Rapid Identification and Classification of *Enterococcus* and *Staphylococcus* by Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy

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SUGGESTED SHORT TITLE

Rapid Identification and Classification of Enterococcus and Staphylococcus by ATR-FTIR Spectroscopy

For my family

Abstract

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) is a proposed method for in-depth identification and discrimination of the bacterial microorganisms. Comparing this method with other clinically available identification methods such as polymerase chain reaction (PCR), pulsed field gel electrophoresis (PFGE) and automated systems including the Vitek II identification system, ATR-FTIR requires little to no sample preparation, rapid acquisition times, no reagent needed, cost effective, and identification can be performed at various taxonomic levels ranging all the way down to the pathotype level. *Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus* and coagulase negative *Staphylococci* (CoNS) are some of the most prevalent pathogens causing both nosocomial and lethal hospital-acquired infections. ATR-FTIR was the method chosen for the research due to its incredible specificity to overcome the challenge which arises when differentiating between *Enterococcus* and *Staphylococcus* species due to their high degree of genetic similarity. A total of 837 isolates, composed of *Enterococcus faecalis* (n=142), *Enterococcus faecium* (n=200), *Staphylococcus aureus* (n=392) and Coagulase-negative *Staphylococcus* (n=103) of fecal and blood origin were obtained from the McGill University Health Center (MUHC), Laboratoire de Santé Publique du Québec (LSPQ), Health Canada (HC), Centre Hospitalier Universitaire Sainte-Justine, New Brunswick (NB) and Nova Scotia (NS). Each bacterial culture analyzed was initially frozen in a 40-50% glycerol solution at -80°C and was then always plated on Oxoid 5% Columbia blood agar and incubated at 37°C for 18-24 h. A sub-culture was taken once the initial incubation time was completed and a second incubation period with identical parameters followed. Sub-culturing was done for the purpose of isolating pure bacterial colonies for analysis. Once the sub-culturing incubation time was complete, a single bacterial colony was collected and smeared onto the ATR crystal of the ATR-FTIR instrument to acquire a spectrum. Each bacterial strain was collected in triplicates and the spectral data was then analyzed by using principal component analysis (PCA) and hierarchical cluster analysis (HCA) of the ATR-FTIR spectral data. *Enterococcus* was successfully discriminated from *Staphylococcus* based on their whole region of selection 1480-980 cm^{-1} and more specifically the regions 1600-1160 cm^{-1} and 1064-985 cm^{-1}. Furthermore, *E. faecalis* was successfully discriminated from *E. faecium* by using the precise spectral regions of 1039-1054 cm^{-1} and 1061-1099 cm^{-1}. Finally, *Staphylococcus aureus* and Coagulase-negative *Staphylococcus* were successfully separated using the region of 1057-1072 cm^{-1}. These results

suggest that ATR-FTIR spectroscopy can be used as a valuable tool for rapidly identifying and discriminating the nosocomial bacteria *Enterococcus* and *Staphylococcus* at the species and sub-species level.

Résumé

La spectroscopie infrarouge à transformée de Fourier par réflectance totale atténuée (ATR-FTIR) est une méthode suggérée pour l'identification et la discrimination approfondi des micro-organismes bactériennes. En comparant cette méthode a d'autre méthode d'identification cliniquement disponible telles que la réaction en chaîne par polymérase (RCP), l'électrophorèse sur gel en champ pulsé (EPCP) et les systèmes automatisés incluant le système d'identification Vitek II, la spectroscopie ATR-FTIR est rapide en termes de temps d'analyse, les échantillons n'ont pas besoin beaucoup de préparations, pas besoin de réactifs, rentable et l'identification a la capacité d'identifier des microorganismes jusqu'au niveau du sous-espèce. *Entérocoques faecalis, Entérocoques faecium, Staphylocoque aureus* et *Staphylocoque* à coagulasse négative (CNS) sont certains des pathogènes les plus répandus qui causent des infections nosocomiales et des infections contractées à l'hôpital. ATR-FTIR a été la méthode choisit pour cette recherche à cause de sa spécificité incroyable, capable de surmonter le défi de différencier les espèces *Entérocoques* et *Staphylocoque* qui ont un haut degré de similarité génétique. Un total de 837 souches, composé d'*Entérocoque faecalis* (n=142), *Entérocoque faecium* (n=200), *Staphylocoque aureus* (n=392) et *Staphylocoque* à coagulasse négative (n=103) d'origine fécal et sanguin ont été obtenu du Centre Hospitalier de l'Université de McGill (CHUM), Laboratoire de Santé Publique du Québec (LSPQ), Santé Canada (SC), Centre Hospitalier Universitaire Sainte-Justine, (CHU Sainte-Justine), Nouveau-Brunswick (NB) et la Nouvelle-Écosse (NE).
Tous les échantillons sont prélevés des cultures congelées dans une solution de 40-50% de glycérol à -80°C et ont été toujours étalés sur des milieux à base Oxoid de 5% de sang Columbia et ont été incubés à 37°C pendant 18 à 24 heures. Après la période d'incubation, les échantillons ont été sous-cultivés et incubé une autre fois à 37°C pendant 18 à 24 heures. La sous-culture des échantillons est faite pour isoler des colonies bactériennes pures pour l'analyse. Une seule colonie isolée de l'échantillon a été prise et étalée sur le cristal ATR du spectromètre ATR-FTIR pour générer un spectre. Chaque échantillons a été recueilli en trois exemplaires et ensuite l'analyse en composantes principales (PCA) et l'analyse de groupement hiérarchique (HCA) ont été performés pour analyser les données spectrales. Les *Entérocoques* ont été différencié avec succès des espèces de *Staphylocoques* basé sur leurs différences spectrales en utilisant toutes les régions de sélection 1480-980 cm^{-1} et plus précisément dans les régions 1600-1160 cm^{-1} et 1064-

985 cm^{-1}. En outre, l'*Entérocoque faecalis* a été différencié avec succès des espèces *d'Entérocoque faecium* en utilisant les régions spectrales spécifique 1039-1054 cm^{-1} and 1061-1099 cm^{-1}. Enfin, *Staphylocoque aureus* et *Staphylocoque* à coagulasse négative ont été séparé avec succès en utilisant la région 1057-1072 cm^{-1}. Ces résultats suggèrent que la spectroscopie ATR-FTIR peut être utilisée comme un outil d'identification précieux pour rapidement identifier et différencier les bactéries nosocomiales *Entérocoques* et *Staphylocoques* au niveau du genre, de l'espèce et de la sous-espèce.

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LIST OF ABBREVIATIONS

Agg	Aggregation substance
ATR-FTIR	Attenuated total reflectance – Fourier transform infrared
BA	Blood agar
CDC	Center for Disease Control and Prevention
CoNS	Coagulase-negative *Staphylococcus*
dNTPs	Deoxyribonucleoside triphosphates
E. coli	*Escherichia coli*
E. faecalis	*Enterococcus faecalis*
E. faecium	*Enterococcus faecium*
G+C	Guanine and Cytosine
GP	Gram-positive
HAI	Hospital-acquired infection
HC	Health Canada
HCA	Hierarchical cluster analysis
LAB	Lactic acid bacteria
LSPQ	Laboratoire de Santé Publique du Québec
MALDI-TOF	Matrix assisted laser desorption ionization – time of flight
MLEE	Multilocus enzyme electrophoresis
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin sensitive *Staphylococcus aureus*
MUHC	McGill University Health Center
MurNAc-GlcNAc	*N*-acetylmuramic acid-(β1-4)-*N*-acetylglucosamine
NB	New Brunswick
NS	Nova Scotia
PBPs	Penicillin binding proteins
PC	Principal component
PCA	Principal component analysis
PCR	Polymerase chain reaction
PFGE	Pulse-field gel electrophoresis
VRE	Vancomycin-resistant *Enterococcus*
VSE	Vancomycin sensitive *Enterococcus*
WGS	Whole genome sequencing

1.1 Introduction

The purpose of the research presented in this thesis is to develop a protocol and evaluate ATR-FTIR as a tool for the identification and discrimination of *Enterococcus* and *Staphylococcus* strains at the species level. *Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus* and coagulase negative staphylococci (CoNS) are some of the most prevalent pathogens causing both nosocomial and lethal hospital-acquired infections. The estimated incidence rate in the United States of America was estimated at 4.5%, corresponding to 9.3 infection per 1000 patient-days which bears an annual financial loss of approximately US$ 6.5 billion (World Health Organisation, 2002). With the emergence of vancomycin-resistant enterococci (VRE) and methicillin-resistant staphylococci (MRSA) as worldwide nosocomial pathogens, rapid identification methods for enterococci and staphylococci strains are needed for proper and immediate treatment. The rapid and accurate discrimination between strains would allow clinicians to implement selective treatment in order to decrease diagnostic time and reduce the risk of over-prescribing antibiotics.

Multiple surveillance programs exist in the vast majority of clinical laboratories, yet no single rapid definitive diagnostic system exists for the identification of both staphylococci and enterococci strains. The characterization and discrimination of these bacterial strains in most public health laboratories are still done using phenotypic methods (phage typing and antimicrobial susceptibility panels) as well as genotypic methods (pulse-field gel electrophoresis (PFGE) and polymerase chain reaction (PCR) – based). The optimal method to replace current clinical procedures to rapidly identify and discriminate between enterococcal and staphylococcal strains would require minimal sample handling, produce rapid results within seconds, direct and reagent free analysis, capable of being automated and inexpensive.

Many types of spectroscopy-based methods have been developed since the emergence of sensitive, rapid and increasingly precise microbiological techniques in the 1980's. The most commonly used spectroscopic techniques include mass spectrometry (MS), Raman spectroscopy, proton magnetic resonance spectroscopy and Fourier transform infrared (FTIR) spectroscopy. The use of FTIR spectroscopy was once viewed as time consuming and impractical. (Norris, 1959) The idea of using FTIR spectroscopy for bacterial identification was then revived with the

development of modern interferometric IR spectroscopy, the availability of low-cost mini-computers, and powerful new algorithms of multivariate statistical analysis (MSA) and pattern recognition methodologies such as hierarchical cluster analysis (HCA). Infrared spectroscopy provides a great amount of information about whole organisms because the absorption intensities yield quantitative information while the absorption frequencies yield qualitative information about molecular structure, interaction to environment and growth medium. The infrared spectrum of bacterial cells is a simultaneous combination of infrared spectra of each bacterial cell component (cell wall, cytoplasmic membrane, nucleic acids and proteins). (Nelson, 1991).

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy as a tool for rapid bacterial identification, can be used in clinical laboratories due to adequate sensitivity, speed, cost effectiveness and reproducibility and its easy-to-use capabilities. In 2010, Davis et al. have shown the preliminary work of identifying bacteria using different types of IR techniques such as ATR, transmittance and diffuse reflectance. ATR-FTIR spectroscopy is believed to be able to identify and discriminate the complex infrared bacterial spectra when coupled with the use of chemometrics. Chemometrics can be described as a multivariate statistical and mathematical tool which correlates structural information to bacterial composition by interpreting molecular structural information of the complex spectra (Burgula *et al*., 2007). Differentiating bacterial strains on the basis of their infrared spectra, the subtle differences are revealed by detailed examination, forward search algorithms highlighting specific spectral regions and chemometrics. Chemometrics is often used to pre-process data with the aim to: (i) reduce the amount of data by eliminating that which are irrelevant to the research project, (ii) to keep or optimize enough information within the data to achieve the goal of the project and (iii) to extract the information in, or transform the data into, a form suitable for further analysis (Adams, 2007). Principal component analysis and hierarchal cluster analysis (HCA) will be further applied to the analysis of the infrared spectra to generate organized and comprehensible results. The overall objective of this thesis is to evaluate the potential of employing ATR-FTIR spectroscopy for identification of *Enterococcus* and *Staphylococcus* both at the genus and the species level. The research includes the differentiation between Enterococcal species *E. faecium* and *E. faecalis* and the differentiation between Staphylococcal species *S. aureus* and various coagulase-negative staphylococcus.

Chapter 2 – LITERATURE REVIEW

2.1 Introduction to *Enterococcus faecalis* and *Enterococcus faecium*

Bacteria considered to be part of the genus *Enterococcus*, are Gram-positive cocci that are spherical or ovoid and are lactic acid bacteria (LAB). They vary in length from 0.6 – 2.0 µm and their width vary from 0.6 – 2.5 µm. They characteristically form short chains and cluster together. The major component of the enterococcal cell wall is peptidoglycan. Peptidoglycan consists of the repeating disaccharide *N*-acetylmuramic acid-(β1-4)-*N*-acetylglucosamine (MurNAc-GlcNAc). Peptidoglycan layers of gram positive bacteria differ from one another by their variation in amino acid sequence that forms the interpeptide bridge. The most common interpeptide bridge for the enterococci genus is a single D-Asp residue. *E. faecalis* is one of the few exceptions, its interpeptide bridge is composed of 2-3 L-Ala residue (Hancock *et al*., 2014). Bacteria of the genus *Enterococcus* are chemoorganoheterotrophes that rely on carbohydrates for energy and therefore, produce lactate (Liese *et al*., 2016). They are facultative anaerobes, capable of generating energy through fermentative pathways and by respiration when provided with hemin under aerobic conditions. Enterococcus bacteria are catalase-negative, PYR-positive and LAP-positive (Manero *et al*., 1999). The enterococci specie can be cultured on TSA agar, blood agar 5%, chocolate agar and nutrient agar. Culture plates should be incubated at 37°C for 18-24 hours.

Classification of *Enterococcus* for routine applications is done by the use of simple and rapid phenotypic techniques. To acquire a more in-depth characterization of enterococci, other techniques exist such as; standardised sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), multilocus enzyme electrophoresis (MLEE), antimicrobial susceptibility testing, serotyping, pyrolysis mass spectrometry and vibrational spectroscopy. With the emergence of antibiotic resistant enterococci and enterococci strains deviating from their phenotypical properties, genotypic methods are used to characterize and identify these types of enterococci. Polymerase chain reaction (PCR)-based typing is the “gold standard” of genotypic methods in major hospitals around the world. Many PCR-based tests exist such as; random amplified polymorphic DNA, amplified fragment length polymorphism, specific and random amplification and pulsed-field gel electrophoresis (PFGE) (Domig *et al*., 2003).

Many factors determine the virulence of Enterococcus species: (i) ability to colonize the gastrointestinal tract, which is the normal habitat; (2) ability to adhere to a range of extracellular matrix proteins, including thrombospondin, lactoferrin and vitronectin; and (3) ability to adhere to urinary tract epithelia, oral cavity epithelia and human embryo kidney cells (Fisher et al., 2009). Enterococcal-associated nosocomial infections are primarily due to *E. faecalis* (80-90%) and secondly *E. faecium* (10-15%). An aggregation substance (Agg) has been found on the surface of *E. faecalis*, Agg is a pheromone-inducible surface glycoprotein and mediates aggregate formation during conjugation, thus aiding in plasmid transfer as well as adhesion to an array of eukaryotic surfaces (Koch et al., 2004). Hällgren et al, demonstrated that the gene encoding Agg was not present in *E. faecium* clinical isolates through Pulsed-field gel electrophoresis analysis (Hällgren et al., 2008). Another cell-surface protein only presented in *E. faecalis* is Ace (adhesion of collagen from *E. faecalis*). This is a microbial surface component recognizing adhesive matrix molecules (MSCRAMM) and is said to play a crucial role in the pathogenesis of endocarditis (Koch et al., 2004). Another virulence factor, Hemagglutinin, which contributes to bacterial attachment to host cells, was found in a research conducted by Elsner et al., to be a part of 97% of their clinical *E. faecalis* strains and 0% in their clinical *E. faecium* strains (Elsner et al., 2000). Compiling all the information from these studies, one can conclude *E. faecalis* is more pathogenic than *E. faecium* due to its extra virulent factors.

Enterococci are predominantly found in the gastrointestinal tract of humans, animals, reptiles and insects. Enterococci species are present in natural environments (i.e. soil, air and water bodies), dairy and fermented foods. Enterococci are the 3rd most common nosocomial pathogen to cause 14% of all hospital acquire infections (HAI) (Weiner *et al*., 2016). Enterococcal-associated nosocomial infections are primarily due to *E. faecalis* (80-90%) and secondly *E. faecium* (10-15%). Enterococci cause device-associated infections in the intensive care unit which includes, surgical site infections, ventilator associated pneumonia, catheter associated urinary tract infection and central line associated bloodstream infections. The device-associated infections can lead to infective endocarditis, bacteremia, peritonitis, endophthalmitis, urinary tract infection and prosthetic joint infection (Weiner *et al*., 2016; Garsin *et al*., 2014).

The emergence of antibiotic-resistant strains induces a great concern when treating an *Enterococcus* infection. The mechanism of Vancomycin resistance is well known and there

exists nine Vancomycin resistant operons; vanA, vanB, vanC, vanD, vanE, vanG, vanL, vanM. The resistance gene vanA is the most predominant worldwide and has a high degree of vancomycin and teicoplanin resistance (Kafil *et al*., 2014). The cell wall composition of the antibiotic resistant bacteria is altered from the peptidoglycan precursor D-Ala-D-Ala (vancomycin-susceptible) to D-Ala-D-lactate (vancomycin-resistant). The D-Ala-D-lactate precursor has 1000 times less affinity for vancomycin, thus removing the susceptible target. The vanA resistance operon is acquired through the Tn1546 transposon (Gilmore *et al*., 2002). Many Vancomycin-resistant *Enterococcus* (VRE) show resistance to penicillin, as well as high-level resistance to aminoglycosides. Previous therapeutic options described linezolid, quinupristin/dalfopristin, and daptomycin to combat the infection (Gilmore *et al*., 2014). Resistance is emerging to the latter antibiotics mentioned and therefore, rapid identification methods for the type of antibiotic resistance is required in order to decrease the rate of over-prescription of antibiotics by clinicians and decrease the time of diagnosis.

2.2 *Staphylococcus aureus*

The genus *Staphylococcus* belongs to the bacterial family *Staphylococcaceae*, which also includes *Gamella*, *Macrococcus* and *Salinicoccus*. *Staphylococcus* bacteria are gram positive spherical bacteria (cocci), 1 µm in diameter, and typically form microscopic clusters resembling grapes. They have a unique cell wall peptidoglycan layer composed of murein, teichoic acids, and wall-associated surface proteins. It is a distinctive feature of staphylococci that the observed degree of murein cross-linking, which was determined as a ratio of bridged peptides to the total amount of all peptide ends in general, is extremely high, on the order of 80 to 90% (Dimitriev *et al*., 2004). *Staphylococci* are facultative anaerobes capable of growing by aerobic respiration or by fermentation that yields lactic acid. *S. aureus* are catalase- positive, and usually oxidase-negative. Characteristically *S. aureus* strains are tolerant to NaCl concentrations as high as 15% w/v and can grow at a temperature range of 15-45°C. The G + C content of *S. aureus* is low for gram-positive bacteria, some gram-positive bacteria such as *Micrococcus luteus* can have a mean G + C content as high as 75%, *S. aureus* has a mean G + C content of 32.70% (Wang *et al*., 1986).

Traditionally the method of choice despite the several limitations associated with it, phage typing was the method of choice for the classification of *S. aureus*. As PFGE was developed in the mid 1980's it quickly replaced phage typing as the classification method of *S. aureus*. DNA-DNA hybridization, as for PFGE, provides genomes level comparisons at the chromosomal level. Since *S. aureus* has low G + C content, it favors the use of restriction enzymes that recognize GC-rich sequences and *Sma*I is the enzyme of choice. Since 1998, MLST has been the gold standard for the classification of *S. aureus* and the genes selected include *arc* (carbamate kinase), *aroE* (shikimate 5-dehydrognase), *glpF* (glycerol kinase), *gmk* (guanylate kinase), *pta* phosphate acetyltransferase), *tpi* (triose phosphate isomerase) *and yqiL* (acetyl-CoA acetyltransferase) (Crossley *et al*., 2009).

Members of the genus *Staphylococcus* are common colonizers of the skin in mammals and birds (Kloos, 1986). They can also be isolated from environmental sources such as air, soil and water bodies. The *Staphylococci* specie is the 2nd most common nosocomial pathogen to cause 11.8% of all hospital acquire infections (HAI) (Weiner *et al*., 2016). *S. aureus* has the greatest pathogenic potential and diversity due to its multiple virulence factors. (1) Surface proteins that promote colonization of host tissues. (2) Factors that probably inhibit phagocytosis (capsule, immunoglobulin binding protein A). (3) Toxins that damage host tissues and cause disease symptoms (Foster, 1996). *S aureus* is notorious for causing boils, furuncles, styes, impetigo and other superficial skin infections in humans. Nosocomial *S. aureus* acquired infections include pneumonia, deep abscesses, osteomyelitis, endocarditis, phlebitis, mastitis and meningitis. *Staphylococci* is divided into two groups based on its ability to clot blood, coagulase-positive and coagulase-negative (CoNS). CNS is also associated with nosocomial infections associated with indwelling medical devices which means that isolation of these bacteria from blood is likely to be important. It is important to differentiate *S. aureus* and CoNS in a clinical setting for diagnostic purposes.

Staphylococcus aureus has had the emergence of resistance to antibiotics due to its high adaptability to antimicrobial drugs. Ever since the first use of penicillin, *S aureus* has shown a remarkable ability to adapt and resistance has developed to new drugs within a short time of their introduction by a variety of genetic mechanisms including (1) acquisition of extrachromosomal

plasmids or additional genetic information in the chromosome via transposons or other types of DNA insertion and (2) by mutations in chromosomal genes (Foster, 1996). One of the most common pathogenic antibiotic resistant strain is methicillin-resistant *Staphylococcus aureus* (MRSA). Methicillin, a β-lactam antibiotic, typically functions by inhibiting penicillin-binding proteins (PBPs) involved in peptidoglycan synthesis, which is an essential polymer surrounding the cell (Stapleton *et al*., 2002).

The key factor that distinguishes MRSA from methicillin-sensitive *Staphylococcus aureus* (MSSA) is the the presence of the *mecA* gene. During a six year period the estimated number of *S. aureus*–related hospitalizations increased 62%, from 294,570 to 477,927, and the estimated number of MRSA-related hospitalizations more than doubled, from 127,036 to 278,203. The findings suggest that *S. aureus* and MRSA should be considered a national priority for disease control and there is a need for rapid identification (Klein *et al*., 2007).

2.3 Current Clinical Methods of *Enterococcus* and *Staphylococcus* Detection

2.3.1 Phenotypic Methods

Many well-known phenotypic methods exist and have been used for the detection of nosocomial bacteria. Phenotypic identification analyzes the biochemical profile and metabolic properties of a microorganism by measuring the growth requirements and enzymatic activities (Canadian Food Agency, 2017). Well-known phenotypic methods include antimicrobial susceptibility testing, bacteriophage typing, serotyping, multilocus enzyme electrophoresis (MLEE), the modified Hodge test and the Boronic acid inhibition test. Bacteriophage typing and serotyping are the two most widely used phenotypic methods. These methods have been useful for the understanding of pathogens; however, these methods have drawbacks that limit their ability to highly discriminate microorganisms.

2.3.2 Pulse-Field Gel Electrophoresis (PFGE)

Pulsed-field gel electrophoresis (PFGE) is a laboratory technique which produces a DNA fingerprint of bacterial isolates. Conventional electrophoresis can effectively separate DNA fragments up to ~20 kb, however, larger fragments will migrate together and appear as one large band at the top of the gel when imaged. In 1984, PFGE was developed to overcome this problem (Klotz *et al*., 1972; Schwartz *et al*., 1984). The bacterial isolates are mixed with melted agarose are poured into a mold ("plugs"), and the cells are then typically lysed with a buffer in order to release the DNA in the agarose plugs. Once the DNA has been released, the DNA is cut by the use of a restriction enzyme (e.g. *SmaI*) at restriction sites. The plugs are then loaded into a gel and the DNA is then resolved by alternating the electrical field between spatially distinct pairs of electrodes. On the contrary of conventional electrophoresis, PFGE results in the separation of DNA fragments of up to ~10 Mb by their reorientation and movement at different speeds through the pores of an agarose gel.

PFGE's high concordance with epidemiological relatedness and its stable and reproducible DNA restriction patterns are some of the biggest advantages of this method. PFGE can also be applied as a universal generic subtyping method for many different bacteria with only the choice of the restriction enzyme and electrophoresis conditions optimized for each species. PFGE has more discriminating power for many bacteria compared to other methods such as ribotyping and multi-locus sequence typing. PFGE does have its limitations, it is time consuming and it can't optimize separation in every part of the gel at the same time. DNA restriction patterns can vary slightly between technicians and the change in one restriction site can lead to a change in more than one band. The greatest limitation of PFGE is that it doesn't differentiate isolates to the same degree that can be achieved by whole genome sequencing (WGS) and "relatedness" should be used as a guide and not as a true phylogenetic measure (Singh *et al*., 2006; Kam *et al*., 2017)

2.3.3 Polymerase Chain Reaction (PCR)

Despite the gene similarity between Enterococcus and Staphylococcus, polymerase chain reaction can separate both bacteria. Dr. Kary Mullis at the Cetus Corporation first invented PCR in the 1980s and was awarded the Nobel Prize in Chemistry in 1993 (Mullis and Faloona 1987). PCR is a method for amplifying (copying) small amounts of DNA or RNA which can efficiently and rapidly produce large amounts of a specific DNA or RNA sequence. A PCR reaction mixture contains a double stranded target DNA template, DNA oligonucleotides that can form base pairs at the ends of the desired sequence and serve as primers for DNA synthesis usually 20-30 nucleotides in length, heat-resistant DNA polymerase, and the four deoxyribonucleoside triphosphates (dNTPs). All the reagents and target bacterial DNA sample are mixed together into a vial. The first step is to heat the reaction mixture to a high temperature, approximately 95°C, to break the hydrogen bonds holding the DNA strands together, thereby separating them. The mixture is then cooled to allow the primers to anneal to the denatured DNA at the 3 prime ends of both separated strands. The temperature is once again increased to facilitate the double stranded DNA synthesis by the DNA polymerase enzyme which uses the dNTPs and the denatured DNA as the template, and the oligonucleotides as the primers. These steps are repeated cyclically, resulting in exponential amplification for better analysis (Julin, 2014).

In a reported study completed by Depardieu et al. in 2004, a multiplex PCR assay was developed for detection of the six types of glycopeptide resistance characterized in enterococci and for identification of *Enterococcus faecium*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* at the species level. For this study, primers were developed to target the *vanA, vanB, vanC, vanD, vanE, vanG,* and *ddl* of *E. faecium* and *E. faecalis* and *nuc* of *S. aureus* and a chromosomal portion specific to *S. epidermidis*. The DNA of 19 phenotypically and genotypically well-characterized strains including glycopeptide-susceptible controls as a template were used in this study to confirm the specificity of the PCR primers. Small amounts of DNA were subjected to multiplex PCR amplification in a reaction mixture containing PCR buffer, each of the four dNTP's, the 10 primer pairs developed, and *Taq* polymerase. There were no discrepancies between the results obtained by multiplex PCR and the previously characterized genotypes for resistance and identification. While this PCR

method proved to be robust, sensitive, specific, and fast PCR requires multiple reagents, development of primers specific for each gene of interest for amplification, and one of the limitations proposed, could be the sequencing variability among *van* genes have occasionally been observed (Depardieu et al. in 2004).

2.3.4 Vitek-2 Identification

In 2005, a French company by the name of Biomerieux launched its new technological invention, the Vitek II. The Vitek II is an automated microbiology system utilizing growth-based technology. It is ability to identify gram positive bacteria, gram negative bacteria, yeasts and spore-forming bacilli. Isolates cultured from patients are placed in a sodium chloride solution and linked to a Vitek II ID card via a transfer tube. Each identification card contains 43 wells each containing unique biochemical tests measuring either: carbon source utilization, enzymatic activities and resistance. Once placed inside the Vitek II, the isolate/sodium chloride solution is pumped into the 43 wells and the cards are incubated 35.5 ± 1°C. The reaction wells are then optically read every 15 minutes for a maximum of 8 hours in order to measure the turbidity level or formation of colored substrate metabolism products. The wells are read using a transmittance optical system which allows the interpretation of test reactions using different wavelengths in the visible spectrum. Once each test of each well is completed, usually 6-12 hours, the data is then collected and a report is created with the probable identity of the unknown bacterial strain (Pincus *et al*., 2006). In some cases, the Vitek II offers a variety of possible strains and lists the necessary serological tests needed to be done to identify the unknown bacteria.

2.4 Introduction to ATR-FTIR

2.4.1 A Brief History of Fourier Transform Infrared Spectroscopy

The recent emergence of sensitive, rapid, and increasingly precise physical techniques for microbiological analysis. These new techniques range from mass spectroscopy, molecular spectroscopy [including fluorescence, Fourier-transform infrared, and Raman spectroscopy], the application of laser technologies, and flow cytometry to different separation techniques such as gas chromatography and high-performance liquid chromatography (Nelson *et al*., 1991). In 1911 W.W. Coblentz was the first scientist to suggest that biological material can profitably be analyzed by IR spectroscopy and this led to the use of IR spectroscopy as a means of

differentiating and identifying bacteria in the 1950s and 1960s (Norris *et al*., 1959; Riddle *et al*., 1956). In 1959, a critical review on this subject was published, it summarized that although bacteria exhibit unique IR spectra for each individual strain, the identification of bacteria by IR spectroscopy was impractical because it was too time consuming and the procedures were speculative (Norris *et al*., 1959). Reports on IR applications to microorganisms became less frequent in the 1960's and had virtually ended in the mid 1970's due to the limitations on sensitivity, reproducibility and measurement time of the IR spectrometers. The development of modern interferometric IR spectroscopy, the availability of low-cost mini-computers, and the development of chemometric techniques revived the interest in this technique in the 1990's (Nelson *et al*., 1991).

2.4.2 ATR-FTIR Spectrometer

The ATR-FTIR process begins by the spectrometer emitting an infrared beam form the light source passing it through an interferometer before hitting the sample. The Michelson interferometer, consisting of two perpendicular mounted mirrors, causes interference, by using a semi-reflecting film called a beam splitter, splitting a beam of light into two parts (Burgula *et al*., 2007). Both parts of the light travel different paths and are then brought together to interfere with each other. The beam splitter is made up of potassium bromide to allow all the IR beam through and is coated with germanium to protect it. The greatest advantage of using an interferometer, the Felgett advantage, is that the detector is able to observe all frequencies simultaneously; therefore, the time taken for one complete scan is completed in less than a second (Burgula *et al*., 2007). The IR beam is then directed onto the internal reflection element (IRE), an optically dense crystal, which internally reflects the beam. The internally reflected beam creates an evanescent wave that protrudes (0.5 – 5.0 μ) into the sample placed in contact with the IRE. The sample absorbs energy and the evanescent wave is attenuated, the attenuated energy from each evanescent wave is redirected into the IR beam and the beam then exists the IRE and is passed on to the detector (Elmer, 2005). The detector then measures the interferogram signal and digitizes it. The digitized signal is then sent to a computer which generates an IR spectrum of the sample by the use of a fast Fourier transform algorithm. (Figure 2.1).

