Rapid Identification of Infectious Agents in Human Plasma with Laser-Based Diagnostics

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Abstract

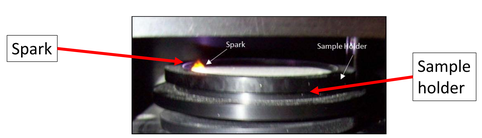
Rapid detection of known pathogens within minutes on-site, with simple specimen preparation, no requirement for highly skilled personnel, and the ability to expand for detecting emerging pathogens is needed to reduce blood donor screening time and risk of transfusion-transmitted disease, and in clinical diagnostics. Laser-induced breakdown spectroscopy (LIBS), a detection method utilizing optical emissions from a sample interrogated by a laser beam, then resolved by spectrometry and processed by programmed analysis, has been effectively used with bloodborne pathogens spiked in blood. Our goal was to further investigate this laser-based technology as a potential diagnostic test for the rapid identification of viral pathogens, HCV and HIV-1, in clinical plasma specimens. We tested clinical specimens and donor plasma spiked with HCV and HIV-1. There was successful differentiation of HCV and HIV-1 in spiked plasma and in clinical plasma with LIBS.

Introduction

The ability to rapidly detect and identify pathogens in bloodborne infections is important for ensuring the safety of the US blood supply and in monitoring and treatment of patients. Currently, the detection of an infection and the identification of the specific organism can typically require up to 72 hr with sample transport to a laboratory, the use of highly skilled personnel, and sophisticated equipment. The development of a blood diagnostic device capable of identifying bloodborne infections on-site within minutes, with minimal sample preparation, portability, and no need for highly skilled laboratory personnel would advance clinical diagnosis. Our research indicates that point-of-care diagnostic instrumentation based on LIBS analysis of spectra from a series of laser sparks formed on a blood sample has this potential. This analysis is based on programming that could be expanded as new algorithms are developed to detect additional infections.

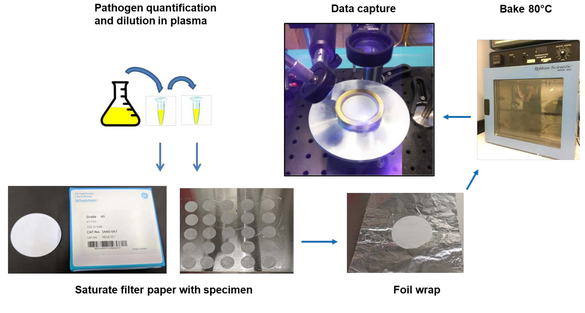
Materials and Methods

The pathogens tested included two viruses, HCV and HIV-1, spiked in donor plasma and clinical specimens from a large donor testing center. HCV genotype 1b and HIV-1 group M subtype B were FDA CBER reference vials with copies per ml validated by CBER. Pathogens were spiked at two concentrations (1000 and 100 copies/ml) in healthy volunteer plasma obtained from the NIH Blood Donor Center. Clinical plasma was obtained from Creative Testing Solutions. Blood donor plasma was previously tested and identified as negative, HCV positive, or HIV-1 positive with FDA licensed tests. The pathogen spiked plasma, negative controls, and clinical plasma were applied (0.5 ml) to 47 mm Grade 40 paper filters. Filters were air dried, aluminum foil wrapped, oven baked, and analyzed with a benchtop assembled LIBS device. Predictive models based on multivariate and statistical analysis were used to develop detection algorithms to differentiate pathogen-free from infected plasma.



Materials and Methods

LIBS Sample Preparation



LIBS Analysis System Components

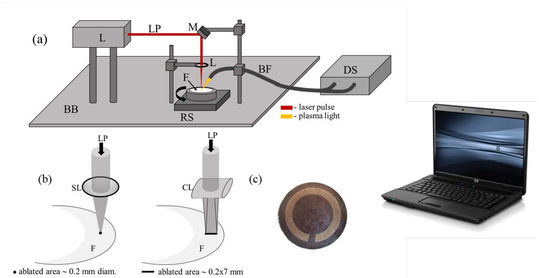
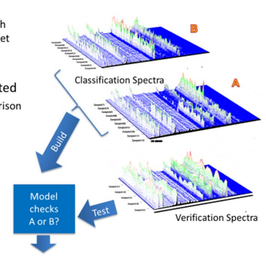


Figure 1. (a) Diagram of the benchtop apparatus used to record spectra. BB: breadboard; L: laser; LP: laser pulse; M: mirror; L: lens; F: filter, BF: bifurcated fiber optic; DS: dual channel spectrometer; RS: rotating stage. (b) Geometry of focusing the laser pulses using a spherical and cylindrical lens. SL: spherical lens; CL: cylindrical lens. (c) Photo of a filter following interrogation by 620 laser pulses (annular light-colored region) focused using the cylindrical lens.

