IN SITU COCAINE DETECTION IN HUMAN SWEAT USING INTEGRATED DIAGNOSTIC SKINPATCHES AND HAND HELD FLUORESCENCE READER

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ABSTRACT

In this paper we present a novel highly sensitive point-of-care (PoC) diagnostic system, called Skinpatch, that allows non-invasive in situ monitoring of human sweat for the presence of cocaine over extended periods. The system is utilizing a wearable skinpatch for collecting and analyzing human sweat samples and a hand held fluorescence reader for measuring and interpreting the assay results. In various studies 320 skinpatches were characterized to determine assay sensitivity, thresholds and to verify the mechanical and fluidic functions during periods of 24 and 48 hours under real conditions. The limit of detection of the skinpatch for cocaine was established at below 2.5 ng, with a threshold sensitivity around 7.5 ng.

KEYWORDS: cocaine, fluorescence

INTRODUCTION

Drug-of-abuse (DoA) detection is important for a broad spectrum of applications ranging from medical diagnostics to law enforcement. There are many low cost rapid PoC devices for DoA testing commercially available. Some microfluidic devices with various methods of analysis are also reported in the literature [1-4]. The common work principle of commercial PoC drug tests is based on immunochromatographic separation of a sample on a lateral flow strip. The readout of the test result is typically based on colorimetric changes of the test and control lines. This technology is well understood and offers low cost, user-friendly formats and short time to test results. However, the majority of commercial devices only work with urine, blood or saliva samples, which can only be obtained by invasive or intrusive sample collection methods, are often susceptible to tampering, or not economically viable or practical for continuous drug use monitoring.

The analysis of sweat may become an effective method for monitoring drug use over extended periods. In this paper we present results of investigations on a novel highly sensitive diagnostic system called skinpatch that allows non-invasive in-situ sweat collection and analysis of human sweat for the presence of cocaine.

EXPERIMENT

The Skinpatch analysis system contains two components: a wearable integrated diagnostic skin-attached patch and a handheld fluorescence reader. The skinpatch is a flexible assay enclosure that can be worn attached to the human skin for up to 5 days (Fig. 1). It employs a multilayer lateral flow assay strip where fluorescence test and control lines (Dylight649 dye was used) are deposited onto a nitrocellulose strip. In the absence of cocaine, the fluorescently-labelled conjugate binds at the test line, and a strong fluorescence signal is obtained. For positive samples, the cocaine present in the sweat binds to the fluorescently-labelled conjugate in the conjugate pad thus binding the available fluorescently-labelled conjugate and inhibiting binding to the benzoylecgonine antigen present at the test line, resulting in a quenching of the fluorescence. For in situ cocaine detection the skinpatch facilitates multiple functions including the collection of sweat samples for up to 5 days, the protection of the assay strip against humidity and environmental influences while the skinpatch is worn, the controlled elution of the sample from the sweat absorber to the assay strip and the provision of optimal assay flow conditions during the immunochromatographic reaction.

a) *b)*

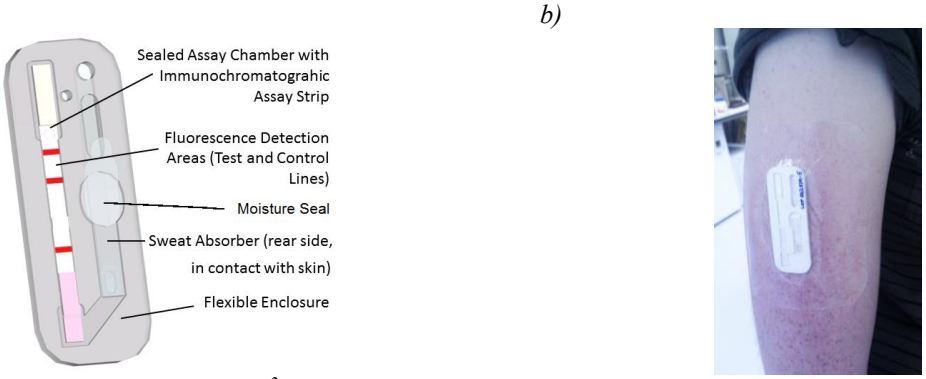
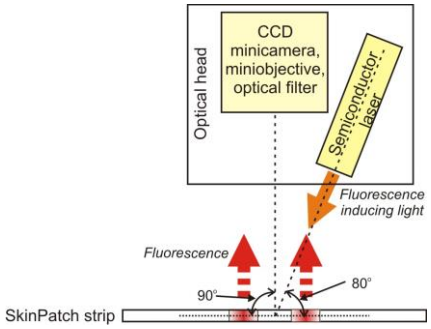


