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Enzyme-based paper test for detection of lactose in illicit drugs†

Jessica Zinna, Tracy-Lynn E. Lockwood and Marya Lieberman *

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Lactose is commonly used as a cutting agent in illicit drugs. Currently, presumptive field color test kits for illicit drugs do not test for the presence of lactose or other cutting agents. A method was developed to detect lactose on a paper-based test card. A three-enzyme system comprised of lactase, glucose oxidase, and peroxidase was used to break down lactose into peroxide, which was then detected with a redox indicator. The test can detect lactose concentrations as low as 5% in solid samples and shows no interference when lactose is mixed with illicit drugs or commercial pharmaceuticals. Prepared test cards were stable on the shelf for up to five months. In a blinded study of samples composed of mixtures of heroin, methamphetamine, cocaine HCl, crack cocaine, fillers, and lactose, the sensitivity for detection of lactose across three readers was 100% and specificity was 96.4% (n = 96). When this test was incorporated into a 12-lane test card for the detection of illicit drugs, readers were correctly able to identify the illicit drug and the presence of lactose with 99.3% sensitivity and 100% specificity (n = 54). This test is a robust and affordable way to detect lactose in illicit drug samples.

1. Introduction

Lactose sugar is abundantly found in human and animal milk. Due to its physiological effects as an energy-carrier, commercially made lactose is now commonly found in many food and pharmaceutical products.[1] Lactose powder can be bought at supermarkets or over the internet in bulk at affordable prices. The widespread availability of lactose powder, low cost, pleasant taste, and white powder appearance have also led to the use of lactose as a cutting agent in illicit drugs.[2,3] As the prevalence of lactose as a cutting agent grows, the need for an easy, affordable, and field-compatible test for lactose becomes apparent.

Illicit drugs are frequently diluted with adulterants or bulking agents to increase dealer volume when dispensing to buyers. Substances are referred to as adulterants when they have biological affects similar to those of the illicit drug being cut. Adulterants are frequently used to improve user experience of the drug and enhance the efficacy. Common adulterants include caffeine, levamisole, procaine, and lidocaine. Diluents/bulking agents are typically inert substances that are used to increase the volume of the sample, since drugs are sold by weight. Diluents commonly found in illicit drug samples include chalk, talcum powder, lactose, and other sugars.[4–6] As drugs pass through various sets of hands in the supply chain, opportunities for multiple cuts arise. The ability to detect cutting agents in street drug samples would allow for better supply chain tracking of illicit materials, such as determining if the drug was cut before or after shipment. If supply reduction methodologies are in place, dealers will be forced to either raise prices on their limited supply or dilute their remaining drug with cutting agents, making cutting agents more common.[7] Tracking the spread of cutting agents across a region can thus provide valuable insight into the efficacy of law enforcement efforts to control supply, as well as provide information on which region batches are from, since dealers are likely to continually use the same cutting agent throughout their samples.

Field drug detection technologies commonly used by law enforcement include liquid reagent-based pouches and swabs, Fourier-transform infrared spectroscopy, and portable mass spectrometers.[8] Complex mixtures such as bulked illicit drugs can pose problems for these testing methods due to interferences from the cutting agents, causing false arrests and delayed identification of unknowns.[9] Additionally, the liquid reagent-based pouches and swabs do not currently test for lactose or other cutting agents.

Several microfluidic devices for presumptive drug detection have been developed,[10–12] but they cannot detect cutting agents, samples must be prepared in a lab setting, and the devices have a short shelf life. For these reasons, these tests are not ideal for use in a field setting, which is important for the application of presumptive drug tests for law enforcement purposes.

A paper analytical device (PAD) capable of detecting multi-component mixtures has previously been developed and optimized in our laboratory for illicit substances (idPAD).[13] The

Department of Chemistry and Biochemistry Notre Dame, University of Notre Dame, IN 46556, USA. E-mail: mlieberm@nd.edu

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idPAD houses 12 independent testing lanes that are separated by hydrophobic barriers and can identify functional groups commonly found in various classes of illicit substances and certain cutting agents. However, there is no readily available chemical test for lactose in solid drug samples.

