CONTRIBUTION OF PCR-BASED METHODS TO DIAGNOSIS AND MANAGEMENT OF IMPORTED MALARIA

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ABSTRACT • Since the first description, in 1990, of the diagnosis of Plasmodium falciparum infection by polymerase-chain-reaction (PCR), the role of this kind of molecular method in laboratory diagnosis of imported malaria is still a topical question. Various molecular assays have been used, the first of which was hybridization using labeled probes in 1984. When compared to thick blood smear, this test displayed a sensitivity ranging from 65% to 81% and specificity was close to 100%. The next technical improvement was the introduction of the so-called polymerase chain reaction (PCR), the principle of which was described in 1985. In 1993, a PCR-based assay detecting all four Plasmodium species was published, followed by different variants of this method. By the turn of the century, novel real-time PCR slashed workarounds time, which dropped from 2 1/2 hours to less than 1 hour. Moreover, automatic reading with no human action on PCR products reduced the risks of contamination. The first application of real-time PCR to the diagnosis of malaria was published in 2001. PCR-based assays were found to be more sensitive than all conventional methods. Variations in sensitivity were probably due to different medical practices as well as to the proportion of various types of subjects (travelers, sojourner chemoprophylaxis, immigrants from malaria-endemic areas) in the population undergoing malaria diagnosis. The target of the primers was also of crucial importance; for the detection of P. falciparum, the most efficient assays amplified either the gene SSUrRNA, or PT15/RESA, or Cox 1. Specificity of PCR results is guaranteed by the nature of the target for primers or probes, as determined by the studies of the Plasmodium genom whose results are available in GenBank. PCR use often confirmed the results of Plasmodium species identification by microscopy and PCR-based methods were found to be the most efficient for the detection of mixed infections. Concerning the diagnosis of imported malaria, it appears clearly that PCR should be considered as second-line method which can be especially interesting, as a negative result rules out malaria in febrile patients. However, the use of PCR assays appears to be restricted to health centers, such as University Hospitals, for whom malaria identification is an important and routine problem. In the future, the detection of mutations related to drug resistance could be used to orient anti-malarial therapy.

KEY WORDS • Imported malaria • Molecular diagnosis • PCR.

