DIAGNOSIS OF *PLASMODIUM FALCIPARUM* MALARIA USING PARASIGHT F®, ICT MALARIA PF® AND MALARIA IgG CELISA® ASSAYS

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Summary :

A battery of sixty-six blood samples from Senegal was analysed by the ParaSight F® test, the ICT Malaria PF® and the Malaria IgG CELISA®. These three assays detect the histidine rich protein 2 antigen of *Plasmodium falciparum*. Thick smear microscopy was used as the reference method.

Sensitivity, specificity, predictive positive and negative values were respectively 89 %, 100 %, 100 %, 88 % for the ICT; 86 %, 93 %, 94 %, 85 % for the paraSight and 88 %, 87 %, 88 %, 87 % for the Malaria IgG CELISA. The three assays failed to detect two positive samples with *P. ovale* and *P. malariae*.

Assays were also compared with regard to the expense of equipment and reagents and speed and ease of use. The rapid ICT and ParaSight F test can be performed with minimal training and may be specially useful in areas where *P. falciparum* is the predominant malaria species, in epidemic malaria regions, and where skilled microscopy is not readily available.

KEY WORDS : thick smear, ParaSight F test, ICT Malaria P.f., Malaria IgG CELISA, histidine rich protein 2, *Plasmodium falciparum*, malaria, Senegal.

Résumé :

DIAGNOSTIC DU PALUDISME À *P. FALCIPARUM* PAR LES TECHNIQUES PARASIGHT F®, ICT MALARIA PF® ET MALARIA IgG CELISA®

Soixante-six échantillons sanguins ont été testés par ParaSight F®, ICT malaria PF® et Malaria IgG CELISA®, trois techniques de détection de l'antigène Pf. HRP-2 en comparaison avec la goutte épaisse.

La sensibilité, la spécificité, les valeurs prédictives positive et négative étaient respectivement de 89 %, 100 %, 100 %, 88 % pour l'ICT; 86 %, 93 %, 94 % et 85 % pour le ParaSight et 88 %, 87 %, 88 % et 87 % pour Malaria IgG CELISA®.

Les trois tests n'ont pas mis en évidence deux échantillons contenant *P. ovale* et *P. malariae*.

L'ICT et le ParaSight F sont de réalisation facile et sont utiles dans les zones où *P. falciparum* domine, où il y a des risques d'épidémie et où un examen microscopique de qualité n'est pas réalisable.

L'interprétation des résultats doit se faire cependant en fonction des données cliniques et épidémiologiques. Leur coût plus élevé que celui de la goutte épaisse pourrait constituer un facteur limitant à une utilisation à grande échelle dans les pays tropicaux.

MOTS CLÉS : goutte épaisse, ParaSight F test, ICT Malaria P.f., Malaria IgG CELISA, histidine rich protein 2, *Plasmodium falciparum*, paludisme, Sénégal.

Malaria remains the most important parasitic disease, causing about 2,000,000 deaths each year, mostly due to *Plasmodium falciparum*. The increase of drug resistant strains and the increasing clinical importance of malaria has led to efforts to improve diagnostic methods. Microscopic examination of blood smears remains the method of choice for diagnosing malaria in most settings, but effective microscopy requires a quality microscope and extensive training. Newer technologies that have been evaluated include hybridization to DNA or RNA (Ambrose Thomas, 1990; Franzen *et al.*, 1984), the polymerase chain reaction PCR (Barker *et al.*, 1992; Mc Laughlin *et al.*, 1987) and the QBC malaria assay (Levine, 1989). However

assays detecting the *Plasmodium falciparum* histidine-rich protein 2 Pf HRP-2 (Howard *et al.*, 1986), a water-soluble protein released from parasitized erythrocytes, are the current major commercial technologies. Related assays include the ParaSight F® test developed by Becton Dickinson, the ICT Malaria P.f.® developed by ICT Diagnostics (Sydney, Australia) and the Malaria IgG CELISA® developed by CellLabs (Sydney, Australia). Pf HRP-2 is identified using monoclonal antibodies in different formats in these assays. The purpose of the present study was to compare thick smears microscopy to these three companies assays which detect Pf HRP-2.

