Abstract: Rapid transmission, high morbidity, and mortality are the features of human infectious diseases caused by microorganisms, such as bacteria, fungi, and viruses. These diseases may lead within a short period of time to great personal and property losses, especially in regions where sanitation is poor. Thus, rapid diagnoses are vital for the prevention and therapeutic intervention of human infectious diseases. Several conventional methods are often used to diagnose infectious diseases, e.g. methods based on cultures or morphology, or biochemical tests based on metabonomics. Although traditional methods are considered gold standards and are used most frequently, they are laborious, time consuming, and tedious and cannot meet the demand for rapid diagnoses. Disease diagnosis using capillary electrophoresis methods has the advantages of high efficiency, high throughput, and high speed, and coupled with the different nucleic acid detection strategies overcomes the drawbacks of traditional identification methods, precluding many types of false positive and negative results. Therefore, this review focuses on the application of capillary electrophoresis based on nucleic detection to the diagnosis of human infectious diseases, and offers an introduction to the limitations, advantages, and future developments of this approach.

Keywords: capillary electrophoresis; diagnosis; gene; pathogen; PCR.

Introduction

The recent outbreak of Ebola virus disease in West Africa caused public panic and aroused great concern in the international community regarding the hazards of infectious diseases to human health [1–4]. Rapid transmission and high morbidity and mortality are features of infectious diseases caused by microorganisms, such as bacteria, fungi, and viruses, which may lead to great personal and property losses in a short period of time, especially in areas where sanitation is poor. Thus, in order to the prevention and therapeutic intervention of human infectious disease [5] as well as the prevention of bioterrorism and biological warfare [6], rapidly diagnosing infectious diseases is of the utmost important for avoiding personal and property losses.

Several methods have been developed to diagnose infectious diseases in humans. Although culture-based methods are the traditional identification technique, these procedures are time consuming, tedious, and laborious, and the growth of microorganisms demands specific nutritional ingredients, growth factors, trace elements, and culture conditions. In addition, several days may be required to produce a quantity of microorganisms sufficient for diagnosis, which may result in rapidly dividing non-target microorganisms displacing the target organism, giving false results. Thus, diagnostics based on growth may yield inaccurate conclusions regarding the presence or absence of pathogens. As a result, the application of culture-based methods is restricted. Biochemical tests for diagnosing infectious disease are also troublesome and time consuming, and the results of these tests may also be uncertain because different strains of the same or closely related species likely possess similar biochemical characteristics, manifest the same symptoms and clinical features, or exhibit cross-reactivity. Morphological methods for diagnosing infectious disease may lead to invalid results because many microorganisms display a similar appearance. Therefore, a rapid, high throughput,
efficient method that produces reliable results for early and accurate diagnose of infectious diseases is urgently needed.

Capillary electrophoresis (CE) is a potential diagnostic technique offering the advantages of high efficiency, high throughput, and high speed. It can be used on a microscale and combined with various nucleic acid detection strategies to avoid many types of false positive and negative results [5], making CE an alternative and promising diagnostic tool for rapid diagnosis of infectious diseases [7, 8]. Thus, this review focuses on CE based on nucleic acid detection in the application of human infectious disease diagnosis. A brief introduction to the limitations, advantages, and future directions of this technique is also provided.

The principles of CE [9]