INTÉRÊT DES MÉTHODES UTILISANT LA PCR POUR LE DIAGNOSTIC ET LA PRISE EN CHARGE DU PALUDISME D’IMPORTATION

RÉSUMÉ • Depuis la première description en 1990 du diagnostic biologique du paludisme à Plasmodium falciparum par polymérase-chain-reaction (PCR), la place exacte de ce type de méthode moléculaire dans le diagnostic de laboratoire du paludisme d’importation est toujours d’actualité. Cette mise au point, qui tente d’évaluer l’intérêt de l’usage en routine de la PCR dans ce cadre, est donc une contribution au débat en cours. Différentes méthodes moléculaires de diagnostic ont été employées, dont la première a été en 1984 l’hybridation avec sondes moléculaires marquées. Comparée à la goutte épaissie, ce test a montré une sensibilité variant de 65 à 81 %, et une spécificité proche de 100 %. L’usage de sondes ARN a amélioré ces résultats, mais la technique d’hybridation n’a pas dépassé le stade expérimental, principalement en raison de la fragilité de la molécule d’ARN, et des difficultés d’extraction et de conservation. Un autre test a été établi avec l’apparition de la PCR, dont le principe avait été décrit en 1985. En 1993, un test était établi qui détectait les 4 espèces plasmodiales, et qui fut suivi par différentes déclinaisons de cette méthode. À la fin du siècle dernier, la mise à disposition d’appareils de PCR en temps réel modifie substantiellement les conditions du diagnostic moléculaire. Cette nouvelle version de la PCR présentait un temps de travail drastiquement réduit, de moins d’une heure contre deux heures et demie auparavant. En sus, la lecture automatique sans aucune intervention humaine sur les produits de PCR réduisait les risques de contamination. La PCR en temps réel a été appliquée en 2001 au diagnostic du paludisme. Les techniques à base de PCR, qui permettent de prédire la nature des amas de parasites, ont été constamment trouvées plus sensibles que les méthodes conventionnelles : après analyse de 15 publications, la sensibilité de la PCR a été trouvée comprise entre 0.001 et 30 parasites / µL. Les raisons de cette sensibilité variable peuvent être rattachées à des différences de pratique médicale, et aussi à la proportion variable de certains groupes de sujets (voyageurs sous chimio prophylaxie, migrants venant de pays d’endémie malarique dans la population objet du diagnostic biologique de paludisme). Un autre point explicatif de ces variations de sensibilité est la cible des amas. Pour la détection de P. falciparum, les tests les plus efficaces ont amplifié soit le gène SSUrRNA, ou le gène PT15/RESA, ou le gène Cox 1. Le type de PCR, classique ou nichée, simple ou multiplex, influe également sur la sensibilité. La spécificité d’un résultat de PCR est garantie par la nature de la cible des amas, déterminée par les études sur le génome des plasmodies, dont les résultats sont accessibles via GenBank. L’examen de la littérature montre que l’emploi de la PCR corrige souvent les résultats du diagnostic de l’espèce plasmodiale fourni par la microscopie, et que les méthodes à base de PCR sont de loin les plus efficaces pour le diagnostic des polyinfections. Concernant le diagnostic du paludisme d’importation, il est clair que la PCR doit être considérée comme une méthode de deuxième intention, particulièrement intéressante du fait d’une valeur prédictive négative de quasiment 100 %, ce qui permet de certifier rapidement en moins de deux heures que la fièvre d’un malade suspect de malaria n’est pas d’origine palustre. Cependant, l’utilisation de la PCR se confie aux centres de soins tels que les HU ou les grands hôpitaux, où le diagnostic du paludisme est un problème important et fréquent. Dans le futur, la détection par PCR en temps réel des mutations liées aux résistances médicamenteuses, dans un délai compatible avec les exigences des cliniciens, pourrait être employée pour orienter la stratégie thérapeutique.

MOTS-CLÉS • Paludisme d’importation • Diagnostic moléculaire • PCR.

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Since the first description, in 1990, of the diagnosis of Plasmodium falciparum infection by polymerase-chain-reaction (PCR) (1), the role of this kind of molecular method in laboratory diagnosis of imported malaria is still a topical question. Albeit considered as the gold standard in regards of sensitivity and specificity, the use of PCR-based assays for routine detection of malaria infection is debatable for many people, who consider the practical requirements (expensive equipment, need for skilled technicians, running costs), together with the risk of contamination and the problems of emergency diagnosis, to be out of proportion for the benefits. However, permanent technological improvement has made PCR faster and more and more user-friendly. Since the introduction of the so-called classical PCR, using a Feltier-effect thermocycler, the technology has evolved to the present real-time PCR. This method represents a real break from the past and provides new perspectives on the advantages versus drawbacks comparison to discuss the pros and cons of PCR for detection of imported Plasmodium infections.

The aim of this article is therefore to contribute to this open debate and attempt to assess the interest of routine PCR use for the diagnosis of imported malaria, which is an increasing health problem in westernized countries (2).

**A RECENT HISTORY**

During the last 15 years, the laboratory diagnosis of infectious diseases has substantially benefited from advances in molecular biology, which have been intensively used by virologists and also, to a lesser degree, by bacteriologists. Mainly due to the lack of major public health problems related to parasites in westernized countries, parasitology has stayed in the background. A routine molecular diagnosis procedure has only been applied to toxoplasmosis, given the difficult problem of the detection of *in utero* transmission and neonatal infection. However, an analysis of the scientific literature suggests that the diagnosis of malaria would also be affected by the use of such molecular techniques.

