Comparison of Rapid Identification of *Staphylococcus aureus* and *Streptococcus pyogenes* by the Optidet ® and the Bruker Biotyper® MALDI-TOF MS.

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Abstract

The performance of the Optidet® FTIR spectroscopy (Optidet) and the Bruker Biotyper MALDI-TOF MS (Bruker) were evaluated using 185 *Staphylococcus* and *Streptococcus sp.* isolates. When compared to Bruker, Optidet identified *S. aureus* and *S. pyogenes* with a specificity and sensitivity of 97.6%, 95% and 98.2%, 97.3%, respectively.

For the vast majority of clinical microbiology laboratories the primary methods of identification are based on phenotypic or direct morphological features, including staining, and biochemical profiling. These methods are usually associated with delays of identification results that hamper initiation of appropriate antimicrobial therapy or infection control interventions. In recent years several methods for rapid, accurate and cost effective methodologies for routine identification have been implemented, most notably Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS).

Following this trend, alternative technologies traditionally associated with Biophotonics or analytical chemistry are now being evaluated for direct diagnostic applications ([1]). In particular, the mid Infra-Red Spectroscopy method uses the absorption spectra within the mid infrared range that is directly related to the vibrational states of the biological substance, [(2)]. The Fourier transform infrared (FTIR) spectroscopy applies interferometric modulation of radiation in order to measure multiple wavelengths simultaneously. The measured signal, called an interferogram, is manipulated mathematically (Fourier transformed) for interpretations. The resulting signal contains absorption data as a function of wavelength [(2)]. Absorption is unique for each molecular bond, and is a result of its vibrational spectra. Therefore, similarly to MALDI-TOF MS, bacterial species have a unique signature in the mid infrared spectral range, specifically between 8 to 12μm[(3)]. This method is associated with rapid acquisitions, relative uniformity within a diverse range of experimental conditions and high specificity. The Optidet® technology (www.opticuldiagnostics.com) relies on the vibrational spectroscopy features, enabling identification directly from overnight plate cultures without preparation or reagents. The instrument only requires an inexpensive disposable component to accommodate the optical analysis of the microbial sample.

The goal of the present paper is to compare the performance of vibrational spectroscopy (Optidet) and Mass spectroscopy (Bruker) in the identification of microbial organisms. As a proof of concept, a group of 185 isolates (100 *S. aureus,* 2 *S. epidermidis,* 1 *S. intermedius,* 1 *S. simulans,* 1 *S. saprophyticus* and 75 *Strep. pyogenes,* 3 *Strep. agalactiae,* 2 *Strep. dysgalactiae*) were selected for this study. All samples were retrieved from archived clinical isolate frozen stocks at Albany Medical Center. All *S. aureus* were previously identified by slide and tube coagulase tests (Remel R21051, KS). All *Strep. pyogenes* isolates were previously identified as *Strep.* Group A (Streptex Kit, Remel, KS) and were positive for the pyrrolidonyl arylamidase (PYR) reaction (Hardy Diagnostics, KS). Organism identifications were confirmed by MALDI-TOF using the Biotyper Microflex LT Instrument (Bruker Daltonic, Inc. Billerica, MA). Specifically, overnight cultures at 35°C under 8% CO_2 atmosphere on Trypticase Soy Broth, 5% Sheep Blood Agar plates (BBL BD Diagnostics, Sparks, MD) were tested using the direct smear method and matrix overlay (1µl saturated solution of α-cyano-4-hydroxycinnamic acid, CHCA in 50% acetonitrile and 2.5% trifluoroacetic acid) using MSP 96 target plates (Bruker Daltonics, Inc. Billerica, MA). Mass spectra acquisition in a linear positive ion mode was performed using default settings for the instrument. Acquisition across 2,000 to 20,000 m/z was performed at a laser pulse frequency of 60Hz with a total accumulation of 240 laser shots per spot, and calibration was performed using the bacterial test standard solution. Automated analysis of the samples was performed using the MALDI-Biotyper software 3.1 against the Biotyper database (DB 5627) all isolates with a score ≥2.0 were considered definitively identified to the species level. Scores ≥ 1.7 were considered genus level identification [(4)].

