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The use of Laser-based Diagnostics for the Rapid Identification of Infectious Agents in Human Blood

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[†]Initial proof-of-principle data collected at Applied Research Associates, Inc.

Abstract

Aims—To investigate the use of a laser-based method of detection as a potential diagnostic test for the rapid identification of infectious agents in human blood.

Methods and Results—In this study, the successful differentiation of blood spiked with viruses, bacteria, or protozoan parasites to clinically relevant levels is demonstrated using six blood types (O+, O-, AB+, A+, A-, B+) using blood from different individuals with blood samples prepared in two different laboratories. Experiments were performed using various compositions of filters, experimental set-ups, and experimental parameters for spectral capture.

Conclusions—The potential for developing a laser-based diagnostic instrument to detect the presence of parasites, bacteria and viruses in human blood capable of providing analysis results within minutes was demonstrated.

Keywords

laser; LIBS; rapid; blood; *S. aureus*; HIV; *T. cruzi*

INTRODUCTION

The ability to detect and identify pathogens in bloodborne infections is an important capability for either patient treatment/monitoring, or ensuring the safety of the donor blood

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CONFLICT OF INTEREST

Stephen Young:

Roche Molecular Systems, Inc. - Advisory Board

Quidel, Inc – Advisory Board

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supply. Today, the detection of an infection and the identification of the responsible organism typically requires up to 72 hours depending on the pathogen; the use of highly skilled personnel; and complex sample preparation. In addition, transport of a blood sample to a microbiology laboratory for analysis is often required. The ability to rapidly (within minutes) diagnose bloodborne infections on-site, with simple sample preparation and no requirement for highly skilled personnel would greatly enhance the ability to identify, contain, and treat bloodborne infections as well as greatly reduce the time needed to screen donated blood for infections. The development of a blood diagnostic with these capabilities would be a significant advancement to laboratory diagnosis as patients could be screened for infections and treatments prescribed immediately. Our research indicates that point-of-care diagnostic instrumentation based on the analysis of spectra collected from a series of laser sparks formed on a blood sample has the potential to provide these capabilities. In addition, as the analysis is based on programming built into the instrument, once blood has been collected, applied to a filter and inserted into the diagnostic instrument, the detection would be accomplished by simply pushing a button to start the analysis. Once the programmed analysis has been completed, results could be readily displayed. In addition, because the analysis is based on programming, the instrument could be programmed for multiplex detection and the programming could be expanded as new algorithms are developed to detect additional infections.

The methodology we are developing uses the same mechanism for signal generation as is used in Laser-Induced Breakdown Spectroscopy (LIBS), an established spectrochemical analysis technique in which a laser pulse is used to simultaneously vaporize a small sample mass and excite the resulting atoms to emit light (Cremers and Radziemski, 2013) via formation of a hot plasma on the sample surface. Light from the plasma is collected, spectrally resolved and recorded. In conventional LIBS, the spectra are analyzed using specific elemental emissions to determine sample composition. The analysis in this work, uses advanced mathematical methods based on multivariate analysis combined with statistics to analyze the entire spectrum with no attempt to link the analysis to specific spectral regions or spectral emissions. Multivariate data analysis methods are designed to extract the structure from the data without any previous knowledge of the data set in the presence of noise and random signals that would normally complicate analysis (Esbensen, 1994; Esbensen and Swarbrick, 2018). In this way, it is possible to develop predictive models capable of separating elementally similar samples with very small variations in concentration by collecting a sufficient number of spectra from the two sample types. The only commonality between conventional LIBS analysis and the analysis presented in this work is the use of a laser spark to generate a signal from the sample. The method of extracting the relevant signal buried in the data is completely different. Using the LIBS technique with a novel analysis approach, the presence of pathogens in the blood has been successfully demonstrated to clinically relevant levels of 10 cells, copies, or parasites per ml.