CE separations are carried out inside a capillary tube, which usually has a diameter of (50–100) μm to facilitate temperature control. The length of the capillary differs in different applications, but it is typically in the region of 20–50 cm. The capillaries most widely used are fused silica covered with an external protective coating. A small portion of this coating is removed to form a window for detection purposes. The ends of the capillary are dipped into reservoirs filled with the electrolyte. Electrodes made of an inert material, such as platinum, are also inserted into the electrolyte reservoirs to complete the electrical circuit. The capillary is filled with running buffer, one end is dipped into the sample, and an electric field (electrokinetic injection) or pressure is applied to introduce the sample inside the capillary. Migration through the capillary is driven by application of a high voltage current (10–30 kV). The molecules are detected as they pass through the window at the opposite end of the capillary. The most frequently used detector is laser-induced fluorescence (LIF), which detects fluorochromes attached to the DNA molecules; alternative detectors include ultraviolet (UV) absorbance and fluorescence. Given the short path, detection requires monitoring by sensitive equipment such as charge-coupled device (CCD) cameras. The detectors are interfaced with computers responsible not only for collecting and displaying the data but also for maintaining the timing between filter wheels and controlling timing exposures, readouts of the CCD cameras, and run-time processing of the CCD images and spectral data. The molecules are detected as fluorescent peaks as they pass through the detector. An electropherogram, which is a plot of the detector response with time, is generated. As the area of each peak is proportional to the concentration of the DNA molecule, integrated peak areas are routinely used for semiquantification due to their greater dynamic range than peak heights (see Figure 1).

Analysis times mostly are in the range of 3–30 min, depending on the complexity of the separation. Modern instruments are relatively sophisticated and may contain fiber optical detection systems, high capacity autosamplers, and temperature control devices. Separation of DNA fragments by CE has advantages over the classical slab gel-based separations in terms of speed and resolution, especially now that instruments that can run more than one sample at a time are available. The principle behind DNA sequencing by an instrument with many capillaries is identical to that of using a single capillary, although the design of the sheath flow cuvet and the fluorescence detection systems is considerably more complicated. At present, 8-, 16-, 48-, 96-, and 384-capillary instruments are commercially available for DNA analysis.

Capillary gel electrophoresis

There is a special situation for biopolymers such as RNA, DNA, or sodium dodecyl sulfate (SDS)-loaded proteins, which have a constant charge-to-size ratio, that is, the increase in the charge is directly related to the increase in size of the molecule. Molecules with a constant charge-to-size ratio may have very similar electrophoretic mobility,
so no electrophoretic separation occurs in free solution. In these cases, separations are performed in capillaries filled with a gel solution. In capillary gel electrophoresis (CGE), a sieving effect occurs as solutes of various sizes migrate through the gel-filled capillary toward the detector. Smaller ions are able to migrate quickly through the gel, whereas larger ions become entangled in the gel matrix, reducing their migration rate. Initially, the gels used in CGE were polyacrylamide covalently bonded to the capillary wall. These fixed gels suffered, however, from problems of shrinkage and blockage and could have relatively short lifetimes. In addition, if sample components contaminated the gel, it could not be reused and would have to be discarded. There has been a recent tendency, therefore, to use pumpable gel solutions, which can be used to fill the capillary with a non-cross-linked liquid gel matrix in which pores are created by the tangling of long linear polymers. These have the advantage of being introduced into the capillary under low pressure, extending the life of the capillary. The use of liquid gels also allows replacement of the gel between injections, reducing the contamination problems encountered with fixed gels.

Resolution

Good resolution in CE data means achieving sharp peaks and optimal separations between peaks. Peak spacing is the separation distance multiplied by the intrinsic velocity difference between two distinct molecules. This intrinsic velocity difference, in turn, is dependent on the physical properties of the molecule itself and on the separation medium (e.g. composition, concentration, ionic strength). Diffusion, thermal gradient, and initial peak width are the major contributors to the peak width. Electric field strength, capillary dimensions, and polymer concentration, in turn, mainly influence these three factors.

