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## Simultaneous Colorimetric Detection of Improvised Explosive Compounds using Microfluidic Paper-Based Analytical Devices ( $\mu$ PADs)

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Abstract:

In this paper the development of microfluidic paper-based analytical devices ( $\mu$ PADs) is described for the rapid, on-site detection of improvised explosives. Five lane  $\mu$ PADs were designed and printed using wax ink on chromatography paper to create hydrophobic channels. Each channel contains colorimetric reagents capable of reacting with one or more explosive compounds resulting in a specific colorimetric reaction. Two devices were prepared, each capable of performing five simultaneous analyses on a single  $\mu$ PAD. The first  $\mu$ PAD was developed to detect inorganic explosives such as black powder, flash powder, and ammonium nitrate. It detects nitrates, nitrites, chlorates, and perchlorate oxidizers, as well as ammonium. The second  $\mu$ PAD was developed to detect military explosives such as TNT and RDX along with other high explosives like urea nitrate. It also detects organic peroxides such as TATP and hydrogen peroxide. All experiments were performed by dissolving the explosives in deionized water or 50:50 acetone/ H<sub>2</sub>O as transport solvents with a detection time of around 5 minutes. Detection limits ranged from 0.39 – 19.8  $\mu$ g of explosive compound. These two customized  $\mu$ PAD devices permit the on-site forensic testing of unknown explosives, thereby supplying law

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3 enforcement and military personnel with a resource for fast, easy detection of military,  
4 commercial, and homemade explosive components at low cost.  
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9 Keywords: microfluidic paper-based analytical devices,  $\mu$ PAD, explosives, colorimetric,  
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14 Introduction:  
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18 In recent years there has been a dramatic increase in the use of improvised explosive devices  
19 (IEDs) due to improved controls placed on commercial and military explosives<sup>1</sup>. IEDs were  
20 once limited to war zones, but have become an increasing concern for law enforcement officials  
21 who may encounter terrorists manufacturing homemade explosives. In these situations, fast and  
22 accurate identification of the explosive material is paramount.  
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31 Commonly used materials for improvised explosive preparations include fertilizers and industrial  
32 chemicals containing oxidizers such as chlorates, perchlorates, and nitrates as well as other less  
33 stable compounds, such as peroxides<sup>2</sup>. These materials encompass a wide range of properties,  
34 such as volatility, polarity, and composition, which require a variety of different analytical  
35 techniques in order to identify the explosive materials. For example, the combination of gas  
36 chromatography-mass spectrometry and liquid chromatography-mass spectrometry is often used  
37 to identify organic compounds while ion chromatography and capillary electrophoresis are used  
38 to determine inorganic ions<sup>3,4,5,6</sup>. Metals can be detected using scanning electron  
39 microscopy/elemental diffraction spectroscopy or x-ray diffraction<sup>7,8</sup>. These devices are large,  
40 expensive pieces of instrumentation that are not portable, so that the sample must be sent to a  
41 laboratory for testing. This cumbersome process increases the amount of time before any  
42 analytical information on the identity of the explosive can be provided to on-site personnel.  
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3 On-site analytical instrumentation, such as ion mobility spectrometry, Fourier transform infrared  
4 spectroscopy, and Raman spectroscopy, can be used to detect explosives in the field; however  
5 these devices commonly rely on the detection of volatile components and can be costly and  
6 bulky, making them unavailable in many situations. These portable instruments also require a  
7 power source such as a battery, which can be drained before the on-site work is finished<sup>12</sup>.

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10 Colorimetric and immunoassay based tests have also been developed, but the current procedures  
11 are not multiplexed and may require multiple tests and reagents for proper use, extending the  
12 time of analysis and increasing the amount of sample that is needed if an unknown explosive is  
13 present<sup>10,11</sup>. Therefore, the development of a simpler, cheaper, and quicker on-site detection  
14 method for multiple explosive compounds is needed.

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17 Paper has become an increasingly attractive substrate for on-site microfluidic testing since it is  
18 cheap, compatible with many chemical applications, and does not require the use of external  
19 pumps in order to transport liquids. Microfluidic paper-based analytical devices ( $\mu$ PADs) permit  
20 the development of inexpensive analytical devices through the fabrication of hydrophobic  
21 patterns on chromatography paper<sup>13,28</sup>. There are many different ways of fabricating  $\mu$ PADs  
22 such as photolithography, plotting, inkjet etching, plasma etching, cutting, and wax printing<sup>16</sup>.

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25 One of the most effective ways to produce  $\mu$ PADs is wax printing on chromatography paper due  
26 to the ease of application and the minimal instrumentation required (commercially available  
27 printer and laminator). The wax channels can be used to compartmentalize chemical reactions  
28 and also direct the liquid samples toward individual sections of the paper containing test  
29 reagents<sup>17</sup>.

