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Review

PCR–Electrospray Ionization Mass Spectrometry

The Potential to Change Infectious Disease Diagnostics in Clinical and Public Health Laboratories

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During the past 20 years, microbial detection methods that are genetically based, such as real-time PCR and peptide nucleic acid fluorescent hybridization, coexisted with traditional microbiological methods and were typically based on the identification of individual genetic targets. For these methods to be successful, a potential cause of infection must be suspected. More recently, multiplex PCR and multiplex RT-PCR were used to enable more broad-range testing based on panels of suspected pathogens. PCR–electrospray ionization mass spectrometry (PCR-ESI/MS) has emerged as a technology that is capable of identifying nearly all known human pathogens either from microbial isolates or directly from clinical specimens. Assay primers are strategically designed to target one or more of the broad pathogen categories: bacterial, mycobacterial, fungal, or viral. With broad-range amplification followed by detection of mixed amplicons, the method can identify genetic evidence of known and unknown pathogens. This unique approach supports a higher form of inquiry, asking the following question: What is the genetic evidence of known or unknown pathogens in the patient sample? This approach has advantages over traditional assays that commonly target the presence or absence of one or more pathogens with known genetic composition. This review considers the breadth of the published literature and explores the possibilities, advantages, and limitations for implementation of PCR-ESI/MS in diagnostic laboratories. ***(J Mol Diagn 2012, 14:295-304; http://dx.doi.org/10.1016/j.jmoldx.2012.02.005)***

Any practicing microbiologist associates microbial detection with microbial stains, culture, and other phenotypic analysis. Throughout the past two decades, genetic-based detection methods, such as real-time PCR and peptide nucleic acid fluorescent hybridization, co-existed with traditional microbiological methods and were typically based on the detection of individual genetic targets; therefore, prior suspicion of the potential causes of infection was typically required. Within the past decade, multiplex PCR and multiplex RT-PCR were implemented to enable partial broad-range testing based on the detection of panels of likely pathogens. In some cases, true broad-range diagnostic testing was achieved using capillary sequencing or pyrosequencing approaches that interrogate clinical samples with consensus primers to both amplify and sequence conserved genetic regions, such as the 16S region of bacteria or the 18S region of fungi. Although previously described methods remain engrained in diagnostic laboratories, emerging new technologies, such as PCR–electrospray ionization mass spectrometry (PCR-ESI/MS), offer a new approach with the capability to identify nearly all known human pathogens directly from clinical specimens and the potential to identify genetic evidence of undiscovered pathogens[1] and genetic changes that may occur to alter sequences used as targets in existing molecular assays. Designed for both broad-range and target-specific identification of pathogens and their genetic characteristics, PCR-ESI/MS can identify minute quantities and mixtures of nucleic acids. Therefore, if a novel microbial genetic sequence is uncovered, it is

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reported and accompanied by information that describes its relationship to closely related organisms. As an example of pathogen discovery, one situation stands out and exemplifies the potential impact of PCR-ESI/MS technology. In April 2009, the technology detected the first US case of the novel H1N1 influenza virus[2] in San Diego, CA. In this instance, the virus was identified as novel but genetically related to influenza A.

A Brief History of PCR-ESI/MS

Because traditional methods for detecting and identifying the presence of biothreats were slow, the Ibis technology was originally developed for biodefense and public health safety, enabling rapid detection and identification of pathogens in clinical and environmental samples. Capable of automated analysis of >1500 PCRs per day, it was deemed a strategic platform in situations when rapid identification, epidemiological surveillance, and mitigation of transmission are concerns.[3] Originally called Triangulation Identification for the Genetic Evaluation of Risks, or TIGER,[4,5] Ecker et al[6] reported the first use of this technique in 2005. The technology was further advanced in 2008.[5]

The Ibis T5000 Biosensor System was the PCR-ESI/MS prototype instrument (Figure 1A).[7] Originally conceived by Ibis Biosciences (Carlsbad, CA), the system couples equipment that desalts PCR products and sends them to a time-of-flight (TOF) analyzer, providing mass accuracy of sufficient resolution to discern the base compositions of the amplicons produced by multiplex PCR.

In 2008, Abbott Molecular (Des Plaines, IL) acquired the Ibis technology and advanced to the current version of the instrument, now called the PLEX-ID (Figure 1B). Abbott Molecular supports reagents that are classified as not for diagnostic use (NFDU) and assumes all instrument and product distribution responsibilities and technical support for the instrument. A list of available NFDU assays is provided in Table 1. The PLEX-ID enables the rapid identification (approximately 6 to 8 hours, depending on the nucleic acid type) and characterization of bacterial, viral, fungal, or other infectious organisms and analysis of human DNA. Because the PLEX-ID is relatively new, in all but one case, the published literature summarized in this review describes research performed on its prototype, the Ibis T5000.[4,5,8]

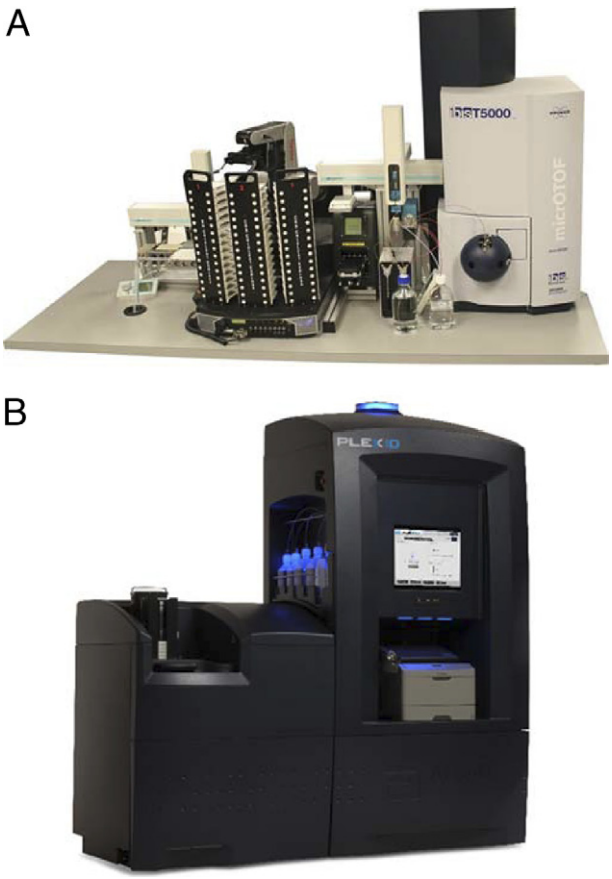

Figure 1. A: The Ibis T5000 Biosensor System is the PCR-ESI/MS prototype. **B:** The Abbott PLEX-ID system is the current instrument. Reprinted with permission from Ibis Biosciences.

Principles of PCR-ESI/MS

Sample Preparation

As with other molecular sample preparation methods, nucleic acids must be extracted, either directly from clinical specimens or from cultivated microbial isolates. A wide variety of DNA or RNA extraction methods, both manual and automated, were successfully used to effectively isolate nucleic acid from bacteria, viruses, mycobacteria, and fungi upstream of the PCR-ESI/MS assays. Even simple inexpensive methods, such as boiling followed by centrifugation or magnetic bead-based purification, were sufficient for some applications, including genotyping of isolated bacterial colonies. Extraction methods are available for a variety of sample types for which efficient cell lysis was achieved, either through mechanical means (zirconium beads) or via chemical lysis involving the use of chaotropic agents, such as commercial reagents[8–25] obtained from companies such as Qiagen (Valencia, CA), KingFisher (Thermo Scientific, Waltham, MA), or Ambion (Austin, TX).

