JOURNAL OF CLINICAL MICROBIOLOGY, May 1987, p. 791–795
0095-1137/87/050791-05$02.00/0

Vol. 25, No. 5

Comparison of Genomic, Plasmid, Synthetic, and Combined DNA Probes for Detecting *Plasmodium falciparum* DNA

G. L. McLAUGHLIN,* W. E. COLLINS, AND G. H. CAMPBELL

Malaria Branch, Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333

Received 17 October 1986/Accepted 19 January 1987

Total genomic *Plasmodium falciparum* DNA, the plasmid clone pRepHind, and a 21-base-long synthetic DNA probe (PFR1), the sequence of which was derived from pRepHind, were hybridized with DNA from various species of the phylum *Apicomplexa*. The genomic probe hybridized with *P. reichenowi* and *P. falciparum* DNA and significantly cross-hybridized with DNA of all the other *Plasmodium* species tested. The synthetic and plasmid probes hybridized to *P. falciparum* DNA and at reduced levels to *P. reichenowi* but did not hybridize to *P. vivax*, *P. malariae*, *P. ovale*, *P. fragile*, *P. inui*, *P. knowlesi*, *Babesia bovis*, *B. microti*, *B. bigemina*, *Anopheles* sp., *Pan* sp., *Aotus* sp., or human DNA. Southern blot analysis indicated that approximately 60 distinct restriction enzyme fragments from *P. falciparum* DNA were similarly detected by PFR1 and pRepHind. A method was developed by using a second brief hybridization with synthetic DNA to amplify signals from samples that were previously hybridized with plasmid-borne repetitive DNA. This amplification procedure was shown to allow the detection of 0.005% *P. falciparum* parasitemias from 10-μl samples of blood from patients in Kenya.

With few exceptions, biological functions have not been determined for the highly repetitive DNA sequences that constitute a large percentage of eucaryotic genomes (1). Such repetitive DNA, however, is an excellent target for DNA-based diagnosis of infections caused by eucaryotic pathogens, because its abundance and rapid evolution allow sensitive and specific detection. Several plasmid clones have been isolated that may be suitable as oligonucleotide-based diagnostic probes for detection of *Plasmodium falciparum* (3, 4, 7–9, 16). Several of these clones have been sequenced and have been shown to contain a family of highly repetitive, somewhat degenerate 21-base-long DNA sequences (3, 8, 16). These tandemly repeated sequences are estimated to be present in about 10^4 to 10^5 copies per nucleus, or about 1 to 10% of the DNA of the *P. falciparum* genome (3), and apparently are represented on all of the resolved *P. falciparum* chromosomes (16). It is possible that this 21-base-long repeat family represents the major group of species-specific repetitive DNA in the *P. falciparum* genome.

We have demonstrated previously that a single-stranded synthetic DNA oligomer derived from one of the sequences from the plasmid pRepHind originally reported by Franzen et al. (8) could also hybridize with and detect *P. falciparum* DNA (14). No hybridization was detected with *P. vivax*, host, or *Babesia bovis* or *B. microti* DNA. The synthetic DNA probe displayed an unusually low background, allowing exposures for at least 1 week without detection of heterologous DNA. Also, hybridizations and washes could be performed at convenient temperatures, such as 37°C or room temperature.

In this study we compared the ability of genomic (19), synthetic (14), and plasmid-borne (8) DNA probes to hybridize with DNA of 10 species of organisms in the phylum *Apicomplexa*, 4 species of primates, and 1 species of insect. We also describe the use of a combination of plasmid and synthetic DNA probes to detect low numbers of *P. falciparum* parasites from field specimens.