Over the last decade, the use of the laser spark and traditional LIBS analysis to detect biological material was demonstrated by a number of researchers. Morel et al. revealed that LIBS could detect and sort species of six bacteria and two pollens (Morel et al., 2003). Rehse et al. and Diedrich et al. showed that both pathogenic and nonpathogenic *Escherichia coli* (*E. coli*) cultured strains grown in both nutrient-rich and nutrient-free media can be

differentiated using a statistical analysis of LIBS spectra of these targets (Rehse et al., 2007a; Rehse et al., 2007b; Diedrich et al., 2007). Multivariate methods of analysis similar to the analysis presented in this manuscript have been applied to the laser spark generated spectra to identify and discriminate as follows: a) bacterial species as well as strains of *E. coli* (Multari et al., 2010); b) to identify and discriminate bacteria, parasites, and viruses on substrates (Multari et al., 2012); c) to detect the presence of pesticides and dioxins in tissue fats and rendering oils (Multari, Cremers, Scott, and Kendrick, 2013); as well as d) to discriminate bacterial pathogens and methicillin-resistant *Staphylococcus aureus* (*S. aureus*) strains grown on blood agar (Multari et al., 2013a). It has been shown in both published and unpublished work by two of the authors that this method of analysis can differentiate bacteria by both type and strain, by concentration, and by metabolic state (Multari et al., 2013b; Multari and Cremers, 2014). First proof-of-principle experiments demonstrating the use of LIBS for the differentiation of pathogens spiked into human blood and Herpes Simplex virus in cerebral spinal fluid was presented at the 2012 Biodetection Conference (Multari, Cremers, Duncan, and Young, 2013). Subsequent work was presented at the 2017 International Biodetection Technologies Conference (Fisher, et al., 2017) and the 2018 International Biodetection Technologies Conference (Fisher, et al., 2018).

The goal of this study is to demonstrate the ability to apply advanced mathematical methods of analysis to the entire emission spectrum of a laser spark to identify blood samples spiked at clinically relevant levels with selected pathogens. Although not identical to clinical samples, the use of spiked samples was the first step in the demonstration of this technique and these can be assumed similar enough to infected blood samples for the purposes of this investigation as has been done in other similar detection method validations (Dong 2016, Hockman 2016). Spiked samples are useful to demonstrate the basic ability to detect pathogens at low concentrations in human blood using multivariate methods on carefully controlled samples. Results will be presented demonstrating the ability to detect blood samples spiked with the bacteria *S. aureus* and *Yersinia pseudotuberculosis* (*Y. pseudotuberculosis*), the parasite *Trypanosoma cruzi* (*T. cruzi*) and the Human Immunodeficiency Virus (HIV-1) by analysis of LIBS spectra.

MATERIALS AND METHODS

Sample preparation

The samples used in this study were prepared in the same way in two different laboratories: the Food and Drug Administration Center for Biologics Evaluation and Research and TriCore Reference Laboratories. Two bacterial species, Gram-positive *Staphylococcus aureus* (ATCC #29213) and Gram-negative *Yersinia pseudotuberculosis* (ATCC #29833) were cultured in Super Broth (Quality Biological, Gaithersburg, MD) and quantified as previously described (Dong et al., 2016). Briefly, the bacteria were quantified by measuring OD600 and converting to cells ml⁻¹ by the formula $5.0 \times 10^8 \text{ cells ml}^{-1} = 1 \text{ absorbance unit}$ in a spectrophotometer (SmartSpec 3000, Bio-Rad, Hercules, CA) or by preparing a bacteria suspension of turbidity equivalent to 0.5 McFarland unit in a densitometer (DENSICHEK™ PLUS STANDARDS, bioMérieux, Durham, NC). HIV-1 group M clade B was prepared from CBER reference material with copies ml⁻¹ validated by CBER (Grigorenko et al.,

2014). Concentrations in the spiked specimens were used without further verification. The extracellular trypomastigotes of the parasite, *Trypanosoma cruzi* (Tulahuen strain), were cultured in 3T3 cells as previously described (Nagarkatti et al., 2012) and quantified with a cellometer (Vision, Nexcelom Bioscience, Lawrence, MA) and by real-time PCR as previously described (Kourout et al., 2016). Spiking of *Staphylococcus aureus*, *Yersinia pseudotuberculosis*, HIV-1 (HIV) and *Trypanosoma cruzi* was performed as previously described (Grigorenko et al., 2017). Briefly, pathogen stock was serially diluted 10-fold into whole heparinized human blood for spiking at concentrations of 0, 10, 100, 1000, and 10,000 cells, copies, or parasites per ml. The blood was obtained from de-identified and tested donors for the research program at the Department of Transfusion Medicine, NIH under an approved FDA research protocol and volunteers at TriCore reference laboratories.