When designing a test for cutting agents in illicit drugs, it is imperative that the test meets certain criteria in order to be utilized by law enforcement officers in a field setting. It is important that a test for cutting agents be portable, cheap, quick, and safe. The World Health Organization (WHO) has established similar criteria for the design of effective diagnostic tests for resource-limited environments that we can use as a benchmark for designing other field devices.[14] Previous studies in lactose detection involved the use of amperometric biosensors,[15–19] which require electricity, laboratory space, and significant sample preparation. Therefore, these tests are not viable for a field setting. A paper-based enzymatic biostrip for lactose detection was also designed,[20] however the test was only evaluated for liquid samples, was not studied in complex mixtures, and could only detect lactose down to 20 mg mL^{-1}. A paper test card for adulterants in milk that was used for a spot test of glucose detection has also been designed by our group.[21] For solid illicit drug samples, a test is needed that requires no sample preparation, is effective in testing complex drug mixtures in the field and can detect lactose in mixtures at concentrations ranging from 9–50% by mass.[3]

In this work, we investigated a 3-enzyme system on paper to detect lactose. The limit of detection for lactose, response to possible interferences in complex illicit drug mixtures, and stability of the test was evaluated. The test was then incorporated into a 12-lane PAD to detect illicit drugs.

2. Experimental

2.1 Chemicals and reagents

The following materials were used to prepare lactose test cards: lactase from *Aspergillus oryzae* (MP Biomedicals), glucose oxidase type X–S from *Aspergillus niger* (Sigma), peroxidase from horseradish (Sigma), *o*-dianisidine dihydrochloride (Spectrum Chemical), acetic acid glacial (VWR), Ahlstrom 319 Paper (Midlands Scientific), desalinized pullulan (Polysciences).

The following materials were used to prepare idPADs: copper(II) sulfate pentahydrate, cobalt(II) nitrate hexahydrate, iodine, 4-nitroaniline, and 4-(dimethylamino)cinnamaldehyde procured from Sigma Aldrich. Polyvinylpyrrolidone (povidone), *p*-toluenesulfonic acid monohydrate (tosic acid), ninhydrin, and dimethylglyoxime were purchased from Alfa Aesar. Tris (base) and sodium nitrite were obtained from J. T. Baker. Potassium carbonate and potassium iodide were purchased from VWR Analytical, potassium thiocyanate from Honeywell, sulfanilamide from Eastman Kodak Co, and 1,2-naphthoquinone-4-sulfonic acid sodium salt (NQS) from Acros Organics. Ferric chloride hydrate, sodium hydroxide, and nickelous chloride were purchased from Fischer Scientific Chemical.

All purchased chemicals were used without further purification.

2.2 Solution preparation

1000 units per mL stocks of lactase, glucose oxidase, and peroxidase enzymes in 50 mM acetate buffer (pH 5) were prepared. Pullulan was also incorporated as a stabilizing sugar at a concentration of 25 mg mL^{-1}. A solution of 25 mg mL^{-1} *o*-dianisidine dihydrochloride was prepared in distilled water. The enzyme solution is stable at 4 °C for up to 6 months. The *o*-dianisidine solution was made fresh each time tests were fabricated.

2.3 Test card fabrication

Test card fabrication. Paper test cards used for the lactose test cards and the idPADs were fabricated by wax and laser inkjet printing on Ahlstrom 319 paper following the method described in Weaver, *et al.* 2013.[22] A 48-spoke inoculating manifold (8 × 6 array, DanKar Corp.) was used to spot solutions from a 96-well plate onto the paper test cards, depositing approximately 2 μL of solution per lane. The test cards were dried in ambient conditions.

Lactose test lane. The enzyme solution and the redox indicator *o*-dianisidine were applied to all 12 lanes of a paper test card using either an automatic pipette set at 2 μL or the spoke inoculating manifold. The enzyme solution was applied below the sample swipe line (indicated by an arrow on the card), and the redox indicator *o*-dianisidine was applied above.

idPAD fabrication. idPADs were prepared following the method described in Lockwood, *et al.* 2020.[13] However, the lactose test was added in lane H of the idPADs in place of the ethambutol test. See Fig. S6 in ESI† for a spotting guide.