**Hybridization using labeled molecular probes**

This method does not require the amplification step, which is specific to PCR. Basically, a labeled probe of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) hybridizes specifically to parasite DNA or RNA.

In 1984, such an assay using a radio-labeled DNA probe was applied to the detection of *P. falciparum* infections (3). When compared to thick blood smear, sensitivity ranged from 65% to 81% and specificity was close to 100% (4-5). Later improvements led to the launch (1993) of a kit for diagnosis of falciparum malaria, with 95% sensitivity, detection threshold of 30 parasites/μL and 99% specificity. Thick smear was the reference method.

The use of RNA probes improved the hybridization method. Ribosomal RNA is more abundant than DNA in eukaryotic cells and has numerous stable and repetitive sequences. An assay detecting the RNA of all four *Plasmodium* species was published in 1989 (6). Sensitivity and specificity were similar to those of blood smear.

However, the hybridization probe method did not go beyond an experimental step, mainly due to the lability of the RNA molecule and to the technical difficulties of RNA harvesting and preserving steps. It became out-dated due to the development of PCR, based upon the principle of gene amplification.

**PCR-based assay**

So-called PCR was first described in 1985 (7) and, for medicine, quickly improved both research and laboratory diagnosis. As early as 1990, PCR was proven useful to detect *P. falciparum* DNA in both fundamental and applied studies (1, 8). In 1993, a PCR-based assay detecting all four *Plasmodium* species was published (9). Following this first publication, several papers described simplex PCR assays including one amplification step (10-11) as well as two-amplification step, or a nested PCR (12-13). Whatever the type of amplification, the PCR products were at that time separated by electrophoresis. The detection step relied upon either DNA staining with ethidium bromide or the use of labeled nucleic probes. Meanwhile, multiple-PCR-based assays, which offered the advantage of a simultaneous detection of the four *Plasmodium* species, were also set up (14-15).

By the turn of the century, the launch of a real-time PCR device dramatically improved PCR-based diagnosis. This novel version of PCR technology slashed workarounds time, which dropped down from 2 1/2 hours to less than 1 hour. Moreover, automatic reading with no human action on PCR products reduced the risk of contamination. The first application of real-time PCR to the diagnosis of malaria was published in 2001 (16), followed by 6 other papers (17-22).

**A SENSITIVE ASSAY**

For laboratory diagnosis of malaria, PCR-based assays have been proven more sensitive than other conventional methods, whatever the type of PCR-based test (see above) or the nature of primers. At its best, the power of detection thick blood smear ranged from 10 to 50 parasites/μL (23-25). Quantitative Buffy Coat (QBC) tests had the same detection threshold (26) and dipstick assays displayed a poor sensitivity when parasitemia was lower than 100 parasites/μL (23). Conversely, in 15 studies (Table I), PCR sensitivity for the detection of *P. falciparum* ranged from 0.01 to 30 parasites/μL. However, some of these results should be carefully considered, since they were experimental and dilution ranges were performed in very different ways, using either blood from infected subjects, or from *P. falciparum* culture (often with no indication concerning synchronous, or the presence of multicellular schizonts might be suspected), or from harvested DNA (with different methods of extraction). For species other than *P. falciparum*, comparing sensitivity results was impossible due to the lack of parasite culture and also to the variation of the proportion of schizonts between different patients.
Concerning the diagnosis of imported malaria, only papers that compared PCR and conventional microscopy methods during a limited period of time and in all patients suspected to be infected were analyzed. In 13 studies including 2304 subjects, 1011 patients were found to be malaria positive by conventional microscopy, and 1112 (+11%) by PCR (range: 0 up to +13%). All the patients positive by microscopy were also positive by PCR, whatever the Plasmodium species. When compared to the set of the requests, the gain of sensitivity was 4.4% (range: 0 up to +11%) (15, 18-19, 21-22, 31-32, 34-35, 38-41). This improvement in sensitivity affected mainly falciparum malaria. Plasmodium falciparum is the most frequently detected agent of imported malaria, especially in the European Union where the closest endemic zone is sub-Saharan Africa (2). Moreover, even loosely implemented chemoprophylaxis remains (rather) active on species other than Plasmodium falciparum, apart from the chloroquine-resistant Plasmodium vivax stains, which are still rarely encountered in Europe.