Pathogen spiked or negative control blood was loaded on size 47 mm Whatman™ qualitative grade 1 filters (600 µl of blood applied), quantitative grade 40 filters (500 µl of blood applied), or borosilicate glass microfiber filters (700 µl of blood applied) depending on the experiment. The volume applied to each filter type was optimized to thoroughly saturate the filter and the properties of each filter impacted the amount of blood the filter could absorb. Care was taken to apply the blood across the filter to ensure the most uniform blood coverage possible. The small volume sample sizes chosen minimize the impact on the quantity of a diagnostic blood draw or a sampling of a unit of donated blood. After pathogen spiking, the filters were air dried, wrapped in aluminum foil, and oven baked at 80°C for 2 hours to inactivate viable organisms. The filters were then sent to Creative LIBS Solutions for laser-based analysis. There was typically a 2 to 3-day delay between the sample preparation and the data collection.

Data collection

A diagram of the benchtop apparatus used to collect spectral data on the blood samples is shown in Figure 1a. The benchtop system was assembled using discrete components mounted on an aluminum optical breadboard for mechanical stability. Laser pulses were provided by a pulsed and Q-switched Nd:YAG laser (Quantel CFR200). The shot-to-shot reproducibility of the laser pulse energy is specified to be within 2% of the set value. The laser pulses (1064 nm wavelength; 13 ns duration) were focused on the samples at a repetition rate of 10 Hz using a simple lens. Depending on the experiment, either a spherical lens (25.4 mm diameter; 100 mm focal length) or a cylindrical lens (25.4 mm diameter; 75 mm focal length) were used to focus the laser pulses. The spherical lens focuses the laser pulse on the sample as a circular spot approximately 0.2 mm in diameter whereas the cylindrical lens focuses the pulse as a line approximately 0.2 mm wide and 7 mm long (Figure 1b). An advantage to using the cylindrical lens is that the laser pulse interrogates a substantially larger area on each shot than the spherical lens (1.4 mm² versus 0.031 mm², respectively). For the same laser pulse energy, however, the spherical lens generates higher power densities on the focus area, potentially leading to increased excitation and greater spectroscopic signals. The laser pulse energy used with each lens was adjusted to produce maximum signal strength without saturation of the recorded spectrum.

The samples (47 mm filters coated with dried blood) were positioned on a rotating stage for data collection. The rotation rate was 5 degrees/s allowing one complete rotation in 72 s which was the interrogation time for each sample. During a measurement, an outer annular region of the filter was interrogated. A filter interrogated using the cylindrical lens resulted in an ablated region, visible as a lighter gray ring (Figure 1c). At the 10 Hz pulse repetition rate, the spacing between the centers of adjacent ablated regions was 0.16 mm providing some overlap of the ablated areas.

As shown in Figure 1a, pulses from the laser were directed vertically downward through the focusing lens onto the sample. The distance between the lens and sample surface was approximately equal to the lens focal length but was adjusted slightly to generate maximum optical signals from the sample thereby compensating for the divergence of the laser beam. Plasma light was collected using a bifurcated fiber optic cable pointed at the plasma formed on the sample at a distance of 3 cm. The fiber collects light within a 26-degree acceptance cone centered on the optic axis of the fiber. The distal end of the fiber consisted of two fibers (each 0.6 mm diam.) splitting the collected light into two equal fractions. Each of these fibers was connected to the input of a channel of a dual channel spectrometer (Avantes AvaSpec ULS2048–2-USB2). The two channels monitored separate but adjacent spectral regions (channel 1: 234–494 nm; channel 2: 481–1008 nm). Over the 234 to 1008 nm spectral region spanned by the spectrometer, signal intensities were recorded at 4096 discrete wavelengths. The approximate spectral resolution of channels 1 and 2 were 0.18–0.2 nm and 0.4 nm, respectively. The spectrometer provided gated detection to record plasma light only during a selected time period after plasma formation. The delay time was set to begin recording at a predetermined time after plasma formation. After the start of recording, in all cases the plasma light was collected over a period of 1.05 μ s. The plasma lifetime was on the order of 20 μ s so that all light from the plasma was recorded after the delay time. The spectra were stored on a laptop computer which controlled operation of the dual channel spectrometer and the laser.