Acceptable sample types include environmental samples (eg, air samples from dry filter units, surface swabs, and water samples), biological samples (eg, bacterial colonies and bacterial or viral cultures), clinical samples (eg, throat swabs, nasal swabs, nasopharyngeal swabs, nasal washes, sputum, and skin swabs), and food samples (eg, meat, dairy, and produce). For human forensics samples, protocols are available for hair, teeth, bone, and blood.

Although many extraction methods were successfully used upstream of the Abbott Molecular NFDU assays, not all methods perform equally. For sensitive detection of a broad range of organisms (eg, the broad bacterial reagents), ultraclean sample preparation reagents, available from Abbott Molecular, are recommended to eliminate background nucleic acid contamination. Lack of such purity will affect the results. Reagents acquired from other sources, labeled as DNase or RNase free, do not equate to DNA-free reagents.

Table 1. List of Assays Commercially Available for PCR-ESI/MS

Assay name	Coverage
BAC detection assay	Designed to detect all groups of bacteria, including *Mycoplasma*, *Chlamydia*, *Rickettsia*, and *Candida* species, and antibiotic resistance markers (*bla*KPC, *mec*A, *van*A, and *van*B genes)
Biothreat	Designed to detect and identify 17 bacterial and viral biothreat agents and distinguish them from their near neighbors
Broad bacteria	Designed to detect and identify all groups of bacteria, including intracellular organisms, such as *Mycoplasma*, *Chlamydia*, and *Rickettsia,* as single organisms or simple mixtures from isolates or high-load samples
Broad bacteria	Designed to detect and identify all groups of bacteria, including intracellular organisms, such as *Mycoplasma*, *Chlamydia*, and *Rickettsia*, as single organisms or simple mixtures from isolates
Broad fungal	Designed to identify *Aspergillus*, *Bipolaris*, *Candida*, *Fusarium*, *Penicillium*, *Clavispora*, and *Cryptococcus* species along with common fungi
Broad viral I	Designed to detect and identify individual organisms or mixtures of Herpesviridae, human adenovirus, human enterovirus, polyomavirus, and parvovirus directly from samples
Clostridium difficile	Designed to rapidly identify *C. difficile* and the mutations associated with the *tcdC* locus and NAP-1 strains while simultaneously detecting *tcd*A, *tcd*B, *cdt*A, and *cdt*B genes
Flu detection assay	Detection and characterization of known and newly emerging influenza A, B, and C viruses directly from human, avian, animal, or environmental specimens
Foodborne bacteria	Designed to detect and identify several common foodborne bacteria, including *Escherichia coli* O55:H7 and O157:H7, >250 members of *Salmonella enterica* serotypes, *Shigella*, and *Listeria* species
MRSA detection	Genotyping and characterization (*mec*A, *luk*S-PV, *luk*D, *tsst*-1 and *mup* genes) of *Staphylococcus aureus* with a focus on MRSA strains
MDR TB	Determination of MDR in *Mycobacterium tuberculosis* isolates and identification of NTM
Pneumococcus serotyping	Identification and characterization of *Streptococcus pneumoniae* from culture isolates
Respiratory virus	Detection and identification of common respiratory viruses, including influenza, respiratory syncytial virus, adenovirus with typing, parainfluenza virus 1 to 4, human metapneumovirus, and coronavirus
Vectorborne BPN	Designed to detect and identify bacteria (*Francisella*, *Borrelia*, *Rickettsia rickettsii*, and alphaproteobacteria), protozoa (*Babesia*), and nematodes (*Dirofilaria immitis*) transmitted by ticks

BAC, bacteria, antibiotics, candida; BPN, bacteria, protozoa, nematodes; MDR, multidrug resistant; MRSA, methicillin-resistant *S. aureus;* NAP, North American pulsed field type; NTM, nontuberculous mycobacteria; TB, *Mycobacteria tuberculosis*.

Multiplex PCR or RT-PCR

After sample preparation, nucleic acids are dispensed into wells of a microtiter plate for downstream multiplex amplification. Each well contains one or more pairs of broad-range or target-specific primers (depending on the assay type) and other PCR components to support amplification via multiplex PCR or multiplex RT-PCR. The PCR-ESI/MS assays contain a variety of purposefully designed primer sets that interrogate common conserved and variable sequences found among various classes of organisms. Small amplicons (80 to 150 bp) of various sizes are generated, depending on the species of microbes present in the original sample. Amplification produces genetic products, unique within a group of organisms, microbial domain, or microbial division.

For PCR/ESI-MS, proper amplification relies on the genetic similarities in microbial genomes. For example, bacteria have highly conserved sequences in several chromosomal locations, including the universally conserved regions of ribosomal RNA, other noncoding RNAs, and essential protein-encoding genes. As illustrated in Figure 2, these conserved sequences, intercalated with regions of sequence diversity, serve as priming sites for broad-range primers to amplify sequences of various sizes and compositions. Thousands of genetic possibilities exist, because broad-range primers are used in conjunction with species-, strain-, and characteristic-specific primers that can target identifiable genes for antibiotic resistance or key pathogenic features.

A typical PCR-ESI/MS assay uses a mastermix of reagents, optimized for use in an amplification reaction

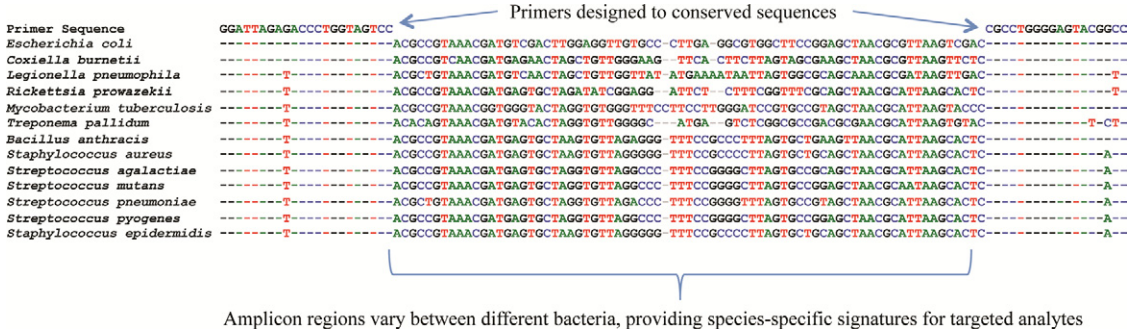

Figure 2. Conserved sequences serve as priming sites on which broad-range primers amplify sequences of various sizes and compositions, because of combinations of conserved regions that flank intercalated regions of sequence diversity, found in the space between common regions. Reprinted with permission from Ibis Biosciences.