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32  $\mu$ PADs have been previously designed for point-of-care testing in medical diagnostics<sup>16</sup> and to  
33 test substandard pharmaceuticals<sup>29</sup> in third world countries. There have also been a number of  
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3 previous attempts to produce  $\mu$ PADs for the analysis of explosives. For example, pyrene excited  
4 with UV light was utilized for the determination of organic explosives<sup>14</sup>, a system for detection  
5 of organic peroxides and nitrobenzenes was developed<sup>15</sup>, and a method was published in order to  
6 detect trinitroaromatic explosives on paper<sup>27</sup>. However, these procedures tend to focus on a  
7 small subset of explosives, and none of them address the important issue of detecting improvised  
8 explosives, particularly those developed from fertilizers and pyrotechnic materials.  
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11 In this project we have developed two different  $\mu$ PADs for the analysis of the widest possible  
12 range of both military and improvised explosives. Both inorganic and organic explosives are  
13 detected. In contrast to previous research, this article demonstrates the capability of multiplexing  
14 the analysis of these explosives. Thus it is possible to detect mixtures of different components  
15 on the same device. Furthermore, the procedure illustrates an interesting application of  
16 organized sequential chemical reactions on  $\mu$ PADs and a single eluent reservoir that is used to  
17 extract the explosives and transport them to the test areas using capillary action.  
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36 Experimental:

### 37 38 39 *Chemicals*

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42 All reagents and chemicals were analytical grade. Explosive samples such as TNT, RDX, and  
43 urea nitrate were prepared as dilute solutions from law enforcement sources. Potassium chlorate,  
44 ammonium nitrate, potassium nitrite, and potassium perchlorate were all purchased from Sigma-  
45 Aldrich (St. Louis, MO, USA). 30% aqueous hydrogen peroxide solution was purchased from  
46 Fisher Scientific (Fair Lawn, NJ, USA).  
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55 The handling of explosives can be hazardous and should be performed with appropriate  
56 laboratory safeguards. All materials were stored as dilute solutions in sealed plastic vials in an  
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explosion proof freezer. All experiments were conducted with appropriate protection such as face shield, gloves, and lab coat. Laboratory hoods were used when appropriate.

### *μPAD Fabrication*

The paper microfluidic devices were designed using Microsoft paint (Microsoft; Redmond, WA, USA) and printed on Whatman no. 1 chromatography paper (GE Healthcare, UK) using a wax-based printer (Xerox ColorQube 8750; Xerox, US). The paper was then placed into an aluminum foil carrier and run through a laminator at 160°C, speed 1 (Tah Hsin Industrial Corp, TCC-600). This process was repeated twice and the μPADs were cut to the appropriate size for use. Two microliters of each colorimetric reagent were spotted onto the paper microfluidic devices and allowed to dry for about 1 minute. This process was used for all colorimetric reagents for both the single lane μPADs and the five lane μPADs.

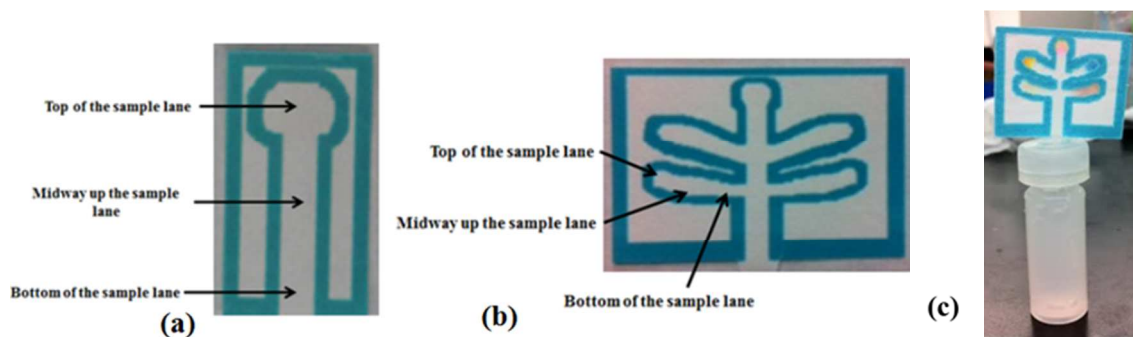


Figure 1. Design and placement of the colorimetric reagents for the μPADs. (a) Single lane μPADs used for optimization of the colorimetric tests and (b) five lane μPADs for multiple explosive analysis. (c) Testing set up for the single lane and five lane μPADs.