Figure 1: Wearable skinpatch (3 x 8 cm^2) for sweat collection and immunochromatographic analysis: a) schematic view of skinpatch, b) skinpatch attached to a person's arm during testing

The hand-held optoelectronic reader is a USB powered device enabling laser-based fluorescence induction and capture of fluorescence images by a miniature non-cooled CCD camera (Fig. 2a). The reader carries out the real-time fluorescence analysis and reports a positive or negative test result instantaneously. The reader communicates with a portable computer and analysis software. The software combines algorithms for data acquisition, calibration and analysis. It automatically calibrates the laser and CCD signals, analyses the scanned images, calibrates the raw data, and calculates the test to control lines ratio. Automatic peak detection recognizes test and control lines as the skinpatch is scanned and displays test results as positive or negative according to pre-defined ratio thresholds (Fig. 2b).

a)



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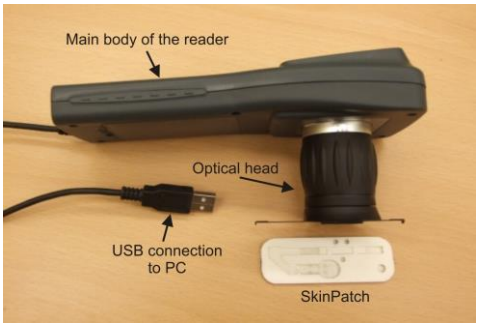

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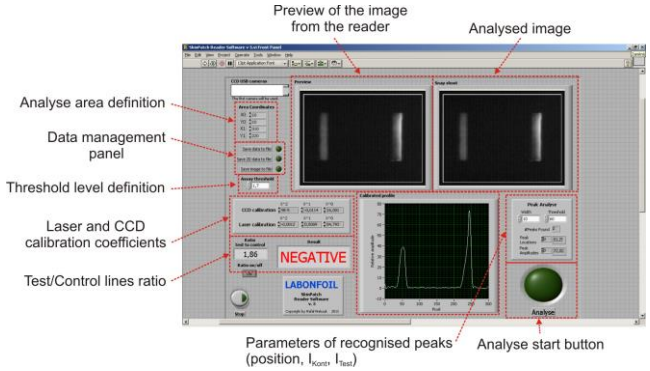
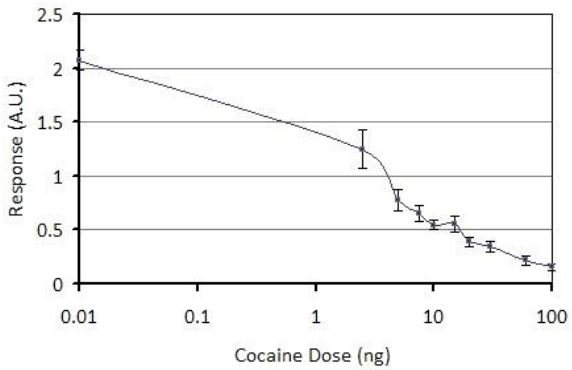


Figure 2: Skinpatch reader: a) schematic principle of the fluorescence detection, b) view of the optoelectronic fluorescence reader, c) screen shot of the image collection and analysis software

RESULTS

To establish the threshold sensitivity of the skinpatch analysis system a dose response curve was generated using spiked human sweat as a sample matrix. Sweat samples were spiked in increments from 2.5 ng to 100 ng of cocaine. The fluorescence analysis confirmed a limit of detection below 2.5 ng. The assays showed clear inhibition with increasing cocaine concentrations (Fig. 3) with a 40% decrease in fluorescent signal at 2.5 ng and 62% and 93% at 5 ng and 100 ng cocaine concentration respectively. This was expected for a competitive assay. As the assay exhibited a normal log-logarithmic response to increasing concentration, these data verified that the assay was operating correctly.

a)



