ABSTRACT: Lateral flow immunoassays (LFIs) are simple, point-of-care diagnostic devices used for detecting biological agents or other analytes of interest in a sample. LFIs are predominantly singleplex assays, interrogating one target analyte at a time. There is a need for multiplex LFI devices, e.g., a syndromic panel to differentiate pathogens causing diseases exhibiting similar symptoms. Multiplex LFI devices would be especially valuable in instances where sample quantity is limiting and reducing assay time and costs is critical. There are limitations to the design parameters and performance characteristics of a multiplex LFI assay with many horizontal test lines due to constraints in capillary flow dynamics. To address some of the performance issues, we have developed a spot array multiplex LFI using Braille format (hence called Blind Spot) and a sensor, MACAW (Modular Automated Colorimetric Analyses Widget), that can analyze and interpret the results. As a proof of concept, we created a multiplex toxin panel, for detecting three toxins, using two letter codes for each. The results indicated that the six-plex, triple toxin assay performs as well as singleplex assays. The sensor-based calls are better compared to human interpretation in discriminating and interpreting ambiguous test results correctly especially at lower antigen concentrations and from strips with blemishes.

INTRODUCTION

There are two general methods for biological agent detection and identification: The first, immuno-diagnostics in which either target-specific antigens expressed by the pathogens/agents or agent-specific host markers such as serum antibodies (the so-called serology assay) resulting from exposure to an agent, are probed; second, molecular diagnostics in which agent-specific nucleic acid signatures are probed. There are other variations of the former paradigm, for example, detecting host-specific biomarkers such as cytokines, in response to exposure to a pathogen.1–3 A common immunoassay format is the lateral flow immunoassay (LFI), which is the primary component in many far forward, point-of-need or point-of-care diagnostics tool boxes.4–8 LFIs have the advantages of being low cost, user-friendly, and rapid, giving results in as little as 15 min. LFIs were described in the 1960s and the first commercialized product was Unipath’s Clearview home pregnancy tests launched in 1988.9,10 LFIs have since been extensively used in many areas, such as environmental detection, biodefense, food safety, drug detection, and medical diagnostics. However, as of yet, there are very few true multiplex LFI devices (single strip with multiple stripes to detect multiple agents or analytes simultaneously in one test) on the market.11–16

There is a need for multiplex LFI devices in instances where sample volume is limited and performing fewer tests to unequivocally identify the potential pathogen is critical. Ultimately, this will enable clinicians to provide appropriate care to save lives and cost as well as cut down time from sample to result, by replacing multiple singleplex tests with a single multiplex test. In health care settings, it would be extremely valuable to use a syndromic multiplex LFI panel to differentiate many different pathogens causing similar symptoms (e.g., respiratory panel or GI (gastrointestinal) panel or fever panel) in order to be able to dispense appropriate care depending on the pathogen. For example, infections caused by viral vs bacterial pathogens exhibiting similar disease symptoms need to be treated differently.

It has been well established that there are inherent limitations to constructing a multiplex assay in the conventional format (horizontal test lines laid one above the other in
the direction of sample flow). The issues mainly stem from drawbacks associated with liquid flow dynamics by capillary action and also due to interference or cross reactivity from sample components and impurities. The limitations are as follows: (1) line bleed; (2) non-specific binding when the sample passes through multiple stripes (cross contamination); (3) membrane size; (4) flow rate; and (5) hook or prozone effect.

The most important factor is the antibody–antigen reaction kinetics, which is influenced by several parameters. The flow rate, length of the flow path, pore size of the medium, and kinetics of interaction between the antigen and antibody (on–off rates) all impact the sensitivity, specificity, and development time of the assay. Because of these various parameters, in a multiplex assay, the time it takes for the reaction to occur and form a test line will increase considerably for lines downstream of the origin of fluid flow, i.e., sample port. Thus, the kinetics and potentially the sensitivity of different assays may decrease with the increase in the distance especially between the lines farthest and closest to the origin.

The pixelation approach used by the Symbolics process addresses many of the limitations outlined above. In the Symbolics process, instead of printing test lines perpendicular to the direction of flow, pixelated (microdots) reagents are printed in the direction of flow to create lines or dots parallel to the flow path. This eliminates many potential issues of line bleed and non-specific binding due to flow retardation. It also ensures that the kinetic features of each assay that are defined by the flow rate are identical between the individual assays. In this format, it is also possible to design quantitative gradient assays by varying the concentration of the pixelated reagents across the gradient. Recently, Lee et al. designed and tested a semiquantitative LFI assay using a gradient spot array approach.