### *Inorganic μPAD*

The five lane inorganic explosives detection μPAD included a test for chlorate, nitrate, ammonium, nitrite, and perchlorate in each respective lane. To detect chlorate, an aniline sulfate reagent (Fisher Scientific; Fair Lawn, NJ, USA) was spotted at the midpoint of the sample lane

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3 and 50% H<sub>2</sub>SO<sub>4</sub> (Fisher Scientific; Fair Lawn, NJ, USA) was spotted at the top of the sample  
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5 lane. For the nitrate test, 3 steps were involved: (1) a solid reducing mixture consisting of 0.08 g  
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7 sulfanilic acid (Sigma-Aldrich; St. Louis, MO, USA), 1.87 g sodium acetate (Fisher Scientific;  
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9 Fair Lawn, NJ, USA), and 0.37 g zinc powder (Alfa Aesar; Ward Hill, MA, USA) was made into  
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11 a slurry using a saturated trehalose (Fisher Scientific; Fair Lawn, NJ, USA) solution in ethanol  
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13 (Fisher Scientific; Fair Lawn, NJ, USA) and pressed into the bottom of the sample lane using a  
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15 small metal spatula; (2) 20% H<sub>2</sub>SO<sub>4</sub> was spotted midway up the lane; (3) 2.5% 1-naphthol  
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17 (Sigma-Aldrich; St. Louis, MO, USA) in ethanol was spotted at the top of the sample lane. In  
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19 order to detect ammonium, Nessler's reagent (La-Mar-Ka, Inc.; Baton Rouge, LA, USA) was  
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21 spotted at the top of the sample lane. For the nitrite test, a Griess reagent was prepared by  
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23 spotting 0.5% aqueous 1-naphthylamine (Sigma-Aldrich; St. Louis, MO, USA) midway up the  
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25 sample lane and 0.1% aqueous sulfanilic acid at the top of the sample lane. To detect  
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27 perchlorate, 0.05% aqueous methylene blue (Fisher Scientific; Fair Lawn, NJ, USA) solution  
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29 was spotted at the top of the sample lane. This  $\mu$ PAD is run using deionized water as the  
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31 solvent.  
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### 39 *High/Organic $\mu$ PAD*

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42 The five lane organic/high explosives  $\mu$ PAD includes tests for RDX/HMX/PETN,  
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44 TNT/TNB/Tetryl, urea nitrate, nitrate, and hydrogen peroxide. For the detection of RDX, three  
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46 steps were utilized. (1) A solid reducing mixture of zinc powder was made into a paste using  
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48 50% acetic acid (Fisher Scientific; Fair Lawn, NJ, USA) and pressed into the bottom of the  
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50 sample lane using a small metal spatula; (2) midway up the sample lane 0.05% sulfanilic acid  
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52 was spotted; (3) 0.1% 1-naphthylamine was spotted at the top of the sample lane. In order to  
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54 detect TNT, 1.5 M potassium hydroxide (Fisher Scientific; Fair Lawn, NJ, USA) was spotted at  
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3 the top of the sample lane. For the urea nitrate test, 0.023 M *para*-  
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5 dimethylaminocinnamaldehyde (Acros Organics; NJ, USA) in ethanol was spotted at the top of  
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7 the sample lane. To test for hydrogen peroxide, 1 M aqueous ammonium titanyl oxalate (Acros  
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9 Organics; NJ, USA) was spotted at the top of the sample lane. This  $\mu$ PAD is run using 50%  
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11 acetone/50% water as the solvent.  
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#### 14 15 16 *Portable Testing System*

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19 One of each  $\mu$ PAD can be generated in less than 15 minutes. These devices can be stored in the  
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21 open at ambient temperature for about 1 month before slight color changes start to occur. The  
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23 portable testing system involved the use of a plastic 1 ml vial (Agilent #5182-0567) or reduced  
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25 volume 250  $\mu$ L (Agilent #5188-2788) vials with a slit cut in the lid (Agilent #5042-6491),  
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27 allowing the insertion of the  $\mu$ PAD device into the solvent (Figure 1c). To perform the analysis,  
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29 a small amount of unknown material is placed into the respective solvent and allowed to  
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31 dissolve. The  $\mu$ PAD is then inserted through the slit in the lid and the lid is placed on the vial.  
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34 The bottom tip of the paper device in the solvent allows capillary action to carry the solvent and  
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36 the analytes up the  $\mu$ PAD into the lanes containing the colorimetric test reagents. This entire  
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38 process, including run time, takes approximately 5 minutes to complete.  
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#### 43 44 *Interference Testing*

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47 Gold Bond blue powder, Gold Bond yellow powder, Gold Bond white powder, Publix baking  
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49 powder, Publix baking soda, salt, iodized salt, Publix powdered whip topping, Publix laundry  
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51 detergent, Publix flour, Crystal Light Pink Lemonade mix, and Crystal Light Iced Tea mix were  
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53 purchased from supermarkets in Miami, FL, United States. Codeine, ephedrine,  
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55 methamphetamine, and cocaine were obtained in powder form from the International Forensic  
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3 Research Institute at Florida International University. One thousand ppm samples were prepared  
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5 for each interferant in the appropriate solvent depending on which  $\mu$ PAD was being tested to  
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7 determine if any of these commonly encountered substances produced interferences when  
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9 present.  
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### 11 12 13 *Real Samples*

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16 The Hodgdon Pyrodex and Triple Seven black powder substitutes were both obtained from  
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18 Hodgdon Powder Company, Inc (Shawnee Mission, KS, USA). GOEX black powder was  
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20 obtained from GOEX (Minden, LA, USA) and Red Dot smokeless powder was obtained from  
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22 Alliant Powder (Radford, VA, USA). American Pioneer Powder FFG and Jim Shockey's Gold  
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24 were obtained from American Pioneer Powder, Inc. (Whitewater, CO, USA). The Lemon Drop  
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26 firework was obtained from a retail store.  
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### 31 32 Results and Discussion:

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35 The goal of this project was to develop a set of  $\mu$ PADs capable of detecting a wide range of  
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37 improvised explosive compositions. For detection purposes, explosives can be divided into two  
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39 main groups: inorganic pyrotechnic compositions and organic explosives. Therefore two  
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41 different devices were created: the first  $\mu$ PAD was designed to detect inorganic materials  
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43 including the important oxidizers used in pyrotechnic manufacturing such as nitrates,  
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45 perchlorates, and chlorates. In addition, this  $\mu$ PAD also contained test lanes for ammonium, to  
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47 detect the common fertilizer based explosive ammonium nitrate and a lane for nitrite, which is  
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49 also a post blast reaction product that appears following the deflagration of nitrate salts. The  
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51 second  $\mu$ PAD was designed for the detection of military explosives such as TNT, RDX and  
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53 PETN, as well as urea nitrate and peroxide based explosives.  
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3 Each device was designed using a five lane template in order to allow multiple tests to be  
4 performed on a single device with minimal run time. The external size of these devices during  
5 developmental stages was 45 mm x 38 mm with lane sizes of 13 mm x 4 mm (Figure 1b). This  
6 size was later reduced to 24 mm x 17 mm to increase speed of analysis. Single lane  $\mu$ PADs were  
7 also designed in order to allow for the development and testing of the individual colorimetric  
8 tests (Figure 1a).  
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18 The printing of the  $\mu$ PAD was completed using a wax based printer on Whatman no. 1  
19 chromatography paper. This type of paper is an ideal substrate for fabricating  $\mu$ PADs due to  
20 faster transfer of solutions, better analytical performance, and high color intensities produced for  
21 colorimetric tests compared to filter paper and other thicker substrates<sup>18</sup>. To ensure that the wax  
22 ink was fully embedded into the paper, the  $\mu$ PADs were run through the laminator twice.  
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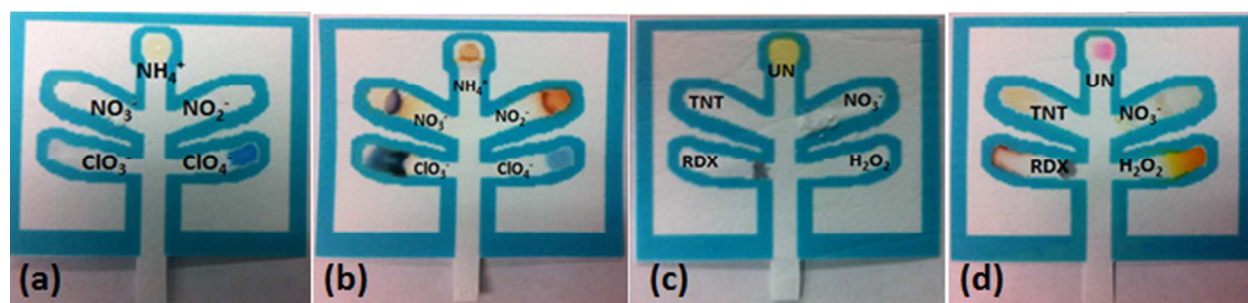
31 The  $\mu$ PADs were originally printed using black ink, but significant bleeding of the ink occurred  
32 due to the effect of the organic solvents that were used. Therefore a comparison of the effects of  
33 the wax ink colors and solvent composition was performed in order to optimize the devices.  
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38 Solvents chosen were also selected based on their ability to maintain the solubility of the  
39 explosive compounds being detected. The optimal wax color chosen was bright blue since none  
40 of the subsequent colorimetric tests generated this color and this color produced minimal  
41 problems with solvent induced bleeding. White ink was not an option since this is not readily  
42 available and the lighter colors (light blue, light pink, lavender, light grey) did not provide a  
43 sufficiently solid barrier to solvent flow.  
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52 Deionized water was used as the optimal solvent for all experiments using the inorganic  
53 explosives  $\mu$ PAD since all of the inorganic explosives are soluble in water. For detection with  
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3 the high/organic explosives  $\mu$ PAD, multiple solvents were tested including acetone, acetonitrile,  
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5 methanol, ethanol, deionized water, 50% DMSO/50% water, 50% acetone/50% water, 75%  
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7 acetone/25% water, 50% methanol/50% water, and 75% methanol/25% water. If the percentage  
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9 of the organic solvent was increased above 50%, a noticeable increase in the bleeding of the wax  
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11 ink occurred due to the dissolution of the dye affecting visualization of the color changes. The  
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13 optimal solvent for this  $\mu$ PAD was determined to be 50% acetone/50% deionized water, in order  
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15 to maintain the solubility of all tested compounds and minimize the bleeding of the wax ink. The  
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17 optimized solvent and wax color were used for all further experiments using the high/organic  
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19 explosives  $\mu$ PAD.  
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26 The first  $\mu$ PAD was developed for the detection of inorganic explosives such as pyrotechnic  
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28 mixtures, black powders, and ammonium nitrate (Figure 2a, 2b) while the second was developed  
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30 in order to detect high explosives such as trinitroaromatics, nitro amines, nitrate esters, and  
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32 organic peroxides (Figure 2c, 2d).  
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47 Figure 2. (a) The inorganic explosives  $\mu$ PAD device prior to analysis. (b) The same  $\mu$ PAD following  
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49 analysis of a 1000 ppm mixture of potassium chlorate, ammonium nitrate, potassium nitrite, and  
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51 potassium perchlorate in deionized water. (c) High/organic explosives  $\mu$ PAD prior to analysis. (d) The  
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53 same  $\mu$ PAD following analysis of a 1000 ppm mixture of RDX, TNT, urea nitrate, and hydrogen peroxide  
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55 in 50% acetone/50% water. Color changes begin to occur after 5 minutes with a total run time of  
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57 approximately 18 minutes for complete visualization.  
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Table 1 lists the relevant expected color changes for each lane in the two developed  $\mu$ PADs.