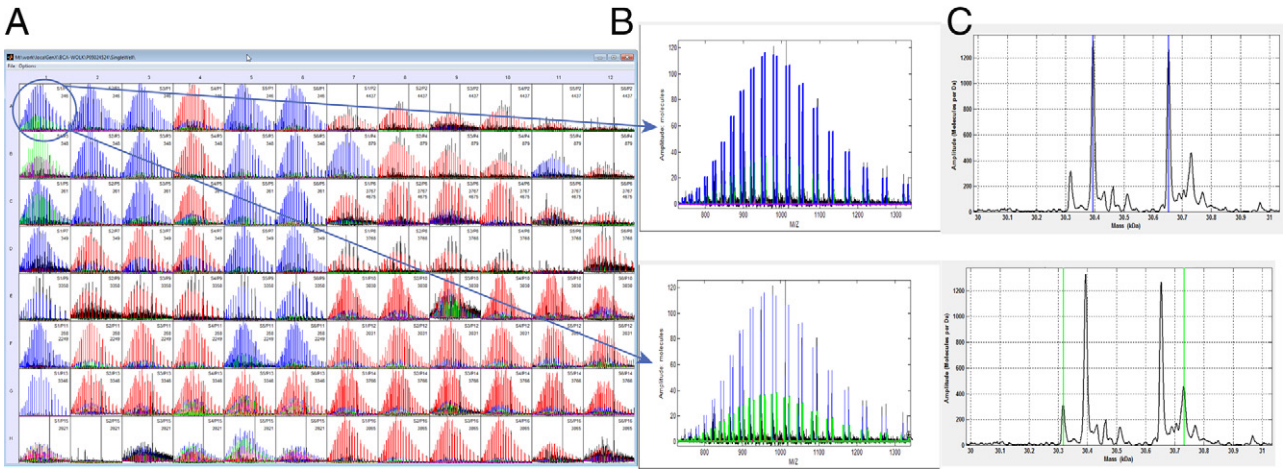

Figure 3. Representative mass spectra from PCR-ESI/MS. Data resulting from ESI TOF MS of each microtiter well containing one primer pair and the target DNA. **A:** The 96-well view of spectra collected. **B:** Two charge-state distributions are seen in products produced with primer 346 for each forward and reverse primer, indicating the presence of two microorganisms in this sample. Blue, *Bacteroides thetaiotaomicron*; green, *S. aureus*. **C:** Deconvolution of collected mass spectra, resulting in a mol. wt. for the forward and reverse strand. The resulting base compositions are as follows: [30A-27G-23C-19T] (**top panel**) and [27A-30G-21C-21T] (**bottom panel**). Reprinted with permission from Kaleta et al,[10] copyright American Society for Microbiology. AI, average intensity.

using multiple primers. Multiplex amplification generally occurs under conditions of low stringency to allow for non-specific primer annealing, a parameter that supports the mismatch amplification required to identify unknown genomic mutations within the targeted regions. Amplification typically requires a two-step PCR protocol to enrich amplification of important genetic targets.

Desalting

After amplification, PCR products undergo an extensive desalting process before injection into a mass spectrometer, which is dedicated to analysis of negatively charged molecules. For the PLEX-ID system to become a reality, various attributes of desalting and ESI required optimization. In 2003, Jiang et al[26] optimized a highly efficient and automated method of purifying and desalting PCR products for analysis by ESI-MS. By using standard laboratory robotics, their protocol used pipette tips packed with an anion-exchange resin and had four primary steps: tip pretreatment, sample loading, rinsing, and sample elution of up to 96 samples at a time. By using an internal mass standard, mass measurement provided an unambiguous base composition for up to a 120-mer PCR product with sub ppm measurement error.[26]

ESI-TOF

Negative ion-mode ESI is used for analysis. Electrospray ionization moves charged amplicons into the mass spectrometer via processes optimized to detect negatively charged oligonucleotide ions. By definition, MS is a sensitive analytical technique that measures the mass/charge (*m/z*) ratio of charged particles and can do so with high accuracy. To optimize the process, the ESI separates double-stranded amplicons and the intact single strands are pulsed into the flight tube of the mass spectrometer under high vacuum. Strands are then separated based on *m/z*, where lower *m/z* ions travel faster and reach the detector earlier than ions with higher *m/z*. The composite distribution of masses appears as a distribution of peaks, corresponding to different charge states, and is called a mass spectrum.

The resulting mass spectrum is deconvoluted, a process by which the spectra are mathematically simplified, as demonstrated in Figure 3, A–C.[10] The mass of the intact amplicon is calculated, and signals from low molecular weight (mol. wt.) chemical noise are eliminated.[27] Once signals are digitally processed, the results are listed as highly accurate mol. wt. (masses) of the forward and reverse strands of each amplicon.

Once the amplicon masses are established, software algorithmically predicts their base composition, as depicted in Figure 4. Calculations rely on the known masses of the four nucleic acids (adenine, guanine, cytosine, and thymine) present in the amplicon and the knowledge of

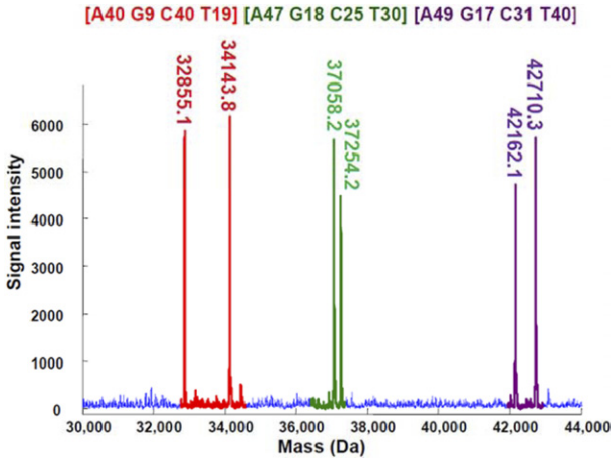

Figure 4. The ESI/MS generates amplicon base composition values from the intact mol. wt. measured by MS for several genetic regions and compare results to the Ibis microbial sequence database. Composition values will vary by microorganism. Reprinted with permission from Ibis Biosciences.

DNA strand complementarity. A joint least-squares algorithm correlates potential organism identifications from across multiple genetic regions. Because several genetic regions are amplified, multiple genetic compositions are compared with a curated database to narrow the genetic possibilities of the amplicons to one unambiguous genetic identity of the microbe. The regions amplified vary by organisms and are assay-type dependent.

Curated Amplicon Reference Database

Undoubtedly, a key element of the PLEX-ID system is the Ibis Biosciences–curated database of genomic information that associates base composition with the identity of thousands of organisms. Despite the enormous class diversity of organisms, the database, containing carefully selected and curated genetic sequences, allows the interrogation to produce relevant and accurate results. The database is populated based on the needs of infectious disease surveillance, diagnostic testing, forensic sciences, and biological research.

The database is regularly updated with information from newly identified microorganisms. Novel base counts pass through the curation process to determine that they are representative of a new microbe or strain. The design of primers and the determination of base compositions for individual organisms to be entered into the database are activities performed by Ibis scientists using sequence alignments and extensive primer scoring over many sequences for the target organisms. Once primers are validated in the laboratory, they are included in the database.

In summary, for microbial identification to occur, genetic sequence matching must occur. The technology's software bases its deductions on several known assumptions: i) the potential genetic targets are known and present in each multiplex primer set; ii) base compositions of forward and reverse amplicon strands must be complementary and, therefore, for a particular *m/z* ratio, there are a limited number of possibilities; iii) for a single, unique base composition, only a few possible base compositions must be consistent with each measured mass, and finally, iv) the unique base composition of an amplicon is compared with the Ibis database of >750,000 entries, which links the base composition for each primer pair to a small list of candidate microbes.