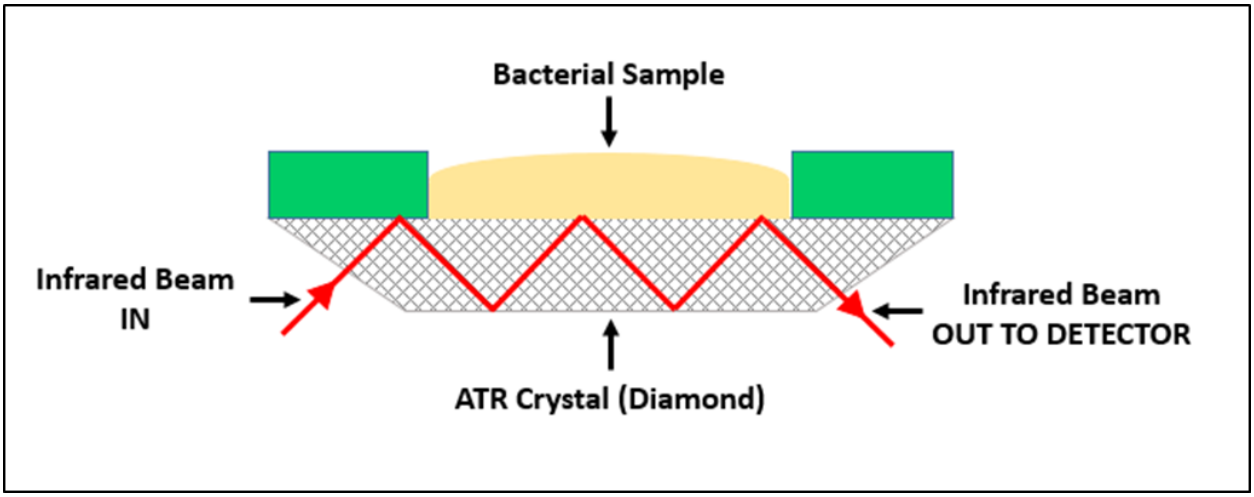

Figure 2.1. Basic principle of ATR-FTIR spectroscopy. Internal reflection of the infrared beam interacts with the bacterial sample at the surface of the ATR crystal.

2.4.3 Principles of IR Spectroscopy

The infrared region of the electromagnetic spectrum ranges from 14000-10 cm^{-1} and is divided into three regions: far IR, mid IR and near IR. The mid IR region, 4000-400 cm^{-1}, is the most commonly used region for analysis as all molecules have characteristic absorbance frequencies that cause excitation of the fundamental vibrational modes of molecules. Specific wavelengths are absorbed by the molecules causing the chemical bonds in the material to undergo various types of vibrations such as stretching, bending and rocking. Fundamental vibrational frequencies occur as molecules are irradiated with a continuous IR beam resulting in an absorption in the IR spectrum. Fundamental vibrational frequencies depend on the bond strength, atomic mass and spatial inter-relationships within the molecule. The absorption bands are due to the energy exchange between discrete light quanta and the mechanical motion (vibrational modes) of the molecules, which are excited by the absorption of IR radiation (Burgula *et al*., 2007). Nonlinear molecules composed of N atoms have 3N-6 fundamental vibrational modes. Vibrations that are accompanied by a change in the dipole moment of the molecule are IR active and are observed in the IR spectrum.

An IR spectrum traditionally plots the absorbance as the Y-axis and the wavenumber units as the X- axis. IR absorption bands are conventionally measured in wavenumbers (cm^{-1}),

waves per centimeter, which correlates to the reciprocal of the wavelength and is proportional to the energy absorbed. The frequency of an absorption band increases as the force constant of the bond(s) involved increases and it decreases as the mass of the atoms involved increases. The IR spectrum can be approximately divided into separate regions. Single bond stretching vibrations are observed between 4000-2500 cm^{-1}, the stretching of double bonds and triple bonds are observed between 2500-1500 cm^{-1}, bending vibrations are observed bonds between 2000-1000 cm^{-1}, and rocking vibrations are observed below 1000 cm^{-1}. The region below 1500 cm^{-1} is referred to as the "fingerprint region". Bonds and functional groups have specific vibrational bands and compounds have unique IR spectra and is thus of immense use to identify unknown compounds.

2.4.4 Characterization of bacteria based on their IR spectra

ATR-FTIR based methods have been used to accurately and rapidly identify, classify, quantify and differentiate between bacteria. The complexity and the variation in the composition and structure of the bacterial cell is important because it enables differentiation of bacteria by FTIR spectroscopy. The bacterial cell wall contains capsular polysaccharides, peptidoglycan (PG), surface proteins, lipopolysaccharides, lipoteichoic acids, capsules, lectins, fimbriae, flagella, outer membrane (OM) proteins (Schaer-Zammaretti *et al*., 2003). Despite all these bacterial components, the interpretation of the ATR-FTIR spectra is key to understanding the structure and composition of the sample, this is done by assigning each peak to specific components of the bacterial cell. Within the desired mid-IR region, from 4,000-400 cm^{-1}, the fundamental vibrational modes of the constituents of the cell wall are responsible for the absorbance peaks (Naumann *et al*., 2000). The fatty acid region is observed between 3000-2800 cm^{-1}; the amide I and amide II absorbance bands of proteins and peptides are located between 1700-1500 cm^{-1}; the region between 1500-1200 cm^{-1} is referred to as the mixed region and contains bands characteristic to fatty acid bending vibrations, proteins and phosphate-carrying compounds; the polysaccharide region which includes the absorption bands of the carbohydrates in microbial cell walls is observed between 1200-900 cm^{-1}; and region between 900-700 cm^{-1} is called the "fingerprint region" and contains weak but very unique bands that are characteristic to specific bacteria. Regions I and II are the most useful for routine bacterial

identification (Burgula *et al*., 2007). The availability of advanced chemometric software and statistical algorithms for analyzing IR spectra is the reason that one can detect the subtle differences between bacterial IR spectra. Once a spectral library is filled with various authenticated reference bacterial strains, the comparison of an unknown bacterial spectra can be compared utilizing mathematical and statistical methods.

2.5 Spectral acquisition and processing

IR spectra of bacteria are taken directly from intact bacterial colonies grown on culture plates. In order to build an adequate spectral library to identify unknown bacterial strains, the authenticated spectra in the library must all contain the exact same microbiological parameters influencing cell growth such as composition of growth media, incubation time and temperature of growth (Puzey *et al*., 2008). In a recent study done by Puzey et al., on the effect of growth media in bacterial classification, they found that the bacteria grown on selective enrichment media exhibited the same spectra as the cells grown in non-selective enrichment broths but when the statistical analysis was performed to evaluate the effect of media on classification, some of the samples were incorrectly classified (Puzey *et al*., 2008). Thus, the sampling of biological species, sample treatment procedures, IR measurement techniques, and IR acquisition parameters have to be controlled and experimental protocols must be standardized.

The reproducibility of the bacterial FTIR spectra is greatly dependant on the sample-handling techniques employed. Spectral acquisition can be done by various modes such as attenuated total reflectance, transmission and diffuse reflectance. The reproducibility of spectra acquired by means of ATR depends on the sample homogeneity and sample thickness (pathlength). The sample non-uniformity leads to variations in the spectra such as shift in the baseline due to the difference in the amount of light absorbed in the sample and then scattered, diffracted back into the crystal. The difference in sample thickness reduces the consistency in peak intensities. Maintaining a high signal-to-noise ratio is crucial in order to minimize variations between spectra by using spectral preprocessing techniques. The main types of preprocessing spectral algorithms that have been applied to the bacterial spectra are listed below.

Outlier can be interpreted as a doubtful or anomalous value; any observation that does not fit a pattern. The term outlier does not signify incorrect, an outlier can be the repercussion of an erroneous human action or simply caused by a real phenomenon that is relevant to the research (Adams, 2004; Bakeev: 2010). Throughout the research, outliers have been truly important for model development, they served as informative indicators for sampling techniques and pattern abnormalities between bacterial strains. During exploratory data analysis using PCA and HCA, the protocol practiced for handling outliers was to detect, assess and remove if appropriate. Outliers were first evaluated by identifying its origin and determining if it was the only one, or if all the triplicates of the same sample were identified as outliers. All samples causing problematic outliers were re-grown on a blood agar medium in the laboratory and further analyzed. The colony morphology was visually verified to ensure no apparent contamination of the sample on the agar plates. Only pure samples were used in this research, but due to improper handling of fresh and frozen samples, they may have been contaminated. During spectral acquisition, an appropriate amount of sample is collected to ensure enough biomass is present per scan and that it doesn't completely dry up before the end of the scan. The plausible bacterial sample is then also identified using the Vitek-2 automated system which simultaneously identifies and provides the antibiotic susceptibility panel. Samples that were visually perceived as mixed or identified as mixed were removed from the data set, only pure samples were used in the research of this thesis.

2.5.1 Principal Component Analysis (PCA)

In spectroscopy, when acquiring a bacterial spectrum, a lot of information and variables pertaining the bacterial sample are collected. For example, we can record a spectrum at thousands of wavelengths at a single time. Once all the data is collected, many correlations can be made between samples and a major problem is to reduce and eliminate the correlations that would lead to invalid results. The goal is to create the simplest structure possible of a data set concerning their patterns and trends and identify which variables contribute the most to the patterns and trends. There exist two general techniques, supervised and unsupervised, that can be used depending on the previous knowledge of the IR spectra and relationships. Unsupervised methods try to disclose naturally occurring groups and structures within the data set without previous knowledge of class assignment. PCA is an example of an unsupervised technique

(Alvarez-Ordóñez *et al*., 2012).

PCA has been widely used in the interpretation of infrared spectra. It reduces a multi-dimensional dataset to its most dominant features, removes the random variation (noise), and retains the principal components (PC) that capture the related variation (Alvarez-Ordóñez *et al*., 2012). PCA identifies the natural clusters in the data set with the first principal component (PC) expressing the largest amount of variation, followed by the second PC which conveys the second most important factor of the remaining analysis, and so forth (Al-Holy *et al*,. 2006). Score plots can be used to interpret the similarities and differences between bacteria. The closer the samples are within a score plot, the more similar they are with respect to the principal component score evaluated (Davis *et al*., 2010).

PCA is an eigenanalysis technique which focuses its attention on the fact that not just the eigenvectors (principal components) are important here, but also the eigenvalues, which underlie the statistical procedures. The eigenvectors are extracted sequentially by the use of nonlinear iterative least squares algorithm (Rodriguez *et al*., 2001). The first eigenvector expresses the largest amount of variation, followed by the second eigenvector which conveys the second most important factor of the remaining analysis. Creation of score plots of eigenvalues can be used to interpret the similarities and differences between bacteria. The closer the samples are within a score plot, the more similar they are with respect to the principal component score evaluated (Davis *et al*., 2010).

2.5.2 Hierarchical Cluster Analysis (HCA)

Cluster analysis divides and classifies data (the spectra of microorganisms) into groups (clusters) based only on information found in the data that describes the objects and their relationships (Tan *et al*., 2013). The similarities between the spectra are analyzed using the distances between the spectra and aggregation algorithms. The greater the similarity within a group and the greater the difference between groups, the more distinct the clustering. Clusters do not always produce crisp separation of clusters, they can also produce overlapping and fuzzy clusters. HCA frequently utilizes the Pearson product moment correlation coefficient and the

Euclidian distance to cluster objects one by one. For bacterial identification, the commonly used cluster analysis algorithms include Ward's algorithm and the average linkage algorithm (also called the unweighted pair group method with arithmetic mean) (Beveridge *et al*., 1991). The spectra are factorized, the most common spectral variations are extrapolated and broken apart by their corresponding scores, which compresses the data and suppresses the noise. HCA is a set of nested clusters that are organized as a tree. HCA and dendrograms are used to show the similarities between spectra of bacteria representing the same species, genus, serotypes, or haplotypes (Davis *et al*., 2010). In addition to finding the similarities between bacteria, an unknown bacterium can be identified by calculating the spectral distance between it and known bacteria or by introducing it in the HCA (Helm *et al*., 1991; Rebuffo-Scheer *et al*., 2007).

2.6 Advantages and Disadvantages of FTIR spectroscopy in microbiological analysis

The following is a comprehensive list of the most notable advantages of FTIR spectroscopy:

1. It's little to no sample preparation and minute amount of sample required (ng-µg), allows for a simple, sensitive and cost-efficient technique.
2. Short acquisition time, spectra of pure colonies can be obtained in minutes.
3. Compact instrumentation takes up small benchtop space and the single instrument includes detection, enumeration, classification and identification.
4. Isolation of a bacterial colony, cultivation and identification can be performed within one working day in a clinical setting.
5. It is a non-destructive technique, the sample is not destroyed (as in most of the techniques) as the bacterial biomass stays intact and can be analyzed further, allowing typing or DNA extraction from the same colony (Alvarez-Ordóñez *et al*., 2012).
6. Detection of specific cell components such as storage materials, spores, bacterial capsules, and properties such as drug resistance and cell–drug interaction (including its monitoring and characterization) is possible (Alvarez-Ordóñez *et al*., 2012).

7. Classification can be performed at various taxonomic levels. IR spectroscopy is useful at the genus, species, and even strain level. The specificity of the technique is extremely high and can even differentiate at the serotype level.

The major disadvantages of the technique include:

1. Microorganisms spectral database is also media specific, only microorganisms capable of growing on that media can be identified using this technique.
2. Classification and identification is highly based on the spectral fingerprint area and the specific information on the specific compounds in the whole bacterial cell is frequently not available.

Only microorganisms capable of being grown in culture can be analyzed. Mixed cultures can't be investigated using this method since mixed biomass would yield overlapping spectra, and erroneous results in identification or characterization.

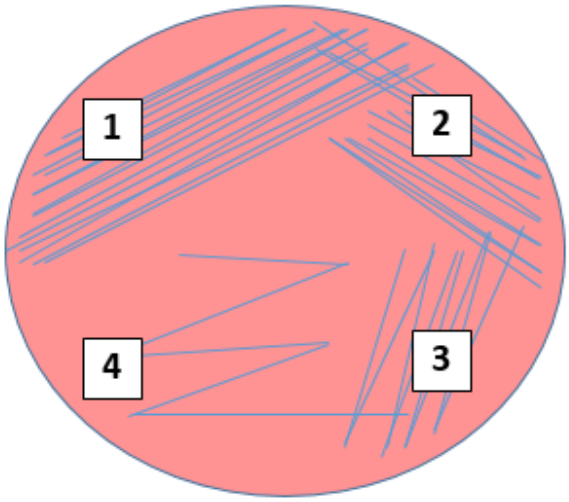

Figure 3.1. Streak plate method for the isolation of pure colonies.

Chapter 3 – METHODS and MATERIALS

Sections of the following chapter will cover the protocols applied to culturing bacteria, acquiring adequate FTIR bacterial spectra and analyzing ATR-FTIR spectra.

3.1 Culturing Technique

The *Enterococcus* and *Staphylococcus* bacterial strains analyzed in this research were graciously provided by the McGill University Health Center (MUHC) and the Laboratoire de Santé Publique du Québec (LSPQ). The bacterial strains obtained were frozen at -80°C in a 40-50% glycerol suspension in 3-mL cryogenic storage vials. Glycerol is used as cryoprotectant in microbial cultures, it adsorbed to the surface of the microorganisms, coating the cells and therefore providing a shield from ice crystals formation during long-term storage. Glycerol conversely acts as a membrane permeant and facilitates the vitrification process by replacing the water in the cells and making hydrogen bonds with water molecules to exert a protective effect (Martin-Desjardin et al., 2013). All samples provided had been isolated from either stool, blood, urine or pus. The bacterial strains were first cultured onto the blood agar growth medium using the streak plate method and the resulting isolated colonies were used for sub-culturing using likewise the streak plate method (Figure 3.1).

The protocol necessitates using sterile wooden stick to scrape the surface of the frozen bacteria. Once a sufficient amount is collected onto the tip of the sterile wooden stick, it is then transferred onto the blood media. Sterile disposable streaking loops were used to gather single colonies for sub-culturing. The bacterial sample was streaked 4 times using the sterile disposable loops in 4 quadrants as demonstrated in Figure 3.1. The purity of the bacterial sample can be determined during the first culture, a mixed culture can be identified visually on the growth media. Only pure colonies were used in the research, with the intention of creating and validating a database, pure colonies should be used in order to avoid inaccurate results. After streaking the bacteria onto the blood agar plates during the culturing and sub-culturing stages, the plates are then incubated for 18-24 hours at 37ºC.

3.2 Sample Preparation

The culturing technique previously described utilizes a blood based bacterial growth medium. The bacterial growth medium is enriched with 5% sheep blood and is a multi-purpose medium suitable for the cultivation of fastidious and non-fastidious organisms. Columbia 5%

sheep blood agar was used to culture and sub-culture all bacterial samples. Columbia blood agar contains both casein hydrolysate and meat digests. The casein hydrolysate encourages the development of large colonies, while the meat digests provide well defined zones of hemolysis and good colonial differentiation. The variety of peptones and extracts makes Columbia Agar especially nutritious, and the addition of defibrinated sheep blood allows for the determination of hemolytic reactions (Ellner et al., 1969). Three types of hemolysis can be differentiated from this media: alpha-hemolysis (α), beta-hemolysis (β) and gamma-hemolysis (γ).

3.3 Data Acquisition

Following the culturing and sub-culturing, the analysis of the bacteria was done by ATR-FTIR. The procedure of acquiring a bacterial spectrum begins by cleaning the 2 mm wide circular sampling window with ethanol and allowing an adequate amount of time for the window to dry. Once the surface is cleaned and dried, a background spectrum was collected and then 2-3 pure bacterial isolates were collected off the surface of the agar plate. The bacterial isolates were smeared onto the sampling window, ensuring a homogenous dispersion of the biomass on the spectrophotometer. A spectrum of the bacterial sample was then taken. (Figure 3.2). Cognisolve1 (Cognisolve Inc., Montréal, Canada) and MicroLab (Agilent technologies, California, US) were the software used for spectral acquisition. The scanning parameters were identical for the background scan and the sample scan. Both are scanned 64 times with 2 times zero filling and 8 cm^{-1} resolution. Each bacterial sample was analyzed in triplicates to evaluate the spectral reproducibility. The superposition of all three spectra was done in order to ensure each spectrum of the same sample were identical.

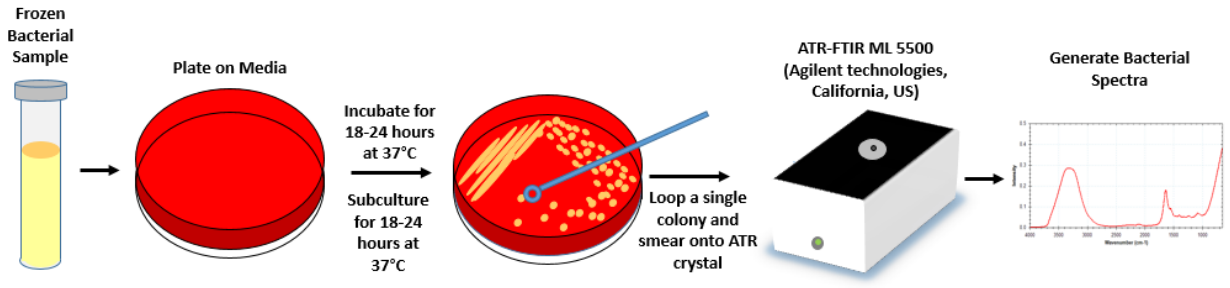

Figure 3.2 Procedure for generating a bacterial spectrum from a frozen bacterial sample.

3.4 Data Analysis

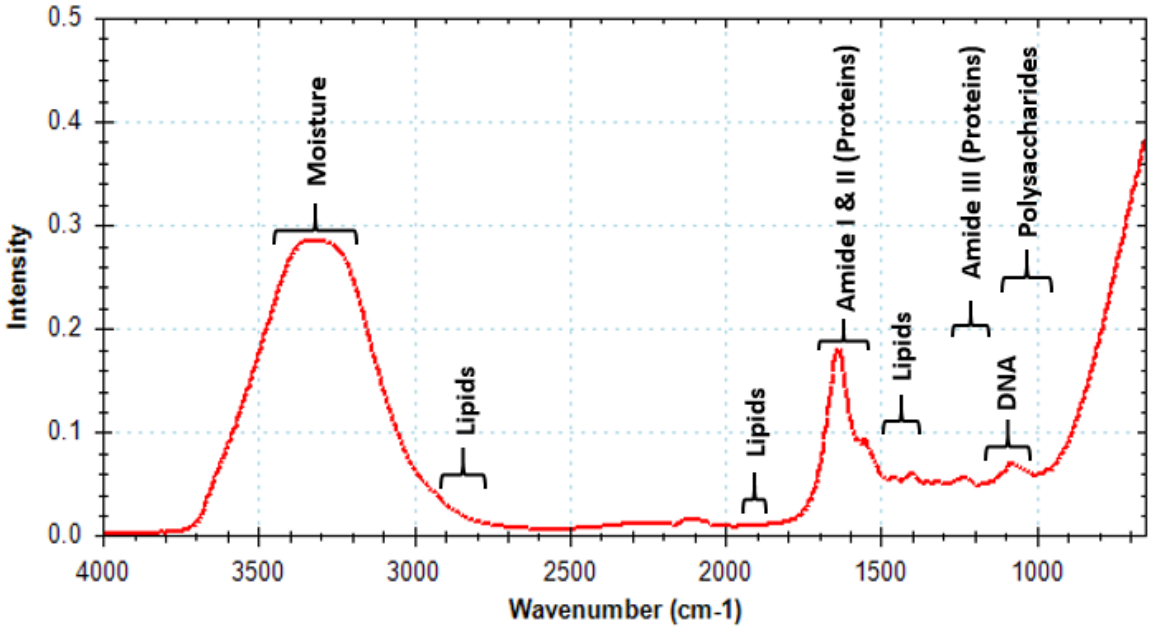

Figure 3.3 A typical bacterial spectrum and the absorbencies of the biomolecular components.

As mentioned previously, a background spectrum is collected before every spectral acquisition. The acquisition of a background spectrum minimalizes the interference caused by water vapor. Through extensive analysis of bacterial spectra, the spectral region at intervals

1480-980 cm^{-1} was established to possess all the important biomolecular components of a bacterial cell. (Figure 3.3). The biomolecular absorptions in this region belong to lipids, DNA, proteins and polysaccharides. The moisture of the bacterial samples is extremely important, the moisture plays a crucial role in the appearance of all the apparent peaks. A strict protocol had to be followed to ensure a certain level of moisture was maintained for each bacterial sample in the database. Once the bacterial isolates were picked off the agar plate, they were immediately smeared onto the sampling surface of the spectrophotometer and a spectrum was taken. Luckily *Enterococcus* and *Staphylococcus* bacteria retain approximately the same amount of moisture. Through spectral analysis, the spectra with too dry or too moist are filtered out using two standard protocols. The first protocol entails normalizing the peak height at 1770-980 cm^{-1} and then analyzing the peak height at 3350 cm^{-1}, this allows for the peak heights to be averaged out

and correlated around the theoretical value of 1. The spectra greatly deviating from this average can be removed from the database. The second procedure begins by taking the first derivative of each spectrum and then normalizing to eliminate biomass variability. Any deviant spectrum from the normalized baseline is removed once again.

CHAPTER 4 - Discrimination of Enterococcus and Staphylococcus

The discriminatory power of ATR-FTIR spectroscopy was first evaluated by separating the *Enterococci* and *Staphylococci* genera. 2098 spectra were analyzed and each genus were grown on BA with 5% sheep blood and incubated for a period of 18-24 hours at 35°C. A total of 138 spectra were removed from the dataset due to inadequate moisture content, the samples were most likely left on the crystal too long before the spectra was collected, allowing the bacterial sample to dry out. Enterococci and Staphylococci make up the most prevalent and lethal nosocomial strains. The objective of this part of the research was to separate the two genera based on their spectral differences. The difference in their biochemical composition should be detected by ATR-FTIR spectroscopy and the separation will be visually demonstrated by PCA.

Table 1 – Spectral Database used to Discriminate *Enterococcus* and *Staphylococcus*

	Total Spectra Collected	Spectra Removed*	Spectra Outlier	Spectra Analyzed
Enterococcus	993	55	0	938
Staphylococcus	1243	83	0	1160
TOTAL	2236	138	0	**2098**

Staphylococcus was discriminated from *Enterococcus* by PCA (Figure 4.1) using the whole region of interest (1480-900 cm^{-1}) based on their spectral differences. Due to their spectral differences a forward search algorithm did not have to be used to remove non-relevant spectral features to enhance the separation between *Enterococcus* and *Staphylococcus*. The average

Staphylococcus and *Enterococcus* spectra were generated using a software and then both spectra were superimposed to visualize their spectral differences (Figure 4.2).

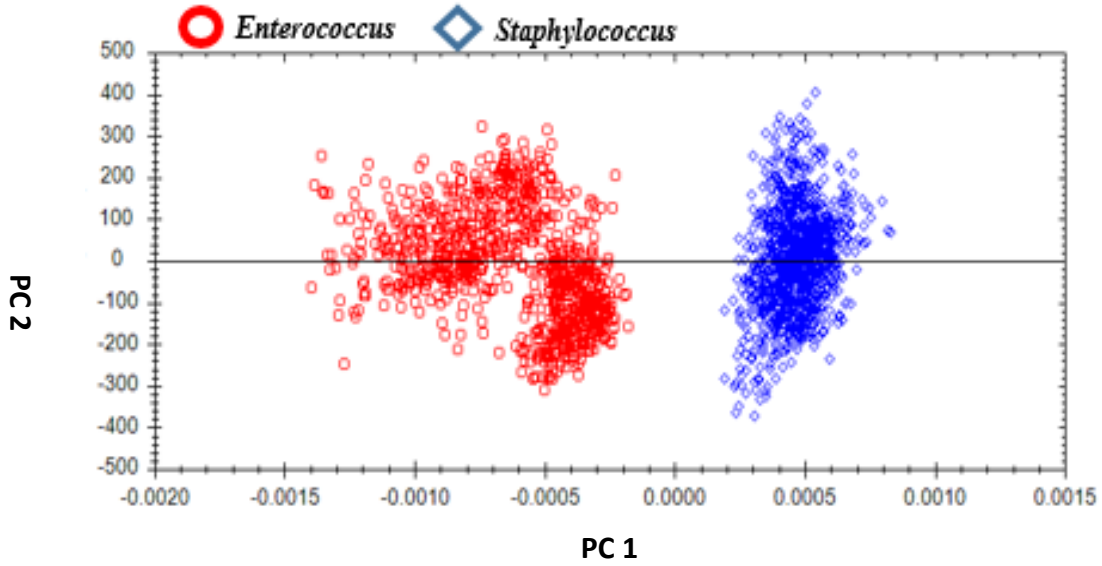

Figure 4.1. Whole spectral region of interest (1480-900 cm-1) principal component analysis of the ATR-FTIR spectra of Enterococcus and Staphylococcus.

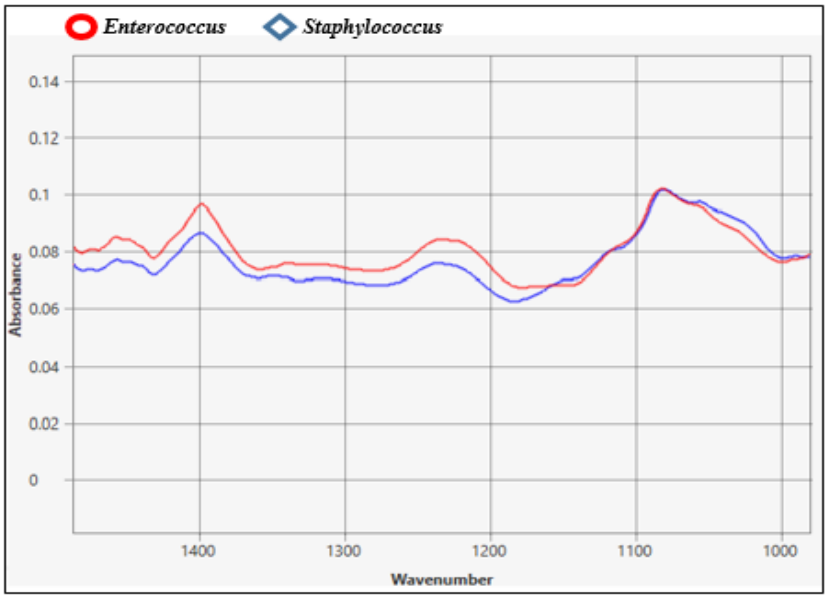

Figure 4.2. Superimposed whole spectral region of interest (1480-900 cm-1) of the average ATR-FTIR spectra of both Enterococcus and Staphylococcus.

The clinical method of discrimination between both genera was done by performing a catalase test. A catalase test helps to distinguish between enterococci (that is catalase-negative) and staphylococci (which are catalase positive). The catalase test can't be performed on bacterial cultures grown on blood agar since the blood itself will react with the catalase. Catalase is an extracellular metabolic enzyme produced by facultative aerobes including *Staphylococcus*. Catalase are heme proteins that degrade the toxic end product of aerobic carbohydrate metabolism, hydrogen peroxide (H_2O_2), into water and oxygen. The Fe (III) heme initially reacts with H_2O_2 to producing an oxyferryl intermediate and releasing a water molecule. The oxyferryl intermediate then reacts with another molecule of H_2O_2 which returns the enzyme to its resting state and releases a molecule of water and oxygen. Enterococci bacteria are aerotolerant and do not possess the catalase enzyme. Grüner et al. recently found that for a clinical isolate of *S. aureus*, the loss of catalase activity was related to a 5-bp deletion leading to a frameshift in the *katA* (catalase) gene.

The average superimposed spectra of *Enterococcus* species (*E. faecalis* and *E. faecium*) and *Staphylococcus* species (*S. aureus* and CoNS) revealed major differences between each other (Figure 4.2). The major spectral differences observed between the two average spectra were visualized in the region of 1600-1160 cm^{-1} and 1064-985 cm^{-1}. This region contains absorption bands of DNA and RNA at 1244 cm^{-1}, assigned to the phosphate antisymmetric stretching. Furthermore, a difference in the region containing absorption bands of DNA at 1225-1220 cm^{-1}, assigned to the main ß-form marker; phosphate antisymmetric stretching, at 1224 cm^{-1}, assigned to the antisymmetric stretch PO_2- sensible to cationic polymer complexed to DNA, at 970-965 cm^{-1}, assigned to the O-P-O bending, at 970-950 cm^{-1} assigned to the ß-form: singlet, and at 938 cm^{-1} assigned to the adenine and thymine base pairs in ß-form helices (Mello & Vidal, 2012).

