JOURNAL OF CHEMICAL EDUCATION
Activity
pubs.acs.org/jchemeduc

Using Paper-Based Diagnostics with High School Students To Model Forensic Investigation and Colorimetric Analysis

Rebekah R. Ravgiala,[†] Stefi Weisburd,*[,‡] Raymond Sleeper,[§] Andres Martinez,[∥,#] Dorota Rozkiewicz,[∥] George M. Whitesides,[∥] and Kathryn A. Hollar[⊥]

[†]Biology Department, Tyngsborough High School, Tyngsborough, Massachusetts 01879 United States
[‡]Center for Biomedical Engineering, University of New Mexico, Albuquerque, New Mexico, 87131 United States
[§]Chemistry Department, Merrimack High School, Merrimack, New Hampshire 03054 United States
[∥]Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts 02138 United States
[⊥]Department of Chemistry, School of Engineering and Applied Sciences, Harvard University, Cambridge, Massachusetts 02138 United States

S *Supporting Information*

ABSTRACT: Emerging paper-based diagnostics is an inexpensive yet elegant approach to medical diagnostics and environmental testing in resource-poor regions of the world. Wicking by capillary action distributes a small drop of sample to reagent or assay zones without the need for pumps, refrigeration, or electricity. The advantages of using paper for medical diagnostics (low expense, availability, portability, easy disposal) also apply to the classroom. This article describes a two-session high school activity that employs a paper device to solve a crime scene mystery. On the first day, students role-play either suspects or a detective and make inferences to predict the guilty party. In the second session, students perform presumptive, paper-based forensic testing of "unknown" liquids retrieved from the suspects' homes and compare these findings with their predictions. The primary goals of this exercise are to demonstrate the similarities between forensic investigation and scientific inquiry and to introduce students to presumptive colorimetric analysis and to the promise of paper-based diagnostics and testing.

KEYWORDS: *High School/Introductory Chemistry, Forensic Chemistry, Learning Theories, Analytical Chemistry, Problem Solving/Decision Making, Qualitative Analysis, Public Understanding/Outreach, Student-Centered Learning, Ethics*

Recent advances in the development of paper-based immunoassays promise to transform medical care, especially in the developing world where electricity, refrigeration, clean water, and medical expertise may be lacking.[1−3] Instead of employing instruments or pumps, paper diagnostic devices rely on paper's microfluidic capillaries to wick a drop of blood, urine, or other biological sample to a set of assay zones where it is subject to colorimetric chemical testing, comparable in sensitivity to commercial dipstick assays (see Figure 1). The resultant color can either be matched to a key or be photographed and sent to a doctor via cell phone or scanner. These postage-stamp-sized devices are inexpensive, fabricated for less than \$0.01,[1] and are thus attractive for use in poor communities. Other key advantages include easy portability and storage, the ubiquitous availability of paper, compatibility with many biological and chemical assays, and simple disposal via incineration. Moreover, the filtering capacity of paper also serves to separate and sequester contaminants that might be encountered in the field. Three-dimensional versions of the paper devices are capable of distributing a sample to thousands of testing zones, in some ways surpassing the capabilities of open-channel, glass, or polymeric fluidic devices.[2]

A recent pioneer of this approach, one of us (coauthor George Whitesides at Harvard University) cofounded Diagnostics for All, a nonprofit that is commercializing easy-to-use paper-based immunoassays for the 60% of the developing world that lives far from hospitals or medical facilities.[4] The Whitesides group foresees other applications, including use in home health care settings, rural areas, and natural disaster sites. Analytical chemistry could also benefit from the continued development of simple paper tests. In spite of the unsurpassed specificity and accuracy of mass spectroscopy and other sophisticated analytical techniques, the need for semiqualitative presumptive "spot tests", using small amounts of reagent and sample, remains vital in forensic, environmental, agricultural, and food safety testing.[5]

■ RATIONALE

The same characteristics that make paper attractive for developing world health care—its portability, universal availability, and low cost—also make it appealing for use in

Published: October 28, 2013

© 2013 American Chemical Society and Division of Chemical Education, Inc.