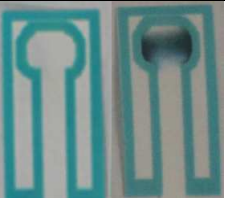



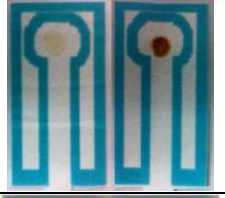
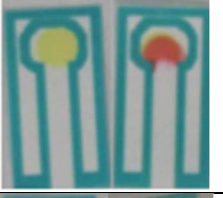


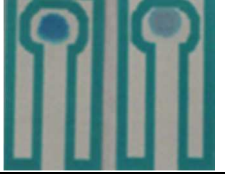

Inorganic Explosives $\mu$ PAD			Organic/High Explosives $\mu$ PAD		
Compound Targeted	Color Change	Results Blank / Sample	Compound Targeted	Color Change	Results Blank/Sample
Chlorate <sup>19</sup> (ClO <sub>3</sub> <sup>-</sup> )	Colorless to dark green		RDX/HMX/ PETN <sup>24</sup>	Colorless to pink/red	
Nitrate <sup>21,22</sup> (NO <sub>3</sub> <sup>-</sup> )	Colorless to orange		Trinitrotoluene (TNT), TNB, Tetryl <sup>19</sup>	Colorless to orange/red	
Ammonium <sup>20</sup> (NH <sub>4</sub> <sup>+</sup> )	Pale yellow to brown		Urea Nitrate <sup>25</sup> (UN)	Yellow to red	
Nitrite <sup>19</sup> (NO <sub>2</sub> <sup>-</sup> )	Colorless to orange/brown		Nitrate <sup>21,22</sup> (NO <sub>3</sub> <sup>-</sup> )	Colorless to orange	
Perchlorate <sup>20</sup> (ClO <sub>4</sub> <sup>-</sup> )	Blue to Purple		Hydrogen Peroxide <sup>23</sup> (H <sub>2</sub> O <sub>2</sub> )	Colorless to yellow	

Table 1. Colorimetric test results for the detection of inorganic explosive compounds and organic/high explosive compounds. These  $\mu$ PADs were prepared using 2  $\mu$ L of each reagent spotted onto the  $\mu$ PAD and allowed to dry (see material and methods section). All tests were run using 1000 ppm of the corresponding explosive compound dissolved in deionized water or 50% acetone/50% deionized water. Deionized water and 50% acetone/50% deionized water were used as the blank. The reaction time for the single lane  $\mu$ PADs is about 5 minutes.

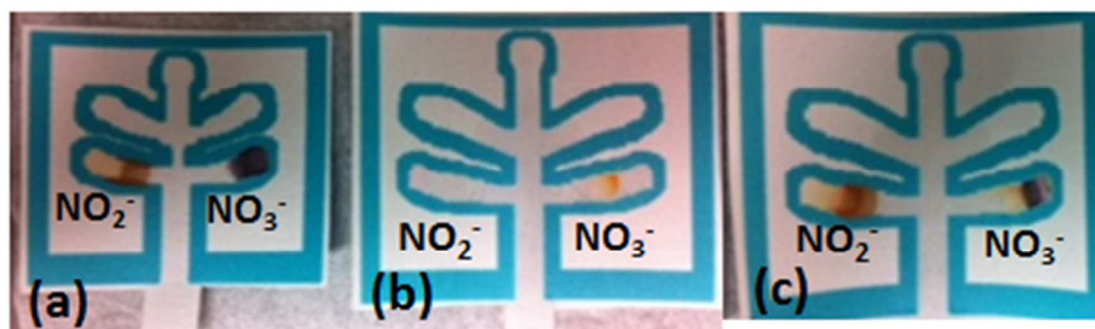
Initially the tests performed on these  $\mu$ PADs were chosen based on a literature study of previously developed liquid based colorimetric tests used in qualitative analysis. However,