The guanine and cytosine (G+C) content of genomic DNA can be related to their phylogeny; the evolution of a genetically related group of organisms. E. faecium and E. faecalis have a G+C content of 38.0% and 38.7% (Ezaki et al., 1990) respectively and S. aureus have a G+C content of 32.7% (Wang et al., 2012). Both genus have a G+C content of their genomic DNA differentiating by approximately 5%. Through literature, cytosine and guanine have specific absorption bands at 1527, 1425, 1374 cm^{-1} (Mello & Vidal, 2012), a difference in these

bands can be observed in figure 4.3.

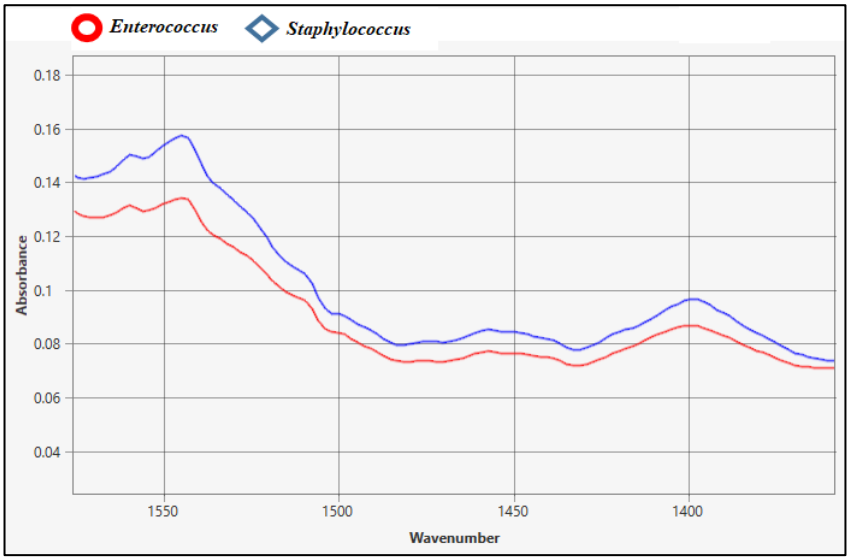

Figure 4.3. Superimposed spectral region of interest (1575-1350 cm-1) of the average ATR-FTIR spectra of both Enterococcus and Staphylococcus.

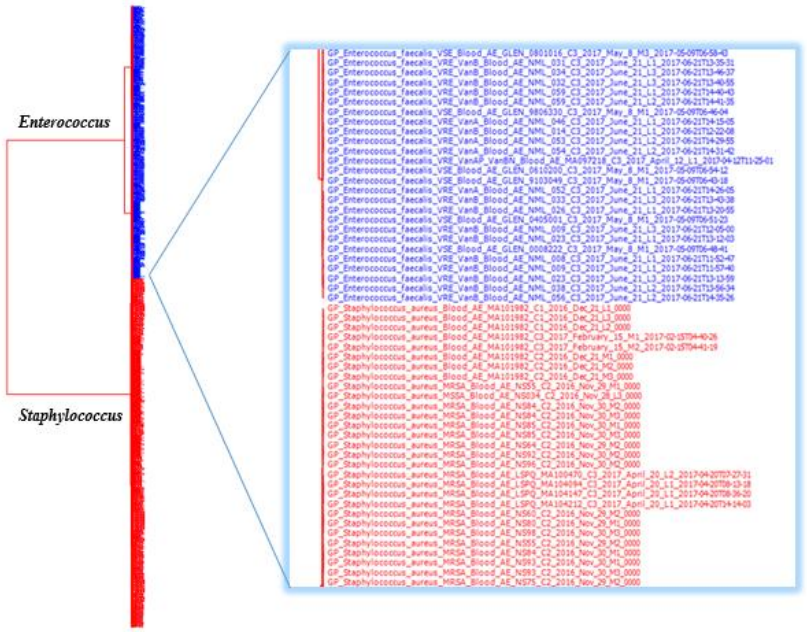

Figure 4.4. Dendrogram generated by HCA using the whole region of selection (1480-980 cm-1) for the separation of Enterococcus and Staphylococcus.

Hierarchical cluster analysis (HCA) was used to generate a dendrogram showing the complete discrimination between Staphylococcus and Enterococcus (Figure 4.4). The dendrogram is based on spectral distances, computed using the cosine metric and the Ward type linkage, within the whole region of selection 1480-980 cm-1. The Enterococcus and Staphylococcus spectra split into two distinct branches of the dendrogram. Within the Enterococcus branch, the preliminary separation of *E. faecalis* and *E. faecium* can be observed.

CHAPTER 5 - Discrimination at the Species Level of Enterococcus faecalis and Enterococcus faecium

In accordance to previously discriminating between genera, ATR-FTIR spectroscopy may also separate two species within the same genus. The discrimination between *Enterococcus faecalis* and *Enterococcus faecium* by ATR-FTIR spectroscopy was investigated in this segment of the study. *E. faecium* and *E. faecalis* are the two most prevalent species of the genus *Enterococcus* that cause nosocomial infections.

Table 2 – Spectral Database used to Discriminate *Enterococcus faecalis* and *Enterococcus faecium*

	Total Spectra Collected	Spectra Removed *	Spectra Outlier	Spectra Analyzed
E. Faecalis	426	37	0	389
E. faecium	598	45	39	514
TOTAL	1024	82	39	**903**

A total of 426 samples of *Enterococcus faecalis* and 598 samples of *Enterococcus faecium* were included in this study. A total of 82 spectra were removed once again from the dataset due to inadequate moisture content, the samples were most likely left on the crystal too long before the spectra was collected, allowing the bacterial sample to dry out. The samples were grown onto

Columbia 5% sheep blood agar and incubated for a period of 18-24 hours at 35°C.

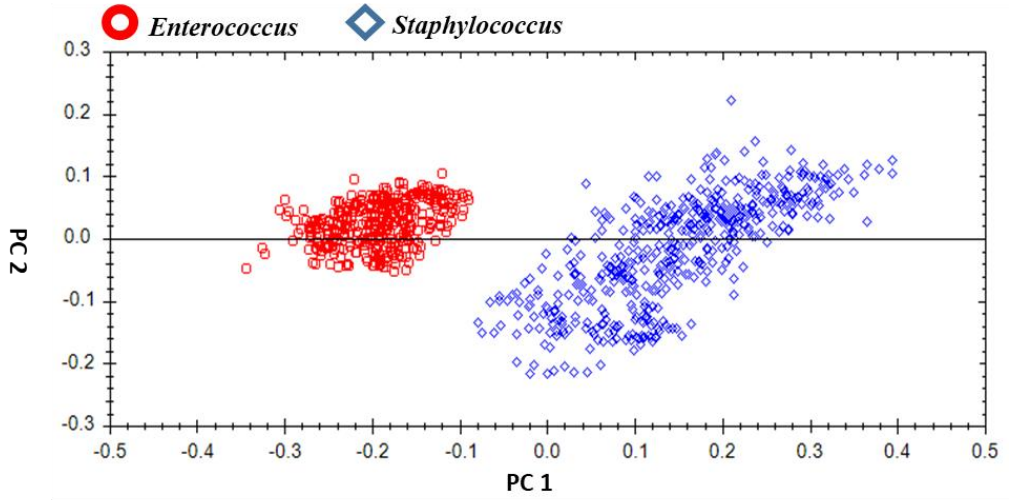

Figure 5.1. Whole spectral region of interest (1480-900 cm-1) principal component analysis of the ATR-FTIR spectra of Enterococcus faecium and Enterococcus faecalis..

E. faecalis was discriminated from *E. faecium* by PCA (Figure 5.1) using the whole region of interest (1480-900 cm-1) based on their spectral differences. Using a forward search algorithm to remove all non-relevant spectral features, the regions 1042-1049 cm^{-1}, 1079-1085 cm^{-1} and 1109-1167 cm^{-1} enhanced the separation between *E. faecalis* and *E. faecium* and led to the identification of three outliers (Figure 5.2). As reported by Kirkwood et al., absorption in these regions originates from carbohydrates (Kirkwood, 2007). The spectral points originating to

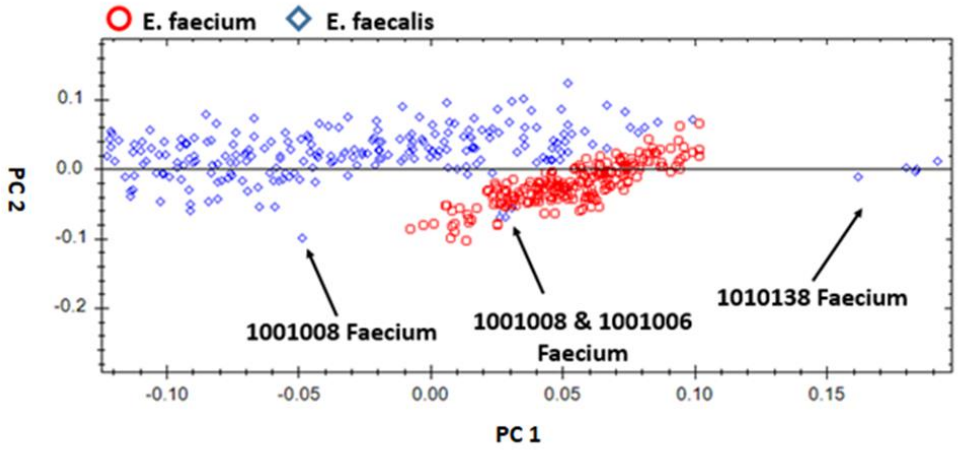

Figure 5.2. Computed principal component analysis using principal components 2 and 3 to discriminate the ATR-FTIR spectra of Enterococcus faecalis and Enterococcus faecium using the regions 1039-1054 cm-1 and 1061-1099 cm-1.

E. faecium clustering with *E. faecalis* belong to the samples 1001006 and 1001008, respectively. Upon further investigation, sample 1001006 and 1001008 were identified using MALDI and Vitek-2, with a 99% confidence level, sample 1001006 and 1001008 were both identified as E. faecalis. The outliers completely to the right belonging to sample 1010138 were identified by MALDI and Vitek-2, with a 99% confidence level, the results showed that it was actually an *Escherichia coli* sample and the spectra were then removed. Spectra with too low absorbance and too great of an absorbance were removed and Figure 5.3 portrays the final successful discrimination between *E. faecalis* and *E. faecium* with sample 1001006 and 1001006 correctly labelled as *E. faecalis* and sample 1010138 discarded.

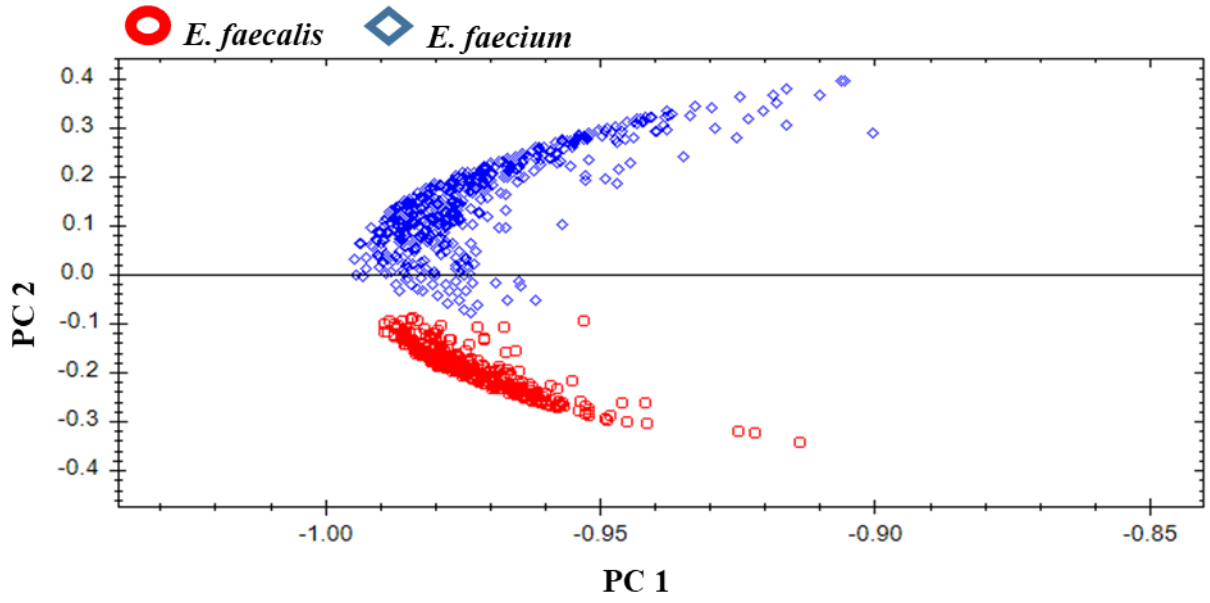

Figure 5.3. Final principal component analysis using principle components 2 and 3 to discriminate the ATR-FTIR spectra of Enterococcus faecalis and Enterococcus faecium using the optimized region of selection 1039-1054 cm-1 and 1061-1099 cm-1.

A possible cause for the separation lies within the cell wall which is composed of a peptidoglycan backbone, anionic polymers (teichoic acids and cell wall polysaccharides) and wall-associated/anchored proteins. The peptidoglycan backbone and anionic polymers comprise nearly 90% of the total cell wall weight, with the protein content comprising less than 10% of the cell wall weight (Coyette & Hancock, 2002). The overall difference in the peptidoglycan structure stems from the variation in the amino acid sequence that forms the interpeptide bridge, also known as the crossbridge. *Enterococcus faecium* as well as most species in the genus *Enterococcus*, has a crossbridge composed of a single D-Asp residue. *Enterococcus faecalis* differs from the rest of the genus, as it possesses 2-3 L-Ala residues (Schleifer & Kandler, 1972). Another difference between both specie lies within the structure of the repeating units of the cell wall teichoic acids (WTA) of *E. faecium* and *E. faecalis*, they were identified using NMR spectroscopy. Bychowska et al., found that the repeating unit of WTA consisted of two residues of 2-acetamido-2-deoxy-D-galactose, glycerol, and phosphate (Bychowska, 2011). Later, Theilacker et al., identified that the repeating units of WTA were composed of d-glucose, d-galactose, 2-acetamido-2-deoxy-d-galactose, 2-acetamido-2-deoxy-d-glucose, d-ribitol, and phosphate in a molar ratio 1:2:1:1:1:1 (Theilacker, 2012). The differences in the peptidoglycan crossbridge and the cell wall associated teichoic acids may be responsible for the spectral

separation.

The forward search algorithm identified three regions of the greatest difference between both specie; 1042-1049 cm^{-1}, 1079-1085 cm^{-1} and 1109-1167 cm^{-1}. The regions 1042-1049 cm^{-1} and 1109-1167 cm^{-1} is associated with C-O-C bending and C-O stretching vibrations of carbohydrates. The difference in WTAs between specie may be linked to this spectral region. (Burgula et al., 2007). Secondly, bands in the region 1079-1085 cm^{-1} are said to belong to the PO_2 stretching of phosphodiester bonds. The phosphodiester bond is the link between the 3' carbon atom of a sugar molecule and the 5' carbon atom of deoxyribose in DNA and ribose in RNA. This being said, the variance in the genetic composition of both specie can be related to this spectral region.

Figure 5.4 illustrates the separation between *Enterococcus faecalis* and *Enterococcus faecium*. The dendrogram was assembled by HCA and optimized by the forward-search algorithm using two spectral regions, 1039-1054 cm^{-1} and 1061-1099 cm^{-1}. Each species is separated by a distinct branch which indicates that the spectral differences were large enough to discriminate between the spectra of *E. faecium* and *E. faecalis*.

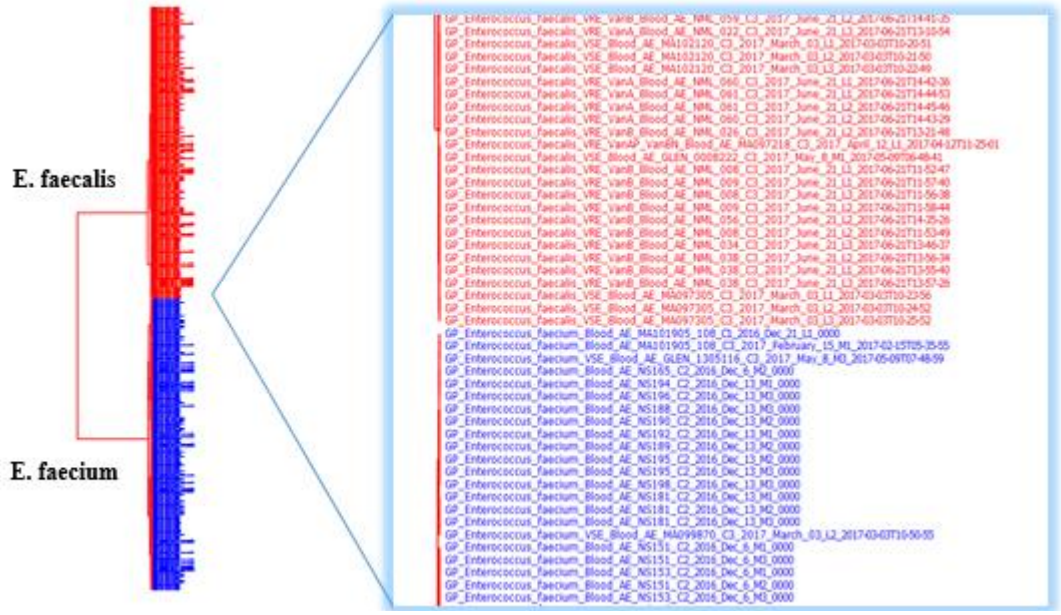

Figure 5.4. Separation of Enterococcus faecalis and Enterococcus faecium depicted by a dendrogram obtained by hierarchical cluster analysis using the optimized spectral regions 1042-1049 cm-1, 1079-1085 cm-1 and 1109-1167 cm-1 of the ATR-FTIR spectral data.

CHAPTER 6 - Discrimination between *Staphylococcus aureus* and Coagulase-Negative *Staphylococci*

In the 1980's the pathogenic potential of CoNS was accepted despite no molecular mechanism were discovered, it was in 1994 that Kloos and Bannerman shed light on the clinical significance of coagulase-negative staphylococci (CoNS) (Kloos et al., 1994). Still today, CoNS represents one of the major nosocomial pathogens, having a substantial impact on the lives of many. The coagulase-negative strains used in the research belong to the normal flora colonizing the skin and mucous membranes of humans. The majority of CoNS infections are of nosocomial origin according to the CDC and National Nosocomial Infections Surveillance System, CoNS belongs to the top five most frequent nosocomial infection. The current identification of CoNS in clinical laboratories is based on morphological, physiological, biochemical, and molecular biological parameters. Many commercial manual identification kits are available such as ID32Staph (bioMerieux), and (semi)-automated instruments for strain identification and susceptibility testing include Vitek II (bioMerieux), Phoenix (BD Becton-Dickinson), and MiscroScan Walkaway (Dade-Behring) (Pace et al., 2005).

For rapid and accurate diagnostic purposes, there is a need for the discrimination and identification of *S. aureus* and CoNS. Currently, in clinical laboratories, one way of discriminating between both is to perform a coagulase test. Coagulase is an enzyme involved in the clotting cascade and is produced by *S. aureus*, but not by CoNS. Coagulase converts soluble fibrinogen in plasma to insoluble fibrin, and coagulase is produced in the bound and free form. The slide coagulase test is performed to detect the bound coagulase form, the clumping factor directly converts fibrinogen to fibrin causing agglutination. Agglutination indicates the presence of S. aureus but has its limitations, some CoNS species can produce positive results. The tube coagulase test is a second coagulase detection test. This test detects staphylocoagulase which reacts with coagulase-reacting factor and indirectly converts fibrinogen to fibrin. Identical to the previous test, some CoNS species can produce positive results.

Table 3 - Summary of Spectra Collected for S. aureus and CoNS Strains.

	Total Spectra Collected	Spectra Removed*	Spectra Outlier	Spectra Analyzed
S. aureus	1175	40	0	1135
CoNS	307	2	0	305
TOTAL	1482	42	0	**1440**

* Spectra having too low moisture content were removed from the dataset.

The strains used in the analysis (Table 3) were grown on 5% Columbia blood agar. Since FTIR is very sensitive and can detect minor differences in the variability of the different manufacturing batches of blood agar, Oxoid 5% Columbia Blood agar was used throughout the entire research project to try and maintain spectral reproducibility. Every strain grown on the blood agar were visually verified for any abnormal morphologies or the presence of mixed colonies. The phylogenetic separation of staphylococcal species and subspecies (ssp.) used are shown in the table below (Table 4). Differentiating at the species level becomes increasingly difficult since the core genome is conserved between the strains. Mathema et al., compared S. aureus with CoNS strains and found that a large proportion of genes are conserved in both sequence and order on the chromosome, comprising the backbone of the staphylococcal genome.

Table 4 - Phylogenetic separation of staphylococcal species and subspecies (ssp.) proposed by Lamers et al. used in this part of the project.

Oxidase	**Negative**					**Positive**
Novobiocin	**Resistant**	**Susceptible**				
Coagulase	**Negative**					**Positive**
Group	**Saprophyticus**	**Epidermis-Aureus**				
Cluster	**Saprophyticus**	**Lugdunensis**	**Haemolyticus**	**Epidermis**	**Warneri**	**Aureus**
Species	S. saprophyticus	S. lugdunensis	S. haemolyticus S. hominis	S. epidermis S. capitis	S. warneri	S. aureus

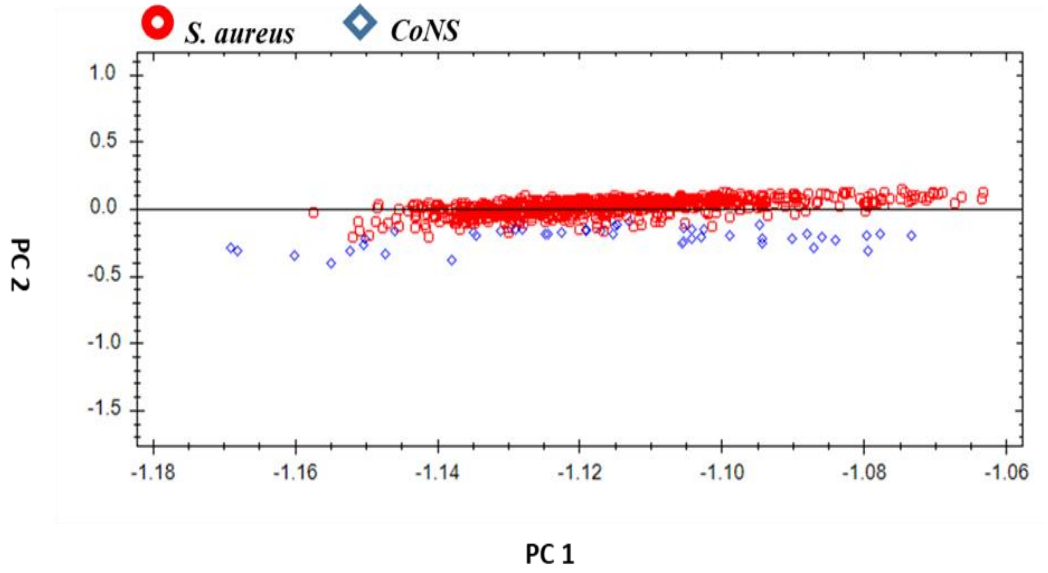

Figure 6.1. Computed principal component analysis using principal components 2 and 3 to discriminate the ATR-FTIR spectra of S. aureus and CoNS using the whole region of selection 980-1480 cm-1.

The discrimination of *S. aureus* and CoNS was attempted by PCA (Figure 6.1) using the whole region of interest (1480-900 cm-1) based on their spectral differences. At first glance, grouping was widespread and there wasn't a complete separation. Using a forward search algorithm to remove all non-relevant spectral features, the regions 1057-1072 cm^{-1} a tighter grouping was observed between *S. aureus* and CoNS (Figure 6.2), complete separation between both was not achieved. The average *S. aureus* and CoNS strain in the database was generated and superimposed to visualize high degree of similarity (Figure 6.3). As reported by Davis and Mauer, absorption in these regions originates from the C-O-C, C-O ring vibrations in various polysaccharides (Davis and Mauer, 2010).

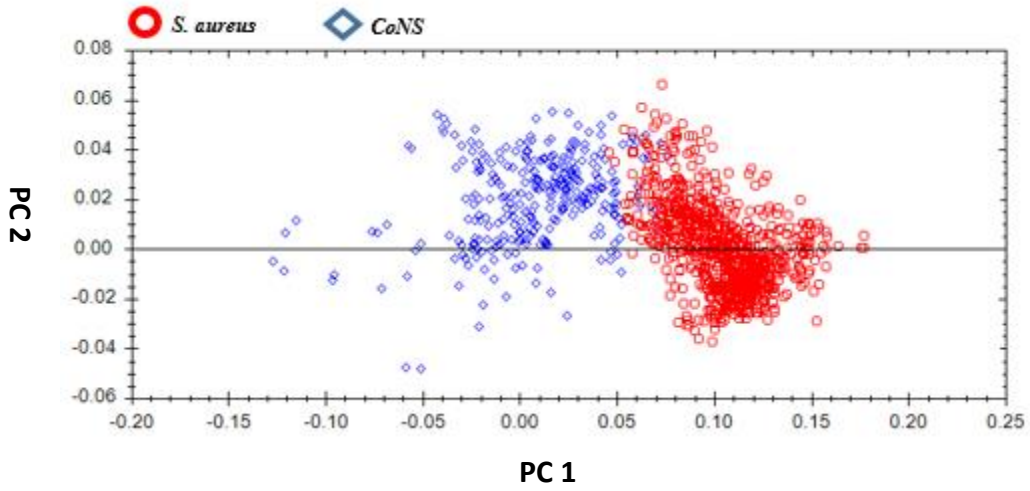

Figure 6.2. Computed principal component analysis using principal components 2 and 1 to discriminate the ATR-FTIR spectra of S. aureus and CoNS using the specific region 1057-1072 cm-1.

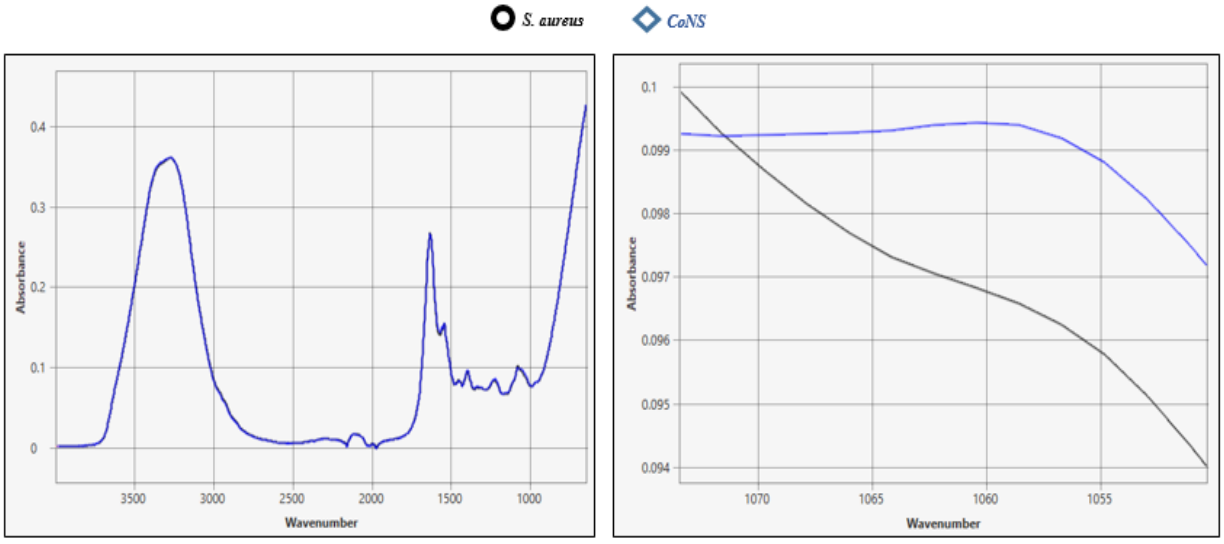

Figure 6.3. Superimposed average spectra of S. aureus and CoNS (Left) and close-up view of the forward search algorithm region 1057-1072 cm-1 (Right).

As a preliminary study of using ATR-FTIR spectroscopy as a means of separating S. aureus and CoNS, additional data and research is needed to perfect the separation. The present study has shown that limitations reside with the small sample size of data used in this research. We were able to form two clusters of data but were unable to achieve full separation from one another. The specificity of the ATR-FTIR spectroscopic technique can be increased with additional data points in the dataset.

CHAPTER 7 – Validation Studies

7.1 2nd Derivative Spectra compared to 1st Derivative Spectra of Enterococcus vs. Staphylococcus Validation Study

In this part of the validation study the importance of using 2nd derivative spectra compared to 1st derivative during spectral analysis will be demonstrated. The average spectra of *E. faecium, E. faecalis, S. aureus* and CoNS were each initially normalized using the spectral region 1480-980 cm^{-1}. The spectra were processed using the first derivative and the variance spectra of each bacterial set was then generated and compared to one another (Figure 7.1-7.5). Figure 6 summarizes the stacked first derivative variance spectra. An average of 10 variance

peaks per variance spectra were identified. The same bacterial set average spectra were then normalized using the same parameters but processed using the second derivative. The variance spectra of the second derivative spectra were then compared to one another (Figure 7.7-7.12). Figure 13 summarizes the stacked second derivative variance spectra. The second derivative spectra had an average of 38 identifiable peaks, 28 more peaks, on average, then the first derivative spectra. The first derivative and second derivative variance spectra peaks were tabulated in Tables 5-9. The second derivative variance isolated peaks were then assigned to bacterial biomolecules found through literature. When using ATR-FTIR as an identification and discrimination method, the second derivative spectra have more unique variant spectral peaks. These results validate that the use of 2nd derivative spectra compared to 1st derivative spectra during spectral analysis detect more variant spectral peaks thus, increases the specificity of identification and discrimination.