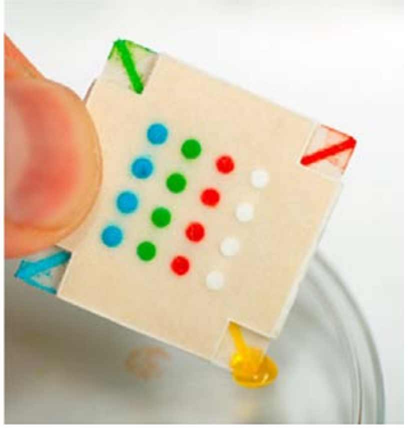

Figure 1. Example of a test paper-based diagnostic device from the lab of George Whitesides. This device can conduct four separate tests on four separate samples. Hydrophobically defined channels are made with photoresist (SU-8).

the chemistry classroom. This article describes an activity designed for high school students that uses a simple paper test adapted from the Whitesides laboratory in the context of a mock crime scene investigation. It was developed and implemented by one of us (coauthor Rebekah Ravgiala) in 2008 as part of Research Experiences for Teachers at Harvard University. Conceived for high school students, it could also be extended to other age groups with suitable modifications.

As has been noted many times in this and other science education journals,[6−9] chemistry labs couched in crime stories are compelling activities for students because of the popularity of *CSI* and other television shows that highlight forensics. Solving a mystery develops analytical skills, motivates group collaboration and problem solving, and can enhance literacy. Our particular activity also incorporates a taste of theater as students role-play the parts of suspects and detectives. This approach to cooperative learning has been shown to increase student interest, creativity, and communication skills.[10,11]

Most significantly, the sleuthing process parallels the practice of science, which a recent National Research Council (NRC) report[12] concludes has received short shift in K−12 education. These practices include asking questions, developing models, carrying out investigations, analyzing and interpreting data, constructing explanations, engaging in argument from evidence, and evaluating and communicating information. The NRC also discusses the importance of investigating the links among engineering, technology, and society. The newly released Next Generation Science Standards,[13,14] based on the NRC report, integrate scientific concepts with these practices. Similarly, our paper-based diagnostic activity requires students to combine science skills and scientific knowledge with scientific reasoning to make inferences and draw conclusions about the perpetrator of a crime. As such, students engage in classroom inquiry in which they work collaboratively to ask scientifically oriented questions, test hypotheses, critically and logically evaluate evidence, reflect on alternative explanations, and communicate and defend their conclusions: in this case, the culprit. In addition to meeting the framework outlined by the NRC, this activity also meets the scientific inquiry skills standards described by the Massachusetts Science and Technology Frameworks (Scientific Inquiry Skills Standards 1−4, 2006)[15] and the New Mexico Science Content Standards, Benchmarks, and Performance Standards (Scientific Thinking and Practice, Standard I, Benchmarks I and II, 2003).[16] Our paper-based approach could easily be extended to other curricula covering acid−base reactions, for example, or in an organic molecule unit to perform glucose and protein tests, which would conserve materials and lower the amount of waste generated in the lab.

■ HAZARDS

The risks associated with this activity are minimal because of the small quantities and low concentrations of chemicals. Nonetheless, gloves and safety glasses should be worn and normal laboratory safety precautions should be taken. Sodium hydroxide is caustic and corrosive; it may pose a hazard to the eyes and skin. Universal indicator is harmful if inhaled or swallowed or if it comes in contact with the eyes or skin, although indicator papers are no more harmful to use than filter paper. Biuret reagent irritates the skin, eyes, respiratory tract, and mucous membranes. Egg albumin is slightly irritating in case of eye contact or inhalation.

■ FORENSIC ANALYSIS

When attempting to identify a substance, forensic chemists often begin with a screening (or presumptive) chemical spot test, which usually relies on a simple colorimetric reaction. If this reaction produces a specific color change, the test indicates that the particular compound might be present. To positively identify the specific substance, more discriminating tests are conducted, most often employing gas chromatography and mass spectroscopy.[7,17] As these instruments are beyond the budgets of the average K−12 classroom, the activity described here emulates the first step only.

■ CRIME SCENE ACTIVITY

Our activity is designed for two, 1-h sessions. In the first session, students are presented with a crime scenario and divided into groups in which they play the roles of suspects or the detective who conducts interviews. The second session (usually on a second day) is spent performing and interpreting chemical tests on suspects' samples. The Supporting Information includes instructor information and a student guide. It also contains two specific crime scene scenarios with "cast cards", which describe each character's history and possibly relevant scientific clues.

Session 1

The crime scenario is a fictitious story in which a chemical engineer, who had filed a patent for a biodiesel technology, is found dead in her apartment by one of her three friends. An autopsy reveals levels of cyanide and creatine, a performance-enhancing supplement, in her system as well as a blunt trauma wound to her head. A vial of liquid is discovered in her apartment. When similar unknown liquids are recovered at the houses or o ces of her three friends, they become suspects.