An increase in electric field strength should lead to a decrease in diffusion due to shorter run times. It should, however, also lead to an increase in peak dispersion as a result of an increase in the heat generated when the electric current is applied through the capillary (Joule heat). Thus, the electric field strength should be 150 V/cm or lower for long sequencing reads. However, the diameter of the capillary influences the efficiency in dissipating the Joule heat. The narrower the capillary, the smaller the thermal gradient and, therefore, the peak width. A reduction of capillary diameter to <50 μm does not improve resolution, indicating that effects other than the thermal gradient determine the peak width in narrower capillaries. The small cross-sectional area of the capillary creates the phenomenon of electroendosmosis. As CE is normally carried out in capillaries made from fused silica, the negatively charged silanol groups on the surface of the capillary result in a high proportion of unbound, hydrated positive ions. Although some of these cations are tightly bound to the immobilized negative charges, the application of an electric field results in movement of the remainder toward the cathode. As they are hydrated, the consequence is a bulk flow of liquid in the same direction, termed electroendosmotic flow (EOF). Many of the CE techniques rely on EOF to pump the solutes toward the detector. The EOF is produced along the entire length of the capillary, generating a constant flow rate at all distances along the capillary. The consequence is that the solutes are being swept along at the same rate throughout their transport along the capillary, minimizing sample diffusion. Separation is improved with increasing polymer concentration, but at the cost of lower mobility and, therefore, increased run times. Low polymer concentrations are useful for fast screenings at low resolution, whereas higher concentrations produce higher resolutions. Whereas in cross-linked gels, the concentration can be lowered easily, in the absence of cross-linking a decrease in the concentration results in a dilute solution. To compensate for this effect, an increase in the polymer length will keep the solution well entangled, allowing for low viscosity at the same time. To cover a large range of separation sizes, it is better to use low polymer concentrations; therefore, the polymer length must be increased to ensure sufficient entanglement. This leads to a more uniform resolution in function of the DNA size and is especially important for DNA sequencing, in which long reads are required. When analyzing the same DNA sample with the same parameters under native and denaturing conditions, the resolution is better in the environment that denatures the DNA molecules. If high resolution is necessary, denaturing conditions (such as the use of formamide in the sample preparation) should be used.

Diagnosing infectious diseases with CE

CE detection of microorganisms can be based on the detection of cells [10–18], proteins [19–21], liposomes [22], or nucleic acids. Among them, nucleic acid detection eliminates many types of false positive and negative results, offering a more precise diagnosis. Thus, this review concentrates on nucleic acid detection using CE. In diagnostics, the selected target genes are generally the conserved, specific, and mutated genes or sequences
Detecting and diagnosing bacteria

Staphylococcus aureus is an important pathogen, causing high morbidity and mortality in hospitals. Thus, epidemiological approaches monitoring S. aureus strains need to be established. Therefore, Sobral et al. [23] developed an MLVA-CE method exploiting 16 VNTR loci amplified in two multiplex PCRs with analyzed PCR products using CE to genotype human S. aureus isolates in a highly efficient and discriminating manner. Du et al. [24] developed and evaluated an improved multicolor VNTR fingerprinting using CGE for detection (CGE-MLVF), which replaced AGE (traditional MLVF) with CGE. This CGE-MLVF method is most discriminating (Simpson’s index of diversity was 0.855) compared with other identification methods and has the prospect to produce portable results. The reproducibility for CGE-MLVF is 100%, much better than 70% for traditional MLVF. Thus, these results indicate that CGE-MLVF, a fast and easy genotyping method that is relatively inexpensive, monitors the outbreak and clonal spread of S. aureus isolates with good reproducibility and discrimination.

Yersinia pestis is the pathogen that causes plague. To establish a genotyping system for tracing the source of this notorious pathogen, Li et al. [25] studied the traits of VNTRs in the Y. pestis genome and established a simple hierarchical genotyping system based on MLVA-CE analysis. Their results demonstrate that the MLVA-CE genotyping method improves the study of Y. pestis with respect to source tracing and microevolution of this pathogen, which compares favorably with single nucleotide polymorphism-based phylogenetic analysis, but costs less time and money.