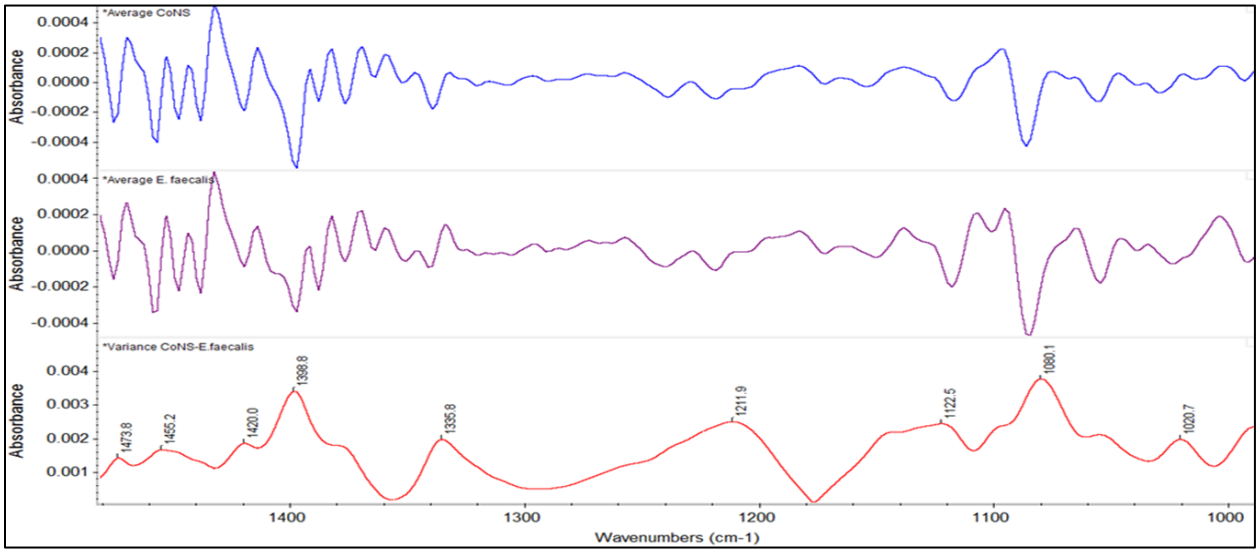

Figure 7.1. Variance spectrum generated using the average CoNS and E. faecalis spectra processed using the region of selection 1480-980 cm-1 and using the 1st derivative.

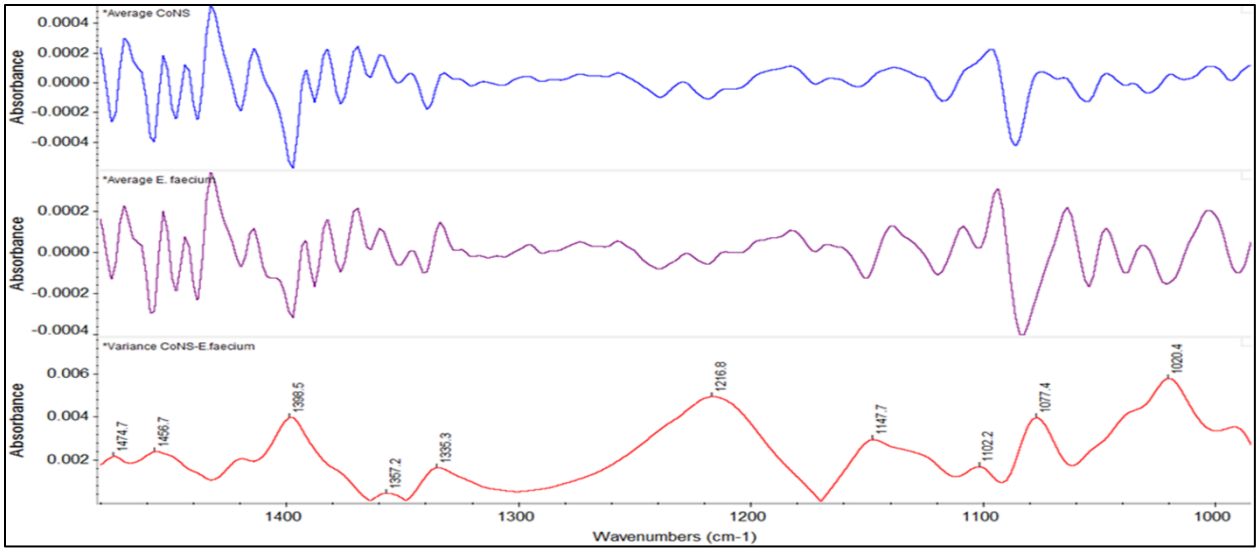

Figure 7.2. Variance spectrum generated using the average CoNS and E. faecium spectra processed using the region of selection 1480-980 cm-1 and using the 1st derivative.

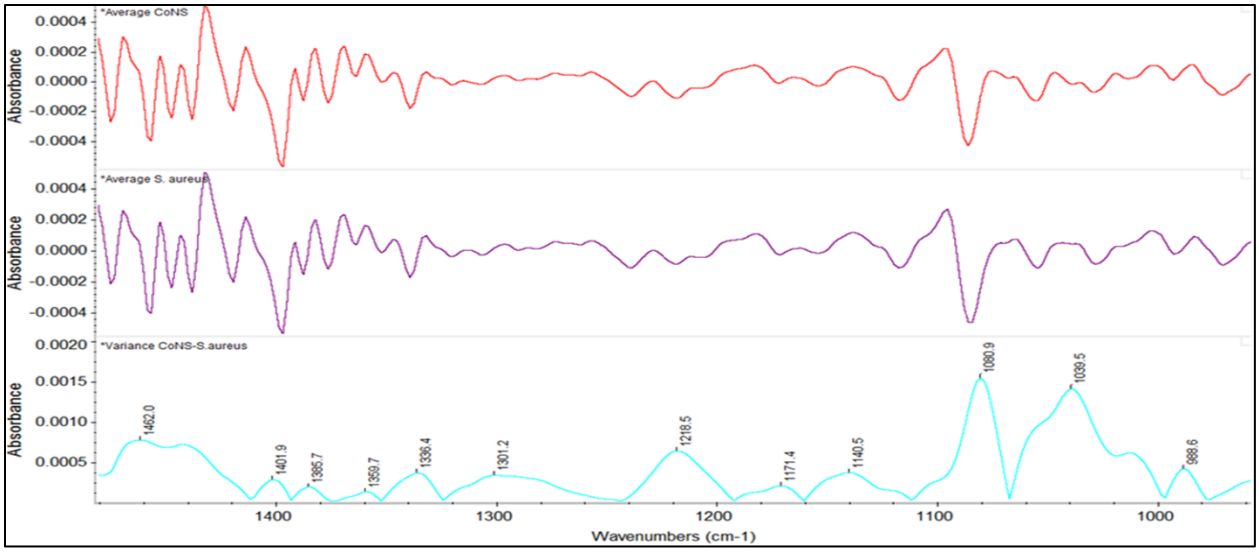

Figure 7.3. Variance spectrum generated using the average CoNS and S. aureus spectra processed using the region of selection 1480-980 cm-1 and using the 1st derivative.

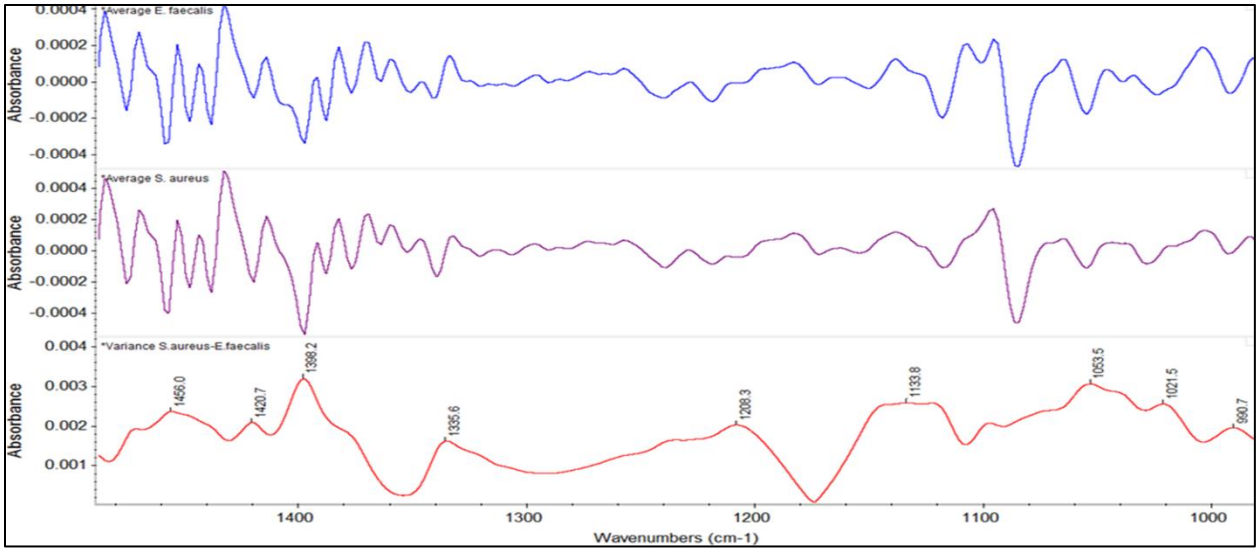

Figure 7.4. Variance spectrum generated using the average E. faecalis and S. aureus spectra processed using the region of selection 1480-980 cm-1 and using the 1st derivative.

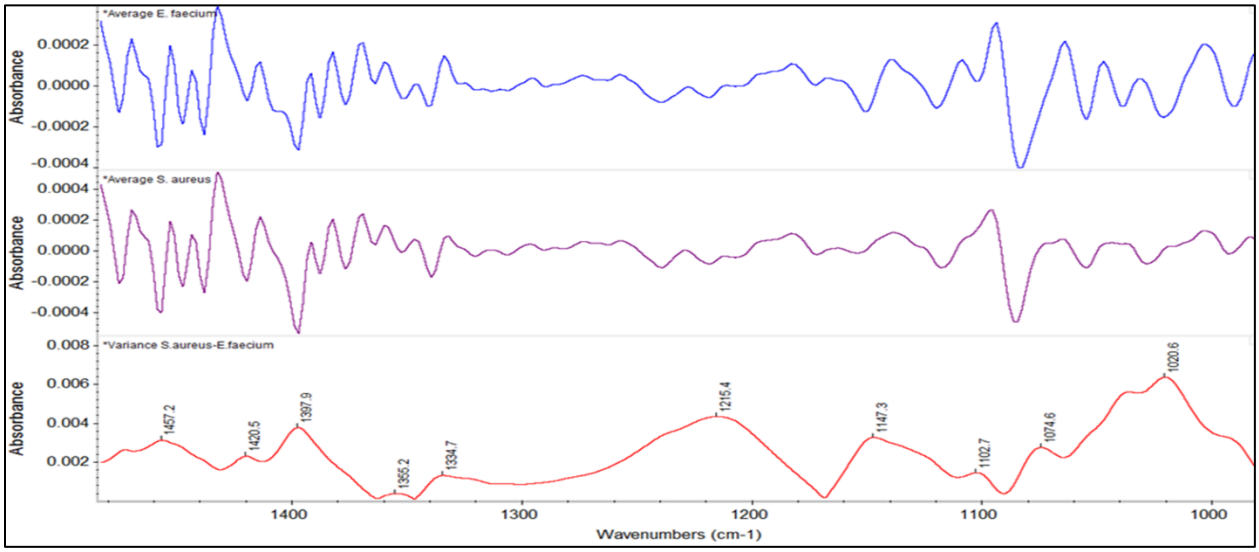

Figure 7.5. Variance spectrum generated using the average E. faecium and S. aureus spectra processed using the region of selection 1480-980 cm-1 and using the 1st derivative.

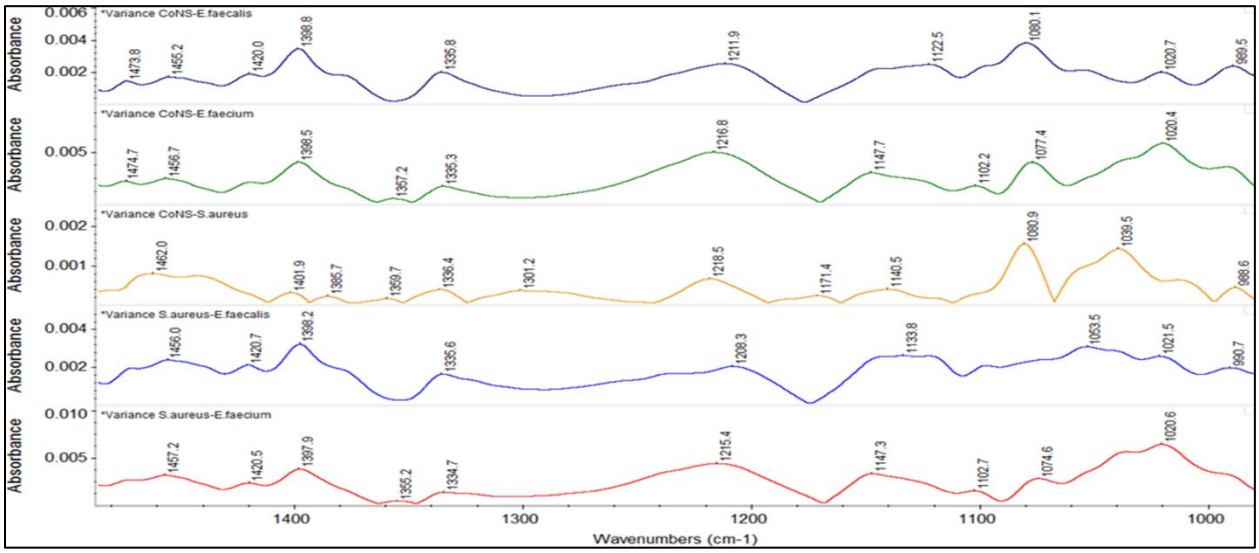

Figure 7.6. Summary compilation of the variance spectra using the average spectra processed using the 1st derivative through the region of selection 1480-980 cm-1.

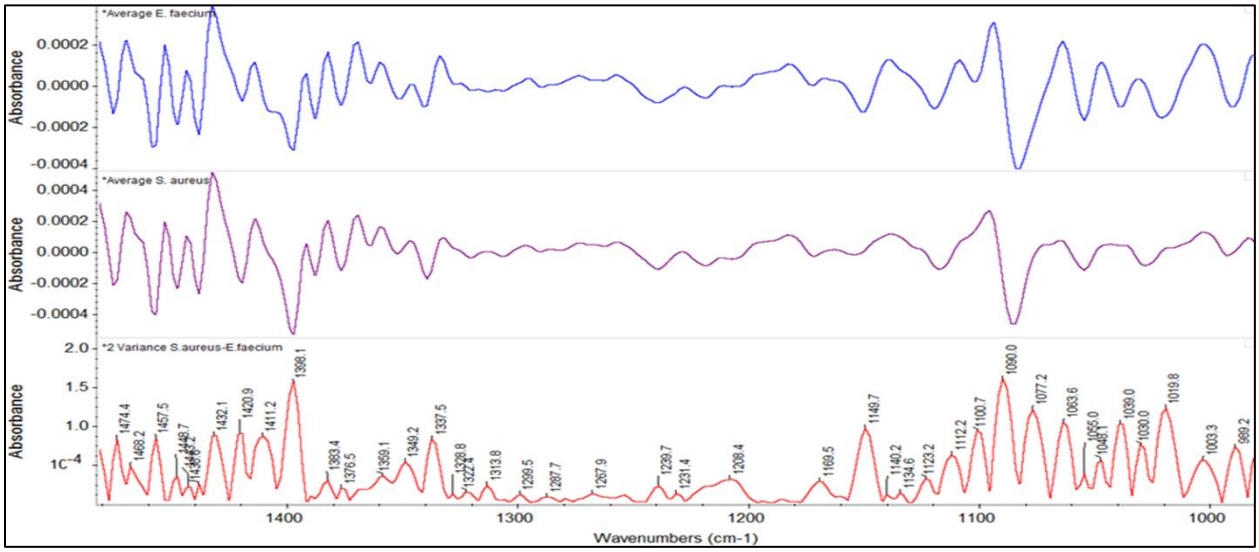

Figure 7.7. Variance spectrum generated using the average E. faecium and S. aureus spectra processed using the region of selection 1480-980 cm-1 and using the 2nd derivative.

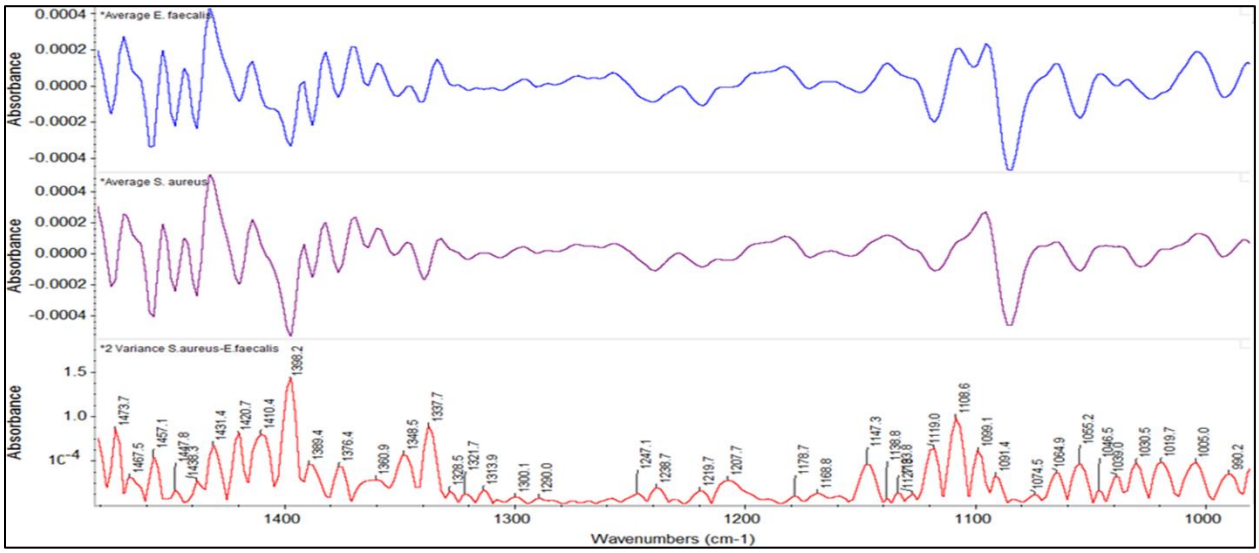

Figure 7.8. Variance spectrum generated using the average E. faecalis and S. aureus spectra processed using the region of selection 1480-980 cm-1 and using the 2nd derivative.

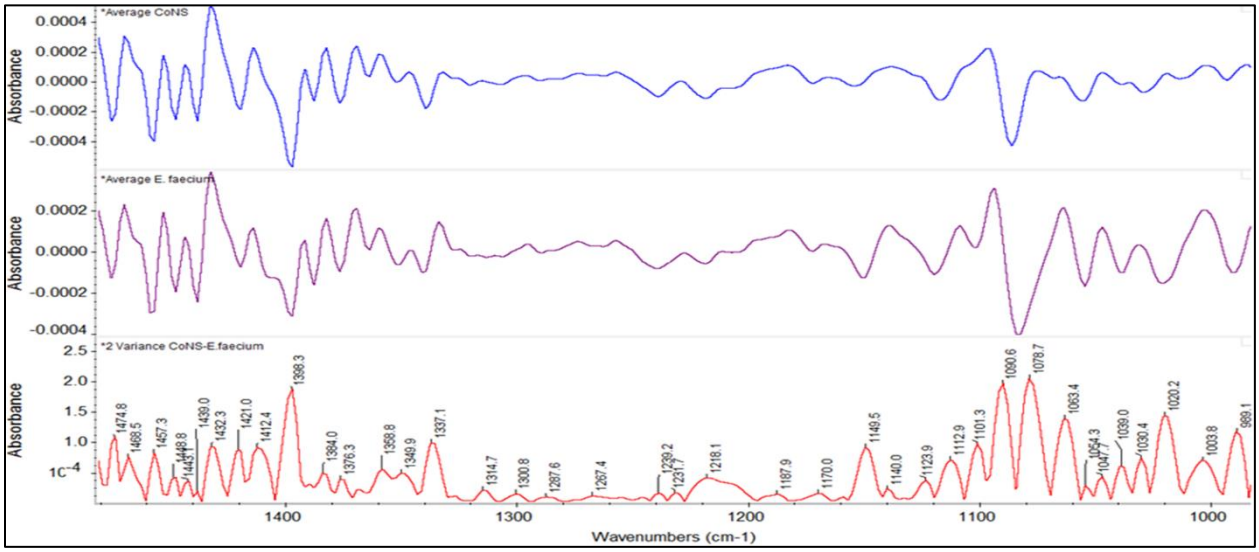

Figure 7.9. Variance spectrum generated using the average CoNS and E. faecium spectra processed using the region of selection 1480-980 cm-1 and using the 2nd derivative.

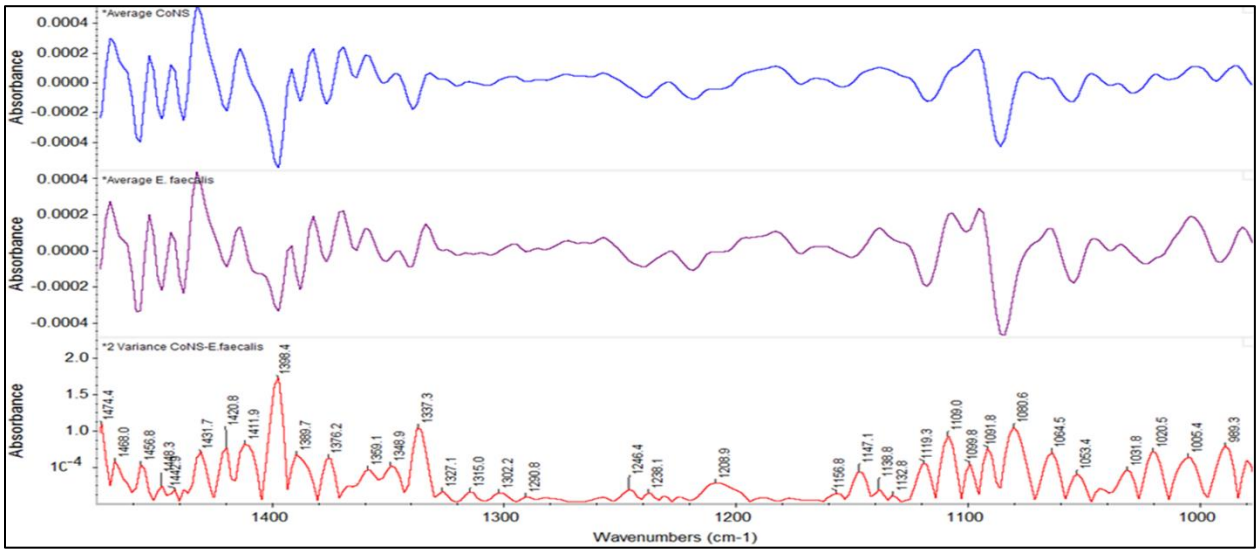

Figure 7.10. Variance spectrum generated using the average CoNS and E. faecalis spectra processed using the region of selection 1480-980 cm-1 and using the 2nd derivative.

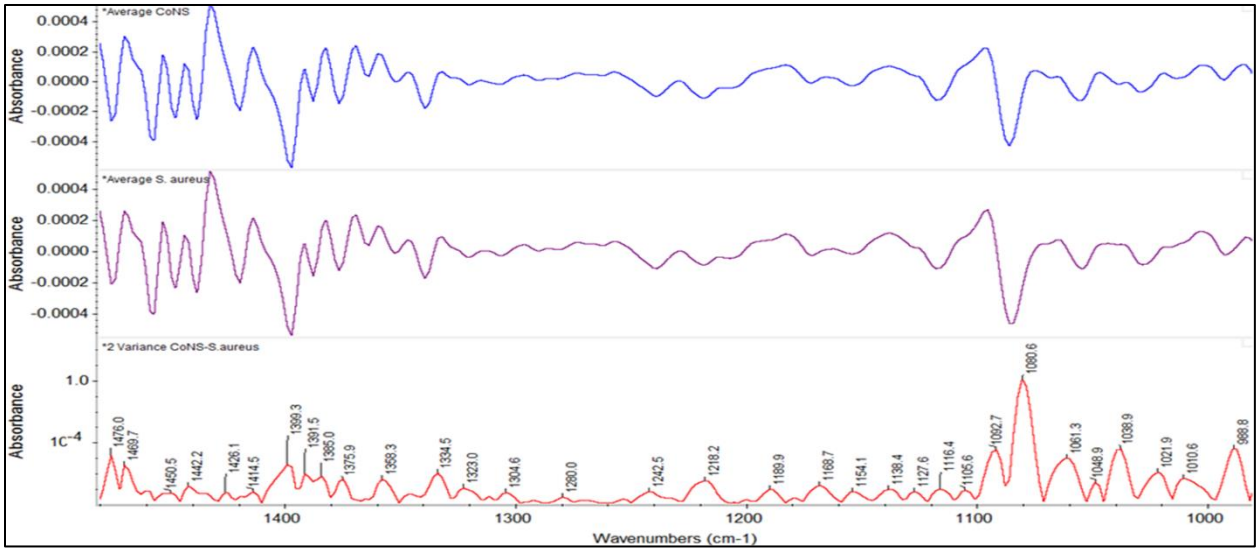

Figure 7.11. Variance spectrum generated using the average E. faecium and S. aureus spectra processed using the region of selection 1480-980 cm-1 and using the 2nd derivative.

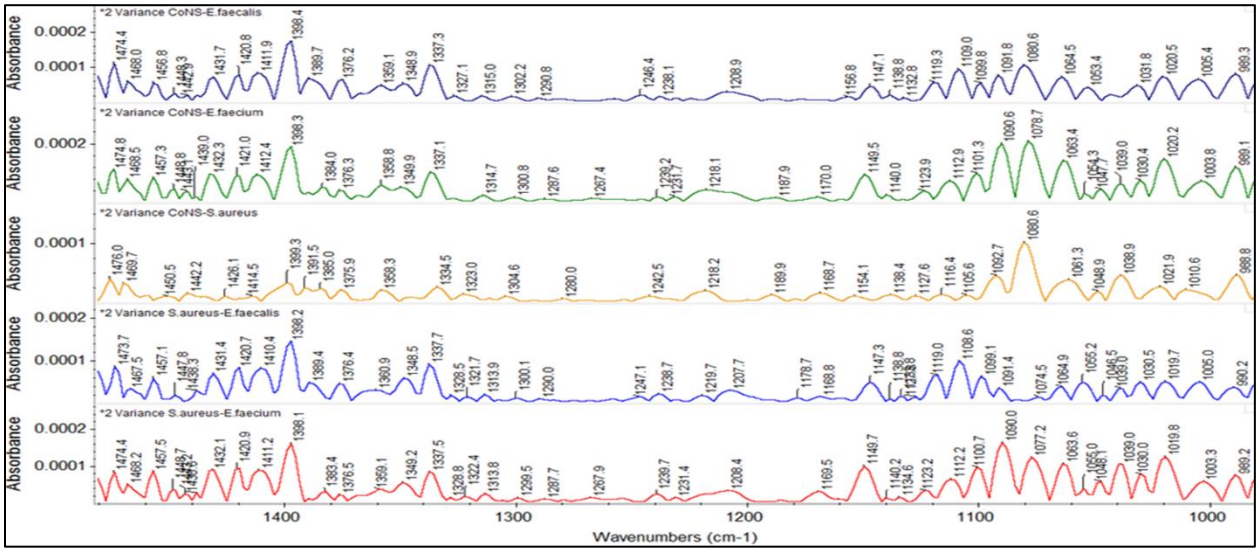

Figure 7.12. Summary compilation of the variance spectra using the average spectra processed using the 2nd derivative through the region of selection 1480-980 cm-1.

Table 5– Summary of Peak Identification between 1st and 2nd Derivative CoNS and *E. faecalis* Variance Spectra and the Biomolecule Peak Assignment for the 2nd Derivative Variance Spectra.

Comparision of CoNS and E. faecalis Variance Spectra				
Peak #	**1st Derivative**	**2nd Derivative**	**Biomolecule Peak Assignment**	**References**
1	1473.8	1474.4	Nucleic Acids	Banyay et al.
2	1455.2	1468.0	Lipid Proteins	Davis et al.
3	1420.0	1456.8	Nucleic Acids	Banyay et al.
4	1398.8	1448.3	Protein	Mello et al.
5	1335.8	1442.9	Nucleic Acids	Banyay et al.
6	1211.9	1431.7	Nucleic Acids	Banyay et al.
7	1122.5	1420.8	Nucleic Acids	Banyay et al.
8	1080.1	1411.9	Nucleic Acids	Banyay et al.
9	1020.7	1398.4	Nucleic Acids	Banyay et al.
10		1389.7	Nucleic Acids	Banyay et al.
11		1376.2	Nucleic Acids	Banyay et al.
12		1359.1	Nucleic Acids	Banyay et al.
13		1348.9	Nucleic Acids	Banyay et al.
14		1337.3	Nucleic Acids	Banyay et al.
15		1327.1	Nucleic Acids	Banyay et al.
16		1315.0	Protein	Kirkwood
17		1302.2	Protein	Davis et al.
18		1290.8	Protein	Davis et al.
19		1246.4	Protein	Davis et al.
20		1238.1	Protein	Davis et al.
21		1208.9	Protein	Davis et al.
22		1156.8	Polysaccharides	Davis et al.
23		1147.1	Polysaccharides	Davis et al.
24		1138.8	Polysaccharides	Davis et al.
25		1132.8	Polysaccharides	Davis et al.
26		1119.3	Polysaccharides	Davis et al.
27		1109.0	Polysaccharides	Davis et al.
28		1099.8	Polysaccharides	Davis et al.
29		1091.8	Polysaccharides	Davis et al.
30		1080.6	Polysaccharides	Davis et al.
31		1064.5	Polysaccharides	Davis et al.
32		1053.4	Polysaccharides	Davis et al.
33		1031.8	Polysaccharides	Davis et al.
34		1020.5	Polysaccharides	Davis et al.
35		1005.4	Polysaccharides	Davis et al.
36		989.3	Polysaccharides	Davis et al.

Table 6 – Summary of Peak Identification between 1st and 2nd Derivative CoNS and *E. faecium* Variance Spectra and the Biomolecule Peak Assignment for the 2nd Derivative Variance Spectra.