MATERIALS AND METHODS

During November 1996, sixty-six patients with malaria symptoms presenting at the Roi Baudouin out-patient clinic in Guediawaye, a suburb of Dakar, Senegal were enrolled in the study. The age distribution was 1-65 years. Thirty-five were female and thirty-one were male; informed consent was

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obtained. Thick blood examination was made using blood from a digital puncture. Additional blood (5 ml) was collected by venipuncture into 10 ml heparinized tubes.

MICROSCOPY

Thick blood smears were lysed in water and were stained with Giemsa. Numbers of malaria parasites per 1,000 white cells were counted and the average parasitemias were calculated assuming 8,000 white cells per microliter of blood.

ICT MALARIA® ASSAY

For each sample, 10 µl of whole blood were added in the purple area of the test strip card containing antibodies where lysis occurs and any Pf HRP-2 antigen present binds to the colloidal gold labelled antibody. One drop of a running buffer reagent A was added above the purple pad, then two drops of reagent A were added below the area where sample was added. Then four drops of reagent A were added on the clearing pad containing the second antibody immobilized in a line across the test strip. The sample was allowed to migrate by capillary action up the membrane. When the red lysed blood complexed with the gold labelled antibody reaches the second antibody on the membrane, the card was closed. For positive samples a pink line appeared at the strip's capture region within 3-5 minutes if the sample contains Pf HRP-2.

PARASIGHT F® TEST

For each sample, 50 microliters of whole blood were mixed in a Dispens Tube R with three drops of lysing agent. The filter tip was added and a drop of lysed filtered blood was expelled onto the base of the Test Strip. After the blood rose by capillary action, one drop of detection agent (pink-loaded dye liposomes with specific antibodies) was applied at the base of each Test Strip. For positive samples a pink color developed as the liposomes were captured at the strip's capture region, which was rapidly (10 sec.-3 mn) identifiable.

MALARIA IGG CELISA®

This assay uses an indirect or sandwich ELISA principle beginning with antibody-coated 96-well polystyrene microtiter plate microwells. 100 µl of blood patients samples were added in the microwells. The plate was incubated covered in a humidified chamber for one hour at 37°. The plate was then washed with 100 ml PBS-Tween. Conjugate (100 µl) was added to all wells. After one hour of incubation at room temperature in the humidified chamber wells were rinsed with PBS-Tween. Fresh substrate solution (100 µl) was added and color was allowed to develop for 15 mn at room

temperature in the dark. The reaction was stopped by adding stop solution and mixing. Absorbance at 450 nm was measured with an ELISA reader.

DATA ANALYSIS

Sensitivity, specificity, positive and negative predictive values were calculated using microscopy as the standard method.

RESULTS

In this study sixty-six patients with clinical symptoms of malaria were examined using thick smear, the ParaSight F® test, ICT Malaria P.f.® and the Malaria IgG CELISA®. Thirty-six samples were detected as positive by examination of stained smears at relative parasitemias ranging from 500 to 86,286 parasites per microliter of blood. Thirty-four were *P. falciparum*. Additionally, one sample contained asexual forms of *P. malaria* and another contained only *P. ovale*.

ICT ASSAY

Each assay run was performed and scored within 3-5 minutes. Strong positives were identified near the top of the Test Strip. A positive control dash was seen at the upper region of all strips. The assay was easy to perform following the manufacturer's instructions, and requires no equipment. Thirty-two samples were detected as positive by ICT. Four missed samples had parasitemias at 12,697, 30,619, 20,418 and 10,542 p/µl blood (Table I). Using thick smear microscopy as the standard method, the sensitivity of ICT was 89 %, the specificity 100 %, positive predictive value 100 %, negative predictive value 88 %.

Samples with *P. malariae*, (n° 1) *P. ovale* (n° 2) were negative with ICT test.

PARASIGHT F ASSAY

Each assay run was performed and scored within 8-15 minutes. Thirty-one positively scored samples using

No	Parasitemia (P/µl)	ICT	ParaSight F	CELISA
1	20,418	—	—	—
2	10,542	—	—	—
3	12,697	—	—	—
4	30,619	—	—	—
5	2,500	+	—	+
6	—	—	+	+
7	—	—	+	+
8	—	—	—	+
9	—	—	—	+

Table I. — Discordant results.

the ParaSight were also positive by thick smear microscopy. The four negative samples by ICT were also negative with ParaSight. One negative sample using ParaSight was scored positive both by thick smear microscopy and ICT (n° 5: 2,500p/µl). Two negative samples (n° 6, n° 7) by microscopy were scored positive by ParaSight (Table I).