Work Flow Overview

The PLEX-ID Rapid Bioidentification System provides complete information management for instrument control, tracking, mass spectral signal processing, and analysis from original samples to organism identification. After the extraction and amplification steps, the PLEX-ID process is fully automated with regard to sample tracking, instrument control and robotics, data processing and analysis, and comparison with the curated database. The entire PLEX-ID process requires approximately 6 to 7 hours after extraction, including approximately 4 hours of amplification. Processing events, such as PCR amplification and sample dispensing, which are the limiting factors, are scheduled in parallel to maximize the throughput of the instrument. Post-PCR on-instrument throughput is approximately 1.25 hours, such that approximately ≥20 plates can be analyzed each day and, depending on the assay, 6 to 12 patient samples can be processed per plate, resulting in maximum throughput ranges from 108 to 216 samples per 24-hour day. The upper range is achieved when up to four thermocyclers are available for the PCR steps.

During the PLEX-ID testing process, robotics and sample tracking register and verify plate barcodes to ensure accurate tracking of the physical transfer of samples during the spray and data acquisition phases. The system also automatically triggers spectral processing and data analysis of the raw data. Coupled together, the instrument enables a continuous, automated work flow from physical sample to the resulting organism identification.

Review of the Literature for the T5000-Based Assays

Across the United States, there are >20 PLEX-ID systems in place, primarily for infectious disease research and surveillance, food or biopharmaceutical testing, and human identification studies. Although the commercial version of the technology is known as PLEX-ID, the proof of principle was established on its earlier prototype, the T5000. The publications that describe PCR-ESI/MS performed with the T5000 instrument are summarized in the following sections.

Select Agents

In the first reported use of this technique,[28] select agents were used to demonstrate its potential. First, isolated colonies of *Bacillus anthracis* were analyzed to illustrate the ability to detect an amplicon and derive base composition by comparing the results from high-resolution Fourier-transform ion cyclotron resonance MS with those obtained by a TOF mass analyzer. The authors concluded that, because of the detection of the reverse compliment of each amplicon, only 20-ppm mass accuracy was required to derive a single putative base composition from a measured mol. wt. The mass accuracy of a typical TOF experiment using post-calibration is approximately 5 to 10 ppm, indicating that TOF-MS is sufficient for mass analysis.

The first reported occurrence of plague in Afghanistan used PCR-ESI/MS for select agent identification. *Yersinia pestis* was identified in a postmortem analysis of an outbreak of acute gastroenteritis associated with the consumption or handling of camel meat. Molecular testing of patient samples and tissue from the camel using PCR-ESI/MS revealed DNA signatures consistent with *Y. pestis*. Confirmatory testing using real-time PCR and immunological seroconversion of one of the patients confirmed that the outbreak was caused by *Y. pestis*.[29]

Clinical and Public Health Settings and Infection Prevention

Bacterial identification and genotyping are useful for the detection of infections and biosurveillance activities of both the clinical and public health sectors. Classical

genotyping is performed by a variety of methods, including pulsed-field gel electrophoresis (PFGE), repetitive element sequence-based amplification (rep-PCR), multilocus sequence typing (MLST), and DNA sequencing of genomic regions that contain genetic variation. Most genotyping methods are tedious and expensive. In contrast, PCR-ESI/MS performs this analysis with relative ease. In the strain genotyping mode, PCR/ESI-MS primers are targeted to genes that contain highly variable regions, which are used to distinguish closely related strains within a given species. The derived genotype has significant practical value at a fraction of the turnaround time of traditional methods and, therefore, could enable rapid tracking of transmission in an infectious disease outbreak and appropriate outbreak intervention and infection prevention measures.

Culture-based testing can be biased toward one organism, thus biasing the true representation of the true cause of infection; however, the ability of PCR-ESI/MS to identify multiple strains and multiple organisms may be valuable in certain clinical and public health settings.[6,18,30] Initial single bacterial isolate identification with a blinded panel of 156 diverse bacterial species of human and/or animal pathogens was tested, with 91% accuracy.[17] Further refinements to the Ibis database have since improved accuracy, as reported for specific bacteria in the rest of this review, depicting the potential of the PLEX-ID system for a variety of applications.

Clinical specimens can be tested directly, and identification of multiple microbes is feasible up to a point. The first report describing testing of mixed microbes directly from patient samples depicts a military-based outbreak investigation. Analysis of respiratory samples revealed high concentrations of pathogenic respiratory species, including *Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pyogenes*. When *S. pyogenes* was identified in samples from the epidemic site, the identical genotype was found in almost all recruits.[31]

Kaleta et al[10] performed PCR-ESI/MS testing directly from blood culture bottles and compared results with conventional methods to identify the genus and species of microorganisms found to cause human bloodstream infections ($n = 234$). This study demonstrated high analytical accuracy, with 98.7% and 96.6% concordance at the genus and species levels, respectively. Mixtures of microbes were identified in 29 blood culture bottles, including mixed species of the same genus, and mixtures containing Gram-positive and Gram-negative organisms, exemplifying the ability to identify multiple organisms simultaneously without the need for further sub-cultivation. The study also reports some microbe combinations that were not accurately detected; therefore, method improvements are still required.[10] In a second study, compared with matrix-assisted laser desorption/ionization–TOF/MS of subcultured microbial isolates, no statistically significant difference in accuracy was observed.[32]

Stoodley et al[33] performed testing and evaluated PCR-ESI/MS for use in bacterial biofilms, which have been observed in many prosthesis-related infections. An ankle arthroplasty culture, negative on preoperative aspiration produced positive results via PCR-ESI/MS, detecting *Staphylococcus aureus*, *Staphylococcus epidermidis*, and the methicillin-resistance gene *mecA* in soft tissues associated with the explanted hardware. This viable multispecies biofilm was confirmed by further micrographic and molecular examinations, such as confocal microscopy using Live/Dead stain, bacterial fluorescence *in situ* hybridization, and RT-PCR for bacterial mRNA. These results suggest that PCR/ESI-MS may be a useful adjunct to routine cultures in the detection of biofilm bacteria in prosthetic joint infections.

Review by Specific Microbes

S. aureus

By using a panel of diverse bacterial isolates collected from the Centers for Disease Control and Prevention that were previously characterized by MLST and PFGE, and clinical isolates from two geographically distinct hospitals, PCR-ESI/MS accurately determined *S. aureus* identification and genotypes. By using a primer designed to amplify the same genes analyzed in MLST methods, PCR-ESI/MS accurately identified the major clonal complex (98.8% accuracy) and US pulsed-field type (100% accuracy). Clustering results were in agreement with those for rep-PCR and PFGE, making the method suitable for use in outbreak investigations.[34]

Furthermore, Wolk et al[35] reported accuracy of the high-resolution genetic signature to identify genotypic determinants, encoding antimicrobial resistance and virulence, consistent with phenotypic traits in well-characterized reference and clinical isolates of *S. aureus*. In this study, the identification of common toxin genes correlated with independent PCR analyses for the toxin genes. Molecular identification of the antibiotic-resistance genes correlated with phenotypic *in vitro* resistance; sensitivity ranged from 90% to 100%, and specificity ranged from 96% to 100%, with the exception of erythromycin (sensitivity, 70%; specificity, 98%). Staphylococcal isolates were correctly classified into genotypic groups that correlated with genetic clonal complexes, rep-PCR patterns, or PFGE types.[35]

Acinetobacter *Species*

Hujer et al[36] reported accurate resistance-determinant identification for *Acinetobacter* species, which could inform therapeutic decision making in the treatment of infections. By using a collection of 75 well-characterized multidrug-resistant *Acinetobacter* species, PCR-ESI/MS accurately identified quinolone resistance mediated by mutations in the quinolone resistance-determining regions of *gyrA* and *parC*, highly conserved essential housekeeping genes. Single point mutations were identified in *parC* for 55 of 75 isolates and in *gyrA* for 66 of 75 isolates. Overall, results correlated with susceptibility testing and DNA sequencing.