Comparision of CoNS and E. faecium Variance Spectra				
Peak #	**1st Derivative**	**2nd Derivative**	**Biomolecule Peak Assignment**	**References**
1	1474.7	1474.8	Nucleic Acids	Banyay et al.
2	1456.7	1468.5	Lipid Proteins	Davis et al.
3	1398.5	1457.3	Nucleic Acids	Banyay et al.
4	1357.2	1448.8	Protein	Mello et al.
5	1335.3	1443.1	Nucleic Acids	Banyay et al.
6	1216.8	1439.0	Nucleic Acids	Banyay et al.
7	1147.7	1432.3	Nucleic Acids	Banyay et al.
8	1102.2	1421.0	Nucleic Acids	Banyay et al.
9	1077.4	1412.4	Nucleic Acids	Banyay et al.
10	1020.4	1398.3	Nucleic Acids	Banyay et al.
11		1384.0	Nucleic Acids	Banyay et al.
12		1376.3	Nucleic Acids	Banyay et al.
13		1358.8	Nucleic Acids	Banyay et al.
14		1349.9	Nucleic Acids	Banyay et al.
15		1337.1	Nucleic Acids	Banyay et al.
16		1314.7	Protein	Kirkwood
17		1300.8	Protein	Davis et al.
18		1287.6	Protein	Davis et al.
19		1267.4	Protein	Davis et al.
20		1239.2	Protein	Davis et al.
21		1231.7	Protein	Davis et al.
22		1218.1	Protein	Davis et al.
23		1187.9	Polysaccharides	Davis et al.
24		1170.0	Polysaccharides	Davis et al.
25		1149.5	Polysaccharides	Davis et al.
26		1140.0	Polysaccharides	Davis et al.
27		1123.9	Polysaccharides	Davis et al.
28		1112.9	Polysaccharides	Davis et al.
29		1101.3	Polysaccharides	Davis et al.
30		1090.6	Polysaccharides	Davis et al.
31		1078.7	Polysaccharides	Davis et al.
32		1063.4	Polysaccharides	Davis et al.
33		1054.3	Polysaccharides	Davis et al.
34		1047.7	Polysaccharides	Davis et al.
35		1039.0	Polysaccharides	Davis et al.
36		1030.4	Polysaccharides	Davis et al.
37		1020.2	Polysaccharides	Davis et al.
38		1003.8	Polysaccharides	Davis et al.
39		989.1	Polysaccharides	Davis et al.

Table 7 – Summary of Peak Identification between 1st and 2nd Derivative CoNS and *S. aureus* Variance Spectra and the Biomolecule Peak Assignment for the 2nd Derivative Variance Spectra.

Comparision of CoNS and S. aureus Variance Spectra				
Peak #	**1st Derivative**	**2nd Derivative**	**Biomolecule Peak Assignment**	**References**
1	1462.0	1476.0	Nucleic Acids	Banyay et al.
2	1401.9	1469.7	Lipid Proteins	Davis et al.
3	1385.7	1450.5	Protein	Mello et al.
4	1359.7	1442.2	Nucleic Acids	Banyay et al.
5	1336.4	1426.1	Nucleic Acids	Banyay et al.
6	1301.2	1414.5	Nucleic Acids	Banyay et al.
7	1218.5	1399.3	Nucleic Acids	Banyay et al.
8	1171.4	1391.5	Nucleic Acids	Banyay et al.
9	1140.5	1385.0	Nucleic Acids	Banyay et al.
10	1080.9	1375.9	Nucleic Acids	Banyay et al.
11	1039.5	1358.3	Nucleic Acids	Banyay et al.
12	988.6	1334.5	Nucleic Acids	Banyay et al.
13		1323.0	Nucleic Acids	Banyay et al.
14		1304.6	Protein	Davis et Al.
15		1280.0	Protein	Davis et Al.
16		1242.5	Protein	Davis et Al.
17		1218.2	Protein	Davis et Al.
18		1189.9	Polysaccharides	Davis et Al.
19		1168.7	Polysaccharides	Davis et Al.
20		1154.1	Polysaccharides	Davis et Al.
21		1138.4	Polysaccharides	Davis et Al.
22		1127.6	Polysaccharides	Davis et Al.
23		1116.4	Polysaccharides	Davis et Al.
24		1105.6	Polysaccharides	Davis et Al.
25		1092.7	Polysaccharides	Davis et Al.
26		1080.6	Polysaccharides	Davis et Al.
27		1061.3	Polysaccharides	Davis et Al.
28		1048.9	Polysaccharides	Davis et Al.
29		1038.9	Polysaccharides	Davis et Al.
30		1021.9	Polysaccharides	Davis et Al.
31		1010.6	Polysaccharides	Davis et Al.
32		988.8	Polysaccharides	Davis et Al.

Table 8 – Summary of Peak Identification between 1st and 2nd Derivative *S. aureus* and *E. faecium* Variance Spectra and the Biomolecule Peak Assignment for the 2nd Derivative Variance Spectra.

Comparision of S. aureus and E. faecium Variance Spectra				
Peak #	**1st Derivative**	**2nd Derivative**	**Biomolecule Peak Assignment**	**References**
1	1457.2	1474.4	Nucleic Acids	Banyay et al.
2	1420.5	1468.2	Lipid Proteins	Davis et al.
3	1397.9	1457.5	Nucleic Acids	Banyay et al.
4	1355.2	1448.7	Protein	Mello et al.
5	1334.7	1438.6	Nucleic Acids	Banyay et al.
6	1215.4	1432.1	Nucleic Acids	Banyay et al.
7	1147.3	1420.9	Nucleic Acids	Banyay et al.
8	1102.7	1411.2	Nucleic Acids	Banyay et al.
9	1074.6	1398.1	Nucleic Acids	Banyay et al.
10	1020.6	1383.4	Nucleic Acids	Banyay et al.
11		1376.5	Nucleic Acids	Banyay et al.
12		1359.1	Nucleic Acids	Banyay et al.
13		1349.2	Nucleic Acids	Banyay et al.
14		1337.5	Nucleic Acids	Banyay et al.
15		1328.8	Nucleic Acids	Banyay et al.
16		1322.4	Nucleic Acids	Banyay et al.
17		1313.8	Protein	Mello et Al.
18		1299.5	Protein	Davis et Al.
19		1287.7	Protein	Davis et Al.
20		1267.9	Protein	Davis et Al.
21		1239.7	Phospholipids	Banyay et al.
22		1231.4	Phospholipids	Banyay et al.
23		1208.4	Polysaccharides	Davis et Al.
24		1169.5	Polysaccharides	Davis et Al.
25		1149.7	Polysaccharides	Davis et Al.
26		1140.2	Polysaccharides	Davis et Al.
27		1134.6	Polysaccharides	Davis et Al.
28		1123.2	Polysaccharides	Davis et Al.
29		1112.2	Polysaccharides	Davis et Al.
30		1100.7	Polysaccharides	Davis et Al.
31		1090.0	Polysaccharides	Davis et Al.
32		1077.2	Polysaccharides	Davis et Al.
33		1063.6	Polysaccharides	Davis et Al.
34		1055.0	Polysaccharides	Davis et Al.
35		1048.1	Polysaccharides	Davis et Al.
36		1039.0	Polysaccharides	Davis et Al.
37		1030.0	Polysaccharides	Davis et Al.
38		1019.8	Polysaccharides	Davis et Al.
39		1003.3	Polysaccharides	Davis et Al.
40		989.2	Polysaccharides	Davis et Al.

Table 9 – Summary of Peak Identification between 1st and 2nd Derivative *S. aureus* and *E. faecalis* Variance Spectra and the Biomolecule Peak Assignment for the 2nd Derivative Variance Spectra.

Comparision of S. aureus and E. faecalis Variance Spectra				
Peak #	**1st Derivative**	**2nd Derivative**	**Biomolecule Peak Assignment**	**References**
1	1456.0	1473.7	Nucleic Acids	Banyay et al.
2	1420.7	1467.5	Lipid Proteins	Davis et al.
3	1398.2	1457.1	Nucleic Acids	Banyay et al.
4	1335.6	1447.8	Protein	Mello et al.
5	1208.3	1438.3	Nucleic Acids	Banyay et al.
6	1133.8	1431.4	Nucleic Acids	Banyay et al.
7	1053.5	1420.7	Nucleic Acids	Banyay et al.
8	1021.5	1410.4	Nucleic Acids	Banyay et al.
9	990.7	1398.2	Nucleic Acids	Banyay et al.
10		1389.4	Nucleic Acids	Banyay et al.
11		1376.4	Nucleic Acids	Banyay et al.
12		1360.9	Nucleic Acids	Banyay et al.
13		1348.5	Nucleic Acids	Banyay et al.
14		1337.7	Nucleic Acids	Banyay et al.
15		1328.5	Nucleic Acids	Banyay et al.
16		1321.7	Nucleic Acids	Banyay et al.
17		1313.9	Protein	Kirkwood
18		1300.1	Protein	Davis et Al.
19		1290.0	Protein	Davis et Al.
20		1247.1	Protein	Davis et Al.
21		1238.7	Phospholipids	Banyay et al.
22		1219.7	Phospholipids	Banyay et al.
23		1207.7	Polysaccharides	Davis et Al.
24		1178.7	Polysaccharides	Davis et Al.
25		1168.8	Polysaccharides	Davis et Al.
26		1147.3	Polysaccharides	Davis et Al.
27		1138.8	Polysaccharides	Davis et Al.
28		1133.8	Polysaccharides	Davis et Al.
29		1127.8	Polysaccharides	Davis et Al.
30		1119.0	Polysaccharides	Davis et Al.
31		1108.6	Polysaccharides	Davis et Al.
32		1099.1	Polysaccharides	Davis et Al.
33		1091.4	Polysaccharides	Davis et Al.
34		1074.5	Polysaccharides	Davis et Al.
35		1064.9	Polysaccharides	Davis et Al.
36		1055.2	Polysaccharides	Davis et Al.
37		1046.5	Polysaccharides	Davis et Al.
38		1039.0	Polysaccharides	Davis et Al.
39		1030.5	Polysaccharides	Davis et Al.
40		1019.7	Polysaccharides	Davis et Al.
41		1005.0	Polysaccharides	Davis et Al.
42		990.2	Polysaccharides	Davis et Al.

7.2 Identification of Enterococcus and Staphylococcus Species Validation Study

In this validation study, 475 unknown bacterial isolates were identified using a prediction software. The 475 isolates did not originate from the spectral database. Table 10 and 11 summarizes the amount of isolates and spectra used in this validation study as unknowns for identification and known species used to create the bacterial database. The database used in the prediction of the unknown isolates consisted of 54 CoNS, 94 *E. faecalis*, 116 *E. faecium* and 211 *S. aureus* combining for a total of 1459 spectra. Table 10 summarizes the name of each strain predicted, the predicted identity, the true identity and the percentage of the three closest spectral matches in the database. The calculation of the spectral matches were based on the selected spectral region 1320-1020 cm^{-1} for the discrimination between *Enterococcus* and *Staphylococcus*.

Table 10 – Summary of the isolates used in the unknown prediction set.

Genus	**Species**	**# of Isolates**	**# of Spectra**
Staphylococcus	*S. aureus*	117	362
CoNS	CoNS	45	138
Enterococcus	*E. faecalis*	41	123
	E. faecium	4	12
	Total	**475**	**635**

Table 11 – Summary of the isolates in the spectral database used for the prediction of the unknowns.

Genus	**Species**	**# of Isolates**	**# of Spectra**
Staphylococcus	*S. aureus*	211	691
CoNS	*S. capitis*	7	28
	S. epidermis	9	26
	S. haemolyticus	3	8
	S. hominis	13	39
	S. lugdunensis	9	31
	S. pseudintermedius	1	2
	S. saprophyticus	3	9
	S. warneri	9	30
Enterococcus	*E. faecalis*	94	256
	E. faecium	116	339
	Total	**475**	**1459**

Illustrated in Figure 8.13 is the results for identification of unknown strain 161150. As shown in Figure 8.13, the computerized identification software provides the method (database) name, date of analysis, sample name, spectral region used and the top 12 spectral matches to the unknown sample which were all *S. aureus*. For this particular strain the percent similarity to the closest spectral match was 94.44% and was fairly constant throughout all 12 spectral matches.

All unknown samples of *Enterococcus* and *Staphylococcus* were correctly identified with a percent similarity of at least 76.4% and at most 95.12%, giving an average of 90.23% Furthermore, there was a total of 6 misidentified unknown bacterial strains. The 6 incorrectly identified unknowns were, unknowns 168601, 154801, 157360, 159263, 166611 and 165885. The identity of each strain were initially assigned using the Vitek II. Each misidentified strain were then plated on 5% Columbia blood agar and incubated for a period of 18-24 hours. The strains were then sub-cultured and incubated once more. The strains were then re-analyzed using the Vitek II and relevant clinical phenotypic methods of identification for each strain. The results depicted that each of the 6 unknown strains were initially misidentified either at the genus level or at the species level. The Vitek II, a gold standard for identification in most major clinical laboratories, can sometimes misidentify bacterial strains for various reasons. In this case, the use

of ATR-FTIR in conjunction with the Vitek II, led to the detection of 6 incorrectly identified strains. The overall results of this validation study demonstrates the positive potential of ATR-FTIR spectroscopy for bacteria identification.

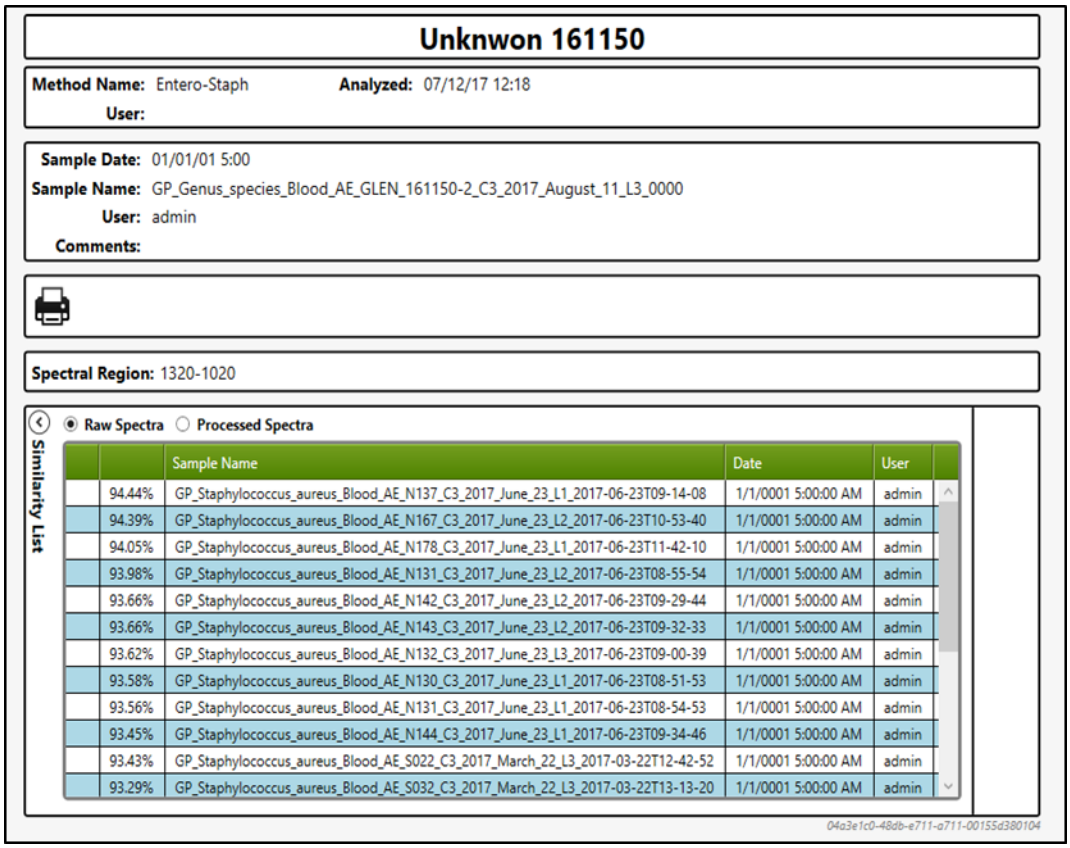

Figure 7.13. Computerized Screenshot of the identification software results for Unknown 161150, listing the 12 closest spectral percentage matches in the database.

Table 12 – List of Unknown Enterococcus and Staphylococcus Validation Results.

ID #	File Name	True ID	C1 ID	1st Similarity %	2nd Similarity %	3rd Similarity %
1	GP_Genus_species_Blood_ABD_AE_GLEN_167363-4_C3_2017_August_23_L1_0000	S. aureus	S. aureus	90.75	90.61	90.47
2	GP_Genus_species_Blood_ABD_AE_GLEN_167363-4_C3_2017_August_23_L2_0000	S. aureus	S. aureus	92.57	92.38	92.3
3	GP_Genus_species_Blood_ABD_AE_GLEN_167363-4_C3_2017_August_23_L3_0000	S. aureus	S. aureus	91.83	91.29	91.15
4	GP_Genus_species_Blood_ABD_AE_GLEN_167535-1_C3_2017_August_23_L1_0000	CoNS	CoNS	88.76	88.26	87.92
5	GP_Genus_species_Blood_ABD_AE_GLEN_167535-1_C3_2017_August_23_L2_0000	CoNS	CoNS	88.75	88.24	88.19
6	GP_Genus_species_Blood_ABD_AE_GLEN_167535-1_C3_2017_August_23_L3_0000	CoNS	CoNS	88.89	88.68	88.45
7	GP_Genus_species_Blood_ABD_AE_GLEN_168091-3_C3_2017_August_23_L1_0001	CoNS	CoNS	91.33	90.79	90.29
8	GP_Genus_species_Blood_ABD_AE_GLEN_168091-3_C3_2017_August_23_L2_0000	CoNS	CoNS	89.92	88.85	88.57
9	GP_Genus_species_Blood_ABD_AE_GLEN_168091-3_C3_2017_August_23_L3_0000	CoNS	CoNS	90.35	90.09	89.81
10	GP_Genus_species_Blood_ABD_AE_GLEN_168312-2_C3_2017_August_24_L1_0000	S. aureus	S. aureus	93.77	93.06	92.74
11	GP_Genus_species_Blood_ABD_AE_GLEN_168312-2_C3_2017_August_24_L2_0000	S. aureus	S. aureus	93.56	93.26	93.14
12	GP_Genus_species_Blood_ABD_AE_GLEN_168312-2_C3_2017_August_24_L3_0000	S. aureus	S. aureus	93.55	93.04	92.98
13	GP_Genus_species_Blood_ABD_AE_GLEN_168322-1_C3_2017_August_24_L1_0000	CoNS	CoNS	90.32	89.8	89.62
14	GP_Genus_species_Blood_ABD_AE_GLEN_168322-1_C3_2017_August_24_L2_0000	CoNS	CoNS	89.18	89.04	88.62
15	GP_Genus_species_Blood_ABD_AE_GLEN_168322-1_C3_2017_August_24_L3_0000	CoNS	CoNS	89.79	89.57	89.05
16	GP_Genus_species_Blood_ABD_AE_GLEN_168322-2_C3_2017_August_24_L1_0000	CoNS	CoNS	91.77	91.25	90.56
17	GP_Genus_species_Blood_ABD_AE_GLEN_168322-2_C3_2017_August_24_L2_0000	CoNS	CoNS	92.03	91.08	90.92
18	GP_Genus_species_Blood_ABD_AE_GLEN_168322-2_C3_2017_August_24_L3_0000	CoNS	CoNS	92.57	91.96	91.76
19	GP_Genus_species_Blood_ABD_AE_GLEN_168322-3_C3_2017_August_24_L1_0000	CoNS	CoNS	89.71	87.19	87.03
20	GP_Genus_species_Blood_ABD_AE_GLEN_168322-3_C3_2017_August_24_L2_0000	CoNS	CoNS	90.47	89.9	89.47
21	GP_Genus_species_Blood_ABD_AE_GLEN_168322-3_C3_2017_August_24_L3_0000	CoNS	CoNS	90.95	88.69	87.62
22	GP_Genus_species_Blood_ABD_AE_GLEN_168601-2_C3_2017_August_24_L1_0000	S. aureus	CoNS	90.57	90	89.75
23	GP_Genus_species_Blood_ABD_AE_GLEN_168601-2_C3_2017_August_24_L2_0000	S. aureus	CoNS	89.5	88.91	88.59
24	GP_Genus_species_Blood_ABD_AE_GLEN_168601-2_C3_2017_August_24_L3_0000	S. aureus	CoNS	89.81	89.41	89.04
25	GP_Genus_species_Blood_AE_GLEN_154801-1_C3_2017_August_11_L1_0000	Staphylococcus	E. faecium	69.25	69.2	68.88
26	GP_Genus_species_Blood_AE_GLEN_154801-1_C3_2017_August_11_L2_0000	Staphylococcus	E. faecium	71.16	70.86	68.6
27	GP_Genus_species_Blood_AE_GLEN_154801-1_C3_2017_August_11_L3_0000	Staphylococcus	E. faecium	65.79	65.74	65.68
28	GP_Genus_species_Blood_AE_GLEN_155227_C3_2017_August_17_L1_0000	E. faecalis	E. faecalis	89.71	89.35	88.85
29	GP_Genus_species_Blood_AE_GLEN_155227_C3_2017_August_17_L2_0000	E. faecalis	E. faecalis	87.8	86.2	85.99
30	GP_Genus_species_Blood_AE_GLEN_155227_C3_2017_August_17_L3_0000	E. faecalis	E. faecalis	87.87	86.99	86.32

31	GP_Genus_species_Blood_AE_GLEN_155387-4_C3_2017_August_08_L1_0000	S. aureus	S. aureus	91.1	90.25	90.06
32	GP_Genus_species_Blood_AE_GLEN_155387-4_C3_2017_August_08_L2_0000	S. aureus	S. aureus	89.83	88.82	88.77
33	GP_Genus_species_Blood_AE_GLEN_155387-4_C3_2017_August_08_L3_0000	S. aureus	S. aureus	89.26	88.98	88.96
34	GP_Genus_species_Blood_AE_GLEN_155387-4_C3_2017_August_09_L1_N_0000	S. aureus	S. aureus	87.8	87.6	85.63
35	GP_Genus_species_Blood_AE_GLEN_155387-4_C3_2017_August_09_L2_N_0000	S. aureus	S. aureus	87.81	87.79	87.28
36	GP_Genus_species_Blood_AE_GLEN_155387-4_C3_2017_August_09_L3_N_0000	S. aureus	S. aureus	86.42	86.39	86.27
37	GP_Genus_species_Blood_AE_GLEN_155660-2_C3_2017_August_08_L1_0000	S. aureus	S. aureus	88.88	87.25	86.93
38	GP_Genus_species_Blood_AE_GLEN_155660-2_C3_2017_August_08_L2_0000	S. aureus	S. aureus	88.08	86.58	86.27
39	GP_Genus_species_Blood_AE_GLEN_155660-2_C3_2017_August_08_L3_0000	S. aureus	S. aureus	88.44	88.36	88.09
40	GP_Genus_species_Blood_AE_GLEN_155660-2_C3_2017_August_09_L1_N_0000	S. aureus	S. aureus	83.47	82.12	82.1
41	GP_Genus_species_Blood_AE_GLEN_155660-2_C3_2017_August_09_L2_N_0000	S. aureus	S. aureus	86.06	85.8	85.8
42	GP_Genus_species_Blood_AE_GLEN_155660-2_C3_2017_August_09_L3_N_0000	S. aureus	S. aureus	87.56	85.03	84.99
43	GP_Genus_species_Blood_AE_GLEN_155663-4_C3_2017_August_08_L1_0000	S. aureus	S. aureus	84.84	84.7	84.57
44	GP_Genus_species_Blood_AE_GLEN_155663-4_C3_2017_August_08_L2_0000	S. aureus	S. aureus	91.09	90.88	90.85
45	GP_Genus_species_Blood_AE_GLEN_155663-4_C3_2017_August_08_L3_0000	S. aureus	S. aureus	92.67	92.53	92.52
46	GP_Genus_species_Blood_AE_GLEN_155663-4_C3_2017_August_09_L1_N_0000	S. aureus	S. aureus	89.14	88.95	88.83
47	GP_Genus_species_Blood_AE_GLEN_155663-4_C3_2017_August_09_L2_N_0000	S. aureus	S. aureus	89.81	89.48	89.11
48	GP_Genus_species_Blood_AE_GLEN_155663-4_C3_2017_August_09_L3_N_0000	S. aureus	S. aureus	88.69	88.58	88.28
49	GP_Genus_species_Blood_AE_GLEN_156686-2_C3_2017_August_08_L1_0000	S. aureus	S. aureus	90.37	89.84	89.79
50	GP_Genus_species_Blood_AE_GLEN_156686-2_C3_2017_August_08_L2_0000	S. aureus	S. aureus	90.26	90.15	90.07
51	GP_Genus_species_Blood_AE_GLEN_156686-2_C3_2017_August_08_L3_0000	S. aureus	S. aureus	89.34	89.09	89.06
52	GP_Genus_species_Blood_AE_GLEN_156686-2_C3_2017_August_09_L1_N_0000	S. aureus	S. aureus	88.5	88.46	88.3
53	GP_Genus_species_Blood_AE_GLEN_156686-2_C3_2017_August_09_L2_N_0000	S. aureus	S. aureus	87.37	87.13	87
54	GP_Genus_species_Blood_AE_GLEN_156686-2_C3_2017_August_09_L3_N_0000	S. aureus	S. aureus	87.95	87.86	87.68
55	GP_Genus_species_Blood_AE_GLEN_156701-2_C3_2017_August_08_L1_0000	S. aureus	S. aureus	91.37	90.95	90.68
56	GP_Genus_species_Blood_AE_GLEN_156701-2_C3_2017_August_08_L2_0000	S. aureus	S. aureus	90.79	90.39	90.17
57	GP_Genus_species_Blood_AE_GLEN_156701-2_C3_2017_August_08_L3_0000	S. aureus	S. aureus	90.79	90.4	90.35
58	GP_Genus_species_Blood_AE_GLEN_156701-2_C3_2017_August_09_L1_N_0000	S. aureus	S. aureus	88.8	88.43	88.37
59	GP_Genus_species_Blood_AE_GLEN_156701-2_C3_2017_August_09_L2_N_0000	S. aureus	S. aureus	92.12	92	91.76
60	GP_Genus_species_Blood_AE_GLEN_156701-2_C3_2017_August_09_L3_N_0000	S. aureus	S. aureus	91.07	90.53	90.29
61	GP_Genus_species_Blood_AE_GLEN_156717-4_C3_2017_August_08_L1_0000	S. aureus	S. aureus	92.3	92.23	92.17
62	GP_Genus_species_Blood_AE_GLEN_156717-4_C3_2017_August_08_L2_0000	S. aureus	S. aureus	93.43	92.91	92.51
63	GP_Genus_species_Blood_AE_GLEN_156717-4_C3_2017_August_08_L3_0000	S. aureus	S. aureus	93.37	93.16	92.04
64	GP_Genus_species_Blood_AE_GLEN_156717-4_C3_2017_August_09_L1_N_0000	S. aureus	S. aureus	87.88	87.75	87.69
65	GP_Genus_species_Blood_AE_GLEN_156717-4_C3_2017_August_09_L2_N_0000	S. aureus	S. aureus	91.13	91.12	90.59

66	GP_Genus_species_Blood_AE_GLEN_156717-4_C3_2017_August_09_L3_N_0000	S. aureus	S. aureus	89.21	89.18	88.94
67	GP_Genus_species_Blood_AE_GLEN_156719-4_C3_2017_August_08_L1_0000	S. aureus	S. aureus	90.29	90.19	90.14
68	GP_Genus_species_Blood_AE_GLEN_156719-4_C3_2017_August_08_L2_0000	S. aureus	S. aureus	91.71	91.69	91.63
69	GP_Genus_species_Blood_AE_GLEN_156719-4_C3_2017_August_08_L3_0000	S. aureus	S. aureus	91.88	91.43	91.24
70	GP_Genus_species_Blood_AE_GLEN_156719-4_C3_2017_August_09_L1_N_0000	S. aureus	S. aureus	92.03	91.04	90.97
71	GP_Genus_species_Blood_AE_GLEN_156719-4_C3_2017_August_09_L2_N_0000	S. aureus	S. aureus	90.82	90.24	90.24
72	GP_Genus_species_Blood_AE_GLEN_156719-4_C3_2017_August_09_L3_N_0000	S. aureus	S. aureus	89.5	89.25	89.2
73	GP_Genus_species_Blood_AE_GLEN_156814-3_C3_2017_August_08_L1_0000	CoNS	CoNS	86.07	85.9	85.78
74	GP_Genus_species_Blood_AE_GLEN_156814-3_C3_2017_August_08_L2_0000	CoNS	CoNS	86.6	86.22	86.13
75	GP_Genus_species_Blood_AE_GLEN_156814-3_C3_2017_August_08_L3_0000	CoNS	CoNS	86.95	86.89	86.6
76	GP_Genus_species_Blood_AE_GLEN_156814-3_C3_2017_August_09_L1_N_0000	CoNS	CoNS	86.53	86.01	85.86
77	GP_Genus_species_Blood_AE_GLEN_156814-3_C3_2017_August_09_L2_N_0000	CoNS	CoNS	86.55	86.3	85.28
78	GP_Genus_species_Blood_AE_GLEN_156814-3_C3_2017_August_09_L3_N_0000	CoNS	CoNS	85.72	85.61	85.23
79	GP_Genus_species_Blood_AE_GLEN_156814-4_C3_2017_August_08_L1_0000	CoNS	CoNS	87.39	87.14	85.95
80	GP_Genus_species_Blood_AE_GLEN_156814-4_C3_2017_August_08_L2_0000	CoNS	CoNS	85.43	84.93	83.9
81	GP_Genus_species_Blood_AE_GLEN_156814-4_C3_2017_August_08_L3_0000	CoNS	CoNS	87.46	87.14	85.88
82	GP_Genus_species_Blood_AE_GLEN_156814-4_C3_2017_August_09_L1_N_0000	CoNS	CoNS	85.1	84.97	84.77
83	GP_Genus_species_Blood_AE_GLEN_156814-4_C3_2017_August_09_L2_N_0000	CoNS	CoNS	86.34	86.13	84.99
84	GP_Genus_species_Blood_AE_GLEN_156814-4_C3_2017_August_09_L3_N_0000	CoNS	CoNS	85.36	85.34	84.23
85	GP_Genus_species_Blood_AE_GLEN_156864-3_C3_2017_August_08_L1_0000	S. aureus	S. aureus	90.84	90.4	90.26
86	GP_Genus_species_Blood_AE_GLEN_156864-3_C3_2017_August_08_L2_0000	S. aureus	S. aureus	92.95	92.46	92.08
87	GP_Genus_species_Blood_AE_GLEN_156864-3_C3_2017_August_08_L3_0000	S. aureus	S. aureus	92.33	91.98	91.61
88	GP_Genus_species_Blood_AE_GLEN_156864-3_C3_2017_August_09_L1_N_0000	S. aureus	S. aureus	89.98	89.55	89.49
89	GP_Genus_species_Blood_AE_GLEN_156864-3_C3_2017_August_09_L2_N_0000	S. aureus	S. aureus	88.7	88.33	88.2
90	GP_Genus_species_Blood_AE_GLEN_156864-3_C3_2017_August_09_L3_N_0000	S. aureus	S. aureus	88.82	88.42	88.34
91	GP_Genus_species_Blood_AE_GLEN_156962_C3_2017_August_10_L1_0000	CoNS	CoNS	82.59	82.38	81.32
92	GP_Genus_species_Blood_AE_GLEN_156962_C3_2017_August_10_L2_0000	CoNS	CoNS	81.48	81.3	79.11
93	GP_Genus_species_Blood_AE_GLEN_156962_C3_2017_August_10_L3_0000	CoNS	CoNS	83.69	83.49	81.23
94	GP_Genus_species_Blood_AE_GLEN_156962_C3_2017_August_11_L1_0000	CoNS	CoNS	92.94	92.45	92.29
95	GP_Genus_species_Blood_AE_GLEN_156962_C3_2017_August_11_L2_0000	CoNS	CoNS	93.43	92.67	92.09
96	GP_Genus_species_Blood_AE_GLEN_156962_C3_2017_August_11_L3_0000	CoNS	CoNS	93.49	91.8	91.48
97	GP_Genus_species_Blood_AE_GLEN_157360-3_C3_2017_August_08_L1_0000	Staphylococcus	E. faecalis	86.19	85.83	85.64
98	GP_Genus_species_Blood_AE_GLEN_157360-3_C3_2017_August_08_L2_0000	Staphylococcus	E. faecalis	87.31	85.98	85.95
99	GP_Genus_species_Blood_AE_GLEN_157360-3_C3_2017_August_08_L3_0000	Staphylococcus	E. faecalis	87.07	85.62	85.53
100	GP_Genus_species_Blood_AE_GLEN_157365_C3_2017_August_08_L1_0000	S. aureus	S. aureus	94.6	94.33	93.7