Running test cards. To run the test cards, solid samples were rubbed across the paper card using a bamboo stirrer across the "swipe" line indicated with an arrow (see Fig. 1). Excess solid that did not stick to the test card was tapped off. This process deposits approximately 10–12 mg of sample per card. The bottom of the card was then placed in shallow water, which moved up the paper, rehydrating the dried reagents and allowing reaction with the sample. Pictures were taken of developed test cards using a Google Pixel 3 cellphone in a lightbox. The lightbox was made from a commercial shoebox containing white light LEDs. The lights were covered with white computer paper to diffuse the light throughout the box. A hole was cut into the top of the box for a cellphone camera. See Fig. S1 in ESI† for a diagram of the lightbox used.

2.4 Sample analysis

Time-dependent studies. Test cards where all twelve lanes were stamped with the lactose colorimetric test were run with pure starch (negative control) or pure lactose (positive control). Freshly prepared test cards and cards stamped five months prior and stored in a foil bag with an oxygen absorber and desiccant paper were studied. After water had traveled up the entirety of the test card, the card was removed and placed in a lightbox for imaging. Cellphone images were taken every 30 seconds for 8 minutes in total. The images were exported, and the color development analyzed following the procedure described in Section 2.5.

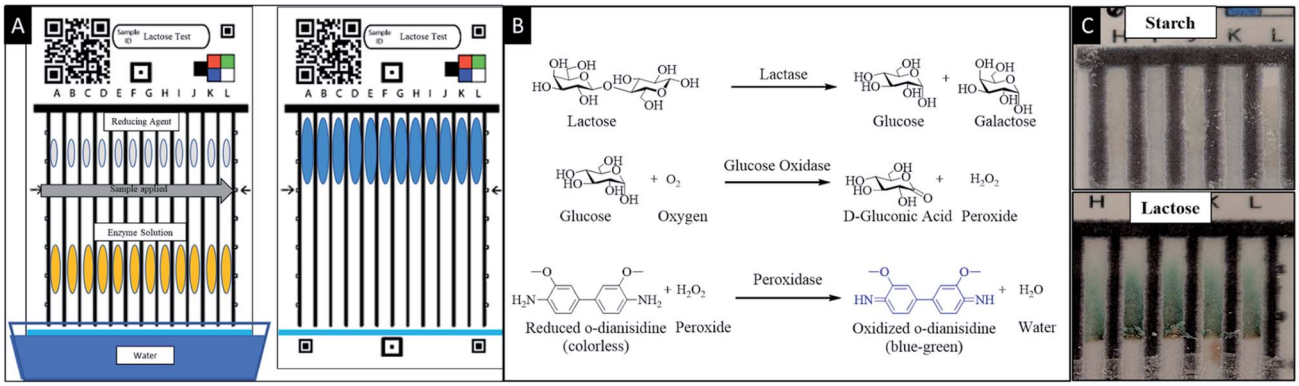

Fig. 1 Diagram of lactose test card showing locations of the enzyme solution and reduced *o*-dianisidine (Panel A), schematic of enzyme reactions utilized for color development (Panel B), and the results of a true positive and true negative color test (Panel C).

Quantification study. Freshly prepared test cards containing the lactose test in every lane were run with varying weight ratios of lactose diluted in starch. Samples of 0, 5, 10, 20, 50, and 100% lactose in starch were studied. After water had traveled up the entirety of the test card, the card was removed and placed in a lightbox for imaging and analyzed following the same procedure as in time-dependent studies.

Blinded studies

Lactose lane studies. One hundred blinded samples were made using various pure samples and mixtures of pharmaceuticals, illicit drugs, lactose, and inert fillers such as starch. Lactose concentrations varied from 10–100% for true positive samples. Four different illicit drugs were represented: cocaine HCl, crack cocaine, methamphetamine, and heroin. The samples were double blinded and run on lactose test cards from three different preparation dates (fresh, one week old, and two weeks old). Cellphone images were taken immediately after removing the cards from water, and approximately five minutes after removal. Three readers (two experts, one novice) were provided with sample images from all three card preparation dates as well as both development times and asked to respond "Yes" or "No" to lactose being present in the samples. Expert readers were users who had experience reading colorimetric test cards as well as extensive experience in developing the lactose color test. Novice users were familiar with reading colorimetric test cards but had no prior knowledge of the lactose color test. Novice and expert readers were both provided with a "reading guide" which provided images of true positive and true negative samples to use as benchmarks for their analysis. Four sets of sample images were not saved, therefore only 47 true negative and 49 true positive samples were analyzed out of the original 100. See ESI S.3† for full sample compositions, card images, and reader evaluation of each sample.

