RESEARCH NOTE

Stained Smears as a Source of DNA

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The molecular biological analysis of infectious agents requires the availability of a reliable source of microorganisms to be used to recover DNA. Clinical samples can be obtained directly from infected patients or can be propagated using *in vitro* or *in vivo* systems. However, not infrequently, the repeated sampling from patients is not possible because, among other reasons, the procedure is invasive or fastidious, the treatment has been initiated, the patients do not accept the repetition of the procedure or it is not possible to locate them again. Moreover, the *in vitro* culture of some microorganisms is not possible (e.g. *Plasmodium vivax*) or requires weeks to months (e.g. *Mycobacterium tuberculosis*). Neither do all laboratory settings have access to animal models for *in vivo* systems. Several procedures for DNA extraction from stained specimens and paraffin-embedded tissues have recently been reported. These procedures have yielded DNA from microbial agents in blood stained smears containing *P. vivax* (RS Witzig, RH Barker 1994 *Trans R Soc Trop Med Hyg 88:* 198) or *P. falciparum* parasites (M Kimura et al. 1995 *Mol Biochem Parasitol 70:* 194-197); stained tissue sections and cytological smears containing, respectively, herpesvirus (GT Nahass et al. 1995 *Arch Dermatol 131*: 805-808) and human papillomavirus (AM de Roda Husman et al. 1995 *Br J Cancer 72*: 412-417); and from formalin-fixed, paraffin-embedded tissues containing mycobacteria (HH Popper et al. 1994 *Clin Microbiol Infect Dis 101*: 738-741, KJ Sung et al. 1993 *Internat J Dermatol 32*: 710-713) or *Leishmania aethiopica* (T Laskay et al. 1995 *Trans R Soc Trop Med Hyg 89*: 273-275). The advantages of such a procedure include: (1) use of the sample to make both the microscopic and the molecular studies, and (2) the possibility of conducting retrospective studies from archival samples, especially from unusual or rare pathologies.

We here report the results of the recovery and amplification of microbial DNA from fixed and stained clinical samples including blood, sputum and tissue fluid.

DNA was extracted from Field-stained *P. vivax* thick smears (Malaria Control Program Clinic in Florencia, Caqueta and Cali, Colombia), Ziehl-Neelsen-stained sputum smears containing *M. tuberculosis* (Corporación CIDEIM), and Giemsa-stained smears of tissue fluid and inflammatory exudate containing *Leishmania (Viannia) panamensis* amastigotes (Corporación CIDEIM) using a 5% (wt/vol) solution of Chelex-100^{R} (K Kain et al. 1992 *Am J Trop Med Hyg 46*: 473-479, Bio-Rad-Richmond, CA) as follows: 500 µl of 1% Saponin (Sigma-St.Louis, MO) in water was added to 1.5 ml Eppendorf tubes containing sample material obtained by scrapping the surface of the stained smear using a razor blade. Samples were incubated on ice for 60 min, vortexed 3-4 times and centrifuged for 4 min at 12,000 x g at room temperature. After discarding the supernatant, the pellets were washed in PBS (pH 7.2) until the supernatants were clear, usually 2-3 times. Following the addition of 50 µl of 5% (wt/vol) Chelex-100^{R}, the samples were vortexed, incubated at 56^{o}C for 15 min, vortexed again, and incubated in boiling water for 10 min. After centrifuging at 12,000 x g for 4 min, the supernatant (sometimes colored bluish) was collected and stored at -20^{o}C until used. Refer to the Table for primer description and amplification conditions. All reactions included the use of 5 µl of extracted DNA and were performed on a MJ Research PTC-100 cycler at Corporación CIDEIM.

All samples containing parasites were amplified by the specific set of primers. The Fig. shows the different PCR products analyzed on agarose gel electrophoresis and stained with ethidium bromide. PCR experiments included the following:

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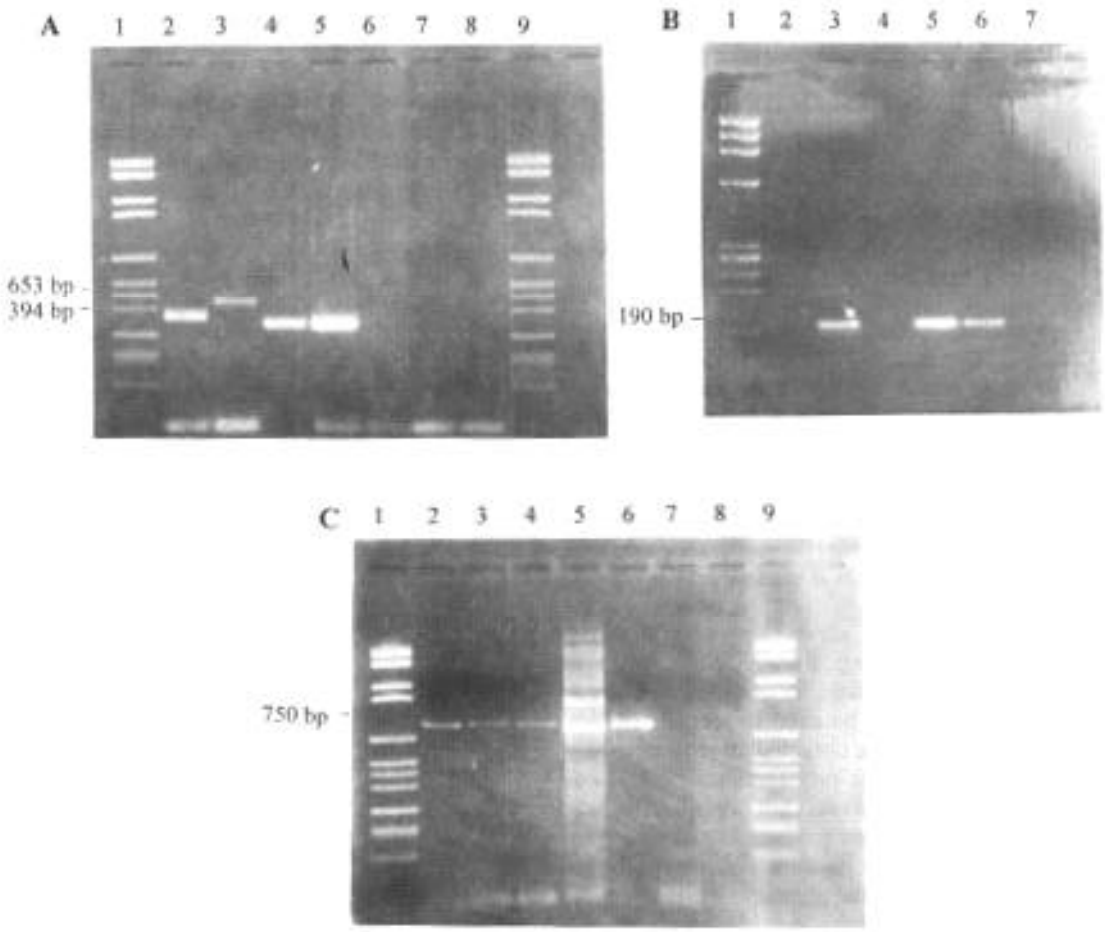

A. Field-stained *Plasmodium vivax* thick smears. Lanes 1,9: molecular weight (MW) standard. Lane 2: 3 parasites/100 white blood cells (WBC). Lane 3: 132 parasites/100 WBC. Lane 4: 54 parasites/100 WBC. Lane 5: filter paper blot (50 µl of blood) of specimen in Lane 4. Lane 6: microscopically negative stained-smear. Lane 7: filter paper sample of negative specimen. Lane 8: reaction control. B**.** Ziehl-Neelsen-stained *Mycobacterium tuberculosis* sputum smears. Lane 1: MW standard. Lane 2: reaction control. Lane 3: 50 fg of purified *M. tuberculosis* DNA. Lane 4: microscopically negative stained smear. Lane 5: stained smear with >10 bacilli/field. Lane 6: stained smear with <1 bacilli/field. Lane 7: *M. fortuitum* stained smear. C. Giemsa-stained *Leishmania (V) panamensis* smears. Lanes 1,9: MW standard. Lanes 2-4: three stained smears with low amount of amastigotes. Lane 5: stained smear with moderate amount of amastigotes. Lane 6: 1 fg of purified *L. (Viannia)* kDNA. Lane 7: microscopically negative stained-smear. Lane 8: reaction control.

TABLE

Primers used in PCRs

Primers	Gene (amplified fragment)	Amplification conditions	Reference
P. vivax MSP-1 5' MSP-1 3'	MSP-1 (polymorphic) Size fragment range 300-480 bp	Denaturing (D):96°C Annealing (A): 66°C Extension (E): 72°C Cycles: 35	J Alger et al. manuscript in preparation.
M. tuberculosis T4 - 5' T5 - 3'	IS6110 (conserved) Size fragment 190 bp	D: 95°C A: 68°C E: 72°C Cycles: 35	KD Eisenach et al. 1991 *Am Rev Respir Dis 144*: 1160-1163.
L. (*V.*) *panamensis* B1 - 5' B2 - 3'	Minicircle kDNA (conserved) Size fragment 750 bp	D: 95°C A: 60°C E: 72°C Cycles: 35	MHL de Bruijn & DC Barker 1992 *Acta Trop 52*: 45-58.

purified target DNA, microscopically negative smear, a smear of other etiology, microscopically positive smear, and a reaction control tube (without target DNA). There was not cross contamination as shown by the results obtained with the negative control specimens. We have compared the results obtained by extracting DNA from *P. vivax* Giemsa-stained thin blood smears and Field-stained thick smears from the same patient and obtained a more intense signal from the thick smear which contains more parasites (data not shown). Similar results were obtained when using stained smears containing different numbers of *M. tuberculosis* bacilli or *Leishmania* amastigotes. Furthermore, the different stains did not interfere with the reaction. The use of saponin has improved our results and the PBS washings have not precluded efficient DNA extraction as has been previously reported (Kimura *loc.cit*). Our results show that stained-smears are a readily available source of DNA of adequate quality and quantity for use in PCR. This resource offers an alternative to molecular studies requiring clinical samples, and facilitates the application of new technologies to diagnosis when microscopy does not allow a sensitive detection or does not allow a complete identification of the etiologic agent.

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