101	GP_Genus_species_Blood_AE_GLEN_15736 5_C3_2017_August_08_L2_0000	S. aureus	S. aureus	94.06	93.81	93.81
102	GP_Genus_species_Blood_AE_GLEN_15736 5_C3_2017_August_08_L3_0000	S. aureus	S. aureus	93.98	93.7	93.03
103	GP_Genus_species_Blood_AE_GLEN_15736 5_C3_2017_August_09_L1_N_0000	S. aureus	S. aureus	91.91	91.12	90.77
104	GP_Genus_species_Blood_AE_GLEN_15736 5_C3_2017_August_09_L2_N_0000	S. aureus	S. aureus	89.64	89.46	89.2
105	GP_Genus_species_Blood_AE_GLEN_15736 5_C3_2017_August_09_L3_N_0000	S. aureus	S. aureus	91.72	91.6	91.42
106	GP_Genus_species_Blood_AE_GLEN_15736 6_C3_2017_August_08_L1_0000	S. aureus	S. aureus	94.4	93.95	93.69
107	GP_Genus_species_Blood_AE_GLEN_15736 6_C3_2017_August_08_L2_0000	S. aureus	S. aureus	92.74	91.73	91.61
108	GP_Genus_species_Blood_AE_GLEN_15736 6_C3_2017_August_08_L3_0000	S. aureus	S. aureus	90.37	90.16	89.94
109	GP_Genus_species_Blood_AE_GLEN_15736 6_C3_2017_August_09_L1_N_0000	S. aureus	S. aureus	90.56	90.34	90.32
110	GP_Genus_species_Blood_AE_GLEN_15736 6_C3_2017_August_09_L2_N_0000	S. aureus	S. aureus	90.72	90.31	90.23
111	GP_Genus_species_Blood_AE_GLEN_15736 6_C3_2017_August_09_L3_N_0000	S. aureus	S. aureus	91.61	91.53	91.51
112	GP_Genus_species_Blood_AE_GLEN_15740 5-3_C3_2017_August_08_L1_0000	E. faecalis	E. faecalis	84.88	84.8	84.74
113	GP_Genus_species_Blood_AE_GLEN_15740 5-3_C3_2017_August_08_L2_0000	E. faecalis	E. faecalis	84.92	84.48	84.04
114	GP_Genus_species_Blood_AE_GLEN_15740 5-3_C3_2017_August_08_L3_0000	E. faecalis	E. faecalis	85.94	85.73	85.53
115	GP_Genus_species_Blood_AE_GLEN_15743 7-2_C3_2017_August_08_L1_0000	S. aureus	S. aureus	91.41	90.14	90.06
116	GP_Genus_species_Blood_AE_GLEN_15743 7-2_C3_2017_August_08_L2_0000	S. aureus	S. aureus	90.47	90.4	90.17
117	GP_Genus_species_Blood_AE_GLEN_15743 7-2_C3_2017_August_08_L3_0000	S. aureus	S. aureus	90.99	90.97	90.77
118	GP_Genus_species_Blood_AE_GLEN_15743 7-2_C3_2017_August_09_L1_N_0000	S. aureus	S. aureus	87.89	87.7	87.2
119	GP_Genus_species_Blood_AE_GLEN_15743 7-2_C3_2017_August_09_L2_N_0000	S. aureus	S. aureus	88.86	88.58	88.41
120	GP_Genus_species_Blood_AE_GLEN_15792 0-5_C3_2017_August_10_L1_0000	CoNS	CoNS	84.62	84.3	83.97
121	GP_Genus_species_Blood_AE_GLEN_15792 0-5_C3_2017_August_10_L2_0000	CoNS	CoNS	84.08	84.03	83.58
122	GP_Genus_species_Blood_AE_GLEN_15792 0-5_C3_2017_August_10_L3_0000	CoNS	CoNS	89.96	89.68	89.65
123	GP_Genus_species_Blood_AE_GLEN_15805 5-2_C3_2017_August_08_L1_0000	E. faecalis	E. faecalis	90.4	90.37	90.21
124	GP_Genus_species_Blood_AE_GLEN_15805 5-2_C3_2017_August_08_L2_0000	E. faecalis	E. faecalis	89.96	89.68	89.65
125	GP_Genus_species_Blood_AE_GLEN_15805 5-2_C3_2017_August_08_L3_0000	E. faecalis	E. faecalis	90.91	90.87	89.9
126	GP_Genus_species_Blood_AE_GLEN_15813 7-1_C3_2017_August_08_L1_0000	E. faecalis	E. faecalis	87.73	87.62	87.22
127	GP_Genus_species_Blood_AE_GLEN_15813 7-1_C3_2017_August_08_L2_0000	E. faecalis	E. faecalis	89.09	88.84	88.32
128	GP_Genus_species_Blood_AE_GLEN_15813 7-1_C3_2017_August_08_L3_0000	E. faecalis	E. faecalis	88.62	88.54	88.35
129	GP_Genus_species_Blood_AE_GLEN_15822 1-2_C3_2017_August_10_L1_0000	S. aureus	S. aureus	92.79	92.29	92.11
130	GP_Genus_species_Blood_AE_GLEN_15822 1-2_C3_2017_August_10_L2_0000	S. aureus	S. aureus	90.25	89.97	89.86
131	GP_Genus_species_Blood_AE_GLEN_15822 1-2_C3_2017_August_10_L3_0000	S. aureus	S. aureus	92.81	91.98	91.9
132	GP_Genus_species_Blood_AE_GLEN_15831 4-2_C3_2017_August_08_L1_0000	S. aureus	S. aureus	90.93	90.77	89.91
133	GP_Genus_species_Blood_AE_GLEN_15831 4-2_C3_2017_August_08_L2_0000	S. aureus	S. aureus	91.56	91.49	91.37
134	GP_Genus_species_Blood_AE_GLEN_15831 4-2_C3_2017_August_08_L3_0000	S. aureus	S. aureus	93.11	92.4	92.24
135	GP_Genus_species_Blood_AE_GLEN_15831 4-2_C3_2017_August_09_L1_N_0000	S. aureus	S. aureus	86.33	86.19	86.14

136	GP_Genus_species_Blood_AE_GLEN_158314-2_C3_2017_August_09_L2_N_0000	S. aureus	S. aureus	87.33	85.94	85.88
137	GP_Genus_species_Blood_AE_GLEN_158314-2_C3_2017_August_09_L3_N_0000	S. aureus	S. aureus	87.11	86.02	85.71
138	GP_Genus_species_Blood_AE_GLEN_158359-2_C3_2017_August_08_L1_0000	S. aureus	S. aureus	90.18	90.13	89.8
139	GP_Genus_species_Blood_AE_GLEN_158359-2_C3_2017_August_08_L2_0000	S. aureus	S. aureus	90.59	90.13	89.76
140	GP_Genus_species_Blood_AE_GLEN_158359-2_C3_2017_August_08_L3_0000	S. aureus	S. aureus	89.67	89.62	89.42
141	GP_Genus_species_Blood_AE_GLEN_158359-2_C3_2017_August_09_L1_N_0000	S. aureus	S. aureus	87.16	87.14	86.7
142	GP_Genus_species_Blood_AE_GLEN_158359-2_C3_2017_August_09_L2_N_0000	S. aureus	S. aureus	86.62	86.52	86.46
143	GP_Genus_species_Blood_AE_GLEN_158359-2_C3_2017_August_09_L3_N_0000	S. aureus	S. aureus	86.48	86.22	86.15
144	GP_Genus_species_Blood_AE_GLEN_158389-3_C3_2017_August_08_L1_0000	E. faecalis	E. faecalis	88.32	88.09	87.88
145	GP_Genus_species_Blood_AE_GLEN_158389-3_C3_2017_August_08_L2_0000	E. faecalis	E. faecalis	89.58	88.61	88.25
146	GP_Genus_species_Blood_AE_GLEN_158389-3_C3_2017_August_08_L3_0000	E. faecalis	E. faecalis	90.23	89.61	88.54
147	GP_Genus_species_Blood_AE_GLEN_158444-3_C3_2017_August_10_L1_0000	S. aureus	S. aureus	90.31	89.11	89.07
148	GP_Genus_species_Blood_AE_GLEN_158444-3_C3_2017_August_10_L2_0000	S. aureus	S. aureus	93.24	92.14	91.94
149	GP_Genus_species_Blood_AE_GLEN_158444-3_C3_2017_August_10_L3_0000	S. aureus	S. aureus	92.17	92.02	91.82
150	GP_Genus_species_Blood_AE_GLEN_158449-2_C3_2017_August_10_L1_0000	S. aureus	S. aureus	91.98	90.21	88.71
151	GP_Genus_species_Blood_AE_GLEN_158449-2_C3_2017_August_10_L2_0000	S. aureus	S. aureus	91.38	91.11	91.11
152	GP_Genus_species_Blood_AE_GLEN_158449-2_C3_2017_August_10_L3_0000	S. aureus	S. aureus	92.57	92.51	91.98
153	GP_Genus_species_Blood_AE_GLEN_158450-2_C3_2017_August_10_L1_0000	S. aureus	S. aureus	93.16	92.64	92.6
154	GP_Genus_species_Blood_AE_GLEN_158450-2_C3_2017_August_10_L2_0000	S. aureus	S. aureus	94.04	93.24	93.01
155	GP_Genus_species_Blood_AE_GLEN_158450-2_C3_2017_August_10_L3_0000	S. aureus	S. aureus	91.92	91.8	91.72
156	GP_Genus_species_Blood_AE_GLEN_158488_C3_2017_August_10_L1_0000	S. aureus	S. aureus	92.39	92.34	92.25
157	GP_Genus_species_Blood_AE_GLEN_158488_C3_2017_August_10_L2_0000	S. aureus	S. aureus	91.16	90.85	90.78
158	GP_Genus_species_Blood_AE_GLEN_158488_C3_2017_August_10_L3_0000	S. aureus	S. aureus	91.9	91.84	91.71
159	GP_Genus_species_Blood_AE_GLEN_158561_C3_2017_August_08_L1_0000	E. faecalis	E. faecalis	91.57	91.26	91
160	GP_Genus_species_Blood_AE_GLEN_158561_C3_2017_August_08_L2_0000	E. faecalis	E. faecalis	91.63	91.47	91.19
161	GP_Genus_species_Blood_AE_GLEN_158561_C3_2017_August_08_L3_0000	E. faecalis	E. faecalis	92.55	92.04	91.31
162	GP_Genus_species_Blood_AE_GLEN_158595-2_C3_2017_August_10_L1_0000	S. aureus	S. aureus	89.17	88.68	87.87
163	GP_Genus_species_Blood_AE_GLEN_158595-2_C3_2017_August_10_L2_0000	S. aureus	S. aureus	90.97	88.58	88.33
164	GP_Genus_species_Blood_AE_GLEN_158595-2_C3_2017_August_10_L3_0000	S. aureus	S. aureus	90.71	88.38	88.21
165	GP_Genus_species_Blood_AE_GLEN_158666-6_C3_2017_August_10_L1_0000	S. aureus	S. aureus	91.53	91.19	91.09
166	GP_Genus_species_Blood_AE_GLEN_158666-6_C3_2017_August_10_L2_0000	S. aureus	S. aureus	92.16	91.86	91.74
167	GP_Genus_species_Blood_AE_GLEN_158666-6_C3_2017_August_10_L3_0000	S. aureus	S. aureus	91.54	91.47	90.51
168	GP_Genus_species_Blood_AE_GLEN_158708_C3_2017_August_10_L1_0000	E. faecalis	E. faecalis	89.24	88.78	88.69
169	GP_Genus_species_Blood_AE_GLEN_158708_C3_2017_August_10_L2_0000	E. faecalis	E. faecalis	88.46	88.2	87.98
170	GP_Genus_species_Blood_AE_GLEN_158708_C3_2017_August_10_L3_0000	E. faecalis	E. faecalis	87.53	87.31	86.72

171	GP_Genus_species_Blood_AE_GLEN_158773-4_C3_2017_August_10_L1_0000	E. faecalis	E. faecalis	91.19	92.19	91.05
172	GP_Genus_species_Blood_AE_GLEN_158773-4_C3_2017_August_10_L2_0000	E. faecalis	E. faecalis	93.37	92.65	92.15
173	GP_Genus_species_Blood_AE_GLEN_158773-4_C3_2017_August_10_L3_0000	E. faecalis	E. faecalis	92.77	91.99	91.76
174	GP_Genus_species_Blood_AE_GLEN_158774_C3_2017_August_08_L1_0000	S. aureus	S. aureus	93.69	93.13	92.76
175	GP_Genus_species_Blood_AE_GLEN_158774_C3_2017_August_08_L2_0000	S. aureus	S. aureus	92.59	91.91	91.64
176	GP_Genus_species_Blood_AE_GLEN_158774_C3_2017_August_08_L3_0000	S. aureus	S. aureus	93.36	93.3	93.27
177	GP_Genus_species_Blood_AE_GLEN_158774_C3_2017_August_09_L1_N_0000	S. aureus	S. aureus	91.32	91.23	90.92
178	GP_Genus_species_Blood_AE_GLEN_158774_C3_2017_August_09_L2_N_0000	S. aureus	S. aureus	90.96	90.72	90.71
179	GP_Genus_species_Blood_AE_GLEN_158774_C3_2017_August_09_L3_N_0000	S. aureus	S. aureus	90.52	90.2	90.11
180	GP_Genus_species_Blood_AE_GLEN_158787_C3_2017_August_08_L1_0000	S. aureus	S. aureus	93.69	93.13	92.76
181	GP_Genus_species_Blood_AE_GLEN_158787_C3_2017_August_08_L2_0000	S. aureus	S. aureus	94.17	93.94	93.92
182	GP_Genus_species_Blood_AE_GLEN_158787_C3_2017_August_08_L3_0000	S. aureus	S. aureus	93.31	93.03	92.9
183	GP_Genus_species_Blood_AE_GLEN_158787_C3_2017_August_09_L1_N_0000	S. aureus	S. aureus	92.4	92.29	91.98
184	GP_Genus_species_Blood_AE_GLEN_158787_C3_2017_August_09_L2_N_0000	S. aureus	S. aureus	92.69	92.6	92.52
185	GP_Genus_species_Blood_AE_GLEN_158787_C3_2017_August_09_L3_N_0000	S. aureus	S. aureus	92.56	92.12	92.09
186	GP_Genus_species_Blood_AE_GLEN_158801_C3_2017_August_08_L1_0000	S. aureus	S. aureus	89.97	89.8	87.92
187	GP_Genus_species_Blood_AE_GLEN_158801_C3_2017_August_08_L2_0000	S. aureus	S. aureus	90.41	90.1	89.75
188	GP_Genus_species_Blood_AE_GLEN_158801_C3_2017_August_08_L3_0000	S. aureus	S. aureus	89.97	89.89	89.79
189	GP_Genus_species_Blood_AE_GLEN_158801_C3_2017_August_09_L1_N_0000	S. aureus	S. aureus	90.24	89.99	89.98
190	GP_Genus_species_Blood_AE_GLEN_158801_C3_2017_August_09_L2_N_0000	S. aureus	S. aureus	89.1	88.91	88.57
191	GP_Genus_species_Blood_AE_GLEN_158801_C3_2017_August_09_L3_N_0000	S. aureus	S. aureus	88.33	88.32	88.1
192	GP_Genus_species_Blood_AE_GLEN_158802-1_C3_2017_August_08_L1_0000	S. aureus	S. aureus	93.84	92.84	92.55
193	GP_Genus_species_Blood_AE_GLEN_158802-1_C3_2017_August_08_L2_0000	S. aureus	S. aureus	93.29	92.64	92.63
194	GP_Genus_species_Blood_AE_GLEN_158802-1_C3_2017_August_08_L3_0000	S. aureus	S. aureus	91.74	90.94	90.74
195	GP_Genus_species_Blood_AE_GLEN_158802-1_C3_2017_August_09_L1_N_0000	S. aureus	S. aureus	92.07	91.87	91.75
196	GP_Genus_species_Blood_AE_GLEN_158802-1_C3_2017_August_09_L2_N_0000	S. aureus	S. aureus	90.31	90.12	89.73
197	GP_Genus_species_Blood_AE_GLEN_158802-1_C3_2017_August_09_L3_N_0000	S. aureus	S. aureus	91.38	91.25	91.24
198	GP_Genus_species_Blood_AE_GLEN_158805-2_C3_2017_August_08_L1_0000	S. aureus	S. aureus	92.65	92.62	92.45
199	GP_Genus_species_Blood_AE_GLEN_158805-2_C3_2017_August_08_L2_0000	S. aureus	S. aureus	93.19	93.16	93.06
200	GP_Genus_species_Blood_AE_GLEN_158805-2_C3_2017_August_08_L3_0000	S. aureus	S. aureus	94.24	93.78	93.5
201	GP_Genus_species_Blood_AE_GLEN_158805-2_C3_2017_August_09_L1_N_0000	S. aureus	S. aureus	91.88	91.31	91.26
202	GP_Genus_species_Blood_AE_GLEN_158805-2_C3_2017_August_09_L2_N_0000	S. aureus	S. aureus	92.45	92.03	92.03
203	GP_Genus_species_Blood_AE_GLEN_158805-2_C3_2017_August_09_L3_N_0000	S. aureus	S. aureus	91.48	91.23	91.13
204	GP_Genus_species_Blood_AE_GLEN_158825_C3_2017_August_08_L1_0000	S. aureus	S. aureus	93.18	93.01	92.78
205	GP_Genus_species_Blood_AE_GLEN_158825_C3_2017_August_08_L2_0000	S. aureus	S. aureus	93.51	92.87	92.68

206	GP_Genus_species_Blood_AE_GLEN_158825_C3_2017_August_08_L3_0000	S. aureus	S. aureus	93.54	93.2	93.02
207	GP_Genus_species_Blood_AE_GLEN_158825_C3_2017_August_09_L1_N_0000	S. aureus	S. aureus	92.22	91.9	91.81
208	GP_Genus_species_Blood_AE_GLEN_158825_C3_2017_August_09_L2_N_0000	S. aureus	S. aureus	92.24	92.05	91.98
209	GP_Genus_species_Blood_AE_GLEN_158825_C3_2017_August_09_L3_N_0000	S. aureus	S. aureus	92.78	92.35	92.31
210	GP_Genus_species_Blood_AE_GLEN_158892-2_C3_2017_August_10_L1_0000	S. aureus	S. aureus	91.36	91.28	91.19
211	GP_Genus_species_Blood_AE_GLEN_158892-2_C3_2017_August_10_L2_0000	S. aureus	S. aureus	92	91.84	91.65
212	GP_Genus_species_Blood_AE_GLEN_158892-2_C3_2017_August_10_L3_0000	S. aureus	S. aureus	91.61	91.08	91
213	GP_Genus_species_Blood_AE_GLEN_158910-2_C3_2017_August_10_L1_0000	S. aureus	S. aureus	89.81	89.75	89.5
214	GP_Genus_species_Blood_AE_GLEN_158910-2_C3_2017_August_10_L2_0000	S. aureus	S. aureus	91.04	90.51	90.46
215	GP_Genus_species_Blood_AE_GLEN_158910-2_C3_2017_August_10_L3_0000	S. aureus	S. aureus	90.44	90.09	90.01
216	GP_Genus_species_Blood_AE_GLEN_158983-2_C3_2017_August_10_L1_0000	S. aureus	S. aureus	91.51	91.31	89.78
217	GP_Genus_species_Blood_AE_GLEN_158983-2_C3_2017_August_10_L2_0000	S. aureus	S. aureus	91.9	91.44	89.76
218	GP_Genus_species_Blood_AE_GLEN_158983-2_C3_2017_August_10_L3_0000	S. aureus	S. aureus	92.25	92.12	91.94
219	GP_Genus_species_Blood_AE_GLEN_158986_C3_2017_August_10_L1_0000	CoNS	CoNS	92.11	91.58	90.6
220	GP_Genus_species_Blood_AE_GLEN_158986_C3_2017_August_10_L2_0000	CoNS	CoNS	92.07	90.79	90.4
221	GP_Genus_species_Blood_AE_GLEN_158986_C3_2017_August_10_L3_0000	CoNS	CoNS	93.54	91.98	91.47
222	GP_Genus_species_Blood_AE_GLEN_158987-2_C3_2017_August_10_L1_0000	CoNS	CoNS	91.19	91.17	90.34
223	GP_Genus_species_Blood_AE_GLEN_158987-2_C3_2017_August_10_L2_0000	CoNS	CoNS	90.81	90.8	90.48
224	GP_Genus_species_Blood_AE_GLEN_158987-2_C3_2017_August_10_L3_0000	CoNS	CoNS	93.64	91.75	91.65
225	GP_Genus_species_Blood_AE_GLEN_158988_C3_2017_August_10_L1_0000	CoNS	CoNS	90.56	90.29	90.15
226	GP_Genus_species_Blood_AE_GLEN_158988_C3_2017_August_10_L2_0000	CoNS	CoNS	89.28	88.95	88.46
227	GP_Genus_species_Blood_AE_GLEN_158988_C3_2017_August_10_L3_0001	CoNS	CoNS	90	89.58	88.81
228	GP_Genus_species_Blood_AE_GLEN_159110-2_C3_2017_August_10_L1_0000	S. aureus	S. aureus	85.48	85.26	85.23
229	GP_Genus_species_Blood_AE_GLEN_159110-2_C3_2017_August_10_L2_0000	S. aureus	S. aureus	91.46	92.36	92.31
230	GP_Genus_species_Blood_AE_GLEN_159110-2_C3_2017_August_10_L3_0000	S. aureus	S. aureus	89.94	89.93	89.76
231	GP_Genus_species_Blood_AE_GLEN_159135-4_C3_2017_August_11_L1_0000	S. aureus	S. aureus	91.79	91.79	91.62
232	GP_Genus_species_Blood_AE_GLEN_159135-4_C3_2017_August_11_L2_0000	S. aureus	S. aureus	92.58	92.51	92.28
233	GP_Genus_species_Blood_AE_GLEN_159135-4_C3_2017_August_11_L3_0000	S. aureus	S. aureus	91.11	90.98	90.97
234	GP_Genus_species_Blood_AE_GLEN_159263-1_C3_2017_August_10_L1_0000	CoNS	S. aureus	78.43	78.39	78.12
235	GP_Genus_species_Blood_AE_GLEN_159263-1_C3_2017_August_10_L2_0000	CoNS	S. aureus	81.19	81.07	81.03
236	GP_Genus_species_Blood_AE_GLEN_159263-1_C3_2017_August_10_L3_0000	CoNS	S. aureus	82.47	82.29	82.13
237	GP_Genus_species_Blood_AE_GLEN_159274-1_C3_2017_August_10_L1_0000	S. aureus	S. aureus	94.62	94.25	94.2
238	GP_Genus_species_Blood_AE_GLEN_159274-1_C3_2017_August_10_L2_0000	S. aureus	S. aureus	93.48	93.28	93.12
239	GP_Genus_species_Blood_AE_GLEN_159274-1_C3_2017_August_10_L3_0000	S. aureus	S. aureus	92.79	91.9	91.66
240	GP_Genus_species_Blood_AE_GLEN_159393-2_C3_2017_August_11_L1_0000	CoNS	CoNS	93.04	92.77	92.4

241	GP_Genus_species_Blood_AE_GLEN_159393-2_C3_2017_August_11_L2_0000	CoNS	CoNS	86.49	86.37	86.26
242	GP_Genus_species_Blood_AE_GLEN_159393-2_C3_2017_August_11_L3_0000	CoNS	CoNS	93	92.78	92.55
243	GP_Genus_species_Blood_AE_GLEN_159639_C3_2017_August_10_L1_0000	E. faecalis	E. faecalis	91.09	90.74	90.39
244	GP_Genus_species_Blood_AE_GLEN_159639_C3_2017_August_10_L2_0000	E. faecalis	E. faecalis	91.74	91.35	90.86
245	GP_Genus_species_Blood_AE_GLEN_159639_C3_2017_August_10_L3_0000	E. faecalis	E. faecalis	90.8	90.47	90.34
246	GP_Genus_species_Blood_AE_GLEN_159689_C3_2017_August_10_L1_0000	E. faecalis	E. faecalis	91.85	91.67	91.34
247	GP_Genus_species_Blood_AE_GLEN_159689_C3_2017_August_10_L2_0000	E. faecalis	E. faecalis	92.42	91.52	90.98
248	GP_Genus_species_Blood_AE_GLEN_159689_C3_2017_August_10_L3_0000	E. faecalis	E. faecalis	92.73	92.28	92.11
249	GP_Genus_species_Blood_AE_GLEN_159691_C3_2017_August_10_L1_0000	E. faecalis	E. faecalis	91.14	91.08	90.11
250	GP_Genus_species_Blood_AE_GLEN_159691_C3_2017_August_10_L2_0000	E. faecalis	E. faecalis	91.46	91.25	91.12
251	GP_Genus_species_Blood_AE_GLEN_159691_C3_2017_August_10_L3_0000	E. faecalis	E. faecalis	91.15	90.35	90.09
252	GP_Genus_species_Blood_AE_GLEN_159745-3_C3_2017_August_11_L1_0000	S. aureus	S. aureus	93.78	93.56	93.25
253	GP_Genus_species_Blood_AE_GLEN_159745-3_C3_2017_August_11_L2_0000	S. aureus	S. aureus	93.3	93.27	93.04
254	GP_Genus_species_Blood_AE_GLEN_159745-3_C3_2017_August_11_L3_0000	S. aureus	S. aureus	93.34	93.21	92.99
255	GP_Genus_species_Blood_AE_GLEN_159872_C3_2017_August_11_L1_0000	S. aureus	S. aureus	93.56	93.24	92.58
256	GP_Genus_species_Blood_AE_GLEN_159872_C3_2017_August_11_L2_0000	S. aureus	S. aureus	92.67	92.28	92.09
257	GP_Genus_species_Blood_AE_GLEN_159872_C3_2017_August_11_L3_0000	S. aureus	S. aureus	93.66	93.4	93.31
258	GP_Genus_species_Blood_AE_GLEN_159872-2_C3_2017_August_11_L1_0000	S. aureus	S. aureus	94.15	94.01	93.7
259	GP_Genus_species_Blood_AE_GLEN_159872-2_C3_2017_August_11_L2_0000	S. aureus	S. aureus	94.54	94.08	93.9
260	GP_Genus_species_Blood_AE_GLEN_159872-2_C3_2017_August_11_L3_0000	S. aureus	S. aureus	91.68	91.42	90.76
261	GP_Genus_species_Blood_AE_GLEN_160064_C3_2017_August_11_L1_0000	S. aureus	S. aureus	93.21	92.99	92.86
262	GP_Genus_species_Blood_AE_GLEN_160064_C3_2017_August_11_L2_0000	S. aureus	S. aureus	92.1	91.75	91.7
263	GP_Genus_species_Blood_AE_GLEN_160064_C3_2017_August_11_L3_0000	S. aureus	S. aureus	93.18	92.87	92.47
264	GP_Genus_species_Blood_AE_GLEN_160076_C3_2017_August_11_L1_0000	S. aureus	S. aureus	91.02	90.3	90.02
265	GP_Genus_species_Blood_AE_GLEN_160076_C3_2017_August_11_L2_0000	S. aureus	S. aureus	90.28	90.06	89.38
266	GP_Genus_species_Blood_AE_GLEN_160076_C3_2017_August_11_L3_0000	S. aureus	S. aureus	89.51	88.69	88.63
267	GP_Genus_species_Blood_AE_GLEN_160200-2_C3_2017_August_11_L1_0000	CoNS	CoNS	92.78	92.68	92.36
268	GP_Genus_species_Blood_AE_GLEN_160200-2_C3_2017_August_11_L2_0000	CoNS	CoNS	91.7	90.7	90.24
269	GP_Genus_species_Blood_AE_GLEN_160200-2_C3_2017_August_11_L3_0000	CoNS	CoNS	93.62	93.42	93.14
270	GP_Genus_species_Blood_AE_GLEN_160254-3_C3_2017_August_16_L1_0000	CoNS	CoNS	86.6	86.29	86.29
271	GP_Genus_species_Blood_AE_GLEN_160254-3_C3_2017_August_16_L2_0000	CoNS	CoNS	89.82	89	88.81
272	GP_Genus_species_Blood_AE_GLEN_160254-3_C3_2017_August_16_L3_0000	CoNS	CoNS	88.89	88.61	88.34
273	GP_Genus_species_Blood_AE_GLEN_160280_C3_2017_August_11_L1_0000	CoNS	CoNS	92.13	91.95	91.73
274	GP_Genus_species_Blood_AE_GLEN_160280_C3_2017_August_11_L2_0000	CoNS	CoNS	91.62	91.41	91.15
275	GP_Genus_species_Blood_AE_GLEN_160280_C3_2017_August_11_L3_0000	CoNS	CoNS	91.88	91.74	91.59