MATERIALS AND METHODS

DNA isolation. Genomic DNA was isolated from leukocytes of uninfected humans or nonhuman primates (*Pan* and *Aotus* spp.) and from the mosquito host *Anopheles gambiae* by proteinase K digestion and phenol extraction (14). DNA was isolated from *P. falciparum* Honduras I/CDC clone B3, which was grown in cultures (10) from which most of the human leukocyte DNA was removed by three 10-min, 1,100 × *g* centrifugations and aspirations in which 70% of the erythrocytes were also removed. DNA from other malaria species was isolated from 3 to 50 ml of parasitized blood in which parasitemias ranged between 0.1 and 40%. *P. reichenowi*- and *P. malariae*-infected blood was placed in short-term cultures and incubated until most of the parasites matured to the schizont stage before DNA isolation. *P. vivax* and *P. ovale* parasites were separated from the remaining leukocytes by Percoll gradients (2). Infected erythrocytes of other *Plasmodium* species were separated from leukocytes by combinations of Percoll gradients, Whatman columns (CF11; Whatman, Inc., Clifton, N.J.) (5), or alpha-cellulose columns (C8002; Sigma Chemical Co., St. Louis, Mo.) (20). DNA from *B. bovis*, *B. bigemina*, and *B. microti* was isolated as described previously (15). The ratio of parasite nuclei to leukocytes in the final preparations exceeded 100:1, except for samples infected with *P. ovale* (20:1) and *P. malariae* (50:1). After lysis of erythrocytes by treatment with saponin, the parasites were digested with proteinase K in the presence of 0.5% sodium dodecyl sulfate and extracted with phenol, phenol-chloroform, and chloroform; and DNA was precipitated several times with ethanol. The plasmid clone pRepHind was supplied by Ulf Pettersson and Lena Franzen, University of Uppsala, Uppsala, Sweden (3, 8). The plasmid was propagated in *Escherichia coli* HB101 in the presence of ampicillin, and plasmid DNA was isolated by the alkaline lysis procedure (13).

* Corresponding author.

Species specificity. DNA preparations were dissolved in 10 mM Tris buffer (pH 8.0) containing 1 mM EDTA. Concentrations were adjusted to 10 ng/μl by determining the A_{260} and UV fluorescence of gel-separated DNA after ethidium bromide staining in comparison with known standards. In the cases of *P. ovale*, *P. malariae*, and *P. reichenowi*, the estimate of 0.05 pg per parasite nucleus (3) was used in the purified infected erythrocyte preparations. For spot-blot experiments, duplicate filters (pore size, 0.45 μm; Pall Biodyne; ICN Pharmaceuticals Inc., Irvine, Calif.) were each spotted with rows of 1-μl fractions containing 10, 1.0, 0.1, and 0.01 ng of host or parasite DNA, as described previously (15), and were processed in parallel.

Filter processing. Filters were incubated for 3 min over blotting paper saturated with 1.5 M NaCl–0.5 M NaOH, neutralized for 3 min over 3 M sodium acetate, (pH 5.2), dried, baked for 1 h at 55°C under vacuum, and incubated for 2 h at 55°C in a modified blocking buffer (11) (0.5% nonfat dry milk, 0.01% Merthiolate, 0.2 mg of sonicated calf thymus DNA per ml, 2× SSPE [14], 0.05% sodium dodecyl sulfate) before labeled probes were added.

Probe labeling and hybridization. The synthetic DNA oligonucleotide PFR1 (5′-AGGTCTTAACTTGACTA ACAT-3′) (0.1 to 1.0 μg) was labeled by using T4 kinase (New England Nuclear Corp., Boston, Mass., or New England BioLabs, Inc., Beverly, Mass.) and 7,000 Ci of [γ-^{32}P]ATP (New England Nuclear) per mM. After treatment with kinase, the synthetic DNA was heated for 5 min in a boiling water bath, cooled to room temperature, and processed through columns (Elutip-D; Schleicher & Schuell, Inc., Keene, N.H.). Specific activities of kinase-labeled probes ranged from 5×10^8 to 1×10^9 dpm/μg. The probes were hybridized to DNA on filters in blocking buffer for 1 h at 37°C, and filters were rinsed at room temperature and washed three times, 5 min per wash, with shaking in 300 ml of 2× SSPE at 39°C before autoradiography was carried out at −80°C with enhancer screens.

