Touch Spray Mass Spectrometry Using Medical Swabs for the Detection of Strep Throat Causing Bacterium and Illicit Drugs in Oral Fluid

Introduction

Touch Spray (TS) is a spray-based ambient ionization technique in which a sample is transferred to a substrate (i.e. probe) from which direct ionization occurs, thus allowing rapid and straightforward sampling and analysis of complex biological samples with minimal-to-no pretreatment [1]. Original TS-MS experiments used metallic probes with rough texture and a sharp tip for cancer diagnostics (see Figure 1).

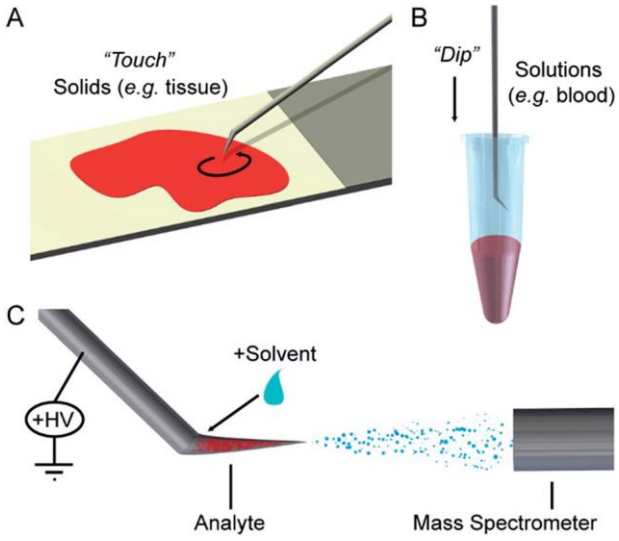

Figure 1. Sampling of **(A)** solids and **(B)** liquids by touch spray ionization using an angled teasing needle. **(C)** Application of high voltage and solvent at the mass spectrometer causes release of analyte-containing charged droplets.

In such experiments, the metallic probe was used to remove small amounts of cellular material from the bulk tissue resected during surgery [1,2]. Then the probe was positioned in front of the mass spectrometer. The application of solvent and high voltage generates a strong electric field and results in the emission of analyte-containing charged droplets. The droplets undergo evaporation and coulombic fission by mechanisms similar to those of electrospray ionization. While the features possessed by these metallic probes are advantageous for MS analysis (i.e. easy generation of an electrospray, minimal quantity of sample transferred to the probe tip, low solvent volume and voltage compared to other electrospray-based technique like DESI), they are not suitable for non-invasive procedures [1]. Adaptation of medical swabs as an alternative probe opens the way to new applications, particularly those requiring

harmless and *in-vivo* sampling of biological samples, e.g. oral fluid collected from a patient's oral cavity [3-6]. The use of commercial medical swabs for both sample collection and ion generation has potential for rapid point-of-care testing in clinical and toxicological applications. Here we present proof-of-concept TS experiments with medical swabs for (i) oral detection of bacteria causing strep throat via lipid profiling of pathogenic microorganisms [3], and (ii) qualitative detection of common illicit drugs in oral fluid [4]. For both applications, swabs are already used as sampling devices, but rather than being directly analyzed by MS they are inserted into immunoassay screening devices for on-site testing, as depicted in Figure 2. Then, additional swabs or a more consistent quantity of biofluid (1-2 mL) is collected for the samples providing positive results, and laboratory tests are run for confirmatory purposes (chromatography coupled to mass spectrometry and bacteria culturing, respectively for the two applications listed above), which are more timely and analytically demanding.

