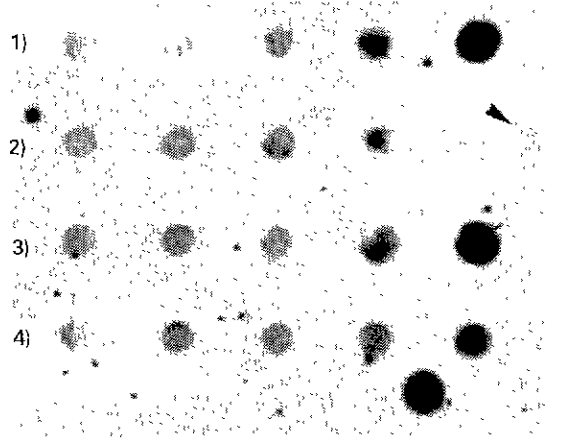


Fig. 1. Autoradiographs of tenfold dilutions of human whole blood infected with *Plasmodium* species. See text for details. 1) *P. vivax*:  $10\text{--}10^5$  parasites/spot. 2) *P. ovale*:  $20\text{--}2 \times 10^4$  parasites/spot (the spot indicated by the arrow is uninfected human blood). 3) *P. malariae*:  $15\text{--}1.5 \times 10^5$  parasites/spot. 4) *P. falciparum*:  $4\text{--}4 \times 10^5$  parasites/spot.

*P. yoelii* could also be detected using the method at similar levels to that of *P. falciparum* (Fig. 2b).

In order to estimate the average copy number of the telomeric sequence per haploid plasmodial genome, serial dilutions of the pBBal10 plasmid DNA were made, and the results are shown in Fig. 2b (row 1). As deduced by comparison of the hybridization spots after densitometric analysis of the film, the copy number ranged from 30 to 60. Since the probe consists of 61 heptamer repeats, these results indicate that one haploid plasmodial genome contains between 1800 and 3600 CCCT $\delta$ AA sequence repeats.

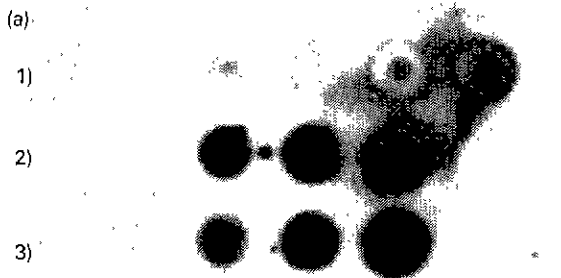


Fig. 2a. Autoradiographs of tenfold dilutions of lysed human infected blood containing *Plasmodium* species. See text for details. 1) *P. ovale*: 40 up to  $4 \times 10^6$  parasites/spot. 2) *P. malariae*: 500 up to  $5 \times 10^6$  parasites/spot. 3) *P. vivax*: 500 up to  $5 \times 10^6$  parasites/spot.

<sup>a</sup> Available from New England Nuclear, Postfach 401240, D-6072 Dreieich, Federal Republic of Germany

<sup>b</sup> Kodak X-OMAT or Fuji.

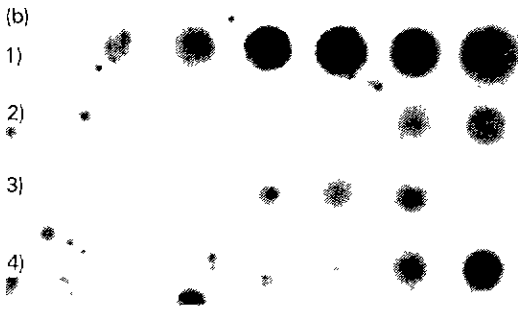


Fig. 2b. Autoradiographs of twofold dilutions of lysed murine or human infected blood containing *Plasmodium* species and, for comparison, a serial dilution of pBBal10 plasmid. 1) pBBal10 plasmid:  $3.1 \times 10^5$  up to  $2 \times 10^7$  molecules/spot. 2) *P. falciparum*:  $6.2 \times 10^2$  up to  $4 \times 10^4$  parasites/spot. 3) *P. yoelii*:  $6.2 \times 10^2$  up to  $2 \times 10^4$  parasites/spot. 4) *P. berghei*:  $6.2 \times 10^2$  up to  $4 \times 10^4$  parasites/spot.