The genomic *P. falciparum* probe and the plasmid probe pRepHind were labeled with a nick-translation kit (Worthington Diagnostics, Freehold, N.J.) and [α-^{32}P]ATP (3,000 Ci/mM). The specific activities of nick-translated probes ranged from 6×10^7 to 1×10^8 dpm/μg. Samples were processed through columns (Elutip-D), denatured in a boiling water bath for 10 min, chilled on ice, added to blocking buffer, and hybridized for 12 h at 58°C. After hybridization, filters were rinsed at room temperature and washed three times, 10 min per wash at 58°C in 300 ml of 2× SSPE before autoradiography was carried out at −80°C with enhancer screens.

Southern blot analysis. Genomic *P. falciparum* DNA (1 μg) digested with *Bgl*II, *Hin*dIII, or *Sau*3A was electrophoresed in a 0.7% agarose gel. The gel was blotted to a filter (Pall Biodyne), and the filter was hybridized for 1 h with PFR1. After autoradiography, the PFR1 probe was removed by two 5-min incubations in distilled water at 80°C, and probe removal was confirmed by autoradiography before hybridization with pRepHind and a second autoradiographic exposure.

Sequential hybridization. In preliminary experiments many different mixtures and sequential hybridizations of the three probes were tested. The highest sensitivity and specificity were obtained with an initial plasmid hybridization and wash, followed by a second hybridization with the synthetic DNA probe. After the plasmid-hybridized filter was washed twice at 58°C, the filter was incubated for 10 min in blocking buffer at 58°C, hybridized for 1.5 h at 37°C with PFR1, washed twice at 37°C in 2× SSPE, and exposed for autoradiography. Results of this dual probe hybridization were compared with those of single probe hybridizations using duplicate filters containing dilutions of *P. falciparum* DNA.

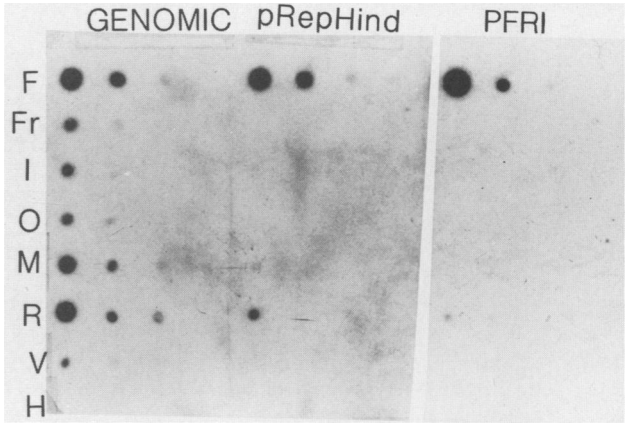

FIG. 1. An autoradiogram of three filters that were processed in parallel and separately hybridized with genomic, pRepHind, and PFR1 probes, respectively. Four 10-fold dilutions of *Plasmodium* (10, 1, 0.1, and 0.01 ng) were spotted for each filter in each row. Rows labeled F, Fr, I, O, M, R, and V rows contain *P. falciparum*, *P. fragile*, *P. inui*, *P. ovale*, *P. malariae*, *P. reichenowi*, and *P. vivax* DNA, respectively. Row H contains 10-ng spots of *Homo* sp., *Aotus* sp., *Pan* sp., and *Anopheles* sp. DNA (left to right).