A total of 620 sequential laser pulses were used to record 62 spectra. Each recorded spectrum was the average of 10 spectra (laser fired at 1 s/spectra). Rotation of the sample stage was initiated about 2 s before the laser was activated to begin recording of the spectra to ensure the stage had achieved the preset rotation speed (5 degrees/s). Measurements were carried out in the vicinity of a HEPA filtered hood to collect ablated material.

Data analysis

The spectral data were analyzed using multivariate analysis combined with statistical analysis to compensate for variations in spectral emissions and sample uniformity. Specifically, Partial Least Squares Discriminant Analysis (PLSDA), a form of multivariate analysis in which partial least squares regression is used iteratively to determine the principal components used for modeling, was used to create predictive models using replicate samples and the quality of the predictive model was then evaluated on other replicate samples not included in the modeling. Various data pre-processing methods (such as simple normalization of the spectrum maximum to unity and omission of selected spectral

regions) were used prior to modeling and the method that produced the best predictive model was selected.

The performance for a predictive model was evaluated using the prediction value output by the model for the test sample analyzed. The modeling prediction range was from 0 to 10. A prediction value of 10 indicated the test sample spectra exactly matched the spectra of the pathogen(s) samples that the model was designed to detect. A prediction value of 0 indicated the test sample spectra exactly matched the spectra of samples the model was designed to distinguish the pathogen(s) samples from. A value between 0 and 10 indicated how well matched the test sample spectra were to the pathogen(s) sample that the model was designed to distinguish. The higher the prediction value, the better the matching. The lower the prediction value, the poorer the matching. All the prediction values obtained from the spectra collected on a test sample were averaged and the average prediction value was used to do the predictive analysis. To differentiate the two groups that were modeled, a threshold prediction value was chosen such that samples with a prediction value above the threshold were identified as being part of the pathogen(s) group the model was designed to detect, whereas samples with average prediction values below the threshold were identified as belonging to the sample group associated with the samples the model was designed to separate the pathogen(s) from. Model quality was evaluated by the position of the threshold and the separation of the prediction values groupings for the test samples. Best models had the largest gaps between the prediction values for the sample groups and a threshold at or very close to 5, the mid-point of the modeling range. However, thresholds in the range of 4–6 (midpoint of the modeling range $\pm$ 10%) also produced acceptable models. Once a satisfactory predictive model was generated, it was used alone or in combination with other models to create a detection algorithm that could be used to determine if the sample was a spiked sample, or a control sample (un-spiked blood) and, if it was a spiked sample, the type of pathogen spiked into the sample.

Two types of controls were used in this study, one was a pure blood sample (no spiking) and another was a blood sample spiked with the microbial culturing media. The media spike was at the concentration of media present in the highest concentration sample used in the experiment (typically 1000 cells, parasites, or copies ml^{-1} for most of the study). It should be noted that this latter control sample is not indicative of any sample which might be encountered in an actual clinical sample but was included to investigate the contribution the culture media might be making to the ability to discriminate between un-spiked and spiked blood samples. In most cases, the media control samples were identified as un-spiked samples even when not included in the modeling indicating the culture media did not play a major role in the detection. In the few cases for which a media control sample binned as a spiked sample, it was counted as a false positive for the cumulative statistics being calculated even though no such sample would be encountered in a clinical diagnostic environment.

RESULTS

In this study, 24 separate experiments were run over six different blood types using blood from 29 different individuals. For four experiments, samples were prepared in two different

laboratories. Initial experiments were focused on determining the best timing parameters, best substrate, best sample preparation method, and the best focusing lens for detecting blood spiked with pathogens at clinically relevant levels of 10–10,000 cells, parasites, or copies ml^{-1} . Table 1 lists the samples used in the 24 experiments. Hundreds of detection models were created and evaluated to determine those producing the best detection algorithms. Overall, 532 test samples (spectra from the test samples were not included in any modeling) were input into the detection algorithms that were developed and, out of these 532 test samples, 100% were correctly identified as spiked samples with 96.6% having the type of spiked pathogen correctly identified. The false positive rate was 2.3% and the false negative rate was 0%. The false positive failures were most often the blood media control samples (approximately 83%) and the pathogen mis-ID's were most often the lowest concentration samples of 10 cells, parasites, or copies ml^{-1} . It should be noted that these statistics cover the entire range of experimental conditions studied and only the latter ten experiments (experiments 15–24) utilized the experimental set-up and parameters found to be optimal for detection. If only the experiments in which the optimized conditions were used are considered, the number of samples in the statistics drops to 244 with 100% of the spiked samples being correctly identified as spiked samples and 93.0% of the spiked samples having the type of spiked pathogen correctly identified. The false positive rate increases to 3.7% and the false negative rate remains 0%. See Table 2 for detailed results.