Pixelation on lateral flow assays have been described before for microarray-based detection of single high-molecular-weight targets or multiple low-molecular-weight targets in a competitive assay format. Arrayed spots have also been used on vertical flow-through assays where the user controls the flow rate. Li and Macdonald described an innovative approach to design a heptaplex, a 7-segment spot array LFI that increases the efficiency of detection without expanding the device dimensions and consuming excess reagents. The development of the 7-segment lateral flow device (LFD) data display concept was inspired by the electronic information display systems used in digital watches and elevators. This approach compresses multiplex LFI data by applying binary encoding to create signature patterns of test dots. Thus, a sample is diagnosed based on the set of test dots that appear on the LFI ticket in the form of a numerical digital display for compact, easy, and intuitive read out and interpretation by the end user. Ultimately, this approach enables higher information density combined with reduced assay time and costs. A recent review article describes the various emerging design strategies for constructing multiplex lateral flow test strip sensors including spot arrays, for enhancing analytical efficiency and precision of on-site point-of-care diagnostics.

**Blind Spot Concept.** Here, we describe a novel spot array LFI termed Blind Spot in which an approach similar to Symbolics and that of Li and Macdonald is used to design a six-plex assay. In the Blind Spot approach, the spot array is formatted and printed in Braille font; i.e., spots in 6 positions (hence called Blind Spot). We also repurposed hardware and methods from a colorimetric chemical sensing project to build a sensor called MACAW (Modular Automated Colorimetric Analysis Widget) that analyzes and interprets the image of the test result and reports the pathogen present in the sample. In the original version of the Blind Spot, each pathogen/analyte is given a letter code (for example, P for *Bacillus anthracis*). The detector antibody corresponding to *B. anthracis* is printed on the LFI strip in Braille font corresponding to the letter P (first four positions/spots are filled with antibody and the fifth and sixth positions are empty, thus representing the letter P). This pattern will depend on the letter assigned for a given pathogen. For practical reasons, however, we have modified the usual Braille font in 3 rows x 2 columns for each letter into 1 row and 6 columns or positions. If the test results show the expected spot pattern for the letter P, the software analyzes the image in the modified Braille format, interprets it as the letter P, and makes a positive call for the presence of *B. anthracis* in the sample. The letters of the alphabet and the corresponding spot patterns in modified Braille format and several examples of test patterns are depicted in Figure S1.

In this study, we have established a proof of concept with a six-plex assay for detecting three toxins using two letter codes for each toxin as shown in Figure 1. We created the Blind Spot triple toxin LFI devices and compared their performance to the gold standard singleplex assays that are currently in use.

![Figure 1. Six-plex triple toxoid assay and MACAW sensor. Left: Six-plex triple toxoid assay, with spotting pattern of two letter codes for the three toxoids described. Antibodies corresponding to each toxoid are spotted in the corresponding positions determined by the letter code assigned (PC—positive control). Right: MACAW: Modular Automated Colorimetric Analysis Widget (MACAW) system is intended to provide rapid, on-site identification of unknown toxin and biological agents at a low cost point using the Blind Spot cassettes. Custom multiplex lateral flow immunoassays have been developed for this system, which transport a liquid sample to 42 unique spots, including assay control spots. The color intensity induced in these spots by the addition of samples is monitored by the reader and matched against a database to identify the agent in the sample. The MACAW system consists of three major parts: the reader itself, the stage, and the cassette.](https://doi.org/10.1021/acsomega.1c02938)

**RESULTS AND DISCUSSION**

**Performance of Singleplex Toxin Assays.** The gold standard for the toxin assays evaluated here is a singleplex LFI used for environmental sample detection of each toxin. To establish the baseline of singleplex LFI performance with representative capture and detector antibodies, we conducted SEBv (Staphylococcus Enterotoxin B vaccine), RicA (Ricin A
Figure 2. Singleplex LFI toxin assay performance evaluation. Results of conventional singleplex LFI assays for the three toxoids and the concentrations/mL of toxoids are indicated. The toxoids are as follows: SEBv, RicA, and BotA (chemically inactivated). S—sample port; C—control line; T—test line. Note that the test lines at the lowest concentrations shown appear darker if visually viewed in a laboratory setting with ideal lighting conditions compared to these images.