Cronobacter spp. (Enterobacter sakazakii) is an opportunistic pathogen that causes a 40%-80% death rate among infected infants and immunocompromised patients. Duplex PCR combined with CE-LIF was developed for detecting Cronobacter spp. In food samples [26]. The specific gene sequences of the 16S-23S rDNA internal transcribed spacer (ITS) and the outer membrane protein A of Cronobacter spp. were amplified using duplex PCR, and the PCR products were analyzed using CE-LIF. The PCR products could be detected within 12 min with the relative standard deviations of migration time were 2.01%-2.91%. The detection limit for Cronobacter spp. was 1.6×10^7 cfu/mL. These results demonstrate that duplex PCR-CE-LIF is a fast and sensitive approach for routinely detecting Cronobacter spp. in food samples.

Shigella, a Gram-negative invasive enteropathogenic bacterium, frequently causes bacillary dysentery. Thus, Rawal et al. [27] analyzed this important pathogen using a fast and reliable MLVA-CE based on seven polymorphic VNTRs of all Shigella species. In total 194 distinct MLVA-genotypes were obtained for 235 Shigella spp, and they found that MLVA-CE was fast and robust for detecting Shigella.

Detection of Legionella pneumophila is important for preventing its infection. Thus, Kahlisch et al. [28] genotyped L. pneumophila isolates with MLVA-CE. They extracted DNA from drinking water and then used PCR-VNTR to MLVA genotype L. pneumophila. The PCR amplimers were analyzed using CE to obtain the respective MLVA electrophoretograms. The results demonstrate that MLVA-CE is a valuable method to identify outbreak strains and can be used directly for clinical detection.

A high throughput method for the identification of pneumococcal capsular types was established using multiplex PCR-CE (FAF-mPCR) [29]. The automation of the process can genotype 30 isolates in a few hours and carry out 90 reactions in 90 min, three-fold less time than that of Quellung reaction, these results indicated that FAF-mPCR was a useful and alternative method to determine the capsular serotype of Streptococcus pneumoniae. Selva et al. [30] applied FAF-mPCR to identify 40 serotypes/sero-groups from clinical samples and found that FAF-mPCR was able to genotype pneumococcal capsular directly in clinical isolates showing a high bacterial load (cycle threshold values of real-time PCR ≤30), even in patients with negative bacterial culture.

Pseudomonas aeruginosa is a main cause of hospital-acquired pneumonia and is the major pathogen in cystic
fibrosis lung pathology. The MLVA assay can provide detailed information for epidemiological monitoring of the chronic infections caused by *P. aeruginosa*. Thus, an automated MLVA assay was established to analyze 16 VNTRs using two multiplex PCRs with CE and was applied to genotype 83 isolates from eight patients with cystic fibrosis within 4 h. The results determined that most cases showed the same genotype during 8 years of chronic infection [31].

MLVA-CE for DNA detection commonly shows a considerable difference between measured and real fragment lengths. This discrepancy arises from variations within laboratory set-ups used for fragment analysis. To obtain comparable results between laboratories using different set-ups, calibration is a necessity. Larsson et al. [32] used standard strains with known allele sizes to determine the compensation factors required for the selected conditions to obtain the accurate allele sizes. They successfully applied the technique to the analyses of *Salmonella enterica* serovar Typhimurium test strains, demonstrating that accurate inter-laboratory comparisons are possible.

To evaluate the incidence and possible role of *Helicobacter pylori* in the pathogenesis of otitis media with effusion in children, Melake et al. [33] used two methods: culture versus PCR-CE. Middle ear fluid samples were positive for *H. pylori* in 40% of the patients as determined with culture technique and in 56.7% of the patients as determined using PCR-CE method. Similarly, the culture method found that middle ear mucosa samples were positive in 20% of the patients, whereas 26.7% of patients were found to be positive using PCR-CE. Gastric lavage samples were positive in 46.6% of the patients examined with cultures and in 63.3% of the patients examined with PCR-CE. These results demonstrate that PCR-CE is a more sensitive approach than cultures.

