Rapid Detection of Malaria Parasites

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APL 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 parasitemia. When spatially contiguous Hz heme detections are assigned to single clusters, the distribution of cluster counts obeys a Poisson distribution. This mathematical model, when used in conjunction with calibration LDMS data from infected and uninfected blood, permits 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 confirmed 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 infection. 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 peripheral 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 invisible Hz in ring-stage parasites. It also allowed investigation

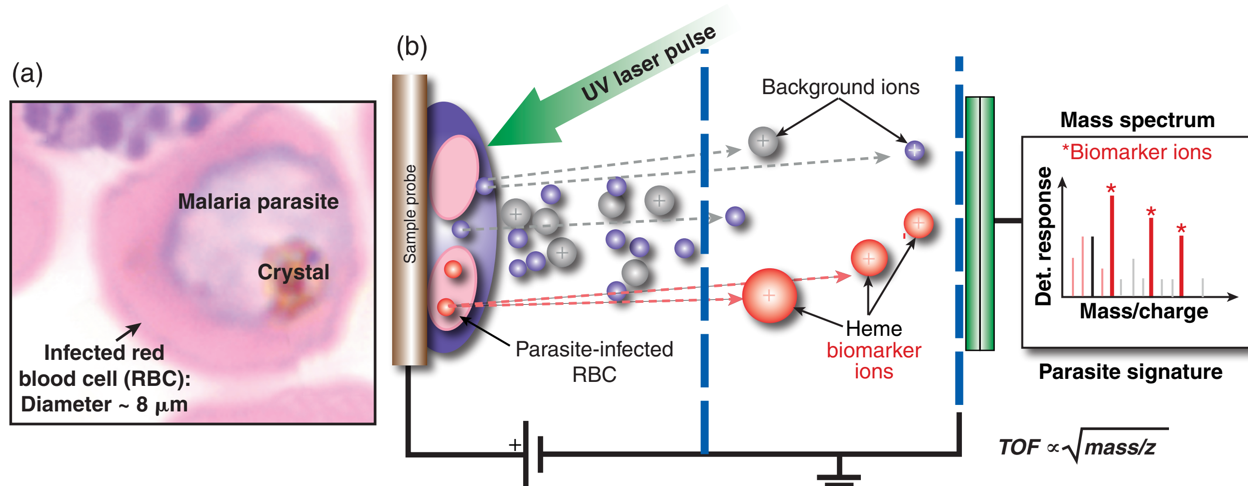

Figure 1. (a) Detection of Hz heme, the crystalline substance accumulated within malaria parasites during their intraerythrocytic growth stage, by LDMS. (b) Principle of operation of a laser desorption time-of-flight mass spectrometer for malaria parasite detection. Heme-specific ions in RBCs are desorbed from the probe and analyzed by their mass/charge ratio, generating a parasite-specific mass spectral signature.

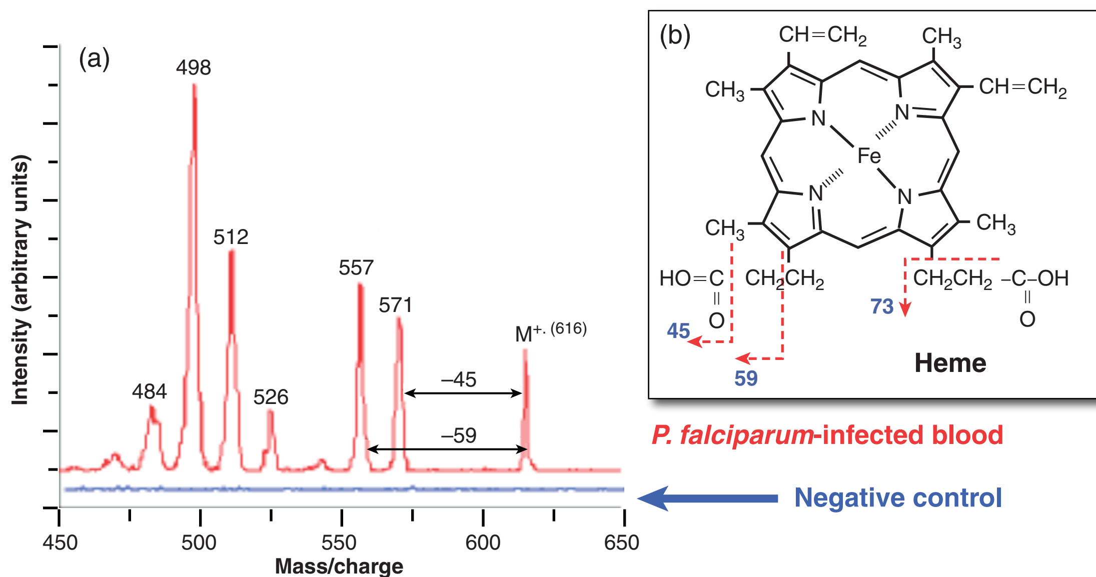

Figure 2. (a) Mass spectral signature of heme (red trace) originating from infected blood. Only heme from Hz in infected blood is detected. (b) Characteristic structure-specific fragmentation pattern of heme.

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.

[1]Demirev, P. A. , Feldman, A., Kongkasuriyachai, D., Scholl, P., Sullivan, D. J., and Kumar, N., "Detection of Malaria Parasites in Blood by Laser Desorption Mass Spectrometry," *Anal. Chem.* 74, 3262–3266 (2002).

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