Quantification of the Performance of Singleplex Toxoid Assays Using the Desktop CAMAG Reader. Quantification of the performance of the toxoid assays was performed using a desktop thin-layer chromatography (TLC) densitometric scanner (CAMAG-TLC-3). Based on historical data comparison by manual vs CAMAG scanner, a threshold for making a positive call by manual eye assessment are slightly lower than the lowest concentrations shown in Figure 2. In addition, there are inherent differences in the limits of detection (LoD) between the three-toxoid specific assays. The LoDs based on these tests are 1, 5, and 50 ng/mL for SEBv, RicA, and BotA toxoid respectively.

Fabrication of Six-plex Triple Toxin Blind Spot LFI Devices. Having established the LoDs in the singleplex format, we tested the six-plex Blind Spot format of the triple toxin assay. The devices were fabricated as described in the Materials and Methods section.

The singleplex assay strip has a dimension of 4.32 mm × 89.9 mm of the Blind Spot Assay Performance Evaluation. Results of conventional singleplex LFI assays for the three toxoids and the concentrations/mL of toxoids are indicated. The toxoids are as follows: SEBv, RicA, and BotA (chemically inactivated). S—sample port; C—control line; T—test line. Note that the test lines at the lowest concentrations shown appear darker if visually viewed in a laboratory setting with ideal lighting conditions compared to these images.

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in triplicate. A total of 855 images were captured using the sensor and analyzed using the MACAW software. The various steps in image analyses as well as the detection algorithm for determining the presence of each pattern in the image are described in the Supplemental Methods section. Briefly, the detection algorithm evaluates the signal strength of each pattern, the dissimilarity in shape of the observed signal to the expected patterns (also referred to as “distance”, see the Materials and Methods section for more details), and the probability that each pattern is not observed by chance. Each of the three decision metrics is compared to a threshold, and all must pass for a positive identification (more details are found in the Supplementary Methods).

Using default thresholds for the three decision metrics, we observed the best performance with BotA toxoid assays. All (100%) of the tickets exposed to that toxoid were successfully detected (type II error rate-FN of 0), with a false alarm (type I error-FP) rate of 2.15%. For RicA, 100% of the exposed tickets were detected (type II error rate of 0), but there was a significant false alarm rate of 19%. All of the RicA false alarms occurred from assays that included SEBv toxoid in the sample. For SEBv, only about 76% of the assays were detected (type II error-FN of 24%), with a false alarm rate of 0.51%.

Three-dimensional scatter plots of the decision metric values for each analyte (Figure 5) illustrate the differences in performance. Each point in the plot, representing a Blind Spot image, is colored green if the corresponding toxoid was not present in the sample and is colored red if the ticket was exposed to that toxoid. The plot for SEBv (Figure 5, left) is qualitatively different with about a quarter of positive responses being weak in intensity with correspondingly poor distance and probability values. However, all negative samples have insignificant strengths or probabilities. For RicA (Figure 5, middle) and BotA (Figure 5, right), nearly all positive exposures have a probability value of 1 and a distance of 0.

For BotA, strength values varied from less than 0.1 (10% of the control line strength) up to about 0.5 (50% of the control line strength). For RicA, there were some stronger responses (up to about 80% of the control line strength). Some negative samples for RicA showed weak but statistically significant strength values sufficient to cause false alarms.

We postulate that the false alarms for RicA are actually caused by a cross reactivity between the BotA antibody and the RicA antigen. This stems from the polyclonal nature of the BotA antibodies used in the Blind Spot assay fabrication and

Figure 4. Blind Spot triple toxin LFI assays. Six-plex Blind Spot triple toxoid assay (SEBv, RicA and BotA) tested either as a singleplex, duplex, or triplex against a mix of all three toxoids. The letter codes, the corresponding agents, and the spot patterns are shown in the mock up tile on top. The results of singleplex, duplex, and triplex testing are shown in the three rows. Numbers in parentheses indicate concentrations of toxoids in ng/mL.

Figure 5. Evaluation of the performance metrics of the triple toxin Blind Spot assay. Scatter plot showing the value of the three decision metrics for the SEBv, RicA, and BotA response on all Blind Spot tickets. Points are colored red for positive samples (i.e., exposed to toxoid, either by itself or part of a mixture) and are green for negative samples (not exposed to agents).
could potentially be improved in future iterations using different antibodies. The SEBv signal was weaker than initial testing in early prototypes of the Blind Spot. We suspect this is due to antigen degradation over the time of this work. In the meantime, we considered modifications to the default thresholds to improve performance.