By using isolates collected from infected and colonized soldiers and civilians involved in outbreaks in the military health care system, six housekeeping genes (*trpE*, *adk*, *efp*, *mutY*, *fumC*, and *ppa*) were sequenced, and results were compared with analysis by the PCR-ESI/MS using nine

primer pairs targeting the most information-rich regions of the genes. *Acinetobacter baumannii* predominated this sample set (189/216 isolates); however, at least 18 *Acinetobacter* species were accurately identified. Results for genotyping were in agreement with those obtained by PFGE analysis, distinguishing 47 of 48 *A. baumannii* genotypes previously identified by sequencing. Ultimately, 111 isolates had genotypes identical or similar to those associated with well-characterized *A. baumannii* isolates from European hospitals. Twenty-seven isolates had genotypes representing different *Acinetobacter* species, including eight representatives of *Acinetobacter genomospecies* 13 and 13 representatives of *A. genomospecies* 3.[37]

Schuetz et al[38] investigated the relatedness of multidrug-resistant *A. baumannii* isolates from a burn intensive care unit and other outbreak and nonoutbreak strains. Genotypes were compared by three molecular typing methods: rep-PCR, PCR/ESI-MS, and PFGE. Results of PCR/ESI-MS and PFGE genotyping were most closely correlated, whereas rep-PCR and PCR/ESI-MS systems were the most rapid. Molecular typing analysis showed that 17 of 19 outbreak isolates were indistinguishable or closely related to each other, and the outbreak strain represented a novel strain type, ST96, with a new combination of alleles previously not seen in the United States.[38]

Other Bacteria and Bacteria-Like Organisms

In a challenge panel that comprised 25 strain types of *Campylobacter jejuni* and 25 strain types of *Campylobacter coli*, PCR-ESI/MS accurately genotyped *C. jejuni* strain types with a resolving power comparable to MLST and readily distinguished *C. jejuni* from *C. coli*. From pure bacterial isolates, accuracy was achieved with as little as 10 genomes per PCR.[22]

For *Ehrlicia* species, identification was achieved directly from blood specimens. The results were compared to a colorimetric microtiter PCR enzyme immunoassay for 213 whole-blood samples collected from patients with a clinical suggestion of ehrlichiosis. Of those samples, 40 (18.8%) were positive for an *Ehrlichia* species by PCR-ESI/MS, with sensitivity, specificity, and positive and negative predictive values of 95.0%, 98.8%, 95.0%, and 98.8%, respectively. Of 38 specimens that tested positive by both PCR-ESI/MS and PCR–enzyme immunoassay, species-level agreement was 100%. In addition, *Rickettsia rickettsii* was detected by PCR-ESI/MS from four specimens that were confirmed retrospectively by serological findings and PCR–enzyme immunoassay. Another three specimens contained clinically relevant bacterial pathogens, specifically *Pseudomonas aeruginosa*, *Neisseria meningitidis*, and *S. aureus*, confirmed by culture and/or clinical diagnosis.[14]

In one case study, *Bartonella quintana* was identified as a rare cause of abdominal aortic mycotic aneurysm in a 69-year-old man experiencing chronic back pain but otherwise exhibiting no signs of infection.[39]

Mycobacteria *Species*

To simultaneously determine isoniazid and rifampin resistance in the *Mycobacterium tuberculosis* complex and to determine the species of nontuberculous mycobacteria, Massire et al[11] described PCR-ESI/MS using a collection of 1340 DNA samples from cultured specimens collected in New York, Russia, and South Africa. Compared with phenotypic data, PCR-ESI/MS achieved only 89.3% sensitivity and 95.8% specificity for the determination of isoniazid resistance and 96.3% sensitivity and 98.6% specificity for the determination of rifampin resistance. Based on a set of 264 previously characterized liquid culture specimens, the PCR-ESI/MS method had 97.0% sensitivity and 99.9% specificity for determination of nontuberculous mycobacteria identity.[11] Finally, in high- and low-prevalence regions in China, PCR-ESI/MS determined the genotype and drug-resistance profiles for 96 *M. tuberculosis* isolates.[40]

Fungi

To date, few publications have described the use of PCR/ESI-MS for identification of fungi. Gu et al[41] evaluated well-characterized strains of clinically relevant *Candida* species on the PLEX-ID instrument. Of the set, 61 isolates were tested by two methods, rep-PCR and PCR/ESI-MS, and compared with a reference standard, gene sequencing of the internal transcribed spacer ribosomal RNA gene. Both rep-PCR and PCR/ESI-MS correctly identified 51 of 61 isolates to the species level.[41]

In a study describing identification of bloodstream pathogens, Kaleta et al[32] included a few *Candida* species (n = 11) recovered from blood cultures and documented 100% agreement of PCR/ESI-MS versus Vitek 2 identification.

Respiratory Viruses

With promise in the field of influenza surveillance, pandemic preparedness, and as an aid to vaccine strain selection, rapid RT-PCR–ESI/MS is a broad-range influenza identification tool that can be used to simultaneously identify all types of influenza viruses from clinical specimens. Viruses are identified with clade-level resolution of viral populations, which is useful for monitoring global spread and emergence of novel viral genotypes. Base compositions of RT-PCR amplicons from influenza core gene segments (PB1, PB2, PA, M, NS, and NP) are analyzed to provide subspecies identification and infer influenza virus H and N subtypes. In one report,[42] the assay correctly identified 92 mammalian and avian influenza isolates, representing 30 different H and N types, including 29 avian H5N1 isolates. Furthermore, direct analysis of 656 human clinical respiratory specimens collected during a 7-year period (1999 to 2006) showed correct identification of the viral species and subtypes, with >97% sensitivity and specificity. Base composition-derived clusters inferred from this analysis showed 100% concordance to previously established clades. Ongoing surveillance of samples from the recent influenza virus seasons (2005 to 2006) showed evidence for emergence and establishment of new genotypes of circulating H3N2 strains

worldwide. Mixed viral quasispecies were found in approximately 1% of these recent samples, providing a view into viral evolution.[42]

In subsequent work, the assay differentiated novel H1N1 from seasonal and other nonhuman host viruses, demonstrating its usefulness and robustness in influenza virus surveillance and detection of novel and unusual viruses with previously unseen genomic prints.[43] In this study, 757 samples collected between 2006 and 2009 were tested, including 302 seasonal H1N1, 171 H3N2, 7 swine triple reassortants, and 277 H1N1 viruses. Of the 277 H1N1 samples, 209 were clinical specimens (eg, throat, nasal and nasopharyngeal swabs, nasal washes, blood, and sputum).