*Clostridium difficile* is a main pathogen of hospital-acquired diarrhea, and *C. difficile* infection (CDI) often recurs. PCR ribotyping is an effective method for diagnosing CDI [34]. Therefore, Hell et al. [35] applied PCR-CE ribotyping to determine whether CDI originated as a monoclonal or polyclonal pathogen. Their results demonstrated that most CDI is monoclonal in origin. To investigate whether the patients’ stool samples showed more than one *C. difficile* ribotype, Behroozian et al. [36] ribotyped *C. difficile* in each stool sample using PCR-CE. They found that most infections possessed more than one *C. difficile* ribotype, and further study of the stool samples using CE-based PCR ribotyping discovered a high number of cases with mixed *C. difficile* infections.

It is important to monitor the bacterial number and community structure in freshwater used for human consumption because this is closely associated with human health. Thus, the development of the analytical techniques for this purpose is critical. Yamaguchi et al. [37] used MCE for rapid microbial community analysis and developed a microchip-based terminal restriction fragment length polymorphism (T-RFLP) to analyze bacterial communities in freshwater within 15 min, by which time the limit of detection (LOD) of the targeted bacteria was reduced to 1% (10^3 cells/mL). Their results indicate that T-RFLP-MCE analysis is a sensitive tool for rapidly analyzing bacterial number and community structure in freshwater sources used for human activities.

The long repeat regions in many genes correlating with infectivity in non-typeable *Haemophilus influenzae* are associated with phase variations in the expression of the respective gene. Thus, PCR-CE combined with pyrosequencing was used to analyze 13 phase-variable genes and the number of repeats in each gene during 6-day periods of human nasopharyngeal colonization [38]. The results of this study suggest that the phase-on genotypes of licA and igA are important for early human nasopharynx colonization and that lex2A has a potentially important role in the colonization process [38].

Rapidly identifying and genotyping *Mycobacterium tuberculosis* is important for disrupting disease transmission. However, genotyping *M. tuberculosis* using traditional methods is laborious. Thus, Matsumoto et al. [39] presented a new approach that used the QIAxcel CE system for VNTR analysis, in which allelic ladders representing the loci of 15 mycobacterial interspersed repetitive units (MIRU)-VNTR were used to define the peaks and the samples were continuously analyzed in a 96-well plate. A high agreement (99.2%) was found between the results of Hitachi i-chips and that of the QIAxcel CE system. Freidlin et al. [40] applied MIRU-VNTR-CE typing to find the origin of the new tuberculosis cases in Israel. They discovered that the polymorphic exact tandem repeat A (PETRA) lineage is large and a main contribution to the new cases of tuberculosis. Gray et al. [41] used the mycobacterial 16S-23S rRNA ITS as targets and developed PCR-CGE to identify rapidly growing mycobacteria. In total 47 electropherograms for the 178 clinical isolates could differentiate 175 isolates, including closely related species (*M. massiliense* and *M. abscessus*). They successfully studied and identified 19 American Type Culture Collection mycobacterium strains and 178 clinical isolates of rapidly growing mycobacteria (12 species). These results indicated that PCR-CGE of the 16S-23S ITS gene region is a simple, fast, accurate and reproducible approach for identifying and characterizing rapidly growing mycobacteria.

Sajduda et al. [42] applied PCR-restriction analysis (PRA) of the hsp65 gene with CE to identify and
differentiate the most frequently isolated clinical strains of non-tuberculous mycobacteria of *Mycobacterium kansasii* and *Mycobacterium chelonae–Mycobacterium abscessus* group strains. Their results indicate that PRA-CE is a fast, easy, and robust method for identifying and differentiating both clinically relevant and environmental isolates of *M. kansasii* and the *M. chelonae–M. abscessus* group. The PRA-CE approach was also compared with three other methods for rapidly identifying *Mycobacterium* species. Five newly discovered hsp65 alleles in seven isolates were identified, indicating hsp65 PRA-CE is a faster, easier, and more robust approach than the others examined for identification of mycobacteria [43]. PRA can identify mycobacteria quickly and easily, but misidentification may occur because of the similarities in band sizes that are crucial for differentiating among species. To accurately identify mycobacteria, Huang et al. [44] developed an algorithm after combining rpoB duplex PRA (DPRA) and hsp65 PRA with CE. This innovative and efficient identification algorithm rapidly identified mycobacteria and discovered new sub-types. The accuracies reached 100% and 91.4% for *M. tuberculosis* complexes and non-tuberculous mycobacteria, respectively.