idPAD studies. idPADs stamped with the lactose test in lane H were prepared and stored in foil heat sealed bags with oxygen absorber and desiccant paper. The idPADs were stored in a 4 °C cold room for five days before running. Samples were run with 55 blinded samples containing various lactose, illicit drug, and filler concentrations. Lactose concentrations varied from 10–100% for true positive samples. Four different illicit drugs were represented: cocaine HCl, crack cocaine, methamphetamine, and heroin. Cellphone images were taken immediately after removing the cards from water, and approximately five minutes after removal. See ESI S.4† for full sample composition information and idPAD images. The photos for one sample run were not saved, leading to a total sample size of 54. The sample images were analyzed by eye by three different readers (two experts, one novice) who were asked to determine if lactose was present, and if an illicit drug was present in each sample. If they indicated an illicit drug was present, they were asked to identify the drug.

2.5 Image analysis

Although the color change in the lactose lane test is readily apparent to the human eye, images of PADs were analyzed to quantify color changes over time. A rectangle of pixels was sampled from each lane of a PAD (see ESI S.8† for an example of pixels sampled on a PAD). The rectangle was placed just above the sample swipe line on the PAD. The dimensions of the rectangle were the same in all the samples, comprising of a 40 × 150 pixel range with an area of 6000 pixels. The red, green, and blue (RGB) intensity of each pixel was extracted and averaged. This information was used to calculate the standard deviation for each time point sampled.

Color changes can be quantified in a variety of ways. In this study, the color intensity was plotted over time. For every pixel in the sampling rectangle, the RGB value was extracted in a 3-dimensional vector. The change in each element of the vector, corresponding to red, green, and blue, can be measured individually to show color changes over time. Changes in the grayscale intensity, which is equal to the average of the red, green, and blue intensity, can also be measured to show color changes over time. The inverted grayscale intensity was plotted by subtracting 255 from the grayscale intensity (the range for standard RGB values is between 0 and 255) and displaying the absolute value. For the PCA analysis, raw RGB values were used (not inverted).

The code is freely available on the web at http\\www.github.com/ericchansen/padtools.

2.6 Blinded sample preparation

Street drug samples were obtained from the Berrien County Crime Lab in Berrien Springs, Michigan. Samples of cocaine HCl, crack cocaine, heroin, and methamphetamine were of high purity based on FTIR analysis. All blinded samples were made using the same lot of each street drug. See ESI S.5† for FTIR spectra of drug lots used. 155 blinded samples were fabricated; 100 were used for testing the lactose lane only and 55 for testing the lactose lane integrated into the idPAD. Solid solutions were prepared by mixing a known weight of drug and cutting agent together in a mortar for a minimum of 3 minutes to ensure a homogeneous mixture. Additional dilutions were prepared by adding extra cutting agent to a known mass of the initial mixture and grinding as before. Sample compositions are shown in Table 1.

3. Results

3.1 Blinded study of illicit drug samples

We first tested whether illicit drugs or other common cutting agents interfered with the ability of the test to show a positive result for lactose. We prepared complex mixtures containing illicit drugs, pharmaceuticals, and cutting agents, and tested if readers could still identify the blue-green color response that indicates the presence of lactose. Three readers were assigned the samples, with two readers having expert knowledge of the lactose test, and one being a novice. When lactose is present, the lactase enzyme breaks it down into glucose and galactose. The glucose is oxidized by glucose oxidase, which creates peroxide. The peroxide then reacts with the *o*-dianisidine reducing agent to generate a blue-green color. This test does not react with sugars that do not contain glucose (such as mannitol). However, any sugar with a glucose subunit will still read positive. In future studies this will be addressed by changing the tri-enzyme system to remove the glucose oxidase enzyme and replace it with an enzyme that will target the galactose subunit only. In illicit drug samples, lactose and mannitol are the most common sugars observed as cutting agents, and it would be rare to see sucrose or other glucose containing sugars in these samples. To further to determine the impact of possible interfering compounds on the lactose lane test, we ran cards with 24 compounds that are often found as cutting agents in drugs. We found that the card only showed a positive result for compounds containing glucose or lactose. No true negative samples showed a color change. The full results of this study are shown in the ESI S.9.†

The lactose test had excellent sensitivity and specificity for all samples surveyed. Full reader data is shown in Table 2. All three readers identified 100% of true positive samples correctly. False positives were rare, with the novice reader having the highest amount of false positive identifications with 3 out of 96 samples. The average sensitivity among all readers was 100%, specificity 96.3%, and accuracy 98.3%. Overall, this test was effective at detecting lactose concentrations as low as 10% in complex samples with high accuracy, which is within the range of expected lactose concentrations found in street drug samples.