Using thick smear microscopy as the standard method, sensitivity, specificity, predictive positive and negative values were respectively 86 %, 93 %, 94 % and 85 %. The intensities of the positive ParaSight reactions were generally correlated with the densities of parasitemias determined by thick smear microscopy.

MALARIA IGG CELISA

The four positively scored samples using blood smears with parasitemias 12,697, 30,619, 20,418 and 10,542 parasites/µl were negative by ELISA. Two false negative samples with ELISA contained *P. malariae* and *P. ovale* (n° 1, n° 2), while four microscopic scored negative were positive using ELISA. These four false positive samples were retested by thick microscopy and were again found to be negative. Using thick smear microscopy as the standard method sensitivity, specificity, positive and negative predictive values were respectively 88 %, 87 %, 88 % and 87 % (Table I).

DISCUSSION

A battery of sixty-six blood samples from Senegal was analysed with three different malaria diagnosis methods detecting HRP-2, blood smears examination used as standard method. Two positive multiple freeze-thawed samples (n° 3, n° 4) were scored as negative by all three methods. These false negative samples were retested by thick microscopy and were again found to be positive. Although histidine 2 antigen is apparently stable multiply freeze-thawed samples may decrease the sensitivity of the assay. An other possibility could be the omission of the lysis step of the blood.

The two blood samples with *P. malariae* and *P. ovale* were both scored negative with the three methods. This confirm the antigen histidine-rich protein 2 is specific of *P. falciparum*.

The correlation between parasites densities by thick smear microscopy and signal intensity scores of the ICT and ParaSight F assay suggests that these techniques provided a semiquantitative estimation of parasitemias. A sample with 500p/µl parasitemia was detected by ICT.

Even though the detection limit was not determined during this study we might consider ICT and ParaSight can detect at least 1,000 p/µl.

Four samples scored positively by Malaria IgG CELISA were negative with thick smear examination. These false positive samples were retested by thick microscopy and were again found to be negative.

These patients reported having had malaria and having been treated. It is known that histidine 2 antigen may be detectable following drug treatment even when parasites are no longer visible in the blood by microscopy (Houze, 1996). Nevertheless, all these four samples were scored negative by ICT and two of them by ParaSight which both detect the same antigen. This might suggest the antihuman globulin IgG antibodies used in the ELISA assay binds strongly the Pf HRP-2 and can detect low antigenemia persisting in the blood better than the two previous techniques. Nevertheless we might consider possible cross reactions with other diseases such as visceral leishmaniasis, DEL and HIV syndrom (Houze *et al.*, 1996).

Transportation, speed, convenience, equipment, labor, and supply costs are often critical factors in malaria diagnosis. Thick smear microscopy when correctly done is inexpensive with regard to supply costs and can detect as low as ten parasites per microliter of blood, but it requires a good microscopy and a skilled technician. The ICT and ParaSight assays do not require the initial equipment cost. The antigen detection is simple, rapid, suitable for use by relatively less-trained personnel, and the reagents are relatively stable and portable. When malaria diagnosis is not readily available as in peripheral areas, and where *P. falciparum* malaria could be epidemic and presents to a largely non immune population as in the case with hypoendemic and seasonal malaria in Senegal (Gaye *et al.*, 1989) the ICT and ParaSight assays may be specially useful.

For the Malaria IgG CELISA, since one of the antibodies is an IgM, the assay doesn't work on a dipstick and requires an ELISA reader which makes it much less useful than ParaSight and ICT.

The limitation of these three methods is that Histidine 2 antigen may be detectable following drug treatment even when parasites are no longer visible in the blood by microscopy. It is an inconvenience for the surveillance of chemoresistance and therapeutic failures which arise in Senegal (Gaye *et al.*, 1991).

In the other hand these methods are specific of *P. falciparum*, the other species present in Senegal as *P. malariae* and *P. ovale* could be under-evaluated.

Another limitation of these tests is due to their higher cost compared to thick smear microscopy : 1.75 \$ for microscopy examination in the public health services vs 2.5 \$ for ParaSight and ICT as proposed by the fabricant.

Globally these techniques are practical and rapid. Final diagnosis should be made with correlation with other clinical and laboratory findings, and epidemiological criterias.

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