To assess SnaPshot minisequencing method for identifying *M. tuberculosis* complex isolates at the level of the species and for further genotyping *M. tuberculosis* isolates. Bouakaze et al. [45] developed a SNaPshot minisequencing approach composed of two steps. First, PCR amplification was followed by a minisequencing reaction using the SnaPshot multiplex kit. The PCR products were then analyzed using CE. This approach simultaneously differentiated closely-related strains of the *M. tuberculosis* complex. The discrimination among the principal genetic species characterizing *M. tuberculosis* isolates into SNP cluster groups indicated that this technique is useful for diagnostic and epidemiological purposes.

The *M. tuberculosis* bacterial *rpoB* gene mutations and mabA-inhA promoter region mutations are associated with tuberculosis drug resistance to rifampicin and isoniazid, respectively. Thus, the rapid, accurate, and cost-effective identification of these mutations causing tuberculosis drug resistance is imperative for designing a correct therapeutic regimen. Krothapalli et al. [46] applied PCR-CE-SSCP to detect genetic mutations involving drug resistance in *M. tuberculosis*. An *rpoB* gene mutation and two mabA-inhA promoter region mutations were successfully detected, with the method simultaneously differentiating a tuberculosis-causing mycobacterium from a non-tuberculosis bacterium in <60 min. These results demonstrated that PCR-CE-SSCP is an inexpensive and rapid analytical tool for the diagnosis and therapy of tuberculosis.

Interferon γ is active against intracellular pathogens and is a primary cell factor activated by macrophages during *M. tuberculosis* infections. To determine whether genes that regulate interferon γ affect tuberculosis susceptibility, Möller et al. [47] applied PCR-CE-LIF to study polymorphisms in eight candidate genes. They found that the results of previous research may have given false positives or showed a stronger genetic effect than those of their study.

Brucellosis is a zoonotic disease, and *Brucella* is a major human pathogenic bacterium that contains little characteristic diagnostic information. The MLVA-16, involving singleplex PCR and AGE, is considered the standard method for genotyping *Brucella* spp. However, the procedure is time consuming and the simultaneous detection of multiple strains is obstructed, restricting the future application of this technique because analyzing multiple organisms in a single run is required for large scale analysis. Therefore, Gurfido et al. [48] developed an alternative MLVA-16 method that utilized multiplex PCRs and multicolor CE to reliably analyze *Brucella* in a high throughput manner, the standard deviation for VNTR fragment measurement was <2.01%, demonstrating that MLVA-16-CE was a powerful tool for analyzing *Brucella*. Foster et al. [49] developed a CE-based assay that included a 1000 SNP microarray and a selective primer-extension system, targeting 17 valuable SNPs across eight major branches of the phylogeny to identify *Brucella* subspecies. Their results indicate that SNP-CE genotyping approaches can accurately determine the evolutionary relationships of bacterial isolates. Multiplexed PCR for molecular identification is the most cost-effective, fastest, and simplest method. However, the disadvantages are that the system must be re-validated when a new marker is added and that polymorphic markers are unable to analyze at the single-nucleotide level. As new *Brucella* species are discovered continuously, open methodologies accommodating new markers while maintaining the previous parameters are needed. Thus, Wattiau et al. [50] presented a ligase chain reaction (LCR)-CE approach to simultaneously analyze multiple genetic markers at the single-nucleotide level and yield characteristic CE electrophoretograms of 10 *Brucella* species. In addition, multiple oligonucleotides in a single mixture generally do not interfere with the results of LCR-CE, which allows for easier discovery of new *Brucella* species using this technique [50].