The spectral characteristics of a subset of the samples under investigation were studied using one of the initial data sets. The spectrum of a sample of HIV (10^3 copies ml^{-1}) in blood on a paper filter is shown in Figure 1d. This spectrum is an average of 10 individual spectra and the spectral region from 234 to 900 nm is displayed. The intensities corresponding to wavelengths on channel 1 have been adjusted so the signal intensity of channels 1 and 2 at the common wavelength (480 nm) agree. Elements corresponding to some of the stronger emission features have been labeled.

To determine the best experimental configuration for optimizing the detection of pathogens in human blood, three experimental parameters were investigated: 1) the type of lens used to focus pulsed laser light onto the sample; 2) the delay time between the initiation of the laser spark and the recording of the spectrum; and 3) the filter substrate on which the blood was placed for analysis. The characteristics of the two focusing lenses evaluated are described above and illustrated in Figure 1b. The intensity of the spectrum is dependent on the delay time between the initiation of the laser spark and the time period over which the spectrum was recorded. Three delay times were considered: 0.5, 1.0, and 2.0 μs . At 0.5 μs after spark initiation on the sample, the spectrum (discrete atomic and ion emission features and overall background) was most intense. By 2.0 μs , the overall spectrum intensity was decreased and characterized mainly by atomic emissions with low background. For analysis, blood was applied to a filter substrate and then dried. Only easily obtained inexpensive substrate materials were considered: 1) WhatmanTM paper filters, 2) WhatmanTM glass microfiber filters, and 3) WhatmanTM ashless paper filters. To compare lens performance, a starting substrate material and delay time were held fixed and the detection performance of each lens was evaluated. Once the cylindrical lens was selected based on the greater ability to develop stable detection algorithms using this lens, the three delay times being considered were evaluated using this lens and the starting substrate material. To evaluate performance, data

on pathogens at various concentrations was collected and analyzed as described below to determine the delay that produced the best detection results. Once a delay time was selected, the best substrate was then selected in a similar fashion using the optimized breadboard parameters. Experiments were run to evaluate substrate detection performance using various blood types and pathogen concentrations ranging from 10–10,000 pathogens ml⁻¹.

The first of the experimental configuration experiments determined which laser beam focusing lens, spherical or cylindrical, would optimize detection performance. For this evaluation, spectral data were collected on the replicate samples using a fixed timing delay of 1 μ s. To select the focusing lens, multiple experiments were run using either a spherical lens or a cylindrical lens and predictive models were created to distinguish between samples. The modeling performance for the data collected using the two different lenses was compared and the optimum lens type was selected based on the best overall performance in distinguishing pathogen spiked samples from the controls. While both lenses produced good detection performance, it was determined that the cylindrical focusing lens produced the best performance.

A second set of experiments established the best delay time to use for analysis. The atomic, ionic, and molecular emission intensities are time dependent and changing as the laser spark cools, potentially affecting detection performance. Three delay times were investigated: 0.5, 1, and 2 μ s. For each delay time, data were collected using the cylindrical lens and predictive models were created from these data and tested on data collected from samples not included in the model build. Samples for these experiments were prepared by the FDA and *S. aureus* samples in AB+ blood type was used for testing. The results from many predictive models for delay times examined were compared. Based on the best overall performance in separating the pathogen spiked samples from the control blood and blood with media samples, it was determined that a 0.5 μ s delay produced the best performance.