**Origins of the Variable Sensitivity of PCR-Based Assays**

**Epidemiological causes**

- **Medical practices**

The «number of requests for malaria diagnosis / positive results» ratio for patients varied significantly between the studies, from 1.2 (35) to 3.9 (40) (thus indicating different ways of handling febrile travelers or immigrants). This variation appeared to be country-independent, since the range was 1.4 - 3.9 in France (19, 40) and 1.6 - 2.7 in Italy (21, 38). When a malaria diagnosis was requested for a febrile patient returning from a malaria-endemic area within 2 months before the onset of the fever, the ratio observed in Toulouse University Hospital ranged from 3.05 to 3.9 (18, 40).

Moreover, it should be underlined that a delayed request for a diagnosis test may lead to the rise of parasitemia, thus increasing the sensitivity of conventional microscopy and decreasing the interest of the intrinsically more sensitive PCR-based methods.

- **Immigrants, travelers and chemoprophylaxis**

A recent study analyzed the status of 32 subjects who had displayed a «negative microscopy / positive PCR» discrepancy (40). A first group included 14 patients who had received a curative anti-malarial therapy a few days before, but this information was not available in our Department of Parasitology at the time of diagnosis. A second group included 5 patients who were European travelers displaying a clinical picture consistent with malaria. The two cases diagnosed with Plasmodium falciparum infection despite the fact that they were taking chemoprophylaxis, 2 with chloroquine plus proguanil, one with mefloquine. For the last 2 patients, the onset of clinical problems occurred 48 and 90 days after they returned from a malaria-endemic area. PCR detected Plasmodium malariae and Plasmodium ovale infection, respectively. A third group consisted of 10 patients, all immigrants from various African countries. Seven of them had anti-Plasmodium antibodies. Eight of them displayed a positive PCR assay result for Plasmodium falciparum, 1 for Plasmodium malariae and 1 for Plasmodium ovale.
Ndao et Coll investigated 210 asymptomatic immigrants to Canada who were refugees from the Great Lakes region in East Africa. Twenty-four were found to be malaria-positive by both microscopy and PCR, vs. 48 by PCR only (42).

These results demonstrated that the rate of diagnosis discrepancies between microscopy and PCR technique might vary according to the proportion of the above-mentioned groups of subjects in a population undergoing malaria diagnosis. Moreover, these data suggested that the exact prevalence of malaria in endemic areas is probably underestimated when assessed by microscopy only.

**Technical procedures**

- **DNA extraction**

This is the only point of agreement between the protocols descibed in the 13 major papers about for malaria diagnosis by PCR (15, 18-19, 21-22, 31-32, 34-35, 38-41). The extraction step was performed from 200 µL (9/13) of total blood collected in EDTA vials (13/13), sometimes using fast extraction in columns from commercial kits (9/13). The protocol of DNA extraction does not therefore appear to be a cause of sensitivity variation and this point must be underlined.

- **Targets of the primers**

In the *Plasmodium* genus, DNA is found in the nucleus, the mitochondrion and also the plastids. A given gene inside one of these structures should first have its sequence established then a specific primer can be designed. Apart from the quality of primer design, PCR sensitivity also depends on the number of copies of the target gene as well as on the amount of cytoplasmic organelles, namely mitochondria and plastids.

The small subunit ribosomal RNA (SSUrRNA) gene, 4 to 8 copies per parasite (43), has had its sequences intensive by studied. This gene contains conserved regions, so primers common to the 4 *Plasmodium* species could be targeted, as well as variable sequences, which were used to design primers specific to each species. Consequently, a survey of the studies concerning the molecular diagnosis of malaria showed that this gene was targeted 8 times out of 10.