By using base count signatures from one of the first cases of the 2009 H1N1 pandemic (A/California/04/2009), PCR-ESI/MS confirmed an unusual, previously unrecognized influenza A virus, with core genes related to viruses of avian, human, and swine origins. Subsequent analysis of an additional 276 H1N1 pandemic samples revealed that they shared the genomic print of A/California/04/2009, which differed from those of North American swine triple reassortant viruses, seasonal H1N1 and H3N2, and other viruses tested.[44] Moreover, this assay allowed distinction between core genes of cocirculating groups of seasonal H1N1, such as clades 2B and 2C, and their reassortants with dual antiviral resistance to adamantanes and oseltamivir.[43]

In a broader assessment of emerging coronaviruses, 14 isolates of nine diverse *Coronavirus* species, including the severe acute respiratory syndrome–associated coronavirus, were tested.[25] The PCR-ESI/MS method identified and distinguished between severe acute respiratory syndrome and other known coronaviruses, including the human coronavirus 229E and OC43, individually and in a mixture of all three human viruses. The sensitivity of detection was estimated to be approximately 1 plaque-forming unit (PFU)/mL.[25]

Finally, the NFDU Respiratory Viral Panel identifies respiratory syncytial virus; influenza A and B; parainfluenza 1 to 3; adenovirus A to F; coronavirus; and human metapneumovirus. Chen et al[45] tested nasopharyngeal aspirates to detect acute infections of the upper respiratory tract and found only 77.9% sensitivity but identified an additional 7% positive viruses relative to viral culture and antigen detection.

For the rapid detection, identification, and serotyping of human adenoviruses (A to F), the performance of the assay was characterized using quantified viral standards and environmental and human clinical samples collected from a military training facility.[21]

Other Viral Agents

Evaluation of select viral agents, flaviviruses, and alphaviruses was based on RT–PCR–ESI/MS. The first study involved the evaluation of an assay designed to detect both tick– and mosquito–borne flaviviruses. A panel of 13 different flaviviruses was analyzed, with all samples correctly identified to the species level. The limit of detection for the mosquito–borne primer sets, determined using RNA from West Nile virus, was equivalent to a viral titer of 0.2 PFU/mL. The assay accurately identified the virus within infected mosquitoes, including *Aedes aegypti* mosquitoes that were laboratory infected with dengue–1 virus.[6] By using human blood, serum, and urine spiked with West Nile virus and mouse blood and brain tissues from Karshi virus–infected mice, the authors demonstrated that clinical matrices did not inhibit the detection of these viruses.

Finally, testing of field-collected *Ixodes scapularis* ticks revealed 16 of 322 (5% infection rate) ticks positive for deer tick virus, a subtype of Powassan virus. The assay detects multiple tick- and mosquito-borne flaviviruses suitable for epidemiological surveillance.[15] Likewise, members of the genus *Alphavirus* were unambiguously characterized to the species or subtype for 35 of the 36 isolates tested. The assay detected Venezuelan equine encephalitis virus, Eastern equine encephalitis virus, and mixtures of both in pools consisting of laboratory-infected and laboratory-uninfected mosquitoes. Furthermore, the assay was used to detect alphaviruses in naturally occurring mosquito vectors, some with a distinct signature. Subsequent sequence analysis confirmed that the virus was a member of the Mucambo virus species (subtype IIID in the Venezuelan equine encephalitis virus complex).[23]

Limitations to PCR-ESI/MS and Ways to Mitigate Risks

Although PCR-ESI/MS displays potential for rapid multiplexed identification of infectious agents, there are several key areas of concern related to this technology. First, there is the potential for contamination. Because this instrument is an open platform, there is potential for instruments and workspace to be exposed to amplicons and for subsequent contamination of future samples during processing. To mitigate this concern, it is imperative to maintain a separate positive-pressure space for reagent preparation before PCR and a strict unidirectional work flow. Furthermore, in January 2012, an exhaust adaptor will be available to direct TOF emissions out of the work area.

Second, the PCR-ESI/MS method is capable of identifying multiple organisms in a single sample using broad-range PCR; however, there are limitations for identification of mixed infections. Because PCR-ESI/MS is semiquantitative, a relative intensity of multiple organisms can be determined, allowing for selection of a treatment that manages multiple organisms. A confidence score is also assigned to both organisms, depending on the number of primers making the identification and goodness of fit to database compositions. Interpretation of mixtures relies on examining a combination of factors, including the quality of the result (Q score), the signal intensity, the match to the expected base count, and the number of primers that produce a result. Improvements in assessing the signals derived from mixed infection will be required prior to clinical implementation. A future clinical software version will interpret the score automatically, but the NFDU software allows users to see all of the contributing factors and perform the analysis themselves.

Moreover, because of the sensitivity of the mass spectrometric detection method, the potential for PCR contamination with environmental organisms is of some concern. A few published studies[22,25] address the limit of detection in terms of colony-forming unit/mL or PFU/mL, reported as low as 1 to 10/mL. In general, the limit of detection for PCR/ESI-MS will be similar to that of other multiplex PCR assays. Low-level environmental contamination may be mitigated by the use of DNA-free and ultrapure reagents. In addition, inspection of the semiquantitative measurement of the relative number of genomes per well will be important. Environmental contamination would be expected to produce results associated with low genomes per well. Despite these assessments, it may be difficult to distinguish environmental contamination from low-level pathogens. Appropriate cut-off values for positive signals will need to be refined to address this limitation.

Finally, an additional concern for PCR-ESI/MS is the expected cost and the feasibility of nonreference facilities acquiring the technology. The expected start-up cost for the PLEX-ID is higher than that which can readily be supported by most diagnostic laboratories. Additional costs include the attainment of assay kits, approximately $50 to $100 per sample,[32] which is similar to or greater than the cost of existing molecular assays. The combined expense of start-up and kits will make PCR-ESI/MS implementation into general laboratories difficult, but implementation may be within reach for larger clinical and reference laboratories. Further evaluation of the potential cost avoidance that may occur because of rapid identification of a wide variety of infections may offset the costs of PCR/ESI-MS; however, implementation practices remain to be described in the clinical literature.

Summary

As a future technology, PCR-ESI/MS could shift the way microbiologists think about the diagnosis of infections. It appears accurate and flexible, and has potential for implementation in large reference laboratories or for public health surveillance. With adaptation, PCR-ESI/MS has the potential to fundamentally change the way diagnostic laboratories perform microbial detection and identification.

A common microbiological premise, that rapid detection and identification of microbial agents improves clinical and financial outcome for patients suspected of having infections, is arguably the next frontier for evidence-based laboratory interventions. Support for the clinical utility of rapid testing has been mounting during the past decade, and PCR-ESI/MS merits such investigation, lending itself to situations in which other conventional methods prove noninformative. The future holds distinct possibilities for PCR-ESI/MS–based disease diagnostics in certain settings. It is distinguished among other methods by its broad-based direct sample detection capabilities, its ability to detect target gene sequences despite silent mutations or genetic rearrangement, and its ability to simultaneously identify species, genotype, antimicrobial-resistance genes, and virulence factors, a combination of detection capabilities never before achievable in one testing platform.