Table 1 Sample compositions and amounts for samples used in blinded studies

Sample composition	Number of samples
100% cocaine HCl	10
90% cocaine HCl, 10% lactose	10
75% cocaine HCl, 25% lactose	5
50% cocaine HCl, 50% lactose	10
50% cocaine HCl, 25% lactose, 25% starch	5
100% crack cocaine	10
90% crack cocaine, 10% lactose	5
75% crack cocaine, 25% lactose	3
50% crack cocaine, 50% lactose	5
50% crack cocaine, 25% lactose, 25% starch	2
100% methamphetamine	5
100% heroin	10
90% heroin, 10% lactose	10
75% heroin, 25% lactose	5
50% heroin, 50% starch	10
50% heroin, 50% lactose	5
50% heroin, 25% lactose, 25% starch	10
100% dimethyl sulfone	10
100% mannitol	5
100% lactose	10
100% starch	5
100% procaine	5
Total true positive samples[a]	**85**
Total true negative samples[a]	**70**
Total samples	**155**

[a] True positive samples (contain lactose) are shaded in grey, true negative samples (no lactose) are shaded in white. See ESI S.3 and S.4 for full sample data and reader identification.

3.2 Incorporation of the lactose lane test onto a paper analytical device for illicit drug detection (idPAD)

Next, the lactose test was incorporated into a library of other color tests optimized for the detection of narcotics. Readers were now asked to identify not only the presence of lactose, but also to identify the illicit drug present based on color analysis described in Lockwood, *et al.*[13] Reads were classified as correct when readers identified both the absence/presence of lactose and the illicit drug correctly. Missing one of these characteristics was counted as an incorrect read for the purposes of this study. Three readers were assigned the samples, with two readers having expert knowledge of the idPAD and lactose lane, and one being a novice.

When incorporated into the idPAD, the lactose test did not interfere with color development on adjacent lanes, and lactose was still able to be detected. Full reader data is shown in Table

Table 2 Sensitivity and specificity results for lactose detection in a 100-sample blinded study

Reader 1, expert	Reader 2, expert	Reader 3, novice
True positive: 49/49	True positive: 49/49	True positive: 49/49
True negative: 45/47	True negative: 47/47	True negative: 44/47
Sensitivity: 100.0%	Sensitivity: 100.0%	Sensitivity: 100.0%
Specificity: 95.7%	Specificity: 100.0%	Specificity: 93.6%
Accuracy: 97.9%	Accuracy: 100.0%	Accuracy: 96.9%

3. For 54 samples, the two expert readers identified 100% of the samples correctly. The novice reader had one false negative for a PAD containing lactose, in which they identified the drug correctly but did not positively identify the presence of lactose. The average sensitivity among all readers was 99.3%, specificity 100%, and accuracy 99.4%. Each reader was able to accurately identify the presence or absence of illicit drugs, the identity of any drugs present, and the presence or absence of lactose.

3.3 Semi-quantitative measurement of lactose by the test card

We next wanted to see if this test could quantify the amount of lactose present in a sample. Varying concentrations of lactose in starch ranging from 0–100% were run and images were taken over an 8 minute time period to observe the color change over time.

As shown in Fig. 2, as lactose concentration increased in samples, the slope of the average inverted grayscale intensity over time also increased. For the sample containing 5% lactose, the color change took longer to occur, with the peak increase in intensity occurring around 300 seconds. Additionally, the blue-green color did not spread as far up the PAD in the 5% lactose sample. This indicates that another way to estimate lactose percentage in samples is to analyze how long the card takes to show a sharp increase in color formation.

For samples containing 10–100% lactose, a blue-green color change was immediately noticeable upon removing the card from the water, and steadily increased over time. Therefore, it is possible to differentiate between a sample of 100% lactose and one of 5% lactose by analyzing the color development over time using image analysis. Samples containing 10% lactose can also be differentiated from higher concentrations by comparing the color intensity of the blue-green color produced. Samples containing 20% and above of lactose show a much darker blue-green color development, as well as a darker brown color near the bottom of the colored region. This is also reflected in the graph of color intensity, where samples of 20–100% lactose have similar intensities, while 10 and 5% samples have much lower intensities. This method can be used to semi-quantitatively analyze the lactose concentration in samples.