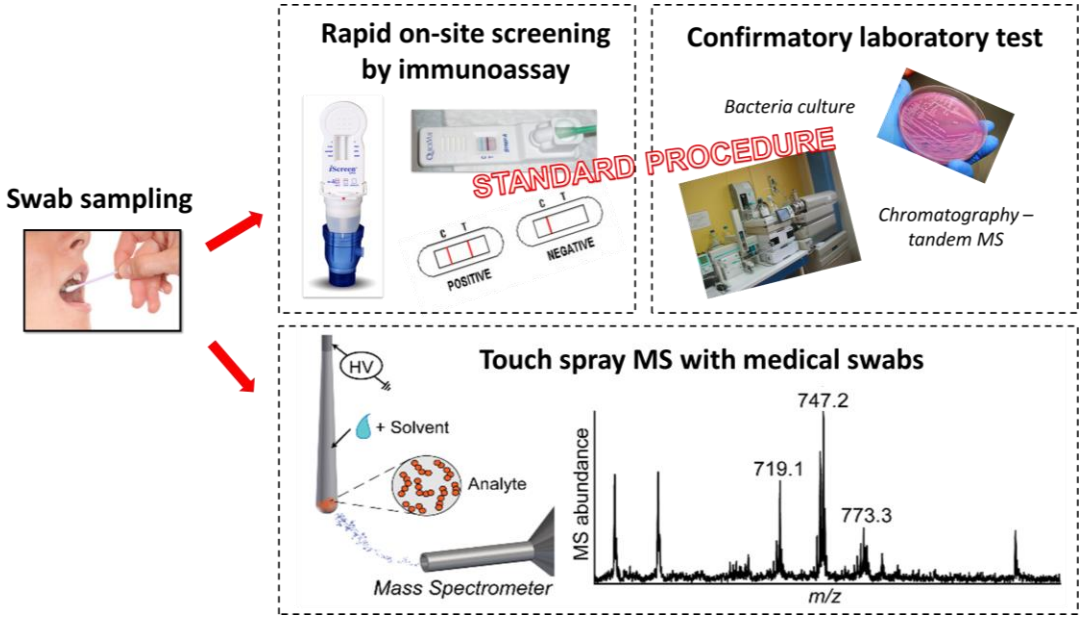

Figure 2. Schematics of a standard dual-step procedure for illicit drug testing and strep throat detection based on rapid immunoassay screening devices and confirmatory lab tests versus an innovative touch spray MS analysis with medical swabs for point-of-care testing.

Aims

- Perform a TS-MS experiment with a metallic teasing probe on *in-vitro* culture of *Streptococcus pyogenes* for the detection of membrane phospholipids. Discuss the results focusing on the identification of the main lipid species using high mass resolution data.
- Perform a TS-MS experiment with a medical swab on *in-vitro* cultured *Streptococcus pyogenes* to determine the pattern of phospholipids unique to this bacteria species and compare the results with those collected previously. Discuss the main analytical differences between the TS-MS experiments run using metallic teasing probes and medical swabs.
- Perform a TS-MS experiment with a medical swab on oral fluid samples spiked with unknown illicit drugs and attempt their identification.

Experimental procedure

1. Introduction to TS-MS and swab TS-MS.
2. Basics of mass spectrometry and exact mass measurements.
3. Perform TS experiments with metallic teasing probes for the identification of bacteria cultured *in-vitro*. Position the probe in front of the MS inlet, apply the high voltage clip to the handle. Apply methanol on the probe tip and start collecting the MS data in full scan. Understand the effects that voltage and solvent have on the formation of an electrospray. Using the instrument software, attempt identification of the main lipid species.
4. Perform TS experiment with medical swabs. Gently touch a colony of *S. pyogenes* with the tip of the swab (Figure 3). Position the swab in front of the MS inlet with the help of a custom-made interface, and apply high voltage clip on the swab metallic handle. Position the silica capillary conveying the solvent in touch with the swab tip. Apply solvent and voltage and wait to visualize the electrospray generation and Taylor cone formation. Understand the effects that voltage, solvent and its flow rate have on the formation of an electrospray. Collect full scan high resolution mass spectra and attempt identification of the main lipid species.