After reading the crime scenario, the class is divided into groups of four. Each member of the group is randomly assigned to play either one of three suspects or the detective. Students read about their character on a laminated cast card. The detective's card includes facts about cyanide: its use in various industries, its health e ects, and its almond scent, which only a subset of the population is capable of detecting.[17] The detective interviews the three suspects, filling out a lead investigator's report and wound chart (see student guide in the Supporting Information). As the crime scene scenario is explored, students are challenged with determining who within the group is the most likely perpetrator and why. Each group presents its findings to the class, which is encouraged to evaluate each presenting group's assumptions and reasoning. In our experience, the groups are fairly mixed as to their conclusions of guilt.

Session 2

The activity culminates on the second day with the testing of four "unknown" liquids collected from the crime scene and the suspects' homes or o ces. Through a simulated chemical forensic analysis, students test each sample for "cyanide" and "creatine".

Obviously, handling actual cyanide would present an egregious safety hazard. Cyanide is highly toxic; a medical examiner who opens the stomach of a person who has swallowed potassium cyanide can be killed by the released gas.[17] There are numerous colorimetric tests for cyanide. For example, a solution containing iron(II) sulfate added to cyanide produces Prussian blue, and a violet color results from another common color reagent containing *p*-nitrobenzaldehyde and *o*-dini-trobenzene.[18]

To simulate the latter violet test, we replaced cyanide with 0.1 M NaOH, which turns universal indicator purple. Likewise, the "creatine" is simulated by LB Nutrient Broth or powdered egg albumin, which turns light blue biuret reagent to violet.

Students analyze these samples with a paper forensic device cut out from chromatography paper with a five-leafed scrapbook punch (see Figure 2). In the Whitesides devices,

Figure 2. Scrapbook punch makes five-leafed paper forensics devices.

fluid flow is directed by lithographically patterned hydrophobic material that defines channels. We attempted to replicate this approach by placing a sheet of wax paper over the chromatography paper and using a pen to inscribe wax channel walls, but the channels proved leaky. Instead we chose to shape the paper in a way that contains the reagents and directs sample flow without the need for patterning. This approach is similar to work conducted at the University of New Mexico, in which Fenton et al. shaped lateral-flow devices in branching and multiarmed configurations.[3] See Figure 3.

In our clover-shaped device, one leaf is treated with universal indicator and another is saturated with biuret reagent and allowed to dry. To test a crime scene sample, students wet a swab with the unknown liquid, provided in a microtube, and blot it down on the center of the clover. From there, the liquid is wicked to the two leaves to react with each reagent. The universal indicator reaction is instantaneous, but the biuret reaction requires about 5−10 min to finalize.

Each group then tapes its paper devices to a summary chart and interprets the results, noting color changes and indicating which suspects tested positive for cyanide and creatine.

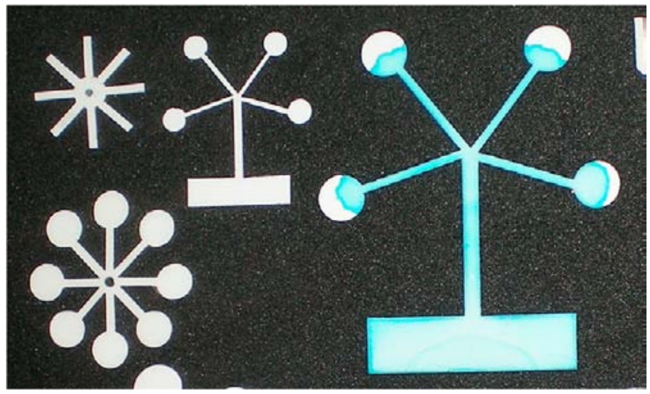

Figure 3. The paper devices used in this activity are partially based on these multibranched, lateral-flow devices that do not require patterning to channel liquids to assay areas at the tips of the arms.

Students are also asked to record possible motives for each character and discuss the implications. A suspect organization chart is included in the student guide as part of the Supporting Information. Table 1 summarizes the intended results.

■ DISCUSSION

This activity works particularly well at the very beginning of the high school semester as it "breaks the ice" by promoting group interaction and introduces students to the scientific method in a low-stress format. It is an especially e ective exercise in elucidating the di erence between inference and observation. In our experience, only one or two groups (out of five or six) correctly predict the most likely guilty party based on interviews.

With younger students, it is advantageous for the class as a whole to generate the kinds of questions the detective should ask before they split up into groups. These questions should be written on the board. We suggest modifying the story and character cards to fit the reading level and interests of the class. For example, when the activity was implemented in a New Mexico middle school summer camp (see Figure 4), the character cards were simplified and local elements, such as green chili, the University of New Mexico, and the locally filmed TV series *Breaking Bad* were integrated into the crime scenario.