The last set of optimization experiments selected the best substrate to use for analysis. Glass microfiber, paper, and ashless paper substrates were evaluated. It was found that the cylindrical lens could not be used with the glass microfiber filter because the surface power density of the focused laser pulse produced by the cylindrical lens was insufficient to generate a laser spark on the filter surface. Based on these experiments, the best results were obtained using a cylindrical lens, 0.5 μ s delay, and ashless Whatman™ filters. Figure 2 shows the algorithm structure for detecting *S. aureus* and *T. cruzi* in blood using these parameters and substrate. Figure 3 shows the results obtained when spectra of test samples of blood, blood spiked with culture media, and blood samples spiked with either *T. cruzi*, or *S. aureus* at concentrations up to 1000 parasites or cells ml⁻¹ respectively, were input into each of the two predictive models of the algorithm of Figure 2 which successfully identifies all of the test samples. The first predictive model in the detection algorithm successfully separates the pathogen spiked samples from the un-spiked samples (Figure 3a). It was created by modeling only over the spectral data collected from the laser sparks generated on the three *S. aureus* concentration samples (10–1000 cells ml⁻¹) and the un-spiked blood samples (0 cells ml⁻¹). The second predictive model in the detection flow successfully separates the *S. aureus* spiked samples from the *T. cruzi* spiked samples (Figure 3b).

The ability to identify, using a single detection algorithm, pathogen spiked blood samples generated in two different laboratories using blood from different donors was investigated. Samples for this investigation were prepared in the FDA Center for Biologics Evaluation and Research (Lab 1) and TriCore Reference Laboratories (Lab 2). Using samples from these laboratories prepared with blood of the same type obtained from donors living in different parts of the country and at different altitudes (96 m versus 1500 m) it was possible to build detection algorithms that identified the pathogen spiked blood and the types of spiked pathogens with an accuracy of 92–94%. Best results were obtained by including blood samples from donors living at different locations in the modeling.

Lastly, the ability to detect blood with a mix of pathogens was investigated. Samples were prepared that included only *S. aureus*, only HIV, and a 1:1 mix of *S. aureus* and HIV. Using 3 different models, it was possible to build a detection algorithm that successfully identified all samples as follows: Using only the *S. aureus*, HIV, and blood control samples, it was possible to build a model that successfully identified the samples with no pathogen, both blood and blood with media control samples (Figure 4a). Using only the *S. aureus* and HIV samples, it was possible to build a model that successfully identified the *S. aureus* samples (Figure 4b). To detect the samples with a mix of both *S. aureus* and HIV, it was necessary to include some of the mix samples in the modeling to differentiate the mix from the HIV samples (Figure 4c).

DISCUSSION

The results of these experiments indicate that the use of a laser-based method for detecting pathogens in blood has the potential to produce reliable results at clinically relevant levels of pathogen concentration. In all tests, the pathogen spiked samples were correctly identified as spiked samples with 96.6% of the spiked pathogens being correctly identified. There were no false negatives and, of the 2.3% false positives, most of these were the media control samples (blood spiked with the culturing media at the volume that is added for the 1000 cells, parasites, or copies ml^{-1} samples) which would not be encountered in a clinical diagnosis situation.

Models capable of detecting at the lowest concentration of 10 cells, parasites, or copies ml^{-1} were challenging to build and the detection algorithms often required multiple models to successfully screen these samples. This suggests that this concentration is close to the detection limit for our methodology (sample preparation methods, the breadboard configuration, and the modeling). At 10 cells, parasites, or copies ml^{-1} , the majority of the 620 laser sparks generated on the sample surface do not contain the spiked pathogen. The ability to detect the pathogen at the lowest concentration may be due to changes in the blood sample resulting from the presence of the pathogen, such as the presence of diffusible metabolites.

These results represent the best detection that could be achieved using the breadboard experimental set-up chosen for this study. Changes in equipment (spectrometer, laser, focusing optics), could result in increased detection sensitivities. Using the equipment selected for this study with a 0.5 μs delay, a cylindrical lens, the blood samples applied to an

ashless Whatman™ filter, detection of pathogens (bacteria, virus, and parasite) in different blood types has been demonstrated.

The results of this study indicate the potential for a laser-based diagnostic to detect pathogens in blood. The next steps would be to replace the breadboard setup with an enclosed module device incorporating the laser, spectrometer, sample rotation stage and controlling computer in a fixed configuration. The device would be calibrated to simultaneously detect multiple pathogens in blood. Such a device, once successfully demonstrated and deployed, would enable more rapid blood analysis without the need for specialized laboratory facilities or highly-skilled personnel and would greatly enhance the ability to identify, contain, and treat bloodborne infections and reduce the time needed to screen donated blood for infections. The development of a blood diagnostic with these capabilities would be game-changing for medical diagnosis.