3.4 Test card stability

As this test is designed for use in a field setting, we wanted to understand the stability of prepared test cards over time. In order to be viable for field use, cards should be stable for several months when stored at ambient conditions. Test cards were run using pure lactose (true positive) or pure starch (true negative) on fresh and five-month-old PADs.

Table 3 Sensitivity and specificity results for lactose and illicit drug detection and identification in a 54-sample blinded study

Reader 1, expert	Reader 2, expert	Reader 3, novice
True positive: 45/45	True positive: 45/45	True positive: 44/45
True negative: 9/9	True negative: 9/9	True negative: 9/9
Sensitivity: 100.0%	Sensitivity: 100.0%	Sensitivity: 97.8%
Specificity: 100.0%	Specificity: 100.0%	Specificity: 100.0%
Accuracy: 100.0%	Accuracy: 100.0%	Accuracy: 98.1%

Although the differences between true positive and true negative samples are readily apparent to the human eye, principal component analysis (PCA) was also used to analyze the differences between true positive and true negative samples. We used the average red, green, and blue intensities from old and new PADs as the features in our PCA. Principal component 1 (PC1) is positively correlated with the lactose concentration. PC1 is negatively correlated with the average red, green, and blue intensities, and PC1 accounts for 97.0% of the variance in the data.

For the PCA analysis, we did not invert the raw pixel intensities. For example, a particular shade of green has the RGB code (0, 205, 102). In this case, the intensities all decreased from the white value of (255 255 255), which would indicate a color change from white to green on paper.

Principal component 2 (PC2) accounts for 2.7% of the variance in the data. Collectively, just two principal components account for 99.6% of the total variance in the data. PC2 is negatively correlated with the blue intensity, relatively neutral to the green intensity, and positively correlated with the red intensity. This result is expected because as the PAD ages, a brown color develops due to slight degradation of the reducing agent. Browning can be represented in the RGB color space by an increase in the red intensity, with a simultaneous decrease in the blue intensity. See S.8 in ESI† for the PCA contribution to variance.

Logistic regression was used to locate the decision boundary between positive and negative samples.[23] We treated positive samples (regardless of lactose concentration) as one class and negative samples as a separate class. We then used logistic regression to fit the decision boundary to form a line to separate the two classes, depicted as the blue line in Fig. 3. As seen in Fig. 3 Panel B, there is a clear distinction between 0 and 5% lactose samples *versus* the 10% and above samples, with a small number of outliers. This indicates the test to be semi-quantitative for lactose with the ability to differentiate between samples of 5% or less lactose compared to 10% and above.

When stored on the shelf for multiple months, the *o*-dianisidine reducing agent turns brown. This color does not affect the performance of the indicator, but it does provide background noise. The blue-green color change seen in positive samples still occurs but is combined with the brown background from the reducing agent. When pure lactose was run on five-month-old test cards, a noticeable blue-green color change was still observed against the brown background color. The difference in a true positive and true negative is still detectable by eye, as seen in the Panel A of Fig. 3.

Five-month-old test cards were also run with pure starch, as a negative control. The brown background color remains, however there is no development of blue-green color over time. The left panel of Fig. 3 shows the test card development over time. Compared to five-month-old cards run with lactose, the starch card shows no blue-green color formation. Through this