Care must be taken when preparing the devices to prevent the reagents from staining the center of the clover. Coupled with overenthusiastic sample wetting of the center, this can lead to the biuret reacting with universal indicator, causing the latter to turn purple. However, these occasions have precipitated a discussion of false positives, the importance of hygienic lab techniques and sample preparation, and the need for further confirmatory testing.

As originally envisioned, the activity takes two days, but it could be expanded in several ways. Students could design and test their own paper devices, experimenting with di erent shapes and paper types to control fluid flow. Another possibility is to study the reagent chemistry that is taking place on the devices and how it causes color change. The social aspects of science may also be explored by investigating the power and limits of forensic evidence in light of reports of incompetently obtained, scientifically unsubstantiated, falsified, or omitted forensic data[19−26] and by researching the needs of rural and resource-poor health delivery systems and how going "low tech" might advance health care.

Table 1. Summary Chart of Presumptive Results

Person	Evidence Obtained (Prepared As)	Paper Diagnostic Reading	Interpretation of Lab Result for "HCN" (Left Leaf)	Interpretation of Lab Result for "Creatine" (Right Leaf)
Victim	Yellowish Liquid (1.3 g albumin in 25 ml 0.1M NaOH)		(+) Purple	(+) Violet
Suspect 1	Yellowish Liquid (1.3 g albumin in 25 ml apple juice)		(–) Orange	(+) Violet
Suspect 2	Yellowish Liquid (1.4 g albumin in 0.1M NaOH)		(+) Purple	(+) Violet
Suspect 3	Yellow Liquid (1 drop yellow food coloring in 25 ml water)		(–) Orange	(–) Blue

Figure 4. After the activity, middle school students at the University of New Mexico made a poster presenting their forensic results and subsequently also filmed a short video of the murder scene for which they also wrote theme music.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Instructor guide; standards; student guide (including lead investigator's report and suspect organizational charts); crime scenario 1: suspect and detective cast cards 1; crime scenario 2: suspect and detective cast cards 2. This material is available via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Corresponding Author E-mail: weisburd@unm.edu.

Present Address

#Andres Martinez is now at the Department of Chemistry and Biochemistry, California Polytechnic State University, San Luis Obispo, California 93407, United States.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We gratefully acknowledge the support of the National Science Foundation's Research Experiences for Teachers (RET), Partnership for Research and Education in Materials (PREM) between the University of New Mexico and Harvard University's MRSEC, and the National Nanotechnology Infrastructure Network (NNIN). We also thank the students at Tyngsborough High School and in UNM's Pre-College Science and Engineering Program's Majik Camp for their feedback. Rebekah Ravgiala extends her deepest gratitude to George Whitesides and members of his research group for providing her with the tools, guidance, and support in the development of this project. She especially acknowledges then-graduate student Andres Martinez and postdoctoral fellow Dorota Rozkiewicz and UNM student and Harvard REU Monica Mascarenas, for sharing their research and experiences. Thank you to the "cast" including Marc Abelard, Darlene Marcano, and Tray Sleeper, whose reflections, storyline ideas, and constructive problem solving made commuting a pleasure, and to Kathryn Hollar for her encouraging words, innovative ideas, and commitment to establishing and maintaining a nurturing atmosphere for teachers conducive for creative thinking and professional growth. Funding for this activity was provided by NSF PREM Grant DMR-0611616.

■ REFERENCES

(1) Martinez, A. W.; Phillips, S. T.; Whitesides, G. M. Diagnostics for the Developing World: Microfluidic Paper-Based Analytical Devices. *Anal. Chem.* **2010**, *82*, 3−10.

(2) Martinez, A. W.; Phillips, S. T.; Whitesides, G. M. Three-Dimensional Microfluidic Devices Fabricated in Layered Paper and Tape. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 19606−19611.

(3) Fenton, E. M.; Mascarenas, M. R.; Lopez, G. P.; Sibbett, S. S. Multiplex Lateral-Flow Test Strips Fabricated by Two-Dimensional Shaping. *ACS Appl. Mater. Interfaces* **2009**, *1*, 124−129.

(4) Diagnostics for All. http://www.dfa.org/ (accessed Sep 2013).

(5) Jungreis, E. Spot Test Analysis. Entry in *Encyclopedia of Analytical Chemistry*; John Wiley & Sons: New York, 2006; DOI: 10.1002/9780470027318.a8111.

(6) Harmon, K. J.; Miller, L. M.; Millard, J. T. Crime Scene Investigation in the Art World: The Case of the Missing Masterpiece. *J. Chem. Educ.* **2009**, *86*, 817−819.

