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Poor Accuracy of Rapid Diagnostic Tests and Misdiagnosis of Imported Malaria: Are PCR-Based Reference Laboratories the Answer?

The recent article by Rubio et al. reports poor accuracy of rapid malaria diagnostic tests (RDTs) and promotes PCR-based reference laboratories to avoid potential misdiagnosis (6). Both points deserve comment.

The authors' reported low sensitivity is mainly due to poor detection of *Plasmodium* species other than *Plasmodium falciparum*. Although "pan-specific" antibodies have been included in some RDTs, it is the diagnosis of *P. falciparum* infection that is crucial for reducing mortality. Here, Rubio et al. confirm the findings of many studies, reporting a sensitivity well above 90% (7). Moreover, an unusually high number of false-positive results are presented, without attempts to identify a reason. For example for an average of more than 17 days, 44.4% of OptiMAL (pLDH-based assay) results were reported to be false positives. However, pLDH is only produced by live parasites, and studies have shown very good correlations with declining parasitemias during follow-up (4). The reported number of pretreatment false positives (>20%) even exceeds numbers reported from studies where the influence of rheumatoid factor on RDT performance was investigated in nonmalarious patients (1).

The authors use this unduly dark picture to justify the need for PCR-based reference laboratories, although they fail to elucidate the clinical usefulness of such tests in the diagnosis of acute malaria. Following their argument of limited accuracy of malaria diagnostic tests, confirmation by a reference center would have to include positive as well as negative samples. Hence, there would be considerable financial and logistic hurdles for such a policy: urgent transport, turnaround time of PCR runs, weekend service, etc. From the authors' country (Spain), a mean annual number of 175 cases per year and for Europe 5,000–8,000 cases per year were reported from 1985 to 1995 (5). However because millions of tourists visit countries where malaria is endemic, each year many of them may present at health service facilities with unspecific symptoms after return, prompting a malaria diagnostic test. For each positive specimen there will be many negative ones. Unfortunately, the feasibility of such a "fast-track" reference laboratory service is not discussed (e.g., in a cost-benefit analysis).

Furthermore, lack of clinical suspicion may be more important in the misdiagnosis of imported malaria than poor performance of laboratory tests. In Canada, more than 50% of malaria cases were clinically not suspected upon first presentation (3). RDTs or PCR-based reference laboratories do not address this problem. Development of common routine laboratory tests that alert to the presence of *Plasmodium* spp. may be more useful. One example is the detection of hemozoin during automated routine full-blood counts, which allowed the diagnosis of six clinically unsuspected malaria cases in one study (2).

Reference centers play an important role primarily in the posterior confirmation of positive samples, species identification, and comprehensive quality assurance. However, whether the use of presently available RDTs increases the misdiagnosis of imported malaria, as suggested by Rubio et al., remains to be proven by an appropriately designed study. Such a study would have to investigate the routine use of RDTs in normal everyday practice involving non-expert microscopists in peripheral laboratories. Is it not more likely that the judicious use of RDTs would reduce the misdiagnosis of imported malaria in many of these places?

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Authors' Reply

As we report in our study, the percentage of false positives is 24%. Four out of the nine patients that were positive remained positive for an average of 17 days. Some explanations for false positives and negatives can be offered. In the first place, some authors have reported high percentages of false positives related to autoantibody formation (rheumatoid factor and others) for the rapid diagnostic tests (RDTs) (although the numbers for ICT and ParaSight-F tests were higher, than those for OptiMal).

On the other hand, the multiplex PCR was designed from a single-stranded ribosomal DNA gene, a gene with only two copies in asexual stages but that generates multiple copies during asexual schizogony. In exchange, the sexual stages (micro- and macrogametocyte) do not present this multiplicity. This fact and the fact that the PCR was designed with a universal forward primer but four reverse specific primers (one per each *Plasmodium* species) explain that sexual stages cannot be amplified with exception of cases of very high parasitation with gametocytes. A portion of patients, after treatment with antimalarial drugs, presented with gametocytes in their blood that could persist several (≤20) days. The gene of pLDH is

monocopy without introns, and it is present in chromosome 13. This gene could be expressed in gametocytes and result in a radical cure of patient and presumably to be detected the enzyme by RDTs.

Finally, several patients in this study followed a regimen of chloroquine for malaria prophylaxis (in our case, chloroquine plus proguanil). Chloroquine competes with NADH for binding to the enzyme and to interact specifically with pLDH in the transformation from pyruvate to L-lactate in glycolytic process (2), a fact that could implicate the accumulation of pyruvate and the following enzyme hyperactivity. This fact could have implications in increases or decreases of pLDH in blood.

The majority of trials with RDT's do not include PCR and microscopic diagnosis is performed as the "gold standard." Moreover, a certain number of patients in this study were semi-immune patients with clinical symptoms but with low parasitemias (<150 parasites/μl). Studies evaluating RDTs in nonimmune people (travellers) reveal satisfactory results; however, in the case of low parasitemias the results of sensitivity and specificity decrease notably (3). Actually, in our laboratory, parasitemias of 135, 204, 281, and 273 parasites/μl were diagnosed and/or confirmed during the years 1998, 1999, 2000, and 2001 (up to 18 October), respectively. Only data from the year 2000 for our Unit of Tropical Medicine (Hospital Carlos III) have been reported to the European Network of Imported Infectious Diseases Surveillance 130 malaria imported cases (TropNetEurop; www.tropnet.net). If the confirmed cases reported by us correspond to 30 to 40% of the malaria imported cases from Spain, we think that actually the number of imported cases could be around 400. We are in agreement with Hänscheid that to evaluate the feasibility of a PCR-based reference laboratory, a cost-benefit analysis (including a discussion of financial and logistic hurdles, not to mention the cost of the return of patients at hospital due to misdiagnosis of relapsing infections, as well as the cost of labor absenteeism) is advisable. At present, one of the objectives of the National Institute of Health Carlos III (National Center for Microbiology) is to act as the Reference Center for all microbiological laboratories in the country, with functions of reference, diagnosis and basic research, the two first supported by the State. Moreover, an other function of our laboratory, one that is important to medical entomologists, is to study the receptivity and vulnerability of Spanish vectors in the context of malaria reintroduction hypotheses (4), now especially important, given the increase in Spain of migrant populations from areas where malaria is endemic (Central America and sub-Saharan areas). Actually, the migrant population in Spain corresponds to a population of continuous comings and goings and this fact increase the possibility of malaria recurrence, increasing at the same time the access to health sector, including the blood banks of the hospitals and centers for transfusion. In accordance with these facts, and without discussing the cost-benefit analysis, recently we studied if PCR could be a reference technique for screening units of blood in hospitals and centers of transfusion from donor at risk (1). In conclusion, we suggest the use of RDTs with great caution; at present, these tests alone cannot replace microscopic diagnosis. We also report the experience of the national reference laboratory, where PCR, and other molecular epidemiological markers (e.g., resistance and diversity) are used for confirmation and surveillance of imported malaria in Spain with satisfactory results.

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