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3 many of these original colorimetric tests required acid concentrations that were high enough to  
4 digest paper. Therefore, reagents were modified to permit the development of distinctive color  
5 changes without the use of strong acids. For example, the first colorimetric test for chlorate  
6 utilized concentrated sulfuric acid and an aniline sulfate solution. In order to adapt this test for  
7 use with a  $\mu$ PAD, the dilution of the concentrated sulfuric acid was varied from 0 to 80% and the  
8 colorimetric test was performed in a test tube in order to determine the minimum acid  
9 concentration that could be used while still being able to detect a color change for chlorate. The  
10 optimal level was determined to be about 50% sulfuric acid and then tested on the  $\mu$ PAD. Fifty  
11 percent concentrated  $\text{H}_2\text{SO}_4$  was spotted onto multiple  $\mu$ PADs and allowed to sit for about one  
12 month. After one month, there was no visible degradation of the  $\mu$ PADs and the resulting  
13 colorimetric test successfully detected chlorate.  
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30 For the detection of nitrate, a sequential 3 step test was chosen based on the Griess test<sup>21,22</sup>. A  
31 solid mixture consisting of sulfanilic acid, sodium acetate, and zinc powder was used to reduce  
32 the nitrate to nitrite prior to the colorimetric reaction. A saturated trehalose solution was used  
33 to make a paste with the solid reducing mixture for two reasons: (1) it facilitated an easier  
34 application to the paper devices and (2) the trehalose slows down the migration of the liquid  
35 sample on the  $\mu$ PAD allowing more time for the nitrate to interact with the solid reducing  
36 mixture and be converted to nitrite. The paste was placed at the bottom of the sample lane,  
37 allowing for an initial reduction of any nitrates present in the sample lane. Following reduction  
38 to nitrite, the liquid sample continued to travel up the lane where the reduced nitrate reacts with  
39 the 20% sulfuric acid that was placed mid-way up the sample lane and the sulfanilic acid to  
40 produce a diazonium salt. This diazonium salt continued to travel up the sample lane to the 1-  
41 naphthol spotted at the end of the sample lane. The reaction between the diazonium salt and 1-  
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3 naphthol produced an azo dye, resulting in the formation of an orange color. An additional test  
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6 lane was developed that was specific for nitrite and did not include a reducing agent. This  
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9 procedure utilized an alternative version of the Griess test where visualization occurs with 1-  
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11 naphthylamine instead of 1-naphthol<sup>21</sup>.

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14 Following the development of these two lanes, a study was performed to determine if nitrate  
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16 could be differentiated from nitrite utilizing this single  $\mu$ PAD. As shown in Figure 3, when only  
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18 nitrite was present, the nitrite channel appeared orange/brown and the nitrate channel was purple,  
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20 presumably due to reduction of nitrite. When only nitrate was present, the orange color appeared  
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22 only in the nitrate channel and the nitrite channel was blank. When both salts are present, both  
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24 channels appeared colored, with an orange/brown color visible in the nitrite lane, and purple and  
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26 orange colors in the nitrate lane. Therefore, the device successfully differentiated between  
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28 nitrate and nitrite based explosive compositions as well as detected the presence of nitrite in post  
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30 nitrate and nitrite based explosive compositions as well as detected the presence of nitrite in post  
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32 blast samples resulting from the reduction of nitrate.  
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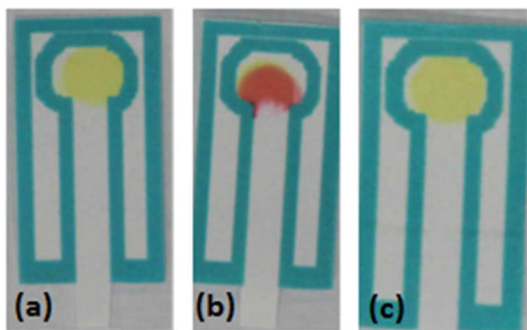
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Figure 3. Nitrite and nitrate tests run with (a) 1000 ppm potassium nitrite in deionized water. The nitrate spot test generates a dark purple color while the nitrite spot test produces an orange-brown color. (b) 1000 ppm potassium nitrate in deionized water. The nitrate spot test generates an orange color while the nitrite spot test does not show any color change. (c) 1000 ppm mixture of potassium nitrate and potassium nitrite. The nitrite spot test produces an orange-brown color while the nitrate spot test shows a dark purple and orange color.