The sequential hybridization procedure was also compared with single hybridizations in a screen of 40 10-μl blood samples from patients in Kenya. These blood samples were processed by a procedure modified from that of Barker et al. (4). Briefly, the infected blood cell pellet was digested in 200 μl of 50 mM Tris buffer (pH 10.0) containing 0.1 mg of proteinase K per ml, 0.2% Triton X-100, and 10 mM EDTA. After the addition of 200 μl of 0.6 M NaOH, samples were filtered through a dot-blot apparatus (Bio-Rad Laboratories, Richmond, Calif.), and the nylon filters were processed for hybridization as described above. Blood samples (10, 50, and 100 μl) from patients in Peru that lacked *P. falciparum* but that contained *P. vivax* or other malaria parasites were processed in parallel as negative controls; and 1.0-, 0.1-, and 0.01-ng amounts of *P. falciparum* DNA were spotted on filters as positive controls.

RESULTS

Specificity and sensitivity. All three probes produced strong autoradiographic signals when hybridized with *P. falciparum* DNA, achieving detection of 0.1 ng of DNA after only a 4-h exposure (Fig. 1, row F). The genomic *P. falciparum* probe also hybridized with the DNA of the other *Plasmodium* species, although not with mosquito DNA or human or nonhuman primate DNA (Fig. 1, row H). The genomic probe hybridized equally well or slightly more to *P. reichenowi* DNA than to *P. falciparum* DNA (cf. rows F and R, Fig. 1). The plasmid and synthetic probes also detected *P. reichenowi* DNA, but with about 10-fold less sensitivity than they detected *P. falciparum* DNA (cf. rows R and F, Fig. 1). None of the other six *Plasmodium* species was detected at this level of sensitivity by either the synthetic or the plasmid probes.

Except for *P. reichenowi*, both the synthetic and the plasmid probes were specific for *P. falciparum*. The other parasite and host DNAs tested were not detected. The ratios

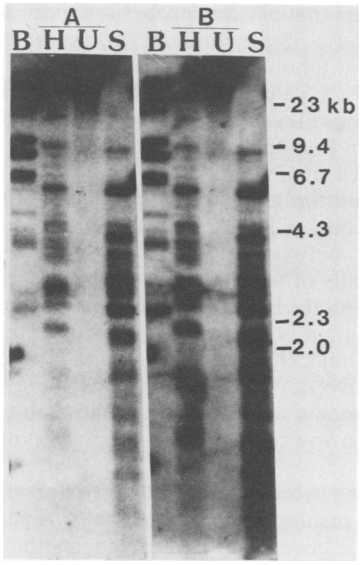

FIG. 2. Two 12-h autoradiograms of a Southern blot hybridized either with pRepHind (A) or with PFR1 (B). *P. falciparum* DNA was cut with *Bgl*II, *Hin*dIII, or *Sau*3A restriction enzymes or was left untreated (lanes B, H, S, and U, respectively) before electrophoresis, transfer, and hybridization. Motility and molecular size (in kilobases [kb]) of *Hin*dIII-digested lambda DNA is indicated to the right of the autoradiograms.

of parasite to host nuclei obtained in the parasite preparations indicate that significant amounts of DNA from these *Plasmodium* species were applied to these filters. Additional evidence that significant amounts of heterologous parasite DNA were contained on these filters is provided by the cross-hybridization of the genomic *P. falciparum* probe with malaria DNA preparations, but not with host DNA preparations.

Southern blot analysis. Two autoradiogram exposures of a Southern blot of *P. falciparum* DNA that was undigested (Fig. 2, lane U) or digested with *Bgl*II, *Hin*dIII, or *Sau*3A restriction enzymes (Fig. 2, lanes B, H, and S, respectively) are shown. The filter was hybridized with PFR1 (Fig. 2B), washed free of probe, and then hybridized with pRepHind (Fig. 2A). At least 60 separate restriction fragments from the *P. falciparum* genome were detectable in the *Bgl*II, *Hin*dIII, and *Sau*3A lanes by either the pRepHind or PFR1 probes. The plasmid and synthetic probes detected DNA fragments that were nearly identical both in mobility and relative intensity of signal.