"Training" the instrument

- Data collected in two sets
  - Classification set (3-5 separate specimens with target pathogen and a set without)
  - Verification set
- Predictive models created
  - Group by Group comparison
- Detection algorithm generated
- Algorithm can be programmed into an instrument to operate as a "black box"

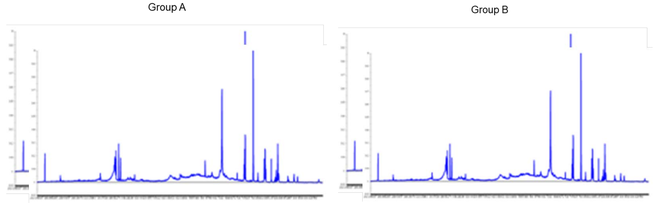


The process results in a numerical value for each sample, called the **prediction value**, that determines the sample's status.

Results and Discussion

In the first study, 65 samples were tested including 20 clinical specimens. Detection algorithms based on multivariate predictive modeling and statistical analysis were developed on 3 of the replicates and 2 replicates tested for distinguishing pathogen-free from pathogen-spiked and clinical plasma. The LIBS system with detection algorithms was 100% correct in differentiating spiked plasma from unspiked plasma and infected clinical plasma from uninfected clinical plasma with identification of HCV or HIV-1. There were no false positives nor false negatives. A repeat study with new additional samples was conducted. In study two, 69 samples including 20 clinical specimens were tested. The LIBS system with detection algorithms was again successful with identification of HCV or HIV-1. There were no false positives nor false negatives.

Detection is not based on specific elemental signals correlated to a particular pathogen.
All optical signals generated from a spark on the sample are used for analysis.
Differentiation of groups is based on a statistically derived prediction value (PV) that represents the characteristics of the average spectra for each sample.



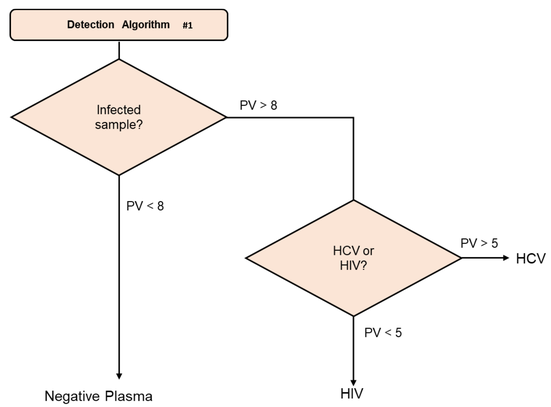


Table 1. The prediction value for each of the test samples from Plasma study 1 and resulting interpretation. (a) shows the model differentiation of infected from uninfected samples for the spiked test samples and the clinical donor test samples. (b) shows the differentiation of HCV from HIV-1 for the spiked test samples and the clinical donor test samples that tested positive in model (a).

Sample	n	Prediction Value	Interpretation
(a)			
Plasma	4	<8	no pathogen present
Plasma+HCV 1000 copies/ml	3	>8	pathogen present
Plasma+HCV 100 copies/ml	6	>8	pathogen present
Plasma +HIV 1000 copies/ml	3	>8	pathogen present
Plasma +HIV 100 copies/ml	6	>8	pathogen present
Clinical negative Plasma	6	<8	no pathogen present
Clinical HCV positive	4	>8	pathogen present
Clinical HIV positive	4	>8	pathogen present
(b)			
Plasma+HCV 1000 copies/ml	3	>5	HCV present
Plasma+HCV 100 copies/ml	6	>5	HCV present
Clinical HCV positive	4	>5	HCV present
Plasma +HIV 1000 copies/ml	3	<5	HIV present
Plasma +HIV 100 copies/ml	6	<5	HIV present
Clinical HIV positive	4	<5	HIV present

Table 2. The prediction value for each of the test samples from Plasma study 2 and resulting interpretation. Format as in Table 1

Sample	n	Prediction Value	Interpretation
(a)			
Plasma	2	<6	no pathogen present
Plasma+HCV 1000 copies/ml	2	>6	pathogen present
Plasma+HCV 100 copies/ml	4	>6	pathogen present
Plasma +HIV 1000 copies/ml	3	>6	pathogen present
Plasma +HIV 100 copies/ml	3	>6	pathogen present
Clinical negative Plasma	7	<6	no pathogen present
Clinical HCV positive	2	>6	pathogen present
Clinical HIV positive	5	>6	pathogen present
(b)			
Plasma+HCV 1000 copies/ml	2	<6	HCV present
Plasma+HCV 100 copies/ml	2	<6	HCV present
Clinical HCV positive	2	<6	HCV present
Plasma +HIV 1000 copies/ml	2	>6	HIV present
Plasma +HIV 100 copies/ml	2	>6	HIV present
Clinical HIV positive	5	>6	HIV present

Conclusion

Our advances in Laser-Induced Breakdown Spectroscopy (LIBS) methodology with differentiation algorithms based on multivariate and statistical analysis allow for effective and rapid (within minutes) differentiation of infected clinical plasma from uninfected. Advantages with LIBS analysis are minimal sample preparation; simultaneous analytical processing, the flexibility to add new algorithms for emerging pathogens, the low cost of consumables, and portability. This laser-based study for multiplex pathogen detection suggests that LIBS has the potential for use as a blood safety diagnostic.

Disclaimer

Our comments are an informal communication and represent our own best judgement. These comments do not bind or obligate FDA.

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