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3 Nitroaromatics such as TNT were detected through the use of 1.5M potassium hydroxide  
4 deposited on the paper, and the subsequent formation of a reddish-orange Meisenheimer  
5 complex<sup>19</sup>. Hydrogen peroxide was detected using ammonium titanyl oxalate with the  
6 formation of a yellow color<sup>23</sup>. This test will also produce a weak orange color in the presence of  
7 triacetone triperoxide (TATP). The overall composition of the organic explosives  $\mu$ PAD is  
8 shown in Table 1 along with the color changes observed for a positive result.  
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18 The p-DMAC colorimetric test for urea nitrate detects the presence of the uronium ion<sup>25</sup> through  
19 a red color change produced by uronium addition to the dye complex; if only urea is present in  
20 the sample, no color change will be produced showing the specificity of this test for the detection  
21 of uronium (Figure 4).  
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40 Figure 4. Urea nitrate test (a) Blank run with just 50% acetone/50% water. (b) 1000 ppm urea nitrate in 50%  
41 acetone/50% water producing a red color. (c) 1000 ppm urea in 50% acetone/50% deionized water.  
42

43 The addition of the nitrate test (described previously) on the five lane  $\mu$ PAD permits the user to  
44 distinguish between urea and ammonium nitrate. If the nitrate test is positive but the urea nitrate  
45 test is not, ammonium nitrate could be present. However, this test is not specific for ammonium  
46 nitrate and will show an orange color in the presence of any nitrate salt, while nitrite salts will  
47 appear purple. The nitrate test also permits the detection of nitrocellulose containing smokeless  
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The test for military explosives RDX/HMX/PETN involves the use of a Griess test with sulfanilic acid and 1-naphthylamine. Therefore, a study was done to determine if nitrite or nitrate would cause a false positive. It was determined that nitrite and nitrate will both produce an orange-brown color change while RDX, HMX, and PETN will produce a pink color change allowing for the compounds to be readily differentiated.

Limits of detection were determined for these  $\mu$ PADs as the lowest concentration that a color change could still visibly be detected (Table 2). Instrumental limits of detection were determined through the use of a Camag Scanner 3 color densitometer plate reader or through Image J software and a digital photograph. It was then calculated by determining the concentration equal to three times the standard deviation of ten replicates at the lowest visible concentration.

Compound being Detected	Visually Minimum Detectable Amount ( $\mu$ g)	Visible LOD (ppm)	Instrumental Minimum Detectable Amount ( $\mu$ g)	Instrumental LOD (ppm)
Chlorate	2.64	100	1.40	53
Nitrite	2.64	100	1.37	52
Ammonium	7.92	300	7.13*	270*
Nitrate	21.12	800	19.8	750
Perchlorate	10.56	400	8.18*	310*
TNT	1.31	50	1.31	50
Hydrogen Peroxide	2.62	100	0.39*	15*
RDX	7.86	300	7.34*	280*
Urea Nitrate	10.48	400	9.17	350

Table 2. Limits of detection determined for each individual colorimetric test using single lane  $\mu$ PADs. Visible limits of detection were determined based on the lowest color change that could be detected on the paper based on the human eye. Instrumental LODs were determined using a color scanner. Those marked with an asterisk (\*) were determined using Image J software due to orientation problems with the color scanner. Experiments were run in triplicate with a run time of about 5 minutes for each blank.

Three different procedures were used for the determination of limits of detection for the colorimetric tests. For detection by eye, single lane  $\mu$ PADs were run for each colorimetric test



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3 from 50 ppm to 1000 ppm and the lowest visible color change compared to the blank was  
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5 determined. Two instrumental procedures were also utilized, the first involving a Camag TLC  
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7 Scanner 3 and the second using a digital camera (Canon Rebel EOS T3i, 18-135 mm lens)  
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9 followed by processing with Image J software.  
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13 For calculations using the Camag Scanner 3 color scanner, measurements were done through  
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15 absorbance detection. The intensity of the color that develops in the test zone is a function of the  
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17 concentration of the analyte and therefore the more analyte present the higher the intensity of the  
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19 color and the higher the absorbance detected. The wavelength used for the analysis was  
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21 determined by scanning a test zone area at 500 nm wavelength to determine the location of the  
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23 test zone with the highest color intensity. This location was then fixed as the wavelengths were  
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25 scanned from 200 nm to 700 nm. The wavelength generating the highest absorbance at this  
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27 location was used for all future measurements for that analyte.  
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33 For the calculations using Image J, measurements were based on the amount of pixels counted  
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35 from pictures of the  $\mu$ PADs using a specific analyte.  $\mu$ PADs were run from 50 ppm to 1000  
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37 ppm and a picture was taken of all of these  $\mu$ PADs. This picture was then loaded into Image J  
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39 and a pixel color range was determined for the measurements by determining the range in which  
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41 the highest concentration generated the most pixels while the blank generated no pixel count in  
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43 that specified range. This range was fixed for all measurements for the specified analyte and the  
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45 pixel count was measured and plotted versus concentration.  
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### 50 *Interference Testing*

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54 These interferences were chosen since they are white powders similar in appearance to many  
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56 explosive powders. It was determined that no false positive were produced. These powders  
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were also run with each explosive compound individually and explosives mixtures; they did not produce false negatives for any of the colorimetric tests.