Synthetic DNA amplification. An autoradiogram exposure of three small filters containing duplicate dilutions of *P. falciparum* DNA is shown (Fig. 3). The plasmid (middle) and dual (lower) filters were hybridized with pRepHind and washed together; and then the lower filter was removed, briefly blocked, and hybridized with labeled PFR1. The synthetic (top) filter was hybridized with PFR1 during the second hybridization of the dual filter. The filters were exposed together for 14 h. As shown in the exposures from the top and middle filters (Fig. 3), the plasmid and synthetic DNA probes strongly detected 0.1 ng of *P. falciparum* DNA. The dual hybridization with plasmid and synthetic DNA probes detected 0.01 ng of *P. falciparum* DNA, and we estimate that signals were amplified about five times compared with those from the single probes.

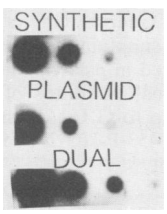

FIG. 3. Duplicate dilutions (10, 1, 0.1, and 0.01 ng) of *P. falciparum* DNA on three separate filters were hybridized either with pRepHind alone (plasmid), PFR1 alone (synthetic), or with both probes in sequence (dual) and were exposed for 12 h.

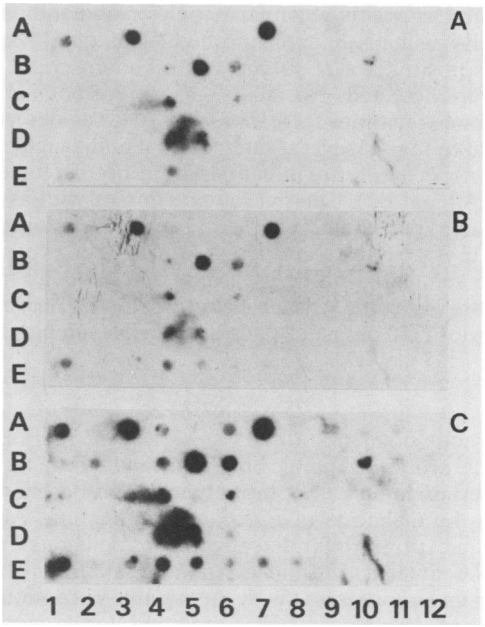

FIG. 4. Three 12-h autoradiogram exposures are shown of the same filter containing 40 10-μl blood samples from patients in the Uriri village, Kenya. (A) PFR1. (B) pRepHind. (C) pRepHind followed by a second hybridization with PFR1. Mixed infections had *P. falciparum* and *P. malariae*. The number of infected erythrocytes per cubic millimeter in samples that contained *P. falciparum* are as follows: A1, 3,300 (mixed); A2, 0; A3, 11,000; A4, 100; A5, 0; A6, 90; A7, 13,500; A8, 50 (mixed); A9, 5,700 (mixed); A10, 600 (mixed); A11, 120; A12, 0; B1, 2,400; B2, 819; B3, 200; B4, 4,500 (mixed); B5, 28,000; B6, 3,200; B7, B8, 0; B9, 800; B10, 2,300; B11, 300; B12, 400; C1, 100; C2, 60; C3, 500; C4, 16,000 (mixed); C5, 120; C6, 1,400; C7, 300; C8, 1400; E1, 3,400; E2, 150; E3, 640; E4, 1,250 (mixed); E5, 1,000; E6, 150; E7, 870; E8, 1,800. Samples that contained only *P. malariae* had 1,600 (A2), 800 (A5), 2,260 (A12), 3,600 (B7), 5,500 (B8), 1,250 (E4) infected erythrocytes per mm^3. Also included were identically processed 10- to 100-μl blood samples from patients in Peru infected with *P. malariae* or *P. vivax* but not *P. falciparum* (C9 to D3; D7 to D12) and 1-μl aliquots containing 1, 0.1, and 0.01 ng of *P. falciparum* DNA (D4 to D6) that were directly spotted without processing.