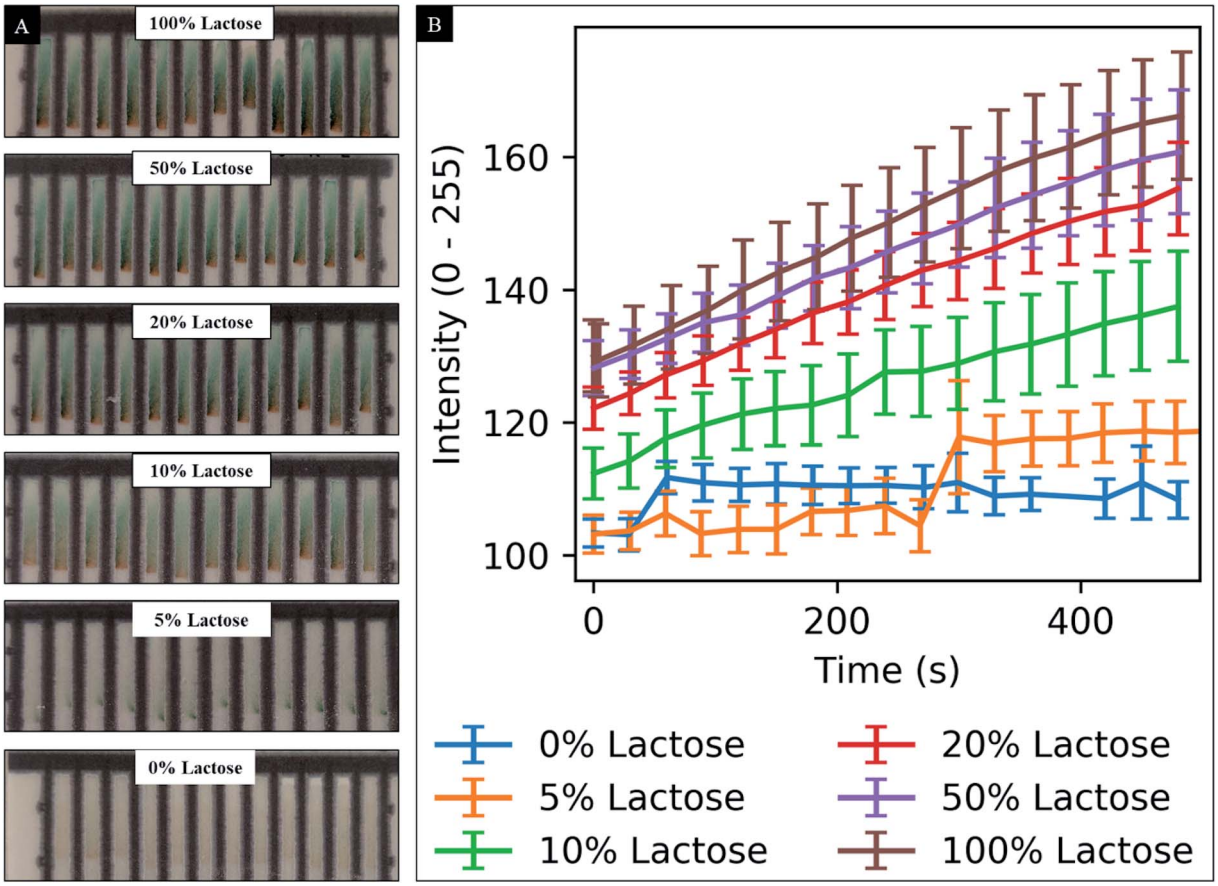

Fig. 2 (A) PAD images of various lactose concentrations (t = 8 minutes). (B) Color development of test cards of various lactose concentrations run over 8 minutes. Lactose and starch mixtures of 0, 10, 20, 50, and 100% lactose were studied.