References

1. Taylor LH, Latham SM, Woolhouse ME: Risk factors for human disease emergence. Philos Trans R Soc Lond B Biol Sci 2001, 356:983–989
2. Metzgar D, Baynes D, Myers CA, Kammerer P, Unabia M, Faix DJ, Blair PJ: Initial identification and characterization of an emerging zoonotic influenza virus prior to pandemic spread. J Clin Microbiol 2010, 48:4228–4234
3. Sampath R, Hall TA, Massire C, Li F, Blyn LB, Eshoo MW, Hofstadler SA, Ecker DJ: Rapid identification of emerging infectious agents using PCR and electrospray ionization mass spectrometry. Ann N Y Acad Sci 2007, 1102:109–120
4. Hofstadler SA, Sampath R, Blyn LB, Eshoo MW, Hall TA, Jiang Y, Drader JJ, Hannis C, Sannes-Lwery KA, Cummins LL, Libby B, Knize DJD, Robbins D, Rudnik K, Desai A, Moradi E, Ecker DJ: TIGER: the universal biosensor. Int J Mass Spectrom 2005, 242:23–41
5. Ecker DJ, Sampath R, Willett P, Wyatt JR, Samant V, Massire C, Hall TA, Hari K, McNeil JA, Buchen-Osmond C, Budowle B: The Microbial Rosetta Stone Database: a compilation of global and emerging infectious microorganisms and bioterrorist threat agents. BMC Microbiol 2005, 5:19
6. Ecker DJ, Sampath R, Blyn LB, Eshoo MW, Ivy C, Ecker JA, Libby B, Samant V, Sannes-Lowery KA, Melton RE, Russell K, Freed N, Barrozo C, Wu J, Rudnick K, Desai A, Moradi E, Knize DJ, Robbins DW, Hannis JC, Harrell PM, Massire C, Hall TA, Jiang Y, Ranken R, Drader JJ, White N, McNeil JA, Crooke ST, Hofstadler SA: Rapid identification and strain-typing of respiratory pathogens for epidemic surveillance. Proc Natl Acad Sci U S A 2005, 102:8012–8017
7. Ecker DJ, Sampath R, Massire C, Blyn LB, Hall TA, Eshoo MW, Hofstadler SA: Ibis T5000: a universal biosensor approach for microbiology. Nat Rev Microbiol 2008, 6:553–558
8. Emonet S, Shah HN, Cherkaoui A, Schrenzel J: Application and use of various mass spectrometry methods in clinical microbiology. Clin Microbiol Infect 2010, 16:1604–1613
9. Chen KF, Rothman RE, Ramachandran P, Blyn L, Sampath R, Ecker DJ, Valsamakis A, Gaydos CA: Rapid identification viruses from nasal pharyngeal aspirates in acute viral respiratory infections by RT-PCR and electrospray ionization mass spectrometry. J Virol Methods 2011, 173:60–66
10. Kaleta EJ, Clark AE, Johnson DR, Gamage DC, Wysocki VH, Cherkaoui A, Schrenzel J, Wolk DM: Use of polymerase chain reaction coupled to electrospray ionization mass spectrometry for rapid identification of bacteria and yeast bloodstream pathogens from blood culture bottles. J Clin Microbiol 2011, 49:345–353
11. Massire C, Ivy CA, Lovari R, Kurepina N, Li H, Blyn LB, Hofstadler SA, Khechinashvili G, Stratton CW, Sampath R, Tang YW, Ecker DJ, Kreiswirth BN: Simultaneous identification of mycobacterial isolates to the species level and determination of tuberculosis drug resistance by PCR followed by electrospray ionization mass spectrometry. J Clin Microbiol 2011, 49:908–917
12. Crowder CD, Matthews HE, Schutzer S, Rounds MA, Luft BJ, Nolte O, Campbell SR, Phillipson CA, Li F, Sampath R, Ecker DJ, Eshoo MW: Genotypic variation and mixtures of Lyme Borrelia in Ixodes ticks from North America and Europe. PLoS One 2010, 5:e10650
13. Endimiani A, Hujer KM, Hujer AM, Sampath R, Ecker DJ, Bonomo RA: Rapid identification of bla KPC-possessing Enterobacteriaceae by PCR/electrospray ionization-mass spectrometry. J Antimicrob Chemother 2010, 65:1833–1834
14. Eshoo MW, Crowder CD, Li H, Matthews HE, Meng S, Sefers SE, Sampath R, Stratton CW, Blyn LB, Ecker DJ, Tang YW: Detection and identification of Ehrlichia species in blood by use of PCR and electrospray ionization mass spectrometry. J Clin Microbiol 2010, 48:472–478
15. Grant-Klein RJ, Baldwin CD, Turell MJ, Rossi CA, Li F, Lovari R, Crowder CD, Matthews HE, Rounds MA, Eshoo MW, Blyn LB, Ecker DJ, Sampath R, Whitehouse CA: Rapid identification of vector-borne flaviviruses by mass spectrometry. Mol Cell Probes 2010, 24:219–228
16. Whitehouse CA, Baldwin C, Sampath R, Blyn LB, Melton R, Li F, Hall TA, Harpin V, Matthews H, Tediashvili M, Jaiani E, Kokashvili T, Janelidze N, Grim C, Colwell RR, Huq A: Identification of pathogenic Vibrio species by multilocus PCR-electrospray ionization mass spectrometry and its application to aquatic environments of the former soviet republic of Georgia. Appl Environ Microbiol 2010, 76:1996–2001