Three 12-h exposures of a filter containing 40 10-μl blood specimens from patients in Kenya are also shown (Fig. 4). In Fig. 4A the filter was hybridized with kinase-labeled PFR1. After removal of the PFR1 probe, the filter was hybridized with pRepHind (Fig. 4B). In Fig. 4C the filter used for Fig. 4B was additionally hybridized for 1.5 h with the same PFR1 probe that was used for the top exposure and was re-exposed for 12 h. With 12-h exposure times the synthetic and plasmid DNA probes detected 22 and 26 samples, respectively, that

were scored as positive by microscopic examination of thick smears. By using sequential probes 32 of the 34 samples scored as positive for *P. falciparum* by microscopic examination were detected. No false signals were obtained from blood samples without *P. falciparum*. Also, as described in the legend to Fig. 4, several samples containing high parasite counts with *P. malariae* infections were not detected (e.g., samples A2 and B7). Ten-microliter samples with as few as 90 parasites per mm^3 (Fig. 4C, sample A6) or 0.003% infected erythrocytes were detected in a 12-h exposure by using the sequential probe hybridization, although the reliable sensitivity limit was probably about 0.005% infected erythrocytes (150 parasites per mm^3; Fig. 4, samples E2 and E6).

DISCUSSION

We directly compared three types of *P. falciparum* oligonucleotide probes: genomic, plasmid, and synthetic DNA. Within certain limits, all of these types of probes may prove useful in the diagnosis of *Plasmodium* infection. Although the genomic probe hybridized with heterologous *Plasmodium* DNA, at least a 10-fold signal differential was observed for other malaria species, with the notable exception of the closely related and rare chimpanzee and gorilla parasite *P. reichenowi* (6). Because the genomic probe hybridized almost identically with *P. falciparum* and *P. reichenowi*, it appears that many of the DNA sequences of these species are closely related. Unlike the genomic probe from our previous *P. falciparum* DNA preparation (14), the genomic probe used here did not detect 10-ng amounts of host DNA, although it did detect 1-μg amounts of human DNA (data not shown). This reflects the importance of the purity of the parasite preparation with respect to contaminating host DNA.

The crucial advantage of plasmid and synthetic DNA probes is that they are specific for the most pathogenic malaria species *P. falciparum*, except for a minimal hybridization with the rare *P. reichenowi* species. Unlike the genomic probe, plasmid and synthetic DNA hybridized at least 10-fold less with *P. reichenowi* DNA compared with *P. falciparum* DNA. Also, for PFR1 this cross-hybridization could be eliminated by using 43°C washes, which had little effect on the hybridization signals from *P. falciparum* (data not shown). It therefore appears that the 21-base-long family of repetitive sequences has changed much more rapidly between *P. falciparum* and *P. reichenowi* than have most of the genomic sequences. Such rapid evolution is frequently reported for highly repetitive DNA (17). It appears, however, that the PFR1 family of sequences remains sufficiently conserved within the species to detect *P. falciparum* from at least three continents (3, 14, 16).

Results of Southern blot analysis suggest that the family of repetitive DNA detected by PFR1 represents the dominant, highly repeated sequence family in the *P. falciparum* genome that is contained in the pRepHind clone. If the pRepHind probe hybridized with abundant classes of *P. falciparum* repeats not found by the synthetic PFR1 probe, it is likely that additional repetitive bands would be detected or that the relative intensity of some of the restriction fragments detected would be significantly different between the two probes. The Southern blot results suggest that PFR1 is able to recognize similarly the repetitive DNA sequences of *P. falciparum*. Because many of the 21-base-long repeats of *P. falciparum* are slightly degenerate (3, 8, 16), many of the oligomers probably remain hybridized with less than 21 hydrogen-bonded nucleotide pairs.

