A. Feldman*, J. Lin*, S. Murphy*, P. Demirev*,
D. Sullivan†, P. Scholl†, and N. Kumar†

*JHU Applied Physics Laboratory, Laurel, MD;
and †JHU Malaria Research Institute, Baltimore, MD

PL and JHU Bloomberg School of Public Health researchers have
shown that laser desorption mass spectrometry (LDMS) is a sensitive
method for detecting malaria parasites in the blood, and they have

validated the test on clinical samples from Zambia. The method is based on the detection of heme in hemozoin
(Hz), the crystalline substance accumulated within malaria parasites during their intraerythrocytic growth
stage (Fig. 1).

The LDMS test requires no consumables other than a lancet and a container for blood collection. Blood is
diluted in water, deposited onto a metal slide, air dried, and then inserted into the mass spectrometer for analysis
(Fig. 1). Hz heme is identified from the pattern of heme-
molecular-structure-specific peaks (Fig. 2). A correlation
filter (CF) algorithm is used to score local mass spectra
for the presence of Hz heme during spatial scanning of
the laser beam across the sample. Spectra with a CF score
exceeding a threshold value are counted, with observed
counts roughly increasing with increasing blood para-
sitemia. When spatially contiguous Hz heme detections
are assigned to single clusters, the distribution of cluster
counts obeys a Poisson distribution. This mathemati-
cal model, when used in conjunction with calibration
LDMS data from infected and uninfected blood, per-
mits tuning of the assay’s parameters (blood volume to
test, CF threshold, etc.) to meet a particular application
requirement, such as a targeted positive predictive value
in a population (low or high parasite prevalence).

Although currently available mass spectrometers
are not optimized for our tissue scanning application,
the modifications needed to make a clinically useful
instrument are straightforward. Our screening assay
was validated for Plasmodium falciparum in a pilot
clinical study in Choma, Zambia, where Hz heme was
detected in 15 of 45 microscopy-negative pregnant
African women. Thirteen of these cases were con-
firmed by detection of P. falciparum-parasite DNA by
using polymerase chain reaction (PCR) testing. The
two LDMS-positive cases in which no parasite DNA
was detected may be attributed to LDMS detection of
released residual Hz from a resolved or resolving infec-
tion. Our test was subsequently validated for all human
malarias in a blind study using archival blood samples
(multiple countries of origin) from Canada’s reference
laboratory at McGill University.

Although use of Hz heme as a diagnostic marker for
malaria infection is appealing as a screening tool, it may
have limitations for disease diagnosis because there are
several potentially confounding sources of Hz in periph-
eral blood (e.g., gametocytes); moreover, LDMS is only
semiquantitative for estimating parasitemia, an important
parameter in clinical diagnosis. The limits on quantitation
arise from the unknown admixture of parasite stages in a
given blood sample and the varying efficiency of Hz heme
detection for different parasite stages. We have quantified
this efficiency using P. falciparum–infected red blood cells
from a highly synchronized in vitro culture. This analysis
clearly demonstrated detection of microscopically invis-
ible Hz in ring-stage parasites. It also allowed investigation
Figure 1. (a) Detection of Hz heme, the crystalline substance accumulated within malaria parasites during their intraerythrocytic growth stage, by LDMS. (b) Principle of operation of a laser desorption time-of-flight mass spectrometer for malaria parasite detection. Heme-specific ions in RBCs are desorbed from the probe and analyzed by their mass/charge ratio, generating a parasite-specific mass spectral signature.

Figure 2. (a) Mass spectral signature of heme (red trace) originating from infected blood. Only heme from Hz in infected blood is detected. (b) Characteristic structure-specific fragmentation pattern of heme.

of the stage-dependence of a novel LDMS-detectable biomarker of malaria parasites, elevated choline phosphate, the presence of which is most likely associated with the parasite’s specific requirements for lipid biosynthesis.

This research has resulted in two U.S. patents and option licensing agreements with two commercial companies. As mass spectrometry moves into hospital diagnostic laboratories as a tool for microbial detection and characterization in clinical specimens, the transition of the LDMS malaria test is likely to be smooth. The test could provide critical diagnostic support in regions where malaria is not endemic (e.g., in Europe, which still has a high number of imported malaria cases) and where the training for microscopic analysis of blood smears is not always adequate to accurately diagnose cases with low to moderate parasitemias.

For further information on the work reported here, see the references below or contact andrew.feldman@jhuapl.edu.