All isolates were submitted blindly for subculture and identification using the Optidet system (Opticul Diagnostics, Rockville, MD). Colonies were collected using a sterile cotton

swab (Copan 150C, Copan, Italy), smeared on the disposable that was placed on its removable holder and inserted into the stationary unit for FTIR spectroscopy. Raw vibrational spectra capture was analyzed in an automated mode. The Optidet identification algorithm is composed of feature extraction from the measured raw vibrational spectra, and creation of compact feature vectors that represent the raw vibrational spectra. These vectors were analyzed using a continuous probabilistic framework[5]. The probability of the feature vector to represent either Strep (*Streptococcus pyogenes*), Staph (*Staphylococcus aureus*) or Other (if neither Strep nor Staph were identified) is calculated based on which a classification decision is made. The overall performance was estimated using a leave-one-out method which is a well-established method for estimating classifiers performance [6].

Tables 1 and 2 present the detection results for *S. aureus* and *Strep. pyogenes*, respectively. The results of the Optidet unit were highly concordant with the MALDI-TOF MS identification results of the whole 185 isolate set.

Table 1: Comparison of results between the Optidet and Bruker for identification of *S. aureus*.

	Optidet Results	
Bruker Results	*S. aureus*	Other
S. aureus	95	5
Other	2	83

Table 2: Comparison of results between the Optidet and Bruker for identification of *Strep. Pyogenes.*

	Optidet Results	
Bruker Results	*S. pyogenes*	Other

S. pyogenes	73	2
Other	2	108

Rapid and reliable species identification of bacteria and yeast improves time to optimal therapy and decreases hospital length of stay and total costs reduction [(7)]. When compared to Bruker, the Optidet specificity and sensitivity was 97.6%, 95% and 98.2%, 97.3% respectively. These results corroborate previous comparisons of these two technologies as reported by Wang et. al [(8)]. Specifically, on the differentiation of the closely related crop pathogens *Acidovorax oryzae* and *Acidovorax citrulli.*

This comparative study shows promising value of FTIR spectroscopy as an alternative method for routine identification of *S. aureus* and *Strep. pyogenes.* Over 95% of all the results were concordant. 5 *S. aureus* and 2 *Strep. pyogenes* were missed by the Optidet system as their detection scores were lower than the pre-determined threshold, whereas one isolate of *S. saphrophyticus* and *S. epidermidis* were miss-identified as *S. aureus,* as they had similar signal features as *S. aureus,* and consequently their detection scores were higher than the threshold.

Two *Strep. dysgalactiae* were miss-identified as *Strep. pyogenes.* The differentiation between *Strep. dysgalactiae/ Strep. pyogenes* is difficult; in fact Bruker issues a warning of possible miss-identification of these species on their RUO/IVR database. However, a positive PYR reaction, a rapid test common in most clinical microbiology laboratories, will definitely allow the distinction between these two species [(9)]. This should not be a major limitation for routine testing in clinical microbiology laboratories. On the other hand, the misclassification of *S. saprophyticus* and *S. epidermidis* is problematic and might be associated with important consequences on clinical management. These errors are due to not as of yet, fully optimized

classifier parameters. It should be noted that this paper presents only preliminary results with a relatively small database. As with any machine learning system, the performance is highly dependent on an appropriate dataset and system optimization. Using a larger pool of training samples and optimizing the algorithm parameters will eliminate such mis-classification errors.

The cost of the Optidet instrumentation is an order of magnitude less than the MALDI-TOF. It is associated with lower services cost and for low volume laboratories will have a lower price-per sample tested as well. It is also noteworthy that thermal source used for FTIR is easy to replace and its usage expectations are well beyond conventional Nitrogen laser source used for commercial MALDI-TOF instrumentation.

An important feature of Optidet is that identification requires less than a minute time-to-results. This compares favorably to Bruker that in our experience might require up to 10 minutes of processing of a single sample. However, for higher identification volumes the Bruker approach will be faster and cost effective. The current RUO databases of the MALDI-TOF includes over 2000 bacterial and fungal species, and the Optidet currently covers only 12 bacterial species [(10)]. However, as a profiling method the Optidet has no theoretical limit on the number of database entries that could be generated [(3)]. Ongoing studies are demonstrating the applicability of this technology to a diverse range of clinically relevant strains.

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