It also appears that plasmid probes are more sensitive than synthetic probes. The synthetic probes had the highest specific activity (about 10^9 dpm/μg), but equal or slightly stronger signals were usually obtained from plasmid probes with specific activities of about 10^8 dpm/μg. Because results of the Southern blot analysis indicated that the plasmid and synthetic probes are able to detect similarly the same regions of the *P. falciparum* genome, the comparable sensitivity of the plasmid probe of lower specific activity may be due to the tailing or networking effect of long oligonucleotide probes (21). Tails of long DNA probes represent sequences that are not directly hybridized but that are labeled during nick translation, and therefore, they contribute to the signal obtained from adjacent covalently bound hybridized regions of the probe DNA. If plasmid probes were labeled with comparable specific activities, they may achieve about 10-fold-greater sensitivity than corresponding synthetic probes alone.

For obtaining acceptable specificity for plasmid-borne DNA probes, results of preliminary experiments indicated that filters must be washed at stringencies that significantly decreased the hybridization efficiency of the complex and variable-length probes, as has been observed by others (12). Synthetic DNA probes are characterized by a rapid hybridization speed and high specificity (18); these are factors that help to make the sequential hybridization procedure practical. The signal amplification may occur both because the labeled synthetic DNA hybridizes with target *P. falciparum* sequences that have lost plasmid sequences during washing and because the synthetic DNA probe hybridizes to the many PFR1 sequences that are found on tails of the long hybridized plasmid DNA probe (3). Consistent with the latter interpretation, pBR322 DNA fixed to filters is detected after sequential hybridizations with unlabeled pRepHind and ^{32}P-labeled PFR1, but not after sequential hybridizations with unlabeled pBR322 and ^{32}P-labeled PFR1 (data not shown). At the reduced temperature of the synthetic DNA hybridization and wash, few additional plasmid sequences would be removed due to the mass contributed by the synthetic DNA that hybridized to plasmid tails. As demonstrated in the comparative hybridizations with field samples, the enhanced signals obtained by the synthetic DNA amplification procedure may be useful in rapidly detecting low *P. falciparum* parasitemias.

ACKNOWLEDGMENTS

We are grateful to Lena Franzen and Ulf Pettersson, Department of Medical Genetics and Microbiology, Uppsala, Sweden, for generously providing us with pRepHind. We thank Pat Andrysiak, Centers for Disease Control (CDC), Malaria Branch, for skillful isolation of many of the *Plasmodium* species and Jimmy Skinner for help with microscopic examinations. We also thank Ira Schwarz, Frank Collins, and Alexander Sulzer, CDC; A. D. Branding-Bennett, CDC, who is assigned to the Kenya Medical Research Institute; and Humberto Guerra, Universidad Peruana Cayetano Heredia, Lima, Peru, for supplying field specimens.

This study was supported in part by Public Health Service grant RR05814 from the National Institutes of Health, a National Research Council Fellowship to G.L.M., and Participating Agencies Service Agreement BST-0453-P-HC-2086 from the U.S. Agency for International Development.

LITERATURE CITED

1. **Adams, D. S., T. H. Eickbush, R. J. Herrera, and P. M. Lizardi.** 1986. A highly reiterated family of transcribed oligo(A)-terminated, interspersed DNA elements in the genome of

Bombyx mori. Cell **43**:465–478.