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Significance and Impact of the Study

There is an ongoing need for clinical diagnostics to adapt to newly emerging agents and to screen simultaneously for multiple infectious agents. A laser-based approach can achieve sensitive, multiplex detection with minimal sample preparation and provide rapid results (within minutes). These properties along with the flexibility to add new agent detection by simply adjusting the detection programming make it a promising tool for clinical diagnosis.

Figure 1.

Top: (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 focused using the cylindrical lens. (d) Spectrum of a sample of HIV at 10^3 copies ml^{-1} in blood with some of the stronger emission lines labelled for the element or molecule producing that spectral line. Elements are labeled with their designation in the periodic table. CN is cyanide.

Figure 2.

Detection algorithm structure for identifying *S. aureus* and *T. cruzi* in blood using the cylindrical lens, a 0.5 μ s delay and ashless Whatman™ filters as substrates (from Experiment 15). The first model separates the pathogen spiked samples from the controls and the second model separates the pathogens by type. The flow of the sample through the model is determined by the numerical prediction value output from the model when the test sample spectrum is input into the model. For this algorithm, a value of 5 is used to determine the branching through the algorithm.

Figure 3.

Test sample results for the detection algorithm presented in Figure 2 from Experiment 12.

(a) Test results for samples when input into the first model of the algorithm. Samples with

prediction values >5 are identified as spiked samples. (b) Results for samples that were

identified as spiked in the first model when input into the second model of the algorithm.

Samples with prediction values >5 are identified as *T. cruzi* spiked samples and samples

with prediction values <5 are identified as *S. aureus* spiked samples.

● Blood ◆ Blood with Media Δ *S. aureus* 10 cells ml⁻¹ ○ *S. aureus* 100 cells ml⁻¹
 □ *S. aureus* 1000 cells ml⁻¹ ● *T. cruzi* 100 cells ml⁻¹ ■ *T. cruzi* 1000 cells ml⁻¹

Figure 4.

(a) Test sample results from the first predictive model in an algorithm created to separate *S. aureus*, HIV samples, and a mix of *S. aureus* and HIV (from Experiment 24). This model was created without any mixed sample data but correctly identifies the mixed samples as spiked samples. (b) Test sample results from the second predictive model in an algorithm created to separate *S. aureus*, HIV samples, and a mix of *S. aureus* and HIV (from Experiment 24). This model was created to separate *S. aureus* from HIV samples and had no mixed samples in the modeling. (c) Test sample results from the third predictive model in an algorithm created to separate *S. aureus*, HIV samples, and a mix of *S. aureus* and HIV. This model was created to separate mixed samples from HIV samples.

- Blood
- ◆ Blood with HIV media
- Blood with Mix Media
- ▲ Blood with *S. aureus* media
- △ HIV 10 copies ml-1
- HIV 100 copies ml-1
- ◻ HIV 1000 copies ml-1
- ✕ Mix 10 cells copies ml-1
- ✕ Mix 100 cells copies ml-1
- ✕ Mix 1000 cells copies ml-1
- ✕ *S. aureus* 10 cells ml-1
- ✕ *S. aureus* 100 cells ml-1
- ✕ *S. aureus* 1000 cells ml-1

Table 1.