Quantification of the Blind Spot Assay Performance. The classic formalism to quantify performance of a detection algorithm is the receiver operator characteristic (ROC) curve. One considers all possible values for the detection threshold and computes probabilities of detection and false alarm. Armed with relative costs for missed detections and false alarms or other requirements, one can tune the threshold to obtain acceptable performance (Figure 6). In the left panel (Figure 6, left), the probability threshold is changed from 0 to 1 while keeping the thresholds for the other metrics at their defaults. This achieves nearly perfect performance for BotA (see green curve, which approaches very closely the coordinates (0,1)). For RicA, modifying the probability threshold can maintain the 100% detection rate while lowering the false alarm rate (to about 5%). For SEBv, lowering the probability threshold below 0.5 can improve the detection rate but comes at the expense of an increased false alarm rate. The ROC curves for varying the strength threshold for each toxin is shown in Figure 6, right panel. Modifying the strength threshold is sufficient to obtain 100% detection with 0 false alarms for both BotA and RicA (for ricin, perfect performance occurs at a more stringent strength threshold that allows the BotA cross reactivity to be ignored while still detecting all of the ricin exposures). For SEBv, changing the strength threshold alone is not sufficient to optimize performance. This highlights the fact that with three detection thresholds, the ROC curve is actually a hypersurface: to fully optimize performance, one would have to investigate all combinations of threshold values for all three of the thresholds, or at least consider changing threshold pairs simultaneously. For this developmental effort, we did not undertake a full optimization, though we did obtain a combination of thresholds for RicA resulting in an 85.7% probability of detection with a 2.6% false alarm rate. The threshold values and corresponding performance estimates for each toxoid that were utilized for the limit of detection study are presented (Table 1).

Establishment of the Limits of Detection of Blind Spot Six-plex Triple Toxin Assay. We established the limit of detection (LOD) of the six-plex triple toxin Blind Spot assay by performing a limiting point dilution of the analytes and evaluating the test results both manually and using the MACAW. Serial dilutions using a 2-fold 50% dilution factor and 9 concentration levels for each toxoid were created. The concentration range for SEBv and RicA spanned 0.391 ng/mL to 1000 ng/mL; for BotA, the concentration range was an order of magnitude higher from 3.91 ng/mL to 1000 ng/mL. Three assays per concentration were performed, and the MACAW sensor collected images in triplicate for each assay.

Thresholds for the three detection metrics were set as in Table 1, and the MACAW software (described in the Materials and Methods section and Supplementary Information) was used to analyze the images. The frequency of detection at each concentration was plotted as a function of concentration and a Gaussian Cumulative Distribution Function (CDF) was fit to the data using least squares (Figure 7). Basic bootstrap confidence intervals were used to estimate the bounds on the estimated CDF, using 10,000 bootstrap iterations. The concentration corresponding to a desired detection rate may be estimated from the point at which the distribution curves cross the desired detection value. Table 2 gives the concentration values at which the detection probabilities are 50 and 90%, termed the LOD50 and LOD90, respectively. Bounds are for an alpha value of 0.05 for a two-sided test, meaning that they represent a 95% confidence interval.

**Table 1. Threshold Settings and Estimated Performance**

| toxoid | S  | P  | D  | PD | PF |
|--------|----|----|----|----|----|
| SEBv   | 0.0025 | 0.037 | 0.011 | 85.68% | 2.56% |
| RicA   | 0.5 | 0.1 | 0.5 | 100% | 0.00% |
| BotA   | default | 0.25 | default | 100% | 0.00% |

*S: strength; P: probability; D: distance; PD: probability of detection; PF: probability of failure.*
Figure 7. Evaluation of the limit of detection using a dilution series of toxoids. Limit of detection plot for the SEBv, RicA, and BotA serial dilution series. Data points represent the frequency of detected assays at each concentration value. The fits (solid curves) are the bias-corrected bootstrap estimate of the best-fit Gaussian CDF, and bounds (dotted curves) correspond to a 95% confidence interval. Limits of detection and bounds may be found from the location each curve crosses a desired detection probability threshold.

| toxoid   | LOD50 [ng/mL] | LOD90 [ng/mL] |
|---------|---------------|---------------|
|         | LB  | Avg | UB  | LB  | Avg | UB  |
| SEBv    | 0.87 | 1.29 | 1.56 | 1.02 | 1.70 | 2.42 |
| RicA    | 3.01 | 3.23 | 3.59 | 3.33 | 3.69 | 4.25 |
| BotA    | 9.45 | 14.50 | 19.44 | 11.68 | 22.37 | 30.51 |

*LB: lower boundary; Avg: average; UB: upper boundary.