2. **Andrysiak, P. M., W. E. Collins, and G. H. Campbell.** 1986. Concentration of *Plasmodium ovale*- and *Plasmodium vivax*-infected erythrocytes from nonhuman primate blood using Percoll gradients. Am. J. Trop. Med. Hyg. **35**:251–254.
3. **Aslund, L., L. Franzen, G. Westin, T. Persson, H. Wigzell, and U. Pettersson.** 1985. Highly reiterated non-coding sequence in the genome of *Plasmodium falciparum* is composed of 21 base-pair tandem repeats. J. Mol. Biol. **185**:509–516.
4. **Barker, R. H., Jr., L. Suebsaeng, W. Rooney, G. A. Alecim, H. V. Dourado, and D. F. Wirth.** 1986. Specific DNA probe for the diagnosis of *Plasmodium falciparum* malaria. Science **231**: 1434–1436.
5. **Beutler, E., C. West, and K.-G. Blume.** 1976. Removal of leucocytes and platelets from whole blood. J. Lab. Clin. Med. **88**:328–333.
6. **Coatney, G. R., W. E. Collins, M. W. Warren, and P. G. Contacos.** 1971. The primate malarias, p. 309–313. U.S. Government Printing Office, Washington, D.C.
7. **Enea, V.** 1986. Sensitive and specific DNA probe for detection of *Plasmodium falciparum*. Mol. Cell. Biol. **6**:321–324.
8. **Franzen, L., R. Shabo, H. Perlman, H. Wigzell, G. Westin, L. Aslund, T. Persson, and U. Pettersson.** 1984. Analysis of clinical specimens by hybridization with a probe containing repetitive DNA from *Plasmodium falciparum*. A novel approach to malaria diagnosis. Lancet **i**:525–528.
9. **Guntaka, R., S. Gowda, A. S. Rao, and T. J. Green.** 1985. Organization of *Plasmodium falciparum* genome. 1. Evidence for a highly repeated DNA sequence. Nucleic Acids Res. **13**:1965–1975.
10. **Jensen, J. B., and W. Trager.** 1977. *Plasmodium falciparum* in culture: use of outdated erythrocytes and description of the candle jar method. J. Parasitol. **63**:853–886.
11. **Johnson, D. A., J. W. Gautsch, J. R. Sportsman, and J. H. Elder.** 1984. Improved technique utilizing nonfat dry milk for analysis of proteins and nucleic acids transferred to nitrocellulose. Gene Anal. Tech. **1**:3–8.
12. **Kafatos, F. C., C. W. Jones, and A. Efstratiadis.** 1979. Determination of nucleic acid sequence homologies and relative concentrations by a dot hybridization procedure. Nucleic Acids Res. **7**:1541–1552.
13. **Maniatis, T., E. F. Frisch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **McLaughlin, G. L., T. D. Edlind, G. H. Campbell, R. F. Eller, and G. M. Ihler.** 1985. Detection of *Plasmodium falciparum* using a synthetic DNA probe. Am. J. Trop. Med. Hyg. **34**:837–840.
15. **McLaughlin, G. L., T. D. Edlind, and G. M. Ihler.** 1986. Detection of *Babesia bovis* using DNA hybridization. J. Protozool. **33**:125–128.
16. **Oquendo, P., P. Goman, M. Machay, G. Langsley, D. Walliker, and J. Scaife.** 1986. Characterization of a repetitive DNA sequence from the malaria parasite, *Plasmodium falciparum*. Mol. Biochem. Parasitol. **18**:89–101.
17. **Peacock, W. J., A. R. Lohe, W. L. Gerlich, P. Dunsmuir, E. S. Dennis, and R. Appels.** 1977. Fine structure and evolution of DNA in heterochromatin. Cold Spring Harbor Symp. Quant. Biol. **42**:1121–1135.
18. **Peterson, E. M., S. L. Aarnaes, R. N. Bryan, J. L. Ruth, and L. M. de la Maza.** 1986. Typing of herpes simplex virus with synthetic DNA probes. J. Infect. Dis. **153**:757–762.
19. **Pollack, Y., S. Metzger, R. Shemer, D. Landu, D. Spira, and J. Golenser.** 1985. Detection of *Plasmodium falciparum* in blood using DNA hybridization. Am. J. Trop. Med. Hyg. **34**:663–667.
20. **Richards, W. H. G., and S. G. Williams.** 1972. Removal of leucocytes from malaria infected blood. Ann. Trop. Med. Parasitol. **67**:249–250.
21. **Singer, R. H., J. B. Lawrence, and C. Villnave.** 1986. Optimization of *in situ* hybridization using isotopic and nonisotopic detection methods. Biotechniques **4**:230–250.