17. Baldwin CD, Howe GB, Sampath R, Blyn LB, Matthews H, Harpin V, Hall TA, Drader JJ, Hofstadler SA, Eshoo MW, Rudnick K, Studarus K, Moore D, Abbott S, Janda JM, Whitehouse CA: Usefulness of multilocus polymerase chain reaction followed by electrospray ionization mass spectrometry to identify a diverse panel of bacterial isolates. Diagn Microbiol Infect Dis 2009, 63:403–408
18. Ecker DJ, Massire C, Blyn LB, Hofstadler SA, Hannis JC, Eshoo MW, Hall TA, Sampath R: Molecular genotyping of microbes by multilocus PCR and mass spectrometry: a new tool for hospital infection control and public health surveillance. Methods Mol Biol 2009, 551:71–87
19. Hall TA, Sannes-Lowery KA, McCurdy LD, Fisher C, Anderson T, Henthorne A, Gioeni L, Budowle B, Hofstadler SA: Base composition profiling of human mitochondrial DNA using polymerase chain reaction and direct automated electrospray ionization mass spectrometry. Anal Chem 2009, 81:7515–7526
20. Wolk DM, Blyn LB, Hall TA, Sampath R, Ranken R, Ivy C, Melton R, Matthews H, White N, Li F, Harpin V, Ecker DJ, Limbago B, McDougal LK, Wysocki VH, Cai M, Carroll KC: Pathogen profiling: rapid molecular characterization of Staphylococcus aureus by PCR/electrospray ionization-mass spectrometry and correlation with phenotype. J Clin Microbiol 2009, 47:3129–3137
21. Blyn LB, Hall TA, Libby B, Ranken R, Sampath R, Rudnick K, Moradi E, Desai A, Metzgar D, Russell KL, Freed NE, Balansay M, Broderick MP, Osuna MA, Hofstadler SA, Ecker DJ: Rapid detection and molecular serotyping of adenovirus by use of PCR followed by electrospray ionization mass spectrometry. J Clin Microbiol 2008, 46:644–651
22. Hannis JC, Manalili SM, Hall TA, Ranken R, White N, Sampath R, Blyn LB, Ecker DJ, Mandrell RE, Fagerquist CK, Bates AH, Miller WG, Hofstadler SA: High-resolution genotyping of Campylobacter species by use of PCR and high-throughput mass spectrometry. J Clin Microbiol 2008, 46:1220–1225
23. Eshoo MW, Whitehouse CA, Zoll ST, Massire C, Pennella TT, Blyn LB, Sampath R, Hall TA, Ecker JA, Desai A, Wasieloski LP, Li F, Turell MJ, Schink A, Rudnick K, Otero G, Weaver SC, Ludwig GV, Hofstadler SA, Ecker DJ: Direct broad-range detection of alphaviruses in mosquito extracts. Virology 2007, 368:286–295
24. Jiang Y, Hall TA, Hofstadler SA, Naviaux RK: Mitochondrial DNA mutation detection by electrospray mass spectrometry. Clin Chem 2007, 53:195–203
25. Sampath R, Hofstadler SA, Blyn LB, Eshoo MW, Hall TA, Massire C, Levene HM, Hannis JC, Harrell PM, Neuman B, Buchmeier MJ, Jiang Y, Ranken R, Drader JJ, Samant V, Griffey RH, McNeil JA, Crooke ST, Ecker DJ: Rapid identification of emerging pathogens: coronavirus. Emerg Infect Dis 2005, 11:373–379
26. Jiang Y, Hofstadler SA: A highly efficient and automated method of purifying and desalting PCR products for analysis by electrospray ionization mass spectrometry. Anal Biochem 2003, 316:50–57
27. Hofstadler SA, Drader JJ, Schink A: Selective ion filtering by digital thresholding: a method to unwind complex ESI-mass spectra and eliminate signals from low molecular weight chemical noise. Anal Chem 2006, 78:372–378
28. Van Ert MN, Hofstadler SA, Jiang Y, Busch JD, Wagner DM, Drader JJ, Ecker DJ, Hannis JC, Huynh LY, Schupp JM, Simonson TS, Keim P: Mass spectrometry provides accurate characterization of two genetic marker types in Bacillus anthracis. Biotechniques 2004, 37:642–644, 646, 648
29. Leslie T, Whitehouse CA, Yingst S, Baldwin C, Kakar F, Mofleh J, Hami AS, Mustafa L, Omar F, Ayazi E, Rossi C, Noormal B, Ziar N, Kakar R: Outbreak of gastroenteritis caused by Yersinia pestis in Afghanistan. Epidemiol Infect 2011, 139:728–735
30. Endimiani A, Hujer KM, Hujer AM, Kurz S, Jacobs MR, Perlin DS, Bonomo RA: Are we ready for novel detection methods to treat respiratory pathogens in hospital-acquired pneumonia? Clin Infect Dis 2011, 52(Suppl 4):S373–S383
31. Ecker DJ, Sampath R, Blyn LB, Eshoo MW, Ivy C, Ecker JA, Libby B, Samant V, Sannes-Lowery KA, Melton RE, Russell K, Freed N, Barrozo C, Wu J, Rudnick K, Desai A, Moradi E, Knize DJ, Robbins DW, Hannis JC, Harrell PM, Massire C, Hall TA, Jiang Y, Ranken R, Drader JJ, White N, McNeil JA, Crooke ST, Hofstadler SA: Rapid identification and strain-typing of respiratory pathogens for epidemic surveillance. Proc Natl Acad Sci U S A 2005, 102:8012–8017
32. Kaleta EJ, Clark AE, Cherkaoui A, Wysocki VH, Ingram EL, Schrenzel J, Wolk DM: Comparative analysis of PCR-electrospray ionization/mass spectrometry (MS) and MALDI-TOF/MS for the identification of bacteria and yeast from positive blood culture bottles. Clin Chem 2011, 57:1057–1067
33. Stoodley P, Conti SF, DeMeo PJ, Nistico L, Melton-Kreft R, Johnson S, Darabi A, Ehrlich GD, Costerton JW, Kathju S: Characterization of a mixed MRSA/MRSE biofilm in an explanted total ankle arthroplasty. FEMS Immunol Med Microbiol 2011, 62:66–74
34. Hall TA, Sampath R, Blyn LB, Ranken R, Ivy C, Melton R, Matthews H, White N, Li F, Harpin V, Ecker DJ, McDougal LK, Limbago B, Ross T, Wolk DM, Wysocki V, Carroll KC: Rapid molecular genotyping and clonal complex assignment of Staphylococcus aureus isolates by PCR coupled to electrospray ionization-mass spectrometry. J Clin Microbiol 2009, 47:1733–1741
35. Wolk DM, Blyn LB, Hall TA, Sampath R, Ranken R, Ivy C, Melton R, Matthews H, White N, Li F, Harpin V, Ecker DJ, Limbago B, McDougal LK, Wysocki VH, Cai M, Carroll KC: Pathogen profiling: rapid molecular characterization of Staphylococcus aureus by PCR/electrospray ionization-mass spectrometry and correlation with phenotype. J Clin Microbiol 2009, 47:3129–3137
36. Hujer KM, Hujer AM, Endimiani A, Thomson JM, Adams MD, Goglin K, Rather PN, Pennella TT, Massire C, Eshoo MW, Sampath R, Blyn LB, Ecker DJ, Bonomo RA: Rapid determination of quinolone resistance in Acinetobacter spp. J Clin Microbiol 2009, 47:1436–1442
37. Ecker JA, Massire C, Hall TA, Ranken R, Pennella TT, Agasino IC, Blyn LB, Hofstadler SA, Endy TP, Scott PT, Lindler L, Hamilton T, Gaddy C, Snow K, Pe M, Fishbain J, Craft D, Deye G, Riddell S, Milstrey E, Petruccelli B, Brisse S, Harpin V, Schink A, Ecker DJ, Sampath R, Eshoo MW: Identification of Acinetobacter species and genotyping of Acinetobacter baumannii by multilocus PCR and mass spectrometry. J Clin Microbiol 2006, 44:2921–2932
38. Schuetz AN, Huard RC, Eshoo MW, Massire C, Della-Latta P, Wu F, Jenkins SG: Identification of a novel Acinetobacter baumannii clone in a US hospital outbreak by multilocus polymerase chain reaction/electrospray-ionization mass spectrometry. Diagn Microbiol Infect Dis 2012, 72:14–19
39. Koo M, Manalili S, Bankowski MJ, Sampath R, Hofstadler SA, Koo J: A "silent culture-negative" abdominal aortic mycotic aneurysm: rapid detection of Bartonella species using PCR and high-throughput mass spectrometry. Hawaii Med J 2010, 69:68–69
40. Wang F, Massire C, Li H, Cummins LL, Li F, Jin J, Fan X, Wang S, Shao L, Zhang S, Meng S, Wu J, Lu C, Blyn LB, Sampath R, Ecker DJ, Zhang W, Tang YW: Molecular characterization of drug-resistant Mycobacterium tuberculosis isolates circulating in China by multilocus PCR and electrospray ionization mass spectrometry. J Clin Microbiol 2011, 49:2719–2721
41. Gu Z, Hall TA, Frinder M, Walsh TJ, Hayden RT: Evaluation of repetitive sequence PCR and PCR-mass spectrometry for the identification of clinically relevant Candida species. Med Mycol 2012, 50:259–265
42. Sampath R, Russell KL, Massire C, Eshoo MW, Harpin V, Blyn LB, et al: Global surveillance of emerging influenza virus genotypes by mass spectrometry. PLoS One 2007, 2:e489
43. Deyde VM, Sampath R, Garten RJ, Blair PJ, Myers CA, Massire C, Matthews H, Svoboda P, Reed MS, Pohl J, Klimov AI, Gubareva LV: Genomic signature-based identification of influenza A viruses using RT-PCR/electro-spray ionization mass spectrometry (ESI-MS) technology. PLoS One 2010, 5:e13293
44. Deyde VM, Sampath R, Gubareva LV: RT-PCR/electrospray ionization mass spectrometry approach in detection and characterization of influenza viruses. Expert Rev Mol Diagn 2011, 11:41–52
45. Chen KF, Blyn L, Rothman RE, Ramachandran P, Valsamakis A, Ecker D, Sampath R, Gaydos CA: Reverse transcription polymerase chain reaction and electrospray ionization mass spectrometry for identifying acute viral upper respiratory tract infections. Diagn Microbiol Infect Dis 2011, 69:179–186