



B9 The Development of a Multianalyte Paper-Based Device for Serological Measurements

Rosa L. Cromartie, BS, 16800 SW 137th Avenue, Apt 1124, Miami, FL 33177; George T. Duncan, PhD, Broward County Crime Lab, 201 SE 6th Street, Rm 1799, Fort Lauderdale, FL 33301; and Bruce R. McCord, PhD, Florida International University, Dept of Chemistry, University Park, Miami, FL 33199*

After attending this presentation, attendees will understand how the development of a paper-based device can be used to obtain serological measurements from various biological samples. This multianalyte device permits a simultaneous analysis of various body fluids obtained from a variety of substrates. This device is rapid, simple, and permits a quick presumptive screening of body fluids collected at crime scenes.

This presentation will impact the forensic science community by demonstrating that multiplexed serological measurements can be made using a microfluidic device developed from paper and wax with colorimetric sensing pads. The design and developmental validation of the system will be discussed, including stability, interference testing, and reproducibility studies. Fieldable test kits are important in evidence screening as they aid in the collection of samples and improve efficiency. Properly designed, they permit simple, fast, and presumptive testing to occur at a lower cost. Unfortunately, many of these tests generally run one body fluid at a time, which can be lengthy in time and potentially destructive to critical samples. This presentation proposes a multianalyte serological screening procedure based on a paper microfluidic platform.

Multianalyte paper-based devices utilize sheets of chromatographic paper and thermal wax to create hydrophilic channels that direct a liquid sample to multiple test wells, each with a different colorimetric sensor. This multianalyte paper-based device can be used to detect various body fluids for field-presumptive testing. The device produces quick and easily distinguishable results without the need for external instrumentation.

In this project, the developmental validation of this multianalyte device will be discussed. Sensitivity studies, interference testing, and experiments involving aged and degraded samples were all performed as part of these validation studies. Colorimetric tests used in this device include modifications of the Kastle-Meyer test for blood, the Urease-Nessler's test for urine, the amylase test for saliva, and the acid phosphatase test for semen. All four tests were implemented on a single paper-based analytical device. Key issues in design included adjusting reagent concentrations for visibility and long-term stability. Tests of single and mixed analytes were performed.

For the determination of blood, a Kastle-Meyer test was used; however, hydrogen peroxide was not an appropriate reagent for this test due to problems with evaporation. Therefore, sodium perborate was used in this test to oxidize phenolphthalein. Although this test is not blood specific, it did provide a generalized test method for the presence of blood. Dilution factors as high as 1:750 still produced results. Interferences included certain acidic foods and bleach. For the determination of urine, urease-based decomposition of urea to ammonia was utilized. The release of ammonia was then detected using the Nessler's reagent. Dilutions of urine up to 1:100 provided a response and urine samples up to 30 days in age still produced results. Interferences included other substances containing urea. For the determination of saliva, the ability of amylase to hydrolyze starch/iodine mixtures was used. This reaction produces color change from a black-purple color to a clear color. Sample dilutions up to 1:100 were still detectable, as were samples aged up to 30 days. Lastly for determination of semen, the acid phosphatase reaction was used. Sample dilutions up to 1:400 were detectable on the chip. Interferences included herbal drinking tea and vaginal secretion fluids.

Experimental results with mixtures and single-source samples demonstrate clear, distinct signals for each serological sample that was present. Results discussed will include the sensitivity of each test, the range of interferences, and how age affects the results. Overall, this presumptive testing method is rapid, reproducible, and easily used in the field for screening unknown fluids during a forensic investigation.

Multianalyte, Serological, Validation