276	GP_Genus_species_Blood_AE_GLEN_160506-2_C3_2017_August_11_L1_0000	S. aureus	S. aureus	91.33	90.61	90.55
277	GP_Genus_species_Blood_AE_GLEN_160506-2_C3_2017_August_11_L2_0000	S. aureus	S. aureus	90.74	90.49	90.46
278	GP_Genus_species_Blood_AE_GLEN_160506-2_C3_2017_August_11_L3_0000	S. aureus	S. aureus	89.81	89.55	89.49
279	GP_Genus_species_Blood_AE_GLEN_160510_C3_2017_August_11_L1_0000	S. aureus	S. aureus	91.15	91.03	90.76
280	GP_Genus_species_Blood_AE_GLEN_160510_C3_2017_August_11_L2_0000	S. aureus	S. aureus	89.55	89.45	89.15
281	GP_Genus_species_Blood_AE_GLEN_160510_C3_2017_August_11_L3_0000	S. aureus	S. aureus	90.49	90.44	89.61
282	GP_Genus_species_Blood_AE_GLEN_160510-1_C3_2017_August_11_L1_0000	S. aureus	S. aureus	89.33	88.27	88.18
283	GP_Genus_species_Blood_AE_GLEN_160510-1_C3_2017_August_11_L2_0000	S. aureus	S. aureus	90.16	89.79	89.46
284	GP_Genus_species_Blood_AE_GLEN_160510-1_C3_2017_August_11_L3_0000	S. aureus	S. aureus	89.48	89.01	88.45
285	GP_Genus_species_Blood_AE_GLEN_160538-2_C3_2017_August_11_L1_0000	S. aureus	S. aureus	93.61	92.55	92.14
286	GP_Genus_species_Blood_AE_GLEN_160538-2_C3_2017_August_11_L2_0000	S. aureus	S. aureus	92.61	92.61	92.56
287	GP_Genus_species_Blood_AE_GLEN_160538-2_C3_2017_August_11_L3_0000	S. aureus	S. aureus	92.94	92.07	92.02
288	GP_Genus_species_Blood_AE_GLEN_160556-2_C3_2017_August_11_L1_0000	S. aureus	S. aureus	93.47	93.26	93.2
289	GP_Genus_species_Blood_AE_GLEN_160556-2_C3_2017_August_11_L2_0000	S. aureus	S. aureus	91.89	91.63	91.55
290	GP_Genus_species_Blood_AE_GLEN_160564_C3_2017_August_10_L1_0000	E. faecalis	E. faecalis	91.66	91.22	91.07
291	GP_Genus_species_Blood_AE_GLEN_160564_C3_2017_August_10_L2_0000	E. faecalis	E. faecalis	91.01	90.84	90.74
292	GP_Genus_species_Blood_AE_GLEN_160564_C3_2017_August_10_L3_0000	E. faecalis	E. faecalis	91.77	91.07	90.97
293	GP_Genus_species_Blood_AE_GLEN_160623_C3_2017_August_11_L1_0000	S. aureus	S. aureus	90.57	90.45	89.75
294	GP_Genus_species_Blood_AE_GLEN_160623_C3_2017_August_11_L2_0000	S. aureus	S. aureus	91.58	91.4	90.87
295	GP_Genus_species_Blood_AE_GLEN_160623_C3_2017_August_11_L3_0000	S. aureus	S. aureus	89.2	88.88	88.6
296	GP_Genus_species_Blood_AE_GLEN_160693_C3_2017_August_10_L1_0000	S. aureus	S. aureus	92.67	92.14	92.08
297	GP_Genus_species_Blood_AE_GLEN_160693_C3_2017_August_10_L2_0000	S. aureus	S. aureus	92.46	92.14	91.63
298	GP_Genus_species_Blood_AE_GLEN_160693_C3_2017_August_10_L3_0000	S. aureus	S. aureus	94.13	94.02	93.91
299	GP_Genus_species_Blood_AE_GLEN_160693_C3_2017_August_11_L1_0000	S. aureus	S. aureus	88.86	88.85	88.59
300	GP_Genus_species_Blood_AE_GLEN_160693_C3_2017_August_11_L2_0000	S. aureus	S. aureus	93.91	93.78	93.75
301	GP_Genus_species_Blood_AE_GLEN_160693_C3_2017_August_11_L3_0000	S. aureus	S. aureus	89.52	89.2	88.45
302	GP_Genus_species_Blood_AE_GLEN_160749_C3_2017_August_11_L1_0000	E. faecalis	E. faecalis	91.36	91.35	91.27
303	GP_Genus_species_Blood_AE_GLEN_160749_C3_2017_August_11_L2_0000	E. faecalis	E. faecalis	92.69	92.22	91.8
304	GP_Genus_species_Blood_AE_GLEN_160749_C3_2017_August_11_L3_0000	E. faecalis	E. faecalis	92.42	91.34	91.25
305	GP_Genus_species_Blood_AE_GLEN_160846_C3_2017_August_11_L1_0000	E. faecalis	E. faecalis	92.9	92.55	92.16
306	GP_Genus_species_Blood_AE_GLEN_160846_C3_2017_August_11_L2_0000	E. faecalis	E. faecalis	93.86	93.52	93.04
307	GP_Genus_species_Blood_AE_GLEN_160846_C3_2017_August_11_L3_0000	E. faecalis	E. faecalis	92.67	92.53	91.93
308	GP_Genus_species_Blood_AE_GLEN_160941-2_C3_2017_August_16_L1_0000	S. aureus	S. aureus	93.99	93.77	93.76
309	GP_Genus_species_Blood_AE_GLEN_160941-2_C3_2017_August_16_L2_0000	S. aureus	S. aureus	93.14	92.68	92.35
310	GP_Genus_species_Blood_AE_GLEN_160941-2_C3_2017_August_16_L3_0000	S. aureus	S. aureus	94.02	93.48	92.66

311	GP_Genus_species_Blood_AE_GLEN_160954-1_C3_2017_August_11_L1_0000	S. aureus	S. aureus	89.31	89.26	88.85
312	GP_Genus_species_Blood_AE_GLEN_160954-1_C3_2017_August_11_L2_0000	S. aureus	S. aureus	92.84	92.71	92.42
313	GP_Genus_species_Blood_AE_GLEN_160954-1_C3_2017_August_11_L3_0000	S. aureus	S. aureus	94.24	93.33	92.85
314	GP_Genus_species_Blood_AE_GLEN_161051_C3_2017_August_11_L1_0000	E. faecalis	E. faecalis	93.82	93.63	93.48
315	GP_Genus_species_Blood_AE_GLEN_161051_C3_2017_August_11_L2_0000	E. faecalis	E. faecalis	92.61	92.32	92.04
316	GP_Genus_species_Blood_AE_GLEN_161051_C3_2017_August_11_L3_0000	E. faecalis	E. faecalis	93.32	92.75	91.69
317	GP_Genus_species_Blood_AE_GLEN_161070-1_C3_2017_August_11_L1_0000	S. aureus	S. aureus	89.38	88.99	88.94
318	GP_Genus_species_Blood_AE_GLEN_161070-1_C3_2017_August_11_L2_0000	S. aureus	S. aureus	92.65	92.33	91.17
319	GP_Genus_species_Blood_AE_GLEN_161070-1_C3_2017_August_11_L3_0000	S. aureus	S. aureus	92.35	92.17	91.46
320	GP_Genus_species_Blood_AE_GLEN_161071-1_C3_2017_August_11_L1_0000	S. aureus	S. aureus	92.39	91.58	91.28
321	GP_Genus_species_Blood_AE_GLEN_161071-1_C3_2017_August_11_L2_0000	S. aureus	S. aureus	93.59	92.59	91.95
322	GP_Genus_species_Blood_AE_GLEN_161071-1_C3_2017_August_11_L3_0000	S. aureus	S. aureus	92.62	92.46	91.03
323	GP_Genus_species_Blood_AE_GLEN_161071-1_C3_2017_August_11_L3_0001	S. aureus	S. aureus	92.95	91.84	91.67
324	GP_Genus_species_Blood_AE_GLEN_161140-1_C3_2017_August_11_L1_0000	S. aureus	S. aureus	93.91	93.7	93.59
325	GP_Genus_species_Blood_AE_GLEN_161140-1_C3_2017_August_11_L2_0000	S. aureus	S. aureus	95.12	94.27	94.11
326	GP_Genus_species_Blood_AE_GLEN_161140-1_C3_2017_August_11_L3_0000	S. aureus	S. aureus	94.15	94.14	94
327	GP_Genus_species_Blood_AE_GLEN_161146-1_C3_2017_August_15_L1_0000	CoNS	CoNS	89.48	88.25	87.78
328	GP_Genus_species_Blood_AE_GLEN_161146-1_C3_2017_August_15_L2_0000	CoNS	CoNS	87.18	86.65	86.24
329	GP_Genus_species_Blood_AE_GLEN_161146-1_C3_2017_August_15_L3_0000	CoNS	CoNS	85.39	81.71	81.34
330	GP_Genus_species_Blood_AE_GLEN_161150-2_C3_2017_August_11_L1_0000	S. aureus	S. aureus	93.93	93.36	93.33
331	GP_Genus_species_Blood_AE_GLEN_161150-2_C3_2017_August_11_L2_0000	S. aureus	S. aureus	93.28	93.22	93.15
332	GP_Genus_species_Blood_AE_GLEN_161150-2_C3_2017_August_11_L3_0000	S. aureus	S. aureus	94.85	94.46	94.45
333	GP_Genus_species_Blood_AE_GLEN_161286-3_C3_2017_August_15_L1_0000	S. aureus	S. aureus	91.63	91.63	91.48
334	GP_Genus_species_Blood_AE_GLEN_161286-3_C3_2017_August_15_L2_0000	S. aureus	S. aureus	93.26	93.11	93.07
335	GP_Genus_species_Blood_AE_GLEN_161286-3_C3_2017_August_15_L3_0000	S. aureus	S. aureus	93.99	93.64	93.42
336	GP_Genus_species_Blood_AE_GLEN_161482_C3_2017_August_11_L1_0000	S. aureus	S. aureus	94.43	94.22	94.16
337	GP_Genus_species_Blood_AE_GLEN_161482_C3_2017_August_11_L2_0000	S. aureus	S. aureus	94.46	94.33	94.26
338	GP_Genus_species_Blood_AE_GLEN_161482_C3_2017_August_11_L3_0000	S. aureus	S. aureus	94.06	94	93.8
339	GP_Genus_species_Blood_AE_GLEN_161482-1_C3_2017_August_15_L1_0000	S. aureus	S. aureus	92.66	91.57	91.18
340	GP_Genus_species_Blood_AE_GLEN_161482-1_C3_2017_August_15_L2_0000	S. aureus	S. aureus	89.46	88.76	88.59
341	GP_Genus_species_Blood_AE_GLEN_161482-1_C3_2017_August_15_L3_0000	S. aureus	S. aureus	89.43	88.47	88.24
342	GP_Genus_species_Blood_AE_GLEN_161691_C3_2017_August_15_L1_0000	CoNS	CoNS	92.66	91.57	91.18
343	GP_Genus_species_Blood_AE_GLEN_161691_C3_2017_August_15_L2_0000	CoNS	CoNS	84.03	83.61	83.13
344	GP_Genus_species_Blood_AE_GLEN_161691_C3_2017_August_15_L3_0000	CoNS	CoNS	81.34	80.22	79.85
345	GP_Genus_species_Blood_AE_GLEN_161691-2_C3_2017_August_15_L1_0000	CoNS	CoNS	86.15	85.9	85.44

346	GP_Genus_species_Blood_AE_GLEN_161691-2_C3_2017_August_15_L2_0000	CoNS	CoNS	86.34	86.33	86.02
347	GP_Genus_species_Blood_AE_GLEN_161691-2_C3_2017_August_15_L3_0000	CoNS	CoNS	87.58	87.07	86.93
348	GP_Genus_species_Blood_AE_GLEN_161692_C3_2017_August_15_L1_0000	CoNS	CoNS	87.35	85.32	85.15
349	GP_Genus_species_Blood_AE_GLEN_161692_C3_2017_August_15_L2_0000	CoNS	CoNS	85.53	85.33	85.01
350	GP_Genus_species_Blood_AE_GLEN_161692_C3_2017_August_15_L3_0000	CoNS	CoNS	87.24	87.11	86.62
351	GP_Genus_species_Blood_AE_GLEN_161692-1_C3_2017_August_15_L1_0000	CoNS	CoNS	87.35	85.32	85.15
352	GP_Genus_species_Blood_AE_GLEN_161692-1_C3_2017_August_15_L2_0000	CoNS	CoNS	85.53	85.33	85.01
353	GP_Genus_species_Blood_AE_GLEN_161692-1_C3_2017_August_15_L3_0000	CoNS	CoNS	83.71	82.64	82.53
354	GP_Genus_species_Blood_AE_GLEN_161698-2_C3_2017_August_16_L1_0000	S. aureus	S. aureus	94.19	94.14	94.02
355	GP_Genus_species_Blood_AE_GLEN_161698-2_C3_2017_August_16_L2_0000	S. aureus	S. aureus	94.1	93.93	93.68
356	GP_Genus_species_Blood_AE_GLEN_161698-2_C3_2017_August_16_L3_0000	S. aureus	S. aureus	93.63	93.61	93.6
357	GP_Genus_species_Blood_AE_GLEN_161727_C3_2017_August_15_L1_0000	E. faecium	E. faecium	90.02	87.57	86.18
358	GP_Genus_species_Blood_AE_GLEN_161727_C3_2017_August_15_L2_0000	E. faecium	E. faecium	90.27	87.94	87.17
359	GP_Genus_species_Blood_AE_GLEN_161727_C3_2017_August_15_L3_0000	E. faecium	E. faecium	88.47	86.45	86.34
360	GP_Genus_species_Blood_AE_GLEN_161805-3_C3_2017_August_15_L1_0000	E. faecalis	E. faecalis	89.03	88.25	87.62
361	GP_Genus_species_Blood_AE_GLEN_161805-3_C3_2017_August_15_L2_0000	E. faecalis	E. faecalis	87.6	86.81	86.45
362	GP_Genus_species_Blood_AE_GLEN_161805-3_C3_2017_August_15_L3_0000	E. faecalis	E. faecalis	86.9	86.69	86.1
363	GP_Genus_species_Blood_AE_GLEN_161814-3_C3_2017_August_15_L1_0000	CoNS	CoNS	89.98	87.67	87.22
364	GP_Genus_species_Blood_AE_GLEN_161814-3_C3_2017_August_15_L2_0000	CoNS	CoNS	90.39	89.64	89.49
365	GP_Genus_species_Blood_AE_GLEN_161814-3_C3_2017_August_15_L3_0000	CoNS	CoNS	90.7	90.64	90.59
366	GP_Genus_species_Blood_AE_GLEN_161915-3_C3_2017_August_15_L1_0000	E. faecalis	E. faecalis	92.68	91.8	91.24
367	GP_Genus_species_Blood_AE_GLEN_161915-3_C3_2017_August_15_L2_0000	E. faecalis	E. faecalis	90.57	89.86	89.67
368	GP_Genus_species_Blood_AE_GLEN_161915-3_C3_2017_August_15_L3_0000	E. faecalis	E. faecalis	91.32	90.57	89.69
369	GP_Genus_species_Blood_AE_GLEN_161957-1_C3_2017_August_15_L1_0000	S. aureus	S. aureus	90.24	89.83	89.77
370	GP_Genus_species_Blood_AE_GLEN_161957-1_C3_2017_August_15_L2_0000	S. aureus	S. aureus	91.38	90.87	90.86
371	GP_Genus_species_Blood_AE_GLEN_161957-1_C3_2017_August_15_L3_0000	S. aureus	S. aureus	90.53	89.98	89.59
372	GP_Genus_species_Blood_AE_GLEN_162001-2_C3_2017_August_15_L1_0000	E. faecalis	E. faecalis	91.41	91.36	91.11
373	GP_Genus_species_Blood_AE_GLEN_162001-2_C3_2017_August_15_L2_0000	E. faecalis	E. faecalis	92.04	91.98	91.92
374	GP_Genus_species_Blood_AE_GLEN_162001-2_C3_2017_August_15_L3_0000	E. faecalis	E. faecalis	92.43	92.41	92.07
375	GP_Genus_species_Blood_AE_GLEN_162257-2_C3_2017_August_15_L1_0000	CoNS	CoNS	92.19	91.85	91.05
376	GP_Genus_species_Blood_AE_GLEN_162257-2_C3_2017_August_15_L2_0000	CoNS	CoNS	92.86	92.6	92.26
377	GP_Genus_species_Blood_AE_GLEN_162257-2_C3_2017_August_15_L3_0000	CoNS	CoNS	93.28	93.15	92.77
378	GP_Genus_species_Blood_AE_GLEN_162287-1_C3_2017_August_15_L1_0000	CoNS	CoNS	93.11	91.39	91.33
379	GP_Genus_species_Blood_AE_GLEN_162287-1_C3_2017_August_15_L2_0000	CoNS	CoNS	92.53	91.66	91.53
380	GP_Genus_species_Blood_AE_GLEN_162287-1_C3_2017_August_15_L3_0000	CoNS	CoNS	92.14	91.08	91.03

381	GP_Genus_species_Blood_AE_GLEN_162287-3_C3_2017_August_15_L1_0000	CoNS	CoNS	93.98	93.23	91.97
382	GP_Genus_species_Blood_AE_GLEN_162287-3_C3_2017_August_15_L2_0000	CoNS	CoNS	93.49	91.88	92.5
383	GP_Genus_species_Blood_AE_GLEN_162287-3_C3_2017_August_15_L3_0000	CoNS	CoNS	92.72	92.48	91.75
384	GP_Genus_species_Blood_AE_GLEN_162352-2_C3_2017_August_15_L1_0000	S. aureus	S. aureus	89.39	88.1	87.83
385	GP_Genus_species_Blood_AE_GLEN_162352-2_C3_2017_August_15_L2_0000	S. aureus	S. aureus	87.97	87.73	87.59
386	GP_Genus_species_Blood_AE_GLEN_162352-2_C3_2017_August_15_L3_0000	S. aureus	S. aureus	88.94	87.46	86.88
387	GP_Genus_species_Blood_AE_GLEN_162445-2_C3_2017_August_15_L1_0000	S. aureus	S. aureus	92.78	92.74	92.72
388	GP_Genus_species_Blood_AE_GLEN_162445-2_C3_2017_August_15_L2_0000	S. aureus	S. aureus	90.47	89.89	89.85
389	GP_Genus_species_Blood_AE_GLEN_162445-2_C3_2017_August_15_L3_0000	S. aureus	S. aureus	91.48	90.65	90.31
390	GP_Genus_species_Blood_AE_GLEN_162445-4_C3_2017_August_15_L1_0000	S. aureus	S. aureus	91.81	91.66	91.35
391	GP_Genus_species_Blood_AE_GLEN_162445-4_C3_2017_August_15_L2_0000	S. aureus	S. aureus	91.02	90.89	90.8
392	GP_Genus_species_Blood_AE_GLEN_162445-4_C3_2017_August_15_L3_0000	S. aureus	S. aureus	91.49	91.3	91.14
393	GP_Genus_species_Blood_AE_GLEN_162446_C3_2017_August_15_L1_0000	CoNS	CoNS	88.86	88.33	88.11
394	GP_Genus_species_Blood_AE_GLEN_162446_C3_2017_August_15_L2_0000	CoNS	CoNS	86.8	84.14	82.7
395	GP_Genus_species_Blood_AE_GLEN_162446_C3_2017_August_15_L3_0000	CoNS	CoNS	89.46	89.04	88.81
396	GP_Genus_species_Blood_AE_GLEN_162446-6_C3_2017_August_16_L1_0000	CoNS	CoNS	79	78.31	77.5
397	GP_Genus_species_Blood_AE_GLEN_162446-6_C3_2017_August_16_L2_0000	CoNS	CoNS	81.91	81.36	81.15
398	GP_Genus_species_Blood_AE_GLEN_162446-6_C3_2017_August_16_L3_0000	CoNS	CoNS	84.69	84.24	84.15
399	GP_Genus_species_Blood_AE_GLEN_162477_C3_2017_August_15_L1_0000	CoNS	CoNS	91.38	90.86	90.5
400	GP_Genus_species_Blood_AE_GLEN_162477_C3_2017_August_15_L2_0000	CoNS	CoNS	87.09	86.94	86.46
401	GP_Genus_species_Blood_AE_GLEN_162477_C3_2017_August_15_L3_0000	CoNS	CoNS	86.9	86.33	84.63
402	GP_Genus_species_Blood_AE_GLEN_162477_C3_2017_August_16_L1_0000	CoNS	CoNS	90.44	89.23	88.93
403	GP_Genus_species_Blood_AE_GLEN_162477_C3_2017_August_16_L2_0000	CoNS	CoNS	90.77	90.69	90.53
404	GP_Genus_species_Blood_AE_GLEN_162477_C3_2017_August_16_L3_0000	CoNS	CoNS	86.48	86.23	86.18
405	GP_Genus_species_Blood_AE_GLEN_162483-1_C3_2017_August_15_L1_0000	S. aureus	S. aureus	93.38	92.87	91.26
406	GP_Genus_species_Blood_AE_GLEN_162483-1_C3_2017_August_15_L2_0000	S. aureus	S. aureus	93.31	92.92	92.48
407	GP_Genus_species_Blood_AE_GLEN_162483-1_C3_2017_August_15_L3_0000	S. aureus	S. aureus	90.79	90.07	88.86
408	GP_Genus_species_Blood_AE_GLEN_162568_C3_2017_August_15_L1_0000	S. aureus	S. aureus	90.98	90.58	90.4
409	GP_Genus_species_Blood_AE_GLEN_162568_C3_2017_August_15_L2_0000	S. aureus	S. aureus	92.01	90.53	90.1
410	GP_Genus_species_Blood_AE_GLEN_162568_C3_2017_August_15_L3_0000	S. aureus	S. aureus	91.65	89.49	89.49
411	GP_Genus_species_Blood_AE_GLEN_162625-1_C3_2017_August_15_L1_0000	S. aureus	S. aureus	90.27	88.98	88.29
412	GP_Genus_species_Blood_AE_GLEN_162625-1_C3_2017_August_15_L2_0000	S. aureus	S. aureus	91.23	91.15	91.06
413	GP_Genus_species_Blood_AE_GLEN_162625-1_C3_2017_August_15_L3_0000	S. aureus	S. aureus	91.64	91.19	90.95
414	GP_Genus_species_Blood_AE_GLEN_162722_C3_2017_August_15_L1_0000	S. aureus	S. aureus	90.24	90.01	89.83
415	GP_Genus_species_Blood_AE_GLEN_162722_C3_2017_August_15_L2_0000	S. aureus	S. aureus	92.29	91.5	91.38

416	GP_Genus_species_Blood_AE_GLEN_162722_C3_2017_August_15_L3_0000	S. aureus	S. aureus	90	89.75	89.32
417	GP_Genus_species_Blood_AE_GLEN_162794-4_C3_2017_August_16_L1_0000	S. aureus	S. aureus	89.8	89.5	89.04
418	GP_Genus_species_Blood_AE_GLEN_162794-4_C3_2017_August_16_L2_0000	S. aureus	S. aureus	88.81	87.36	87.09
419	GP_Genus_species_Blood_AE_GLEN_162794-4_C3_2017_August_16_L3_0000	S. aureus	S. aureus	89.58	88.56	87.91
420	GP_Genus_species_Blood_AE_GLEN_162798-3_C3_2017_August_15_L1_0000	S. aureus	S. aureus	93.69	93.55	93.54
421	GP_Genus_species_Blood_AE_GLEN_162798-3_C3_2017_August_15_L2_0000	S. aureus	S. aureus	91.32	90.78	90.76
422	GP_Genus_species_Blood_AE_GLEN_162798-3_C3_2017_August_15_L3_0000	S. aureus	S. aureus	92.31	91.75	91.7
423	GP_Genus_species_Blood_AE_GLEN_162798-4_C3_2017_August_15_L1_0000	E. faecalis	E. faecalis	90.88	90.36	90.2
424	GP_Genus_species_Blood_AE_GLEN_162798-4_C3_2017_August_15_L2_0000	E. faecalis	E. faecalis	90.6	90.5	90.47
425	GP_Genus_species_Blood_AE_GLEN_162798-4_C3_2017_August_15_L3_0000	E. faecalis	E. faecalis	90.82	90.8	90.79
426	GP_Genus_species_Blood_AE_GLEN_163006-2_C3_2017_August_15_L1_0000	E. faecalis	E. faecalis	92.5	91.53	90.73
427	GP_Genus_species_Blood_AE_GLEN_163006-2_C3_2017_August_15_L2_0000	E. faecalis	E. faecalis	91.17	90.02	90.01
428	GP_Genus_species_Blood_AE_GLEN_163006-2_C3_2017_August_15_L3_0000	E. faecalis	E. faecalis	91.65	91.15	91.1
429	GP_Genus_species_Blood_AE_GLEN_163068-2_C3_2017_August_15_L1_0000	S. aureus	S. aureus	92.06	91.76	91.49
430	GP_Genus_species_Blood_AE_GLEN_163068-2_C3_2017_August_15_L2_0000	S. aureus	S. aureus	87.8	87.65	87.37
431	GP_Genus_species_Blood_AE_GLEN_163068-2_C3_2017_August_15_L3_0000	S. aureus	S. aureus	85.92	85.73	84.75
432	GP_Genus_species_Blood_AE_GLEN_163068-2_C3_2017_August_16_L1_0000	S. aureus	S. aureus	90.27	90.23	90.11
433	GP_Genus_species_Blood_AE_GLEN_163068-2_C3_2017_August_16_L2_0000	S. aureus	S. aureus	92.02	91.64	91.45
434	GP_Genus_species_Blood_AE_GLEN_163068-2_C3_2017_August_16_L3_0000	S. aureus	S. aureus	91.48	91.31	90.71
435	GP_Genus_species_Blood_AE_GLEN_163090_C3_2017_August_16_L1_0000	E. faecalis	E. faecalis	85.17	83.55	83.51
436	GP_Genus_species_Blood_AE_GLEN_163090_C3_2017_August_16_L2_0000	E. faecalis	E. faecalis	87.38	86.61	86.37
437	GP_Genus_species_Blood_AE_GLEN_163090_C3_2017_August_16_L3_0000	E. faecalis	E. faecalis	86.41	85.55	85.41
438	GP_Genus_species_Blood_AE_GLEN_163090-3_C3_2017_August_16_L1_0000	E. faecalis	E. faecalis	85.81	85	84.31
439	GP_Genus_species_Blood_AE_GLEN_163090-3_C3_2017_August_16_L2_0000	E. faecalis	E. faecalis	87.03	84.53	84.48
440	GP_Genus_species_Blood_AE_GLEN_163090-3_C3_2017_August_16_L3_0000	E. faecalis	E. faecalis	86.76	86.67	86.5
441	GP_Genus_species_Blood_AE_GLEN_163149_C3_2017_August_16_L1_0000	E. faecalis	E. faecalis	90.06	89.96	89.67
442	GP_Genus_species_Blood_AE_GLEN_163149_C3_2017_August_16_L2_0000	E. faecalis	E. faecalis	93.28	92.6	92.38
443	GP_Genus_species_Blood_AE_GLEN_163149_C3_2017_August_16_L3_0000	E. faecalis	E. faecalis	92.32	91.33	91.17
444	GP_Genus_species_Blood_AE_GLEN_163149-1_C3_2017_August_16_L1_0000	E. faecalis	E. faecalis	91.81	91.55	91.42
445	GP_Genus_species_Blood_AE_GLEN_163149-1_C3_2017_August_16_L2_0000	E. faecalis	E. faecalis	92.48	92.14	91.8
446	GP_Genus_species_Blood_AE_GLEN_163149-1_C3_2017_August_16_L3_0000	E. faecalis	E. faecalis	90.66	90.35	90.29
447	GP_Genus_species_Blood_AE_GLEN_163182-3_C3_2017_August_17_L1_0000	S. aureus	S. aureus	90.3	89.34	88.33
448	GP_Genus_species_Blood_AE_GLEN_163182-3_C3_2017_August_17_L2_0000	S. aureus	S. aureus	90.36	90.11	89.81
449	GP_Genus_species_Blood_AE_GLEN_163182-3_C3_2017_August_17_L3_0000	S. aureus	S. aureus	93.04	92.72	92.29
450	GP_Genus_species_Blood_AE_GLEN_163195-2_C3_2017_August_16_L1_0000	S. aureus	S. aureus	93.63	93.57	93.52

