B80 The Effect of Organic Acid Influenced by Sample pH on False Positive Test Results Using Immunochromatographic Assays

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After attending this presentation, attendees will better understand how the presence of organic acids at various pH levels can affect the accuracy and reliability of results obtained using immunochromatographic assays for multiple biological fluids from a number of manufacturers.

This presentation will impact the forensic science community by emphasizing the importance of using immunochromatographic tests as a presumptive indication of biological fluids and will illustrate how results from these assays should not be overstated. Instances of non-specific binding events utilizing various immunochromatographic assays as influenced by sample pH and the presence of organic acids will be evaluated.

A majority of forensic biological evidence requires detection of bodily fluids as a means to identify and prioritize items that should be processed for genetic analysis. The screening and confirmatory identification of these bodily fluids can greatly aid an investigation; however, it is important to understand the limits of the test employed. The current methodology most routinely applied to forensic casework for the detection of biological fluids is immunochromatographic assays. Manufacturers of these tests, including Seratec®, Abacus Diagnostics®, and Independent Forensics, market such assays for blood, semen, saliva, and urine detection. Regardless of manufacturer or target analyte, these tests function in a similar manner and therefore suffer from the same limitations. Target biomarkers present in lower concentrations in other biological fluids have demonstrated the potential to produce positive reactions.[1-3] Additionally, false positive reactions due to cross-reactivity with non-target molecules with similar conformational epitopes are possible as are non-specific binding events.[4] This latter category of false positives was further investigated in this study.

Common immunoassay tests utilized in forensic casework, including ABAcard® p30 and ABAcard® HemTrace® by Abacus Diagnostics®; RSID™-Urine, RSID™-Semen, RSID™-Blood, and RSID™-Saliva by Independent Forensics; and PSA Semiquant, HemDirect, and Amylase Test by Seratec®, were evaluated. A 300mM solution of citric acid was prepared across a pH range from 1.78 to 12. Citric acid solution was added according to the manufacturers' recommendations for each test with regard to sample incubation times, volumes, and run times for analysis. Invalid test results, negative results, and false positive results were recorded over the indicated pH range. Repeatability was evaluated by testing the lowest and highest pH citric acid solution to produce a false positive result in triplicate for each test. Deionized water solutions over the pH ranges that produced false positive results for citric acid solutions were also analyzed.

False positive results were observed for each assay evaluated and were seen between a pH of 2 to 12 on various tests. Repeatability was observed for all tests evaluated. All deionized water solutions produced negative test results as expected, indicating the role of organic acids in generating non-specific binding events. It should be emphasized that based on the findings exhibited in this study, immunochromatographic tests display presumptive findings due to lack of specificity. Additional organic acids were also evaluated at multiple concentrations as were possible mechanisms leading to false positive test results.

Reference(s):

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2. Old, Jennifer B. et al. Developmental Validation of RSID™-Saliva: A Lateral Flow Immunochromatographic Strip Test for the Forensic Detection of Saliva. *Journal of Forensic Sciences.* 54.4 (2009): 866-873.
3. Diamandis, Eleftherios P., and He Yu. Nonprostatic sources of prostate-specific antigen. *Urologic Clinics of North America*. 24.2 (1997): 275-282.
4. Seratec®. 2006. PSA in bodily fluids – an overview for users of the SERATEC PSA SEMIQUANT Tests.

Immunochromatographic Assays, False Positives, Organic Acid

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