**CONCLUSIONS**

Blind Spot offers a flexible LFI format that allows for multiplex testing of suspected samples. The focus of this study was to establish a proof of concept for the Blind Spot idea. We have established the baseline comparison for the triple toxin assay to singleplex assays and show equivalent performance by visual reading of results. Using the MACAW software, we demonstrated a better sensitivity and quantitation of the results. The use of multiple spots for each assay (two letters with varying number of spots) allows for sufficient redundancy for the software to make true positive calls. This may not be feasible with visual reading of the assays. However, we note that there are inherent differences in the LODs between assays and cross reactivity in mixed toxoid testing and variations between lots of antigens.

Li et al. used a spot array with visual display of test results that are easily interpretable. The printing of patterns is complicated and requires more reagents, which could make scale up manufacturing impractical. The advantage of the Blind Spot approach is that it is simple and inexpensive to print these patterns on nitrocellulose strips using currently available striping technologies/instruments such as BioDot.

The visual interpretation of the spot results for different assays with different Braille letters/patterns can be achieved by having a print out of the patterns and assay names on the outside of the device itself without having to remember the letters and targets by the user. Use of MACAW obviates that need. Although MACAW is a dedicated reader in its current version, a cell phone app to perform these functions could be created. With a software tool on a phone, the results could be read rapidly and communicated remotely, if desired, with reduced potential for incorrect results due to human error. It is also possible to include redundancies to avoid ambiguous results (for example, 2 or 3 letter codes for each analyte; 2 letters in this study). In addition, membrane dimensions may not be an issue as we have demonstrated that the entire strip can be used in this format without bleed issues or affecting the flow rate. LFI printing in spots will also result in appreciable cost savings from reduced volume of reagents needed compared to stripes.

Clearly, there is a void in the market place for multiplex rapid immunoassays. The few commercial off-the-shelf (COTS) multiplex assays in the conventional line format have the inherent problems described above due to stripes laid over one another. We are presenting a solution that addresses the problems that prevented the development of multiplex LFI panels. We expect the Blind Spot approach to be of tremendous utility in point-of-care diagnostics, particularly in resource-limited settings to rapidly screen for a multitude of pathogens in one assay.

**MATERIALS AND METHODS**

**Reagents.** The nitrocellulose membranes used were purchased from Sartorius, Inc. (Goettingen, Germany). Absorbent pads (wick), conjugate pads, and sample pads were from Ahlstrom-Munksjö (Kaukauna, WI, USA). Antibodies: BotA, SEBv, and RicA antibodies used in this study were produced by Biological Defense Research Directorate, Naval Medical Research Center (BDRD, NMRC), Immunodiagnostics Department with funding from the DBPAO, EB, JPEO. Although these antibodies are part of the DBPAO’s repository, NMRC is the owner of the methodology, classification, and any intellectual property associated with these antibodies. They were obtained as government-furnished materials for this study. Antibodies used consisted of protein A purified mouse monoclonal antibodies for Ricin and SEB and protein G purified polyclonal antibodies for Botulinum toxin A/B. The Ricin polyclonal antibodies were further purified by affinity chromatography using a mixture of Ricin holotoxin, Ricin A chain, and Ricin B chain. Given the nature of the assays (biodefense related toxins), these antibodies are only available to customers within the DoD and other government agencies or to DoD sponsored performers. The control line antibody was anti-mouse IgG. The gold nanoparticles were obtained from Innova Biosciences (Cambridge, UK). Toxoids: Ricin A chain was purchased from Vector Labs, Cat# L-1190-1, Burlingame, California, USA. The BotA toxoid (chemically inactivated) of toxin complex was purchased from Metabiologics (https://www.metabiologics.com/products). SEBv has been referenced elsewhere and was made in-house using a clone in a PET11b expression vector (kind gift to DEVCOM-CBC by NMRC). This construct includes the SEBv open reading frame without the secretion signal, in addition to three point mutations that render the protein functionally inactive and an N-terminal 6× His tag. Production of SEBv was done in Esherichia coli strain BL21 (DE3) using a 500 mL culture of TB Express media (Novagen) grown for 24 h and purified using a HisTrap FF affinity column following the AKTA Xpress (GE) standard protocol. SEBv protein concentration was determined on a NanoDrop 2000 (Thermo Fisher Scientific) and purity and molecular weight were assessed using the Experion Pro260 chip (Bio Rad).
Singleplex Testing. Singleplex assays were obtained from the DBPAO repository and tested using antigens described above. The assays were run for 15 min, and visual evaluation of results utilized manufacturer’s instructions.