#### DISCUSSION

The telomeric probe, which was cloned from the rodent malaria parasite *P. berghei*, cross-hybridized with other rodent malaria parasites and also with the four human *Plasmodium* species, indicating that the telomeric sequence is highly conserved in the plasmodial genome. The probe can therefore be used for interspecific detection of plasmodial DNA. In addition, until specific probes for *P. malariae* and *P. ovale* become available, the probe described can also be used in epidemiological studies of infections

caused also by these two species. The copy number of the telomeric sequence appears to be 2000–4000 per genome, and, as a consequence, its sensitivity for detecting *Plasmodium* species is lower than that reported for highly repetitive specific probes for *P. falciparum*: it is, however, in the same range as that described for other repetitive DNA probes (1). Use of alternative labelling procedures, e.g., random priming or primer elongation, did not increase the specific activity of the probe. Recently, a probe has been described that was cloned from *P. yoelii* DNA and cross-hybridized with several other species, including *P. falciparum* (8). It would be of interest to investigate whether this probe hybridizes with the DNA of the remaining three human plasmodia.

The probe that we have described is the only one yet reported that can recognize all four human *Plasmodium* species. In addition, Vernick & McCutchan have recently shown that the consensus of the telomeric sequence of *P. falciparum* is identical with that of *P. berghei* (9), which confirms the highly conserved character of this structure in the plasmodial chromosomes.

It was also examined whether the telomeric probe hybridized with genomic DNA from several other protozoan blood parasites. For example, with *Trypanosoma brucei rhodesiense* (IBADAN 73) and *Leishmania infantum* (MCAH/IT/82) no hybridization was observed; however, the probe hybridized readily with chromosomal DNA from *Babesia* spp. as indicated by pulsed field gradient electrophoresis. Cross-hybridization with the last-mentioned parasite should not, however, interfere with the practical use of the telomeric probe for epidemiological studies.

#### ACKNOWLEDGEMENTS

We are grateful to Wies van der Star for technical assistance and to Dr S. Panyim for the protocol for preparing the lysed blood spots. Dr Luigi Gradoni, Dr Marina Gramiccia, and Dr Gordon Langsley are thanked for supplying the data on non-plasmodial blood parasites. The work was supported by the WHO Malaria Action Programme and by the Commission of the European Community under the Programme for Science and Technology for Development (contract number TSD.M150).

#### RÉSUMÉ

DÉTECTION DE TOUTES LES ESPÈCES HUMAINES DE PLASMODIES  
PAR UN FRAGMENT D'ADN TÉLOMÉRIQUE CLONÉ À PARTIR DE *PLASMODIUM BERGHEI*

Un fragment télomérique cloné d'ADN de *Plasmodium berghei* a été utilisé comme sonde pour détecter l'ADN de la totalité du génome des parasites du paludisme humain, *P. falciparum*, *P. vivax*, *P. malariae*, et *P. ovale*.

Des échantillons de sang contenant les parasites ont été soit déposés sur des filtres de nitrocellulose et lysés *in situ*, soit lysés en suspension puis déposés sur les filtres. La sonde a reconnu l'ADN génomique de toutes les espèces de

plasmodies humaines avec une sensibilité égale, ce qui indique que le nombre de copies de la séquence télomérique est à peu près le même chez les plasmodies parasites des rongeurs et de l'homme. La sensibilité de la sonde a été de 4000 à 10 000 parasites par échantillon, c'est-à-dire légèrement inférieure à la sensibilité moyenne signalée pour des

sondes hautement répétitives spécifiques de *P. falciparum*. La sonde télomérique peut servir à détecter la présence de parasites du paludisme humain dans le sang périphérique, notamment *P. malariae* et *P. ovale*, pour lesquels il n'existe pas encore de sondes d'ADN spécifiques.

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