Controlled substances such as methamphetamine, cocaine, codeine, and ephedrine were also tested as possible interferences for both  $\mu$ PADs. These substances were tested individually and mixed with explosive compounds. No false positives or false negatives were generated.

It was also determined that the limit of detection for these compounds was not affected when these interferences were tested.

### *Real Samples*

Real samples were also tested using fireworks, black powder, black powder substitutes, and smokeless powders. These powders were tested pre-burned and post-burned in order to determine which compounds were present (Table 3) and the results were compared to those previously determined<sup>23</sup>. All tests were done using 1000 ppm aqueous solutions of the corresponding explosive powders.

Table 3. Real samples analysis with the  $\mu$ PAD for inorganic explosives detection. All tests were performed at 1000 ppm of powder in deionized water.

<b>Powder name</b>	<b>Non-Burned Powder</b>	<b>Burned Powder</b>	<b>Powder content</b> <sup>25</sup>
Hodgdon Pyrodex (The FFFG equivalent)	$\text{NO}_3^-$ , $\text{ClO}_4^-$	$\text{NO}_3^-$ , $\text{NO}_2^-$	$\text{KNO}_3$ , $\text{KClO}_4$
Alliant Powder Red Dot Smokeless Powder	$\text{NO}_3^-$	$\text{NO}_2^-$ , $\text{NO}_3^-$	Nitrocellulose, nitroglycerin
FFFg GOEX Black Rifle Powder	$\text{NO}_3^-$	$\text{NO}_2^-$ , $\text{NO}_3^-$	$\text{KNO}_3$
FFG Hodgdon Triple Seven	$\text{NO}_3^-$ , $\text{ClO}_4^-$	$\text{NO}_2^-$ , $\text{NO}_3^-$	$\text{KNO}_3$ , $\text{KClO}_4$ , 3-nitrobenzoic acid
American Pioneer	$\text{NO}_3^-$ , $\text{ClO}_4^-$	$\text{NO}_2^-$ , $\text{NO}_3^-$	$\text{KNO}_3$ , $\text{KClO}_4$
Jim Shockey's Gold FFG	$\text{NO}_3^-$ , $\text{ClO}_4^-$	$\text{NO}_2^-$ , $\text{NO}_3^-$	$\text{KNO}_3$ , $\text{KClO}_4$
Lemon Drop Firework	$\text{NO}_3^-$ , $\text{ClO}_4^-$	$\text{ClO}_4^-$ , $\text{NO}_2^-$ , $\text{NO}_3^-$	$\text{KNO}_3$ , $\text{KClO}_4$

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3 Nitrite was only detected in the burned powders since it is produced when nitrate is burned. The  
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5 Alliant Powder Red Dot smokeless powder produced a positive result for nitrate, which could be  
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7 due to nitrocellulose, nitroglycerin, or both. A pure nitroglycerin or nitrocellulose sample was  
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9 not able to be obtained at a suitable concentration for detection in order to determine which  
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11 compound is causing this positive result. All of the results obtained for the  $\mu$ PADs correlated  
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13 with previous analysis using varying analytical instrumentation<sup>26</sup>.  
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#### 17 18 *Influence of the $\mu$ PAD dimensions in the reaction time*

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21 The  $\mu$ PAD size and set up was also adjusted in order to allow for faster analysis times and an  
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23 inexpensive, portable testing system to be used. The  $\mu$ PAD size was reduced from 45 mm x 38  
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25 mm to 24 mm x 17 mm for future testing analysis. This dropped the analysis time from  
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27 approximately 18 minutes to less than 5 minutes in order to obtain results. The colorimetric  
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29 changes were still clearly visible and the amount of solvent used was significantly reduced due to  
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31 less time needed to run the  $\mu$ PAD. Therefore, the decrease of the size reduced both the analysis  
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33 time and cost.  
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#### 37 38 39 Conclusion:

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42 Two different five lane  $\mu$ PADs were developed for the analysis of unknown suspected explosive  
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44 materials. The first device is able to identify multiple components of inorganic explosives such  
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46 as chlorate, perchlorate, nitrate, nitrite, and ammonium, using deionized water as the solvent. The  
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48 second device is capable of identifying high/organic explosives such as TNT, RDX, hydrogen  
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50 peroxide, and urea nitrate using 50% acetone/50% water as the solvent. Limits of detection  
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52 ranged from 0.39 – 19.8  $\mu$ g of explosive compound, making the devices well suited for the  
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54 identification of unknown powders that are recovered from improvised explosive manufacturing  
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3 sites. Total analysis time was 5 minutes with very few steps needed in order to process the  
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5  $\mu$ PADs.  
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9 Compared to on-site detection techniques for explosive compound identification utilized today,  
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11 these newly designed  $\mu$ PADs are simpler, smaller, and easily portable. They facilitate the  
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13 identification of combinations of explosive compounds by permitting simultaneous multiplex  
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15 testing. Therefore, these  $\mu$ PADs will provide law enforcement and military personnel with  
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17 inexpensive and portable chemical tests for the rapid determination of suspected explosive  
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19 samples.  
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