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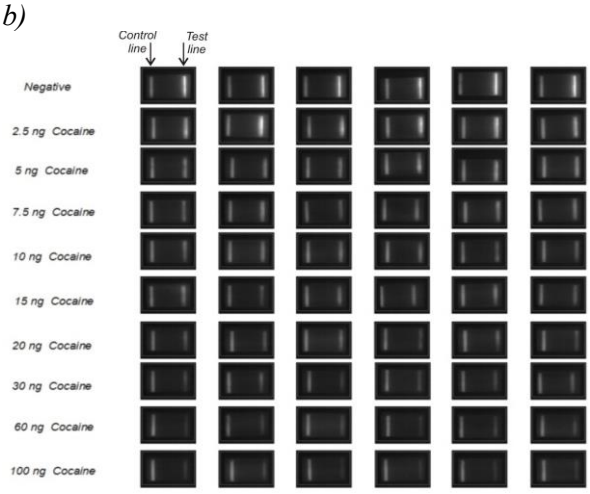

Figure 3: Dose response curve (a) obtained on the base of collected and analyzed images of Skinpatch detection areas (b), response is ratio of fluorescence intensities of control to test line

Based on the dose response the linear range of the curve was estimated around an optimal threshold value of 7.5 ng. At this sensitivity level all samples at 5 ng (67% of threshold) and 10ng (133% of threshold) are estimated as negative and positive respectively. All samples at 0 ng and 100 ng are clearly negative or positive (Fig. 4).

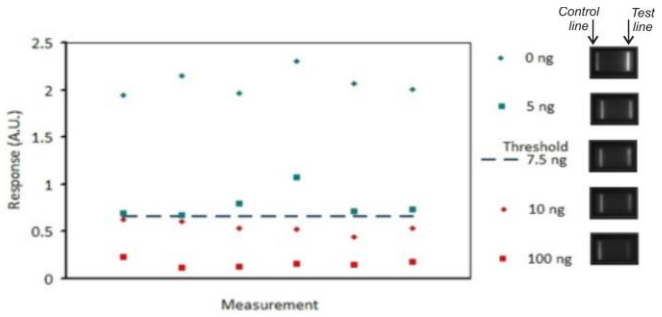

Figure 4: Threshold estimation graph with fluorescence images of skinpatch detection area, response is ratio of fluorescence intensities of control to test line

The in-situ verification of the mechanical and fluidic function was the last validation stage of the skinpatch analysis system. As part of this study the skinpatches were tested for reliability under real life conditions, the cocaine assays were tested with in-situ collected sweat, and the handheld skinpatch reader was characterized against a reference analysis system. A total of 24 skinpatches were worn by volunteers for durations of 24 and 48 hours. The conditions of use were recorded by all volunteers. Results are shown in Figure 5. 12 of the skinpatches were run and characterized in a standard format, whereby a specifically formulated elution buffer was injected into the sample collection chamber, to allow analyte to elute and flow to the assay strip. After 10mins the skinpatches, which were still attached to the volunteers' arms, were analyzed with the reader. The remaining 12 skinpatches were run as simulated "positive" tests. Here the skinpatches were taken off the volunteers and were run with a spiked buffer solution (30ng/ml) prior analysis with the reader. The analysis of worn skinpatches revealed that with the exception of one device, all skinpatches were able to clearly distinguish positive and negative samples after 24h and 48h of continuous wearing.

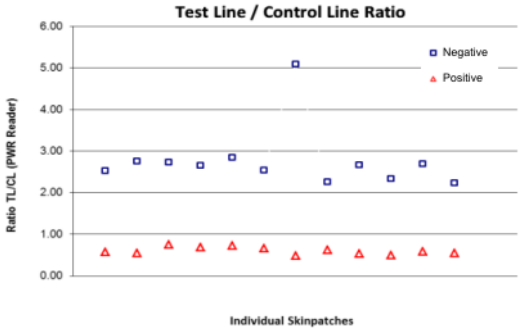

Figure 5: Ratios of test to control lines fluorescence intensities for in-situ tested skinpatch system for positive and negative samples

CONCLUSION

In this paper we present a novel highly sensitive diagnostic system, called Skinpatch, that allows non-invasive in situ sweat collection and analysis of human sweat for the presence of cocaine. The studies demonstrated that the skinpatches are suitable for prolonged drug use monitoring and that the band aids, the sweat collection materials, the moisture sealing features, the cocaine assays and the reader system can function as in situ sweat collection and drug analysis device.

ACKNOWLEDGEMENTS

The works have been financed by EU 7. FP LABONFOIL, contract no 224306.

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