Summary of experiment samples

Experiment	Sample Source	Blood Type	Concentration Range (cells/ parasites, or copies ml ⁻¹)	Bacteria	Parasite	Virus
1	FDA	O+	10, 10 ² , 10 ⁴	<i>Y. pseudotuberculosis</i>	<i>T. cruzi</i>	HIV
2	FDA	O-	10, 10 ² , 10 ³	---	<i>T. cruzi</i>	---
3	FDA	O+, B+, A+	10 ² , 10 ³ , 10 ⁴	---	<i>T. cruzi</i>	---
4	FDA	O-	10, 10 ² , 10 ³	<i>S. aureus</i>	---	---
5	FDA	AB+	10, 10 ² , 10 ³	<i>S. aureus</i>	---	---
6	FDA	AB+	10, 10 ² , 10 ³	<i>S. aureus</i>	---	---
7	FDA	AB+	10, 10 ² , 10 ³	<i>S. aureus</i>	---	---
8	FDA	AB+	10, 10 ² , 10 ³	<i>S. aureus</i>	---	---
9	FDA	O+	10, 10 ² , 10 ³	<i>S. aureus</i>	---	---
10	FDA	O+	10, 10 ² , 10 ³	<i>S. aureus</i>	---	---
11	FDA	A+	10, 10 ² , 10 ³	<i>S. aureus</i>	---	---
12	FDA	A+	10, 10 ² , 10 ³	<i>S. aureus</i>	<i>T. cruzi</i>	---
13	FDA	O+	10, 10 ² , 10 ³	<i>S. aureus</i>	<i>T. cruzi</i>	---
14	FDA	A+	10, 10 ² , 10 ³	<i>S. aureus</i>	<i>T. cruzi</i>	---
15	FDA	A+	10, 10 ² , 10 ³	<i>S. aureus</i>	<i>T. cruzi</i>	---
16	FDA	A-	10, 10 ² , 10 ³	<i>S. aureus</i>	---	---
17	FDA	A-	10, 10 ² , 10 ³ , 10 ⁴	<i>Y. pseudotuberculosis</i>	---	HIV
18 [repeat collection of samples in experiments 15 & 16]	FDA	A+/A-	10, 10 ² , 10 ³	<i>S. aureus</i>	<i>T. cruzi</i>	---
19	FDA	O+	10, 10 ² , 10 ³	<i>S. aureus</i>	---	HIV
20	FDA & TriCore	O+	10, 10 ² , 10 ³	<i>S. aureus</i>	---	HIV
21	FDA	O+	10 ²	<i>S. aureus</i>	---	---
21	FDA & TriCore	O+	10, 10 ² , 10 ³	<i>S. aureus</i>	---	HIV
22	FDA & TriCore	O+	10, 10 ² , 10 ³	<i>S. aureus</i>	---	HIV
23	FDA & TriCore	O+	10, 10 ² , 10 ³	<i>S. aureus</i>	<i>T. cruzi</i>	---
24 *	FDA	O+	10, 10 ² , 10 ³	<i>S. aureus</i>	---	HIV

* includes mixed samples of the two pathogens at the same concentrations

Table 2.

Summary of experimental results

Experiment	Number Samples	Number Control Samples	Number Pathogen Spiked Samples	Number Spiked Samples Correctly Identified as Spiked	Number Spiked Samples with Type of Pathogen Correctly Identified	Number False Positives (Control Identified as Spiked)	Number False Negatives (Spiked Identified as Control)
1	66	33	33	33	33	0	0
2	15	6	9	9	9	0	0
3	30	12	18	18	18	1 [*]	0
4	75	30	45	45	45	0	0
5	5	2	3	3	3	0	0
6	5	2	3	3	3	0	0
7	10	4	6	6	6	0	0
8	10	4	6	6	6	0	0
9	10	4	6	6	6	0	0
10	10	4	6	6	6	0	0
11	10	4	6	6	6	0	0
12	14	4	10	10	10	0	0
13	14	4	10	10	10	1 [*]	0
14	14	4	10	10	10	1 [*]	0
15	10	4	6	6	6	0	0
16	10	4	6	6	6	0	0
17	24	8	16	16	16	0	0
18	18	6	12	12	12	2 [†]	0
19	18	6	12	12	12	0	0
20	58	22	36	36	29	6 [*]	0
21	4	2	2	2	2	0	0
22	36	12	24	24	22	0	0
23 ^{**}	40	16	24	24	22	1	0
24	26	8	18	18	18	0	0
Total	532	205	309	327	316	12	0
Percentage				100.0%	96.6%	2.3%	0.0%

^{*} False positive(s) was(were) media control(s) (samples spiked with pathogen culturing media corresponding to pathogen concentrations of 1000 cells, parasites, or copies/mL) and not representative of a sample that would be encountered in clinical diagnosis

[†] Only one false positive was media control (sample spiked with pathogen culturing media corresponding to pathogen concentrations of 1000 cells, parasites, or copies mL⁻¹) and not representative of a sample that would be encountered in clinical diagnosis, one false positive was a blood control and would be representative of a sample encountered in clinical diagnosis

^{**} Data were collected on samples that were 12 days old and sample aging impacted the differentiation performance of the modeling as indicated by comparing the same model built on data collected after 4 days and 12 days and comparing the model performance.