Other nuclear genes were used, which encoded for DHPS (12), K1-14 (44), MSP (45), CSP (11, 46), Pf155/RESA (47), P126 antigen (48), and STEVOR (31). The latter has 35 copies per parasite, which ensures a good sensitivity for a PCR assay.

Depending on the development stage, malaria parasites have between 1 and 5 mitochondria (49), the genes of which display between 15 and 20 copies per cell (50). The mitochondrial gene encoding for cyclophylase (Cox 1) was targeted to create a PCR assay detecting both *P. falciparum* and *P. vivax* infections (28).

Plastid genes based-PCR to detect *Plasmodium* gene was reported twice (40, 52). Plastid and mitochondrial genes have similar characteristics but there is only one plastid per parasite.

A comparative study, using original technical conditions, was carried out to assess the performance of various PCR tests, previously published (33). The authors quickly screened 17 assays then selected the 5 most efficient tested on 126 samples positive by microscopy. For the detection of *P. falciparum*, the 3 most efficient assays amplified either SSUrRNA gene (54), or Pf155/RESA (47), or Cox 1 (28).

- **PCR characteristics and types**

The choice of the type of amplification greatly influences sensitivity. In «classical» PCR, amplification relies upon a single pair of primers. On the other hand, «nested» PCR includes two successive amplification steps using two different couples of primers. The second couple is designed to allow the amplification of a fragment of the amplon of the first PCR. Nested PCR is apparently more sensitive: in the above-mentioned sensitivity comparative study (53), this type of PCR was quoted 2 times in the 3 best results. However, nested PCR is hardly convenient for routine diagnostic use. The test is time-consuming (two successive PCR) and possible contamination by the amplicons from the first PCR represents a major risk.

Classical PCR assays are also classified as «simplex» or «multiplex». Simplex PCR uses a single couple of primers. Multiplex PCR simultaneously utilizes two or more couples; several targets can thus be amplified in the same step. Multiple detections of malaria agents have been described, namely of *P. falciparum* along with *Plasmodium* genus (18, 40), of *P. falciparum* along with *P. vivax* (28, 32, 33), or of the 4 species (15). Multiplex PCR is an attractive option for routine diagnosis, since mixed infections can be detected in a single round. However, a major technical constraint concerns the fact that the various couples of primers should have similar characteristics, especially for melting temperature (Tm). Calibration of such a PCR assay is therefore awkward and the final compromise often yields a decreased sensitivity of the test. Another difficulty lies in the competition effect when two or more targets are present (mixed infection), with one in a low proportion. This minority population, albeit perfectly detected when there is a single infection, will in this case risk remaining undiagnosed. Assessing the detection of both *P. falciparum* and *P. vivax*, Kho et Coll showed PCR sensitivity for the diagnosis of either of these two species was slashed by a 10-fold ratio when this species was in a very low proportion (33). Conversely, competition effect may be used for an easier analysis of the result. Fabre et Coll (18) have described a multiplex competitive PCR assay for the detection of both *P. falciparum* and *Plasmodium* genus. For a single falciparum infection, only the *P. falciparum* peak is seen.

PCR methods may also be divided into «conventional» or «real-time», according to the type of thermocycler used. Conventional PCR uses a Feltier’s effect thermocycler and PCR products are revealed after electrophoresis. In a real-time PCR device, the whole diagnosis process is performed in a single run and in a single tube, so this method is by far faster and less exposed to contamination hazards. For malaria diagnosis, both conventional and real-time PCR were compared and were found to have identical sensitivity (18).
A SPECIFIC METHOD

The nature of the target for primers or probes, as determined by the studies of the Plasmodium genome whose results are available in GenBank, guarantees the specificity of PCR results. As a further precaution, PCR products may also be sequenced.