(7) Hasan, S.; Bromfield-Lee, D. B.; Oliver-Hoyo, M. T.; Cintron-Maldonado, J. A. Using Laboratory Chemicals To Imitate Illicit Drugs in a Forensic Chemistry Activity. *J. Chem. Educ.* **2008**, *85*, 813−816.

(8) Duncan, K.; Daly-Engel, T. Using Forensics Science as Teaching Tools. *Sci. Teach.* **2006**, *73*, 38−43.

(9) Childs-Disney, J. L.; Kauffmann, A. D.; Poplawski, S. G.; Lysiak, D. R.; Stewart, R. J.; Arcadi, J. K.; Dinan, F. J. A Metabolic Murder Mystery: A Case-Based Experiment for the Undergraduate Biochemistry Laboratory. *J. Chem. Educ.* **2010**, *87*, 1110−1112.

(10) Jackson, P. T.; Walters, J. P. DNA Profiling of the D1S80 Locus: A Forensic Analysis for the Undergraduate Biochemistry Laboratory. *J. Chem. Educ.* **2000**, *77*, 1019−1025.

(11) Grannas, A. M.; Lagalante, A. F. So These Numbers Really Mean Something? A Role Playing Scenario-Based Approach to the Undergraduate Instrumental Analysis Laboratory. *J. Chem. Educ.* **2010**, *87*, 416−418.

(12) Quinn, H.; Schweingruber, H.; Keller, T., Eds. *A Framework for K−12 Science Education: Practices, Crosscutting Concepts, and Core Ideas*; The National Academies Press: Washington, DC, 2012. http://www.nap.edu/catalog.php?record_id=13165 (accessed Sep 2013).

(13) A Next Generation Science Standards. http://www.nextgenscience.org/final-next-generation-science-standards-released (accessed Sep 2013).

(14) National Committee on Science Education Standards and Assessment; BOSE; DBASSE; National Research Council. *National Science Education Standards*; The National Academies Press: Washington, DC, 1996. http://www.nap.edu/openbook.php?record_id=4962&page=145 (accessed Sep 2013).

(15) Massachusetts Department of Education, Massachusetts Science and Technology/Engineering Curriculum Framework, Oct. 2006. http://www.doe.mass.edu/frameworks/scitech/1006.pdf (accessed Sep 2013).

(16) New Mexico State Department of Education, Science Content Standards, Benchmarks, and Performance Standards, and Performance Standards, 2003. http://geoinfo.nmt.edu/museum/science_standards.pdf (accessed Sep 2013).

(17) James, S. H.; Nordby, J. J., Eds. *Forensic Science: An Introduction to Scientific and Investigative Techniques*, 2nd ed.; CRC Press: Boca Raton, FL, 2005; p 54.

(18) Ma, J.; Dasgupta, P. K. Recent Developments in Cyanide Detection. *Anal. Chim. Acta* **2010**, *673*, 117−125.

(19) Schwartzapfel, B. *Newsweek*, February 17, **2011**. http://www.thedailybeast.com/newsweek/2011/02/17/forensics-under-the-microscope.html (accessed Sep 2013).

(20) Chan, S. Scores of Convictions Reviewed as Chemist Faces Perjury Accusations. *Los Angeles Times* online; August 21, **1994**. http://articles.latimes.com/1994-08-21/news/mn-29449_1_lab-tests-fred-zain-double-murder (accessed Sep 2013).

(21) Baldas, T.; Neavling, S. *Detroit Free Press* online; May 29, **2011**. http://www.freep.com/article/20110529/NEWS01/105290502/Detroit-s-%20abandoned-crime-lab-adds-disturbing-U-S-trend (accessed Sep 2013).

(22) Patterson, R. P. A Deadly Twist at Houston's Crime Lab. *Houston Press*; November 26, **2008**. http://www.houstonpress.com/2008-11-27/news/deadly-evidence-at-the-crime-lab/ (accessed Sep 2013).

(23) Dror, I. E.; Charlton, D. Why Experts Make Errors. *J. Forensic Ident.* **2006**, *56*, 600−616.

(24) Giannelli, P. C. Wrongful Convictions and Forensic Science: The Need To Regulate Crime Labs. *N.C. Law Rev.* **2007**, *86*, 163−236.

(25) Bergslien, E. Teaching To Avoid the "CSI Effect". *J. Chem. Educ.* **2006**, *83*, 690−691.

(26) The Innocence Project. http://www.innocenceproject.org/ (accessed Sep 2013).