Quantification of the Singleplex Assay Results Using the CAMAG TLC-3 Scanner. A volume of 100 μL of liquid sample was added to each LFI using a calibrated pipet. After 15 min, the LFI was observed visually and scored on a 0–4 scale with 4 being the most positive (>400 scan value) and then scanned in the CAMAG. Visuals of 0 are negative, and visuals of 1, 2, etc. represent scan values of approximately 100, 200, etc. Prior to scanning, the top of the LFI plastic was removed to allow scanning of the LFI or for photographs. Scanner values were obtained by integrating the test and control line peaks in the CAMAG software and using the peak heights after background subtraction. This device was used to scan up to 10 LFIs at a time. CAMAG v 1.0 was used for quantifying the test and control line intensities. The reader provides the intensity of the bands on a scale of 1–100 arbitrary units.

Fabrication, Cutting, and Assembly of Strips for Blind Spot Devices. The BotA, RicA, and SEBv antibodies were diluted to 2 mg/mL and dispensed at 100 nL per spot onto nitrocellulose membranes with the AD3220 aspirate/dispense platform from BioDot, Irvine, CA. The membranes were dried at 37 °C. BotA, RicA, and SEBv antibodies were conjugated to gold nanoparticles and dispensed onto a conjugate pad. The membrane, wick, gold conjugate pad, and sample pad were assembled onto backing cards and cut to 16 mm with a CM5000 Guillotine Cutter from BioDot in a dry room. The strips were assembled into cassettes and pouched into Mylar foil pouches with a desiccant in a humidity-controlled clean room.

Blind Spot Device Testing. Blind Spot evaluation kits were sent to five different end users from various organizations within the Department of Defense for independent evaluation. Evaluators were asked to make three concentrations for evaluating high, medium, and low levels of toxoid. They also were asked to make a cocktail mixture of each toxoid at similar high, medium, and low concentrations. Independent evaluation by multiple operators was performed at the following antigen concentrations: (1) SEBv at 100, 10, 2, and 5 ng/mL for H, M, Ls, and Lm (high, medium, low single, and low mix, respectively). (2) RicA at 100, 10, 4, and 5 ng/mL for H, M, Ls, and Lm (high, medium, low single, and low mix, respectively). (3) BotA at 1000, 100, 50, and 50 ng/mL for H, M, Ls, and Lm (high, medium, low single, and low mix, respectively). Diluent buffer was used as a negative control for each evaluation. Twenty minutes after adding each sample to the sample port, the end user evaluated them for the presence of the coded spots. For comparison, each toxoid was run on standard singleplex LFIs at the same concentrations.

Sensor-Based Evaluation of Blind Spot Results. MACAW consists of a portable reader based on a Raspberry Pi Zero W using the Camera Module V2 as the optical sensor. A custom 3D printed case houses the Raspberry Pi and two custom daughter boards containing accessories including the camera, LED light source, and OLED screen. A 3D printed removable sample stage was designed to align the LFI assays and block out ambient room lighting. The device was used with the custom Python code to collect images in triplicate of the LFI assays under test. More details are provided in the Supplementary Information.
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Notes
The authors declare no competing financial interest.

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NOMENCLATURE

LFI, lateral flow immunoassay; GI, gastrointestinal; SEBv, Staphylococcus Enterotoxin B vaccine; RicA, Ricin A side chain; BotA, Botulinum toxin A; MACAW, Modular Automated Colorimetric Analysis Widget; TLC, thin-layer chromatography; ROC, receiver operator characteristic; PD, probability of detection; PF, probability of failure; CDS, cumulative distribution function; COTS, commercial off-the-shelf

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