Concerning Plasmodium species identification, literature analysis reports frequent and sometimes substantial discrepancies between microscopy and PCR test. From the last 10 published studies that have compared the performance of thin blood smear examination and PCR, 17.2% (range: 1.5-39) of 823 species identifications by microscopy were corrected after PCR checking (19, 21, 22, 34-35, 38, 40-42, 55). The rate of misdiagnosis varied from 20% to 50% for P. malariae, P. ovale or P. vivax (19, 21-22, 34, 38, 40, 42, 55).

Concerning mixed infections, nearly 100% of the microscopy results were false, either by excess (diagnostic of a mixed infection when there was a single species) or by omission (reverse situation). P. falciparum identification appeared to be more reliable, with no misdiagnosis found in 6 studies (19, 21, 34, 35, 40, 42), a 1.7 to 2.8% misdiagnosis rate in three studies (22) (38, 41) and 8.4% of misdiagnoses in one study (55).

However, the high specificity of PCR methods demonstrated the other side of the coin, namely the intrinsic risk of these tests being unable to detect new molecular variants. This phenomenon was reported concerning P. malariae in China (56, 57), about P. ovale in the Thai - Myanmar border region (13, 58) and also P. vivax in Papua-New Guinea, where the so-called P. vivax-like species was described (59). More over, emergent infections by zoonotic primate Plasmodium can go undiagnosed by species-PCR, which occurred with the Borneo focus of P. knowlesi (60).

When primers or probes are chosen then designed, these two points should be kept in mind and correlated with future uses of the PCR test, e.g. routine diagnosis in a westernized country or, epidemiology surveys from specimens collected on the field.

The concomitant use of an assay detecting the Plasmodium genus, whatever the origin - Man, subhuman Primate or even Rodent - of the species involved, appears to be an excellent safeguard against rare but possible false negative results.

PCR AND THE DIAGNOSIS OF IMPORTED MALARIA

Even now, PCR assay may be considered as the gold standard for the diagnosis of malaria, therefore replacing thick smear examination. This situation is due to both high sensitivity and specificity, as detailed above. While PCR is used more and more in laboratories of parasitology, its exact place in the full range of malaria diagnosis tests has yet to be explicitly established.

Obviously, emergency diagnosis of malaria will for a long time remain the prerogative of microscopy (thin smear or fast thick smear (61) examination, QBC) associated with immunochromatographic methods. Such a test combination can detect all infections displaying a moderate to high parasitemia, which ensures within less than half an hour the diagnosis of severe malaria. PCR should therefore be considered as second-line method.

The first question about the routine use of PCR for diagnosis of imported malaria is about the schedule of use, daily, weekly or bimonthly, and also the type of specimen, all or only those posing a problem. Based on a 4 1/2-year experience, we subsequently believe PCR should be done on a daily basis. In this case, low parasitemia infections due to, e.g. incorrect chemoprophyaxis or an immune status, would be promptly identified, despite having escaped the first-line methods. Anyone who deals with tropical medicine has had a personal example of a patient displaying chronic fever along with a recent history of travel in a malaria-endemic area and who is finally diagnosed as malaria -infected after many days of hospitalization and multiple conventional investigations. A request for PCR would quickly resolve this diagnosis quandary (62-64). Moreover, it should be underlined that, according to our experience, the predictive negative value of a PCR-based result is 100%. A negative result would rule out malaria infection, thus quickly orienting the investigations toward other hypotheses. With the expected reduction of hospitalization time, substantial cost saving could be achieved, as - in France - daily costs are approximately 700 € in a Department of Internal Medicine or Infectious/Tropical Diseases.

P. knowlesi level can be quantified by real-time PCR. However, this can be achieved more easily by combined use of blood count result and thin smear examination.