451	GP_Genus_species_Blood_AE_GLEN_163195-2_C3_2017_August_16_L2_0000	S. aureus	S. aureus	93.72	93.35	93.03
452	GP_Genus_species_Blood_AE_GLEN_163195-2_C3_2017_August_16_L3_0000	S. aureus	S. aureus	94.75	93.94	93.91
453	GP_Genus_species_Blood_AE_GLEN_163321-2_C3_2017_August_16_L1_0000	S. aureus	S. aureus	93.44	93.36	93.32
454	GP_Genus_species_Blood_AE_GLEN_163321-2_C3_2017_August_16_L2_0000	S. aureus	S. aureus	93.04	92.59	92.21
455	GP_Genus_species_Blood_AE_GLEN_163321-2_C3_2017_August_16_L3_0000	S. aureus	S. aureus	93.41	92.9	92.77
456	GP_Genus_species_Blood_AE_GLEN_163359-1_C3_2017_August_16_L1_0000	CoNS	CoNS	90	88.22	88.21
457	GP_Genus_species_Blood_AE_GLEN_163359-1_C3_2017_August_16_L2_0000	CoNS	CoNS	90.34	89.41	89.35
458	GP_Genus_species_Blood_AE_GLEN_163359-1_C3_2017_August_16_L3_0000	CoNS	CoNS	89.95	89.14	89.03
459	GP_Genus_species_Blood_AE_GLEN_163380-2_C3_2017_August_16_L1_0000	S. aureus	S. aureus	88.91	87.95	87.91
460	GP_Genus_species_Blood_AE_GLEN_163380-2_C3_2017_August_16_L2_0000	S. aureus	S. aureus	90.7	90.08	89.25
461	GP_Genus_species_Blood_AE_GLEN_163380-2_C3_2017_August_16_L3_0000	S. aureus	S. aureus	92.42	91.95	91.67
462	GP_Genus_species_Blood_AE_GLEN_163382-4_C3_2017_August_17_L1_0000	E. faecalis	E. faecalis	76.4	74.43	74.2
463	GP_Genus_species_Blood_AE_GLEN_163382-4_C3_2017_August_17_L2_0000	E. faecalis	E. faecalis	88.07	85.58	84.98
464	GP_Genus_species_Blood_AE_GLEN_163382-4_C3_2017_August_17_L3_0000	E. faecalis	E. faecalis	88.57	87.41	86.86
465	GP_Genus_species_Blood_AE_GLEN_163747-2_C3_2017_August_17_L1_0000	S. aureus	S. aureus	92.72	92.23	92
466	GP_Genus_species_Blood_AE_GLEN_163747-2_C3_2017_August_17_L2_0000	S. aureus	S. aureus	93.59	93.38	93.24
467	GP_Genus_species_Blood_AE_GLEN_163747-2_C3_2017_August_17_L3_0000	S. aureus	S. aureus	93.61	93.13	93.09
468	GP_Genus_species_Blood_AE_GLEN_164135_C3_2017_August_17_L1_0000	S. aureus	S. aureus	92.75	92.74	92.69
469	GP_Genus_species_Blood_AE_GLEN_164135_C3_2017_August_17_L2_0000	S. aureus	S. aureus	92.77	92.63	92.55
470	GP_Genus_species_Blood_AE_GLEN_164135_C3_2017_August_17_L3_0000	S. aureus	S. aureus	92.79	92.31	91.84
471	GP_Genus_species_Blood_AE_GLEN_164177_C3_2017_August_17_L1_0000	S. aureus	S. aureus	91.15	90.87	90.43
472	GP_Genus_species_Blood_AE_GLEN_164177_C3_2017_August_17_L2_0000	S. aureus	S. aureus	94.05	94.03	93.8
473	GP_Genus_species_Blood_AE_GLEN_164177_C3_2017_August_17_L3_0000	S. aureus	S. aureus	90.1	88.84	88..71
474	GP_Genus_species_Blood_AE_GLEN_164184-2_C3_2017_August_17_L1_0000	S. aureus	S. aureus	93.4	93.37	93.36
475	GP_Genus_species_Blood_AE_GLEN_164184-2_C3_2017_August_17_L2_0000	S. aureus	S. aureus	93.79	93.75	93.17
476	GP_Genus_species_Blood_AE_GLEN_164184-2_C3_2017_August_17_L3_0000	S. aureus	S. aureus	94.22	93.73	92.44
477	GP_Genus_species_Blood_AE_GLEN_164266-2_C3_2017_August_17_L1_0000	S. aureus	S. aureus	94.6	94.32	94.3
478	GP_Genus_species_Blood_AE_GLEN_164266-2_C3_2017_August_17_L2_0000	S. aureus	S. aureus	93.59	93.75	93.17
479	GP_Genus_species_Blood_AE_GLEN_164266-2_C3_2017_August_17_L3_0000	S. aureus	S. aureus	93.55	93.46	93.24
480	GP_Genus_species_Blood_AE_GLEN_164673-2_C3_2017_August_17_L1_0000	S. aureus	S. aureus	85.82	85.02	83.86
481	GP_Genus_species_Blood_AE_GLEN_164673-2_C3_2017_August_17_L2_0000	S. aureus	S. aureus	93.21	92.59	92.05
482	GP_Genus_species_Blood_AE_GLEN_164673-2_C3_2017_August_17_L3_0000	S. aureus	S. aureus	91.68	91.56	91.45
483	GP_Genus_species_Blood_AE_GLEN_164742_C3_2017_August_21_L1_0000	S. aureus	S. aureus	87.34	87.33	87.31
484	GP_Genus_species_Blood_AE_GLEN_164742_C3_2017_August_21_L2_0000	S. aureus	S. aureus	93.43	91.78	91.24
485	GP_Genus_species_Blood_AE_GLEN_164742_C3_2017_August_21_L3_0000	S. aureus	S. aureus	93.44	93.26	92.91

486	GP_Genus_species_Blood_AE_GLEN_164808_C3_2017_August_21_L1_0000	S. aureus	S. aureus	90.86	90.81	90.32
487	GP_Genus_species_Blood_AE_GLEN_164808_C3_2017_August_21_L2_0000	S. aureus	S. aureus	89.62	88.94	88.76
488	GP_Genus_species_Blood_AE_GLEN_164808_C3_2017_August_21_L3_0000	S. aureus	S. aureus	92.91	92.73	92.59
489	GP_Genus_species_Blood_AE_GLEN_164856_C3_2017_August_21_L1_0000	S. aureus	S. aureus	87.17	87.05	86.73
490	GP_Genus_species_Blood_AE_GLEN_164856_C3_2017_August_21_L2_0000	S. aureus	S. aureus	89.06	87.9	87.74
491	GP_Genus_species_Blood_AE_GLEN_164856_C3_2017_August_21_L3_0000	S. aureus	S. aureus	90	87.83	87.74
492	GP_Genus_species_Blood_AE_GLEN_164856-4_C3_2017_August_21_L1_0000	S. aureus	S. aureus	92.43	92.42	91.86
493	GP_Genus_species_Blood_AE_GLEN_164856-4_C3_2017_August_21_L2_0000	S. aureus	S. aureus	92.94	92.67	92.4
494	GP_Genus_species_Blood_AE_GLEN_164856-4_C3_2017_August_21_L3_0000	S. aureus	S. aureus	93.56	93.31	93.29
495	GP_Genus_species_Blood_AE_GLEN_165062_C3_2017_August_17_L1_0000	E. faecalis	E. faecalis	84.08	83.53	83.52
496	GP_Genus_species_Blood_AE_GLEN_165062_C3_2017_August_17_L2_0000	E. faecalis	E. faecalis	89.95	89.89	89.88
497	GP_Genus_species_Blood_AE_GLEN_165062_C3_2017_August_17_L3_0000	E. faecalis	E. faecalis	84.28	83.7	83.55
498	GP_Genus_species_Blood_AE_GLEN_165077-2_C3_2017_August_17_L1_0000	S. aureus	S. aureus	94.17	93.42	93.24
499	GP_Genus_species_Blood_AE_GLEN_165077-2_C3_2017_August_17_L2_0000	S. aureus	S. aureus	93.88	93.87	93.71
500	GP_Genus_species_Blood_AE_GLEN_165077-2_C3_2017_August_17_L3_0000	S. aureus	S. aureus	93.6	93.6	93.59
501	GP_Genus_species_Blood_AE_GLEN_165309_C3_2017_August_17_L1_0000	E. faecalis	E. faecalis	86.11	85.97	85.08
502	GP_Genus_species_Blood_AE_GLEN_165309_C3_2017_August_17_L2_0000	E. faecalis	E. faecalis	84.03	82.56	82.35
503	GP_Genus_species_Blood_AE_GLEN_165309_C3_2017_August_17_L3_0000	E. faecalis	E. faecalis	87.17	84.6	83.85
504	GP_Genus_species_Blood_AE_GLEN_165349-2_C3_2017_August_17_L1_0000	S. aureus	S. aureus	93.56	92.03	91.77
505	GP_Genus_species_Blood_AE_GLEN_165349-2_C3_2017_August_17_L2_0000	S. aureus	S. aureus	93.92	93.02	92.92
506	GP_Genus_species_Blood_AE_GLEN_165349-2_C3_2017_August_17_L3_0000	S. aureus	S. aureus	92.56	92.19	91.96
507	GP_Genus_species_Blood_AE_GLEN_165357-2_C3_2017_August_17_L1_0000	S. aureus	S. aureus	91.97	91.33	91.1
508	GP_Genus_species_Blood_AE_GLEN_165357-2_C3_2017_August_17_L2_0000	S. aureus	S. aureus	91.27	91.04	90.5
509	GP_Genus_species_Blood_AE_GLEN_165357-2_C3_2017_August_17_L3_0000	S. aureus	S. aureus	92.27	91.59	91.48
510	GP_Genus_species_Blood_AE_GLEN_165885-2_C3_2017_August_21_L1_0000	CoNS	CoNS	85.37	85.14	85.07
511	GP_Genus_species_Blood_AE_GLEN_165885-2_C3_2017_August_21_L2_0000	CoNS	CoNS	88.41	86.59	85.77
512	GP_Genus_species_Blood_AE_GLEN_165885-2_C3_2017_August_21_L3_0000	CoNS	CoNS	84.28	83.61	83.37
513	GP_Genus_species_Blood_AE_GLEN_165885-3_C3_2017_August_21_L1_0000	CoNS	E. faecium	87.44	86.57	85.95
514	GP_Genus_species_Blood_AE_GLEN_165885-3_C3_2017_August_21_L2_0000	CoNS	E. faecium	88.15	87.1	86.85
515	GP_Genus_species_Blood_AE_GLEN_165885-3_C3_2017_August_21_L3_0000	CoNS	E. faecium	88.18	87.81	87.77
516	GP_Genus_species_Blood_AE_GLEN_165961-4_C3_2017_August_21_L1_0000	E. faecalis	E. faecalis	90.86	90.84	90.51
517	GP_Genus_species_Blood_AE_GLEN_165961-4_C3_2017_August_21_L2_0000	E. faecalis	E. faecalis	90.41	89.84	89.8
518	GP_Genus_species_Blood_AE_GLEN_165961-4_C3_2017_August_21_L3_0000	E. faecalis	E. faecalis	90.21	89.26	89.17
519	GP_Genus_species_Blood_AE_GLEN_166046-2_C3_2017_August_21_L1_0000	CoNS	CoNS	83.07	82.6	82.57
520	GP_Genus_species_Blood_AE_GLEN_166046-2_C3_2017_August_21_L2_0000	CoNS	CoNS	85.55	85.39	85.23

521	GP_Genus_species_Blood_AE_GLEN_166046-2_C3_2017_August_21_L3_0000	CoNS	CoNS	86.77	86.66	86.45
522	GP_Genus_species_Blood_AE_GLEN_166229-3_C3_2017_August_21_L1_0000	CoNS	CoNS	87.34	86.64	86.62
523	GP_Genus_species_Blood_AE_GLEN_166229-3_C3_2017_August_21_L2_0000	CoNS	CoNS	87.97	87.87	86.8
524	GP_Genus_species_Blood_AE_GLEN_166229-3_C3_2017_August_21_L3_0000	CoNS	CoNS	88.89	87.37	87.15
525	GP_Genus_species_Blood_AE_GLEN_166309_C3_2017_August_21_L1_0000	E. faecalis	E. faecalis	91.64	90.89	90.76
526	GP_Genus_species_Blood_AE_GLEN_166309_C3_2017_August_21_L2_0000	E. faecalis	E. faecalis	90.73	89.33	88.65
527	GP_Genus_species_Blood_AE_GLEN_166309_C3_2017_August_21_L3_0000	E. faecalis	E. faecalis	91.72	91.23	90.15
528	GP_Genus_species_Blood_AE_GLEN_166407_C3_2017_August_21_L1_0000	E. faecalis	E. faecalis	91.69	91.54	90.62
529	GP_Genus_species_Blood_AE_GLEN_166407_C3_2017_August_21_L2_0000	E. faecalis	E. faecalis	91.96	90	89.87
530	GP_Genus_species_Blood_AE_GLEN_166407_C3_2017_August_21_L3_0000	E. faecalis	E. faecalis	92.59	91.96	91.02
531	GP_Genus_species_Blood_AE_GLEN_166412-2_C3_2017_August_21_L1_0000	S. aureus	S. aureus	91.42	91.21	90.77
532	GP_Genus_species_Blood_AE_GLEN_166412-2_C3_2017_August_21_L2_0000	S. aureus	S. aureus	93.2	92.35	92.12
533	GP_Genus_species_Blood_AE_GLEN_166412-2_C3_2017_August_21_L3_0000	S. aureus	S. aureus	93.56	93.07	93.05
534	GP_Genus_species_Blood_AE_GLEN_166440-1_C3_2017_August_22_L1_0000	CoNS	CoNS	89.69	89.38	89.32
535	GP_Genus_species_Blood_AE_GLEN_166440-1_C3_2017_August_22_L2_0000	CoNS	CoNS	87.92	87.71	87.37
536	GP_Genus_species_Blood_AE_GLEN_166440-1_C3_2017_August_22_L3_0000	CoNS	CoNS	89.48	88.9	88.64
537	GP_Genus_species_Blood_AE_GLEN_166454_C3_2017_August_22_L1_0000	S. aureus	S. aureus	89.21	88.95	88.77
538	GP_Genus_species_Blood_AE_GLEN_166454_C3_2017_August_22_L2_0000	S. aureus	S. aureus	91.33	91.31	89.77
539	GP_Genus_species_Blood_AE_GLEN_166454_C3_2017_August_22_L3_0000	S. aureus	S. aureus	89.55	89.27	88.79
540	GP_Genus_species_Blood_AE_GLEN_166473-2_C3_2017_August_21_L1_0000	S. aureus	S. aureus	89.86	89.79	89.73
541	GP_Genus_species_Blood_AE_GLEN_166473-2_C3_2017_August_21_L2_0000	S. aureus	S. aureus	92.13	90.33	89.53
542	GP_Genus_species_Blood_AE_GLEN_166473-2_C3_2017_August_21_L3_0000	S. aureus	S. aureus	90.42	89.4	89.35
543	GP_Genus_species_Blood_AE_GLEN_166611_C3_2017_August_21_L1_0000	E. faecalis	CoNS	84.82	84.61	84.22
544	GP_Genus_species_Blood_AE_GLEN_166611_C3_2017_August_21_L2_0000	E. faecalis	CoNS	87.4	85.27	84.72
545	GP_Genus_species_Blood_AE_GLEN_166611_C3_2017_August_21_L3_0000	E. faecalis	CoNS	88.24	87.41	87.33
546	GP_Genus_species_Blood_AE_GLEN_166642_C3_2017_August_21_L1_0000	CoNS	CoNS	88.11	87.85	87.35
547	GP_Genus_species_Blood_AE_GLEN_166642_C3_2017_August_21_L2_0000	CoNS	CoNS	88.59	87.8	87.31
548	GP_Genus_species_Blood_AE_GLEN_166642_C3_2017_August_21_L3_0000	CoNS	CoNS	90.11	86.1	86.07
549	GP_Genus_species_Blood_AE_GLEN_166665_C3_2017_August_21_L1_0000	S. aureus	S. aureus	91.11	90.8	89.49
550	GP_Genus_species_Blood_AE_GLEN_166665_C3_2017_August_21_L2_0000	S. aureus	S. aureus	90.23	89.82	89.78
551	GP_Genus_species_Blood_AE_GLEN_166665_C3_2017_August_21_L3_0000	S. aureus	S. aureus	92.22	91.56	90.67
552	GP_Genus_species_Blood_AE_GLEN_166724-1_C3_2017_August_21_L1_0000	CoNS	CoNS	83.08	82.71	82.49
553	GP_Genus_species_Blood_AE_GLEN_166724-1_C3_2017_August_21_L2_0000	CoNS	CoNS	87.54	87.23	87.08
554	GP_Genus_species_Blood_AE_GLEN_166724-1_C3_2017_August_21_L3_0000	CoNS	CoNS	86.19	85.34	85.25
555	GP_Genus_species_Blood_AE_GLEN_166743_C3_2017_August_21_L1_0000	S. aureus	S. aureus	90.92	87.87	87.47

556	GP_Genus_species_Blood_AE_GLEN_166743_C3_2017_August_21_L2_0000	S. aureus	S. aureus	89.67	87.62	87.57
557	GP_Genus_species_Blood_AE_GLEN_166743_C3_2017_August_21_L3_0000	S. aureus	S. aureus	89.77	89.37	89.28
558	GP_Genus_species_Blood_AE_GLEN_166777_C3_2017_August_21_L1_0000	CoNS	CoNS	86.58	86.17	84.69
559	GP_Genus_species_Blood_AE_GLEN_166777_C3_2017_August_21_L2_0000	CoNS	CoNS	86.1	85.53	84.91
560	GP_Genus_species_Blood_AE_GLEN_166777_C3_2017_August_21_L3_0000	CoNS	CoNS	85.3	84.61	84.03
561	GP_Genus_species_Blood_AE_GLEN_166796-1_C3_2017_August_22_L1_0000	S. aureus	S. aureus	91.35	91.14	90.96
562	GP_Genus_species_Blood_AE_GLEN_166796-1_C3_2017_August_22_L2_0000	S. aureus	S. aureus	90.84	90.43	90.28
563	GP_Genus_species_Blood_AE_GLEN_166796-1_C3_2017_August_22_L3_0000	S. aureus	S. aureus	92.39	92.26	92.2
564	GP_Genus_species_Blood_AE_GLEN_166947_C3_2017_August_21_L1_0000	S. aureus	S. aureus	91.87	90.37	90.19
565	GP_Genus_species_Blood_AE_GLEN_166947_C3_2017_August_21_L2_0000	S. aureus	S. aureus	92.08	91.04	88.99
566	GP_Genus_species_Blood_AE_GLEN_166947_C3_2017_August_21_L3_0000	S. aureus	S. aureus	87.08	85.1	84.96
567	GP_Genus_species_Blood_AE_GLEN_166958-1_C3_2017_August_21_L1_0000	S. aureus	S. aureus	89.59	89.11	88.92
568	GP_Genus_species_Blood_AE_GLEN_166958-1_C3_2017_August_21_L2_0000	S. aureus	S. aureus	88.24	87.95	87.91
569	GP_Genus_species_Blood_AE_GLEN_166958-1_C3_2017_August_21_L3_0000	S. aureus	S. aureus	88.75	88.54	88.45
570	GP_Genus_species_Blood_AE_GLEN_166959_C3_2017_August_24_L1_0000	E. faecalis	E. faecalis	86.45	86.45	86.37
571	GP_Genus_species_Blood_AE_GLEN_166959_C3_2017_August_24_L2_0000	E. faecalis	E. faecalis	86.77	86.72	86.13
572	GP_Genus_species_Blood_AE_GLEN_166959_C3_2017_August_24_L3_0000	E. faecalis	E. faecalis	87.5	87.21	87.2
573	GP_Genus_species_Blood_AE_GLEN_166960_C3_2017_August_22_L1_0000	S. aureus	S. aureus	87.88	87.55	87.45
574	GP_Genus_species_Blood_AE_GLEN_166960_C3_2017_August_22_L2_0000	S. aureus	S. aureus	87.75	87.66	86.66
575	GP_Genus_species_Blood_AE_GLEN_166960_C3_2017_August_22_L3_0000	S. aureus	S. aureus	86.65	85.51	85.4
576	GP_Genus_species_Blood_AE_GLEN_166961-4_C3_2017_August_21_L1_0000	CoNS	CoNS	89.15	88.56	87.63
577	GP_Genus_species_Blood_AE_GLEN_166961-4_C3_2017_August_21_L2_0000	CoNS	CoNS	81.75	80.52	80.36
578	GP_Genus_species_Blood_AE_GLEN_166961-4_C3_2017_August_21_L3_0000	CoNS	CoNS	85.33	85	84.7
579	GP_Genus_species_Blood_AE_GLEN_167033-1_C3_2017_August_22_L1_0000	S. aureus	S. aureus	93.86	93.85	93.5
580	GP_Genus_species_Blood_AE_GLEN_167033-1_C3_2017_August_22_L2_0000	S. aureus	S. aureus	93.43	92.82	92.36
581	GP_Genus_species_Blood_AE_GLEN_167033-1_C3_2017_August_22_L3_0000	S. aureus	S. aureus	94.14	92.92	92.9
582	GP_Genus_species_Blood_AE_GLEN_167136_C3_2017_August_22_L1_0000	S. aureus	S. aureus	86.54	85.94	85.2
583	GP_Genus_species_Blood_AE_GLEN_167136_C3_2017_August_22_L1_0001	S. aureus	S. aureus	89.14	88.5	88.47
584	GP_Genus_species_Blood_AE_GLEN_167136_C3_2017_August_22_L2_0000	S. aureus	S. aureus	89.92	89.5	89.15
585	GP_Genus_species_Blood_AE_GLEN_167136_C3_2017_August_22_L2_0001	S. aureus	S. aureus	90.55	90.18	90.18
586	GP_Genus_species_Blood_AE_GLEN_167136_C3_2017_August_22_L3_0000	S. aureus	S. aureus	87.47	87.46	87.34
587	GP_Genus_species_Blood_AE_GLEN_167136_C3_2017_August_22_L3_0001	S. aureus	S. aureus	93.04	92.57	92.45
588	GP_Genus_species_Blood_AE_GLEN_167363_C3_2017_August_22_L1_0000	E. faecalis	E. faecalis	89.89	89.82	89.72
589	GP_Genus_species_Blood_AE_GLEN_167363_C3_2017_August_22_L2_0000	E. faecalis	E. faecalis	90.16	89.86	89.53
590	GP_Genus_species_Blood_AE_GLEN_167363_C3_2017_August_22_L3_0000	E. faecalis	E. faecalis	89.71	89.59	88.65

591	GP_Genus_species_Blood_AE_GLEN_167474_C3_2017_August_21_L1_0000	CoNS	CoNS	89.97	89.78	89.56
592	GP_Genus_species_Blood_AE_GLEN_167474_C3_2017_August_21_L2_0000	CoNS	CoNS	89.71	88.88	88.81
593	GP_Genus_species_Blood_AE_GLEN_167474_C3_2017_August_21_L3_0000	CoNS	CoNS	86.71	86.15	86.1
594	GP_Genus_species_Blood_AE_GLEN_167483_C3_2017_August_22_L1_0000	E. faecalis	E. faecalis	88.96	88.57	88.37
595	GP_Genus_species_Blood_AE_GLEN_167483_C3_2017_August_22_L2_0000	E. faecalis	E. faecalis	88.19	88.09	87.41
596	GP_Genus_species_Blood_AE_GLEN_167483_C3_2017_August_22_L3_0000	E. faecalis	E. faecalis	88.58	88.24	88.08
597	GP_Genus_species_Blood_AE_GLEN_167484-2_C3_2017_August_22_L1_0000	S. aureus	S. aureus	84.84	84.04	83.42
598	GP_Genus_species_Blood_AE_GLEN_167484-2_C3_2017_August_22_L2_0000	S. aureus	S. aureus	87.48	86.47	86.19
599	GP_Genus_species_Blood_AE_GLEN_167484-2_C3_2017_August_22_L3_0000	S. aureus	S. aureus	85.32	85.17	84.44
600	GP_Genus_species_Blood_AE_GLEN_167556_C3_2017_August_22_L1_0000	E. faecalis	E. faecalis	91.31	90.87	89.95
601	GP_Genus_species_Blood_AE_GLEN_167556_C3_2017_August_22_L2_0000	E. faecalis	E. faecalis	90.51	90.32	90.31
602	GP_Genus_species_Blood_AE_GLEN_167556_C3_2017_August_22_L3_0000	E. faecalis	E. faecalis	90.95	90.93	90.7
603	GP_Genus_species_Blood_AE_GLEN_167633_C3_2017_August_22_L1_0000	E. faecalis	E. faecalis	87.2	86.76	86.72
604	GP_Genus_species_Blood_AE_GLEN_167633_C3_2017_August_22_L2_0000	E. faecalis	E. faecalis	88.39	87.71	87.68
605	GP_Genus_species_Blood_AE_GLEN_167633_C3_2017_August_22_L3_0000	E. faecalis	E. faecalis	88.7	88.62	88.58
606	GP_Genus_species_Blood_AE_GLEN_167669-3_C3_2017_August_22_L1_0000	E. faecalis	E. faecalis	87.56	86.73	86.52
607	GP_Genus_species_Blood_AE_GLEN_167669-3_C3_2017_August_22_L2_0000	E. faecalis	E. faecalis	89.03	86.95	86.88
608	GP_Genus_species_Blood_AE_GLEN_167669-3_C3_2017_August_22_L3_0000	E. faecalis	E. faecalis	89.25	86.77	86.63
609	GP_Genus_species_Blood_AE_GLEN_167718-2_C3_2017_August_22_L1_0000	S. aureus	S. aureus	92.2	91.94	91.83
610	GP_Genus_species_Blood_AE_GLEN_167718-2_C3_2017_August_22_L2_0000	S. aureus	S. aureus	92.48	92.45	92.14
611	GP_Genus_species_Blood_AE_GLEN_167718-2_C3_2017_August_22_L3_0000	S. aureus	S. aureus	92.7	92.29	92.13
612	GP_Genus_species_Blood_AE_GLEN_167725_C3_2017_August_22_L1_0000	E. faecalis	E. faecalis	89.02	88.43	88.17
613	GP_Genus_species_Blood_AE_GLEN_167725_C3_2017_August_22_L2_0000	E. faecalis	E. faecalis	89.43	88.39	88.16
614	GP_Genus_species_Blood_AE_GLEN_167725_C3_2017_August_22_L3_0000	E. faecalis	E. faecalis	90.55	89.59	89.18
615	GP_Genus_species_Blood_AE_GLEN_167782_C3_2017_August_22_L1_0000	E. faecalis	E. faecalis	90.92	90.51	90.27
616	GP_Genus_species_Blood_AE_GLEN_167782_C3_2017_August_22_L2_0000	E. faecalis	E. faecalis	89.84	87.76	89.71
617	GP_Genus_species_Blood_AE_GLEN_167782_C3_2017_August_22_L3_0000	E. faecalis	E. faecalis	89.55	89.53	89.03
618	GP_Genus_species_Blood_AE_GLEN_167816_C3_2017_August_22_L1_0000	S. aureus	S. aureus	90.26	90.03	89.48
619	GP_Genus_species_Blood_AE_GLEN_167816_C3_2017_August_22_L2_0000	S. aureus	S. aureus	91.52	89.94	89.78
620	GP_Genus_species_Blood_AE_GLEN_167816_C3_2017_August_22_L3_0000	S. aureus	S. aureus	90.93	90.41	90.36
621	GP_Genus_species_Blood_AE_GLEN_167833_C3_2017_August_24_L1_0000	E. faecalis	E. faecalis	90.92	90.03	89.36
622	GP_Genus_species_Blood_AE_GLEN_167833_C3_2017_August_24_L2_0000	E. faecalis	E. faecalis	89.56	88.13	87.97
623	GP_Genus_species_Blood_AE_GLEN_167833_C3_2017_August_24_L3_0000	E. faecalis	E. faecalis	89.58	88.84	88.39
624	GP_Genus_species_Blood_AE_GLEN_168420_C3_2017_August_23_L1_0000	CoNS	CoNS	90.95	87.1	86.91
625	GP_Genus_species_Blood_AE_GLEN_168420_C3_2017_August_23_L2_0000	CoNS	CoNS	86.95	86.58	86.5

626	GP_Genus_species_Blood_AE_GLEN_168420_C3_2017_August_23_L3_0000	CoNS	CoNS	88.42	88.13	87.1
627	GP_Genus_species_Blood_AE_GLEN_168435_C3_2017_August_24_L1_0000	CoNS	CoNS	83.74	83.24	82.59
628	GP_Genus_species_Blood_AE_GLEN_168435_C3_2017_August_24_L2_0000	CoNS	CoNS	82.22	81.87	81.03
629	GP_Genus_species_Blood_AE_GLEN_168435_C3_2017_August_24_L3_0000	CoNS	CoNS	83.44	82.77	82.4
630	GP_Genus_species_Blood_AE_GLEN_169174_C3_2017_August_24_L1_0000	E. faecalis	E. faecalis	93.13	91.43	91.43
631	GP_Genus_species_Blood_AE_GLEN_169174_C3_2017_August_24_L2_0000	E. faecalis	E. faecalis	90.82	90.81	90.75
632	GP_Genus_species_Blood_AE_GLEN_169174_C3_2017_August_24_L3_0000	E. faecalis	E. faecalis	91.66	91.39	91.39
633	GP_Genus_species_Blood_AE_GLEN_158404 3_C3_2017_August_10_L1_0000	E. faecium	E. faecium	93.18	93.05	92.93
634	GP_Genus_species_Blood_AE_GLEN_158404 3_C3_2017_August_10_L2_0000	E. faecium	E. faecium	93.14	92.44	92.43
635	GP_Genus_species_Blood_AE_GLEN_158404 3_C3_2017_August_10_L3_0000	E. faecium	E. faecium	92.19	91.83	91.55

Chapter 8 – SUMMARY AND CONCLUSIONS

ATR-FTIR spectroscopy has been demonstrated to be a tool for rapid bacterial identification, which can be used in clinical laboratories due to its adequate sensitivity, speed, cost effectiveness, reproducibility and easy-to-use capabilities. Coupled with chemometrics, multivariate statistical and mathematical tools such as principal component analysis and hierarchical cluster analysis, the information embedded in the complex bacterial spectra was successfully obtained. This method of identification was successful at differentiating between closely related Gram-positive genera and two species/group of species from the same genus. The results and conclusions of each levels of discrimination from the research is provided below.

Enterococcus and Staphylococcus species separation

Discrimination between *Enterococcus* and *Staphylococcus* by ATR-FTIR spectroscopy was successfully attained by applying PCA to the whole region of selection 1480-980 cm^{-1}. Due to their spectral differences a forward search algorithm did not have to be used to remove non-relevant spectral features to enhance the separation between *Enterococcus* and *Staphylococcus*. Nonetheless, the major spectral differences observed between the two average spectra were visualized in the region of 1600-1160 cm^{-1} and 1064-985 cm^{-1}. The spectral differences in these regions was found to be related to the differences in RNA/DNA. Moreover, hierarchical cluster analysis (HCA) was used to generate a dendogram to further demonstrate the complete discrimination between *Staphylococcus* and *Enterococcus*. The research conducted establishes the potential of ATR-FTIR spectroscopy for the discrimination between different bacterial species.

Enterococcus faecalis and Enterococcus faecium

E. faecalis was discriminated from *E. faecium* by PCA using the whole region of interest 1480-900 cm^{-1}. After the use of a forward search algorithm to remove all non-relevant spectral features, the regions 1042-1049 cm^{-1}, 1079-1085 cm^{-1} and 1109-1167 cm^{-1} enhanced the

separation between *E. faecalis* and *E. faecium*. The absorption bands in these regions may originate from the carbohydrate composition of the bacterial cell wall. During this study, three outliers were originally detected and with the use of MALDI-TOF and Vitek II, the true identity of the outliers were obtained. It was found that 2 out the 3 were incorrectly labeled *E. faecium* samples and the remaining outlier was found to be *Escherichia coli*. The work completed in this part of the research was able to demonstrate the power of ATR-FTIR to discriminate two species from the same genus and also it was able to detect the presence problematic samples.

Staphylococcus aureus **and Coagulase-Negative** ***Staphylococci***

ATR-FTIR spectroscopy was used in the preliminary study of discriminating *S. aureus* and CoNS by PCA using the whole region of interest 1480-900 cm^{-1}. Using a forward search algorithm to remove all non-relevant spectral features, the region 1057-1072 cm^{-1} was found to create a tighter grouping, complete separation between both was not achieved. Absorption bands in this region were found to originate from polysaccharides. The present study has shown that there lies limitation with the small sample size of data used in this research. We were able to form two clusters of data but were unable to achieve full separation from one another. The specificity of the ATR-FTIR spectroscopic technique can be increased with additional data points in the dataset.

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