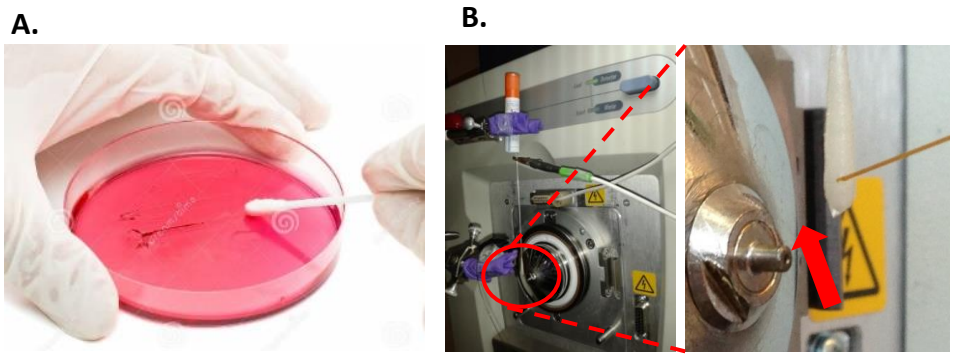

Figure 3. (A) Smearing of the blood agar culture plate with a swab. **(B)** Position of the swab in front of the MS inlet. Application of high voltage and solvent. The zoomed-in figure on the right shows the formation of the Taylor cone from the tip of the swab.

5. Perform TS experiment with medical swabs for the detection of illicit drugs in oral fluid. Analyze a sample of oral fluid spiked with unknown illicit drugs. As done previously, set up the TS experiment and acquire full-scan high resolution mass spectra. Attempt identification of illicit drugs.

Background information

(i) Strep throat is a common illness diagnosed millions of times annually in the United States alone. Bacterial infection caused by *Streptococcus pyogenes* accounts for as much as 30% of all pharyngitis cases. Prompt identification of bacterial infections is necessary to provide proper medical care (antibiotic therapy) especially for children and elderly patients. The remaining fraction of cases is caused primarily by viruses, lacking major risk of disease progression and for which antibiotic therapy is ineffective. Clinical symptoms like a sore throat and fever are not exclusive to bacterial infection, making the development of molecular diagnostic methods of critical importance. Rapid antigen detection tests (RADT) are currently used for point-of-care diagnosis (i.e. doctor's office). The patient's throat is swabbed and then the swab is inserted into the rapid test receptacle. This test is commonly based on lateral flow immunochromatography and provides visual indication of test results similar to pregnancy tests. However, the performances of RADTs are variable and can have a false negative rate of ~10-20%. In clinical practice, a positive RADT supports treatment while a negative result commonly leads to further testing. RADT false negatives contribute to over-prescription of antibiotics in response to the possibility of developing life-threating conditions (*e.g.* chronic rheumatic heart disease). By comparison, the gold standard laboratory technique is throat culture, which minimizes errors in diagnosis but requires 24-48 hours for growth and interpretation of the results, delaying antimicrobial treatment. Mass spectrometry (MS) is one technique which when coupled with ambient ionization allows for easy specimen collection, rapid analysis, and automated data analysis and interpretation. Ambient MS is capable of detecting lipids from complex matrices. Lipids provide cellular structure and contribute to cell functioning. Lipid profiles, unique patterns of lipids and their amount, are characteristic of bacteria genera and species [7], therefore their detection by MS may represent a possible diagnostic method for strep throat.

(ii) Over the past 10 years, oral fluid has progressively gained consideration as a valuable biological matrix for diagnostic purposes. It is well known that oral fluid represents an important alternative to blood because it does not require invasive collection nor complex professional skills to be sampled. Further advantages of oral fluid analysis include minimal risk of contracting infections during sample collection, reduced risk of adulteration, and short detection window, which provides reliable indication of recent drug intake, unlike urine. Therefore, oral fluid analysis is likely to provide a cost-effective approach to the screening of large populations, and a useful tool in several forensic and clinical challenging situations, whenever blood sampling is difficult or impossible, such as in roadside testing, treatment facilities and prisons, and collection from children, handicapped, anxious or chronic pain patients. Workplace drug testing programs also embraced oral fluid as a valuable testing matrix. A major drawback of oral fluid sampling is that an insufficient volume is frequently produced and collected. Therefore, the analytical methods need to be developed with the objective of using a minimal volume of oral fluid, especially when the collected fluid is used for both screening and confirmatory testing or an aliquot is stored for subsequent investigation. The small sample volume available concurrently recalls the need of multi-analyte methods. Confirmation tests need to cover a broad range of drugs and detect low analytes concentration simultaneously, as poly-consumption is an extremely frequent phenomenon [8].

References

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