Since real-time PCR can detect and quantify very low parasite level, it has been hypothesized that this assay could be a valuable tool to monitor falciparum malaria therapy. Two studies indicated that the persistence of positive PCR results after a 5-day treatment was consistent with treatment failure (27, 65). However, such a result can also be due to the presence of gametocytes (66), which removes any purpose from this kind of molecular follow-up.

From a financial point of view, PCR is said to be a costly method. Indeed, technical requirements such as room layout in a laboratory, purchase of a thermocycler and the required ancillaries, are not inexpensive. However, one should consider that this price is on a constantly decreasing slope and these investments are also useful for other diagnosis in parasitology, such as congenital toxoplasmosis. Many hospitals have also chosen the solution of a technical platform shared by different laboratories.

Expenses related to reagents and disposable devices are highly variable, depending on the type of PCR, the number of control samples and the number of specimens per run. In our own experience (18), the cost of a single assay is 10.75 € (all taxes included), including 3 control samples, namely a negative, a P. falciparum positive, and a Plasmodium-genus positive (species other than P. falciparum) specimen. For a 6-sample run, the cost per assay goes down to 5.54 €. If the salary of an experienced technician and a 3-year depreciation on the investments are taken into account, the unitary cost of a PCR assay, on an annual basis of 1000
tests and routine daily use, is 26.75 € for a single assay, decreasing to 17.75 € for a 6-sample run. In comparison, on the same annual basis, the cost of a microscopy diagnosis (thin smear or fast thick smear examination) is 7€, 11 € for a QBC test and between 6.50 € and 8.50 € for the immunochromatographic methods. These analytic cost-accounting results emphasize the salary and the number of specimens per run. If a conventional PCR method is used, the time spent on the electrophorosis detection step increases the salary burden. Conversely, the introduction of affordable automatic DNA extractors should cut the time of human intervention in half and therefore should decrease PCR cost.

In fine, it appears obvious that PCR improves the diagnosis of imported malaria, but also that this method is restricted to health centers, such as University Hospitals, for whom malaria identification is an important and routine problem. For other structures, the combination of conventional microscopy with immunochromatographic methods is certainly the best choice.

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**Prevention of malaria risk in blood supply**

Post-transfusion malaria, which is rarely observed in westernized countries, often becomes a serious or lethal disease, since the diagnosis is often delayed. Strict regulations have been implemented in order to decrease this risk but, e.g. in France, many potential donors who have a history of malaria attacks or who have been found positive at a malaria serology screening are permanently excluded from blood donation. However, most of these subjects do not have any circulating trophozoite, so a PCR-based screening might be relevant. In Spain, Benito et Coll investigated 125 «dangerous» blood donors, 5 of whom tested malaria-positive by PCR (67). These authors suggested PCR could be a useful tool to investigate potentially dangerous blood donors with a history of malaria or exposure to the infection. However, Hanscheid et Coll pointed out that, whatever the sensitivity of a PCR assay, a negative PCR result from a 1000-μL maximum blood sample does not rule out the presence of a single parasite in a 450 mL unit of donated blood (68). Further large-scale prospective surveys are therefore needed to answer this pending question.

**Molecular detection of drug resistance**

Resistance of *P. falciparum* to chloroquine (CQ) has been correlated with a mutation on codon 76 of the PFCr t gene (69). Wild-type strains are CQ sensitive, but some mutated specimens are CQ resistant. K76T mutation is a mandatory but not sufficient condition for CQresistance. Molecular detection of K76T mutation is quick and easy (70-73) and has been used for epidemiological surveys. Moreover, some assays were able to detect a 2-5 % mutant minority population amidst wild type strains (70). The detection of K76T mutation, the turn around time of which meets clinicians requirements for practical use, could therefore be used in the future to orient drug strategy (74, 75), as in virology for HIV infection.


38. PERANDIN F, MANCA N, PICCOLO G et Coll - Identification of *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae* and detection of mixed infection in patients with imported malaria in Italy. *New Microbiol* 2003; 26: 91-100.