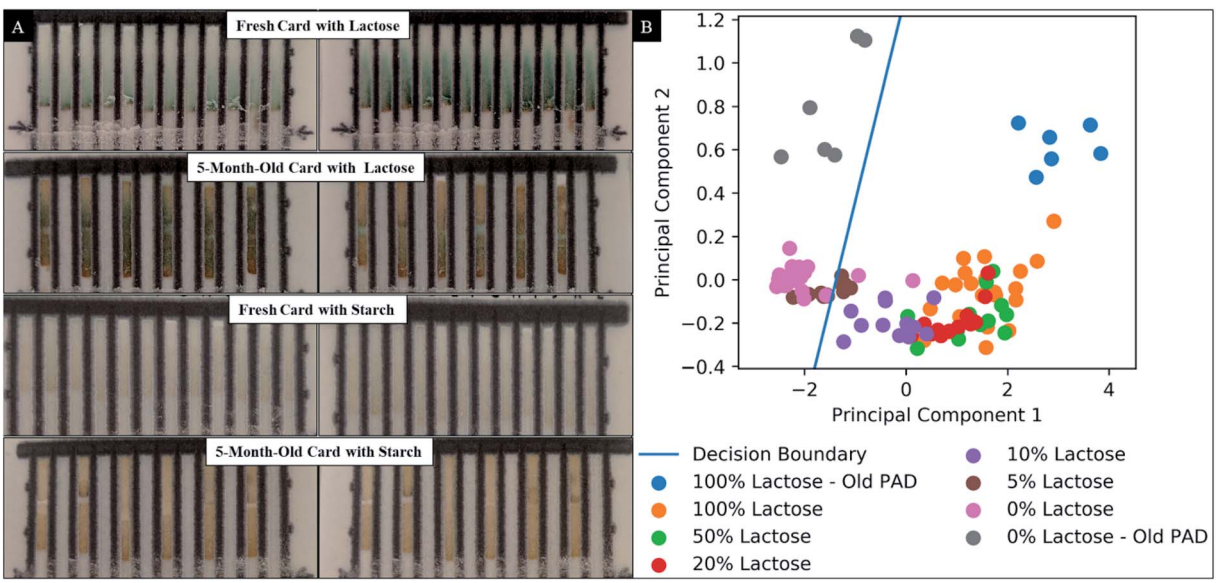

Fig. 3 (A) PAD images of fresh and five-month old cards run with lactose or starch in varying percentages. The image on the left corresponds to t = 0 seconds, the image on the right corresponds to t = 480 seconds. (B) Principal component analysis of true positive (lactose) and true negative (starch) samples. Samples used were run on a mixture of five-month-old and freshly prepared test cards. A decision boundary line was calculated using a least squares regression of the two-dimensional color data set.

comparison, it is still possible to distinguish positive and negative results using test cards stamped up to five months ago and stored on a shelf. Therefore, this test is viable after multiple months on the shelf.

The difference between positive and negative samples on old PADs is clearly visible when the data is interpreted using PCA, as shown in Fig. 3 Panel B. PC2 clearly distinguishes between new PADs and old PADs, while PC1 can be used to simultaneously assess whether the sample is positive or negative for lactose.

4. Discussion

Street drugs are often mixed with cutting agents such as lactose to increase dealer profit margins. Current field tests for illicit drug detection struggle to identify cutting agents in samples[8,9] and these tests typically involve the use of expensive equipment or dangerous reagents, both of which make them difficult for use in a field setting. There is also a lack of good information on what stage in distribution drugs are cut, what cutting agents are used, and in what percentages. This information is crucial to understanding the supply chain of illicit drugs.[2,3] The ability to test drug samples in the field would provide an avenue to quickly and easily determine what cutting agents are present, and the drugs could be tracked at various points in the supply chain to form a more clear view of the spread of cutting agents in illicit drug samples.

Previous work on sensors to detect lactose were not optimized for solid drug mixtures, or for low-resource field settings.[15–19] An effective presumptive field test for cutting agents in drugs should meet the WHO ASSURED criteria[14] to be implemented as a screening tool for illicit drugs. For street samples, it is important that a test is deliverable to law enforcement agencies by being low-cost, safe, and being able to detect common cutting agents as well as illicit drugs.

Using an enzyme-based paper test, we were able to detect lactose in illicit drug samples at concentrations ranging from 5–100% by mass. Positive color results were able to be qualitatively identified by eye within eight minutes after running test cards, even for samples containing as low as 10% lactose. For higher concentration samples, color results were apparent within a minute. The test cards were shelf stable for at least five months. In a 100-sample study, expert and novice readers identified the presence of lactose in complex drug mixtures with an accuracy of 98.3% on average. Color response was also quantified to determine the optimum time point to read the cards, and the code used was made freely available online. When the enzyme test was incorporated into the idPAD for illicit drug detection, readers were able to identify both the presence and identity of illicit drugs and the presence of lactose, with an accuracy of 99.4% on average.

5. Conclusions

The enzymatic paper test described in this paper allows users to detect lactose at very low concentrations (down to 5%) even in complex mixtures and is shelf stable for months. When integrated into a 12-lane system to detect illicit drugs, users can identify specific illicit drugs as well as the presence of lactose. This test provides an affordable, field-friendly, and safe way to detect lactose as a cutting agent in illicit drug mixtures. The low cost, accuracy, and ease of this test provide valuable applications in supply chain monitoring for illicit drugs. In the future, we hope to develop tests for other cutting agents, such as caffeine, levamisole, and other sugars.

Conflicts of interest

The authors declare no conflict of interest.

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