The rapid genotyping of bacteria is very important for preventing the outbreak of infectious disease. However, multilocus sequence typing (MLST) for genotyping cephalosporine-resistant *Escherichia coli* is time consuming and tedious. To quickly genotype bacteria, Nielsen et al.
developed an MLVA analysis based on CE detection (MLVA-CE) and compared the results of this approach with those from MLST. The MLVA-CE could distinguish 100% isolates and showed a high agreement with the results of MLST. Their results indicate that MLVA-CE is a valuable method for rapidly genotyping *E. coli*. Soleimani et al. [52] developed multiplex PCR-CE to specifically detect enteropathogenic *E. coli* and enterohemorrhagic *E. coli* pathotypes based on specific marker genes. The four main pathotypes of *E. coli* were identified in a single reaction, proving multiplex PCR-CE is an easy, reliable, and rapid tool for detecting *E. coli*.

Urinary tract infections make up nearly 40% of all hospital-acquired infections, of which 80% are catheter-associated infections causing by bacteria residing in biofilms. Thus, Choe et al. [53] determined the distribution of fastidious, non-culturable bacteria in the biofilm of urinary catheters by concurrently applying four different 16S rRNA analytical techniques (CE, T-RFLP, denaturing gradient gel electrophoresis, and pyrosequencing). Among the four techniques, CE showed the greatest ability to detect these bacteria, identifying a total of 329 isolates. However, the four techniques detected different types of bacteria, suggesting that the simultaneous application of multiple detection methods improves the accuracy of diagnosis when the bacteria types are complicated.

The human intestinal microorganism system intimately contacts the digestive system and has an important influence on human health and disease states. Therefore, it is important to study the composition and function of the human intestinal microorganism system, and the recovery of intact DNA and RNA is a key step for such studies. Cardona et al. [54] used MCE combined with pyrosequencing to evaluate how storage conditions of fecal samples influence on the quality of extracted nucleic acids and the composition of their microorganisms. They found that the storage conditions affected both the integrity of extracted nucleic acid and the composition of the microbiota. Thus, they suggested that feces samples being stored at room temperature should be taken into the laboratory within 24 h after collection or samples be kept right away at –20 °C and afterward transported while frozen.

A microscale equipment composed of an efficient passive mixer, a magnetic separation chamber, and a capillary electrophoretic microchannel was developed by Jung et al. [55] for the sequential conduct of DNA barcode assays, target pathogen separation, and barcode DNA capillary electrophoretic analyses in multiplex pathogen detection (Figure 2). The principle is that the intestine-shaped, serpentine three-dimensional micromixer offers a high rate of mixing to quantitatively produce magnetic particle–pathogenic bacteria–DNA barcode labeled AuNP complexes. After magnetic separation and purification of the complexes, the barcode DNA strands are released and analyzed using the microfluidic CE. The different sizes of DNA barcodes represent different target bacteria, and the different elution times of the DNA barcode peaks in the electropherogram make the identification of target

Figure 2: Schematic diagram of an integrated passive micromixer–magnetic separation–capillary electrophoresis microdevice that performs a DNA barcode assay and a capillary electrophoretic separation for multiplex pathogen detection [55].
pathogen easy and rapid. The total time for identification is within 30 min and as low as $10^4$ DNA barcode stand per AuNP is enough for detection of multiple pathogens, proving that this novel integrated MCE is rapid and sensitive for multiplex detection of pathogens and has a high value for clinical and environmental testing [55].

Effective bacterial detection and quantification are essential prerequisites for preventing and treating infectious diseases. Thus, Deng et al. [56] developed an N-methylimidazolium modified magnetic particles-assisted (MIm-MPs) high sensitive $E. coli$ detection system based on PCR-CE (Figure 3). $10^1$ cfu/mL of $E. coli$ in 500 mL sample solution can be detected and the capture efficiency reaches 99%, with they successfully identified and quantitated the bacteria in tap water and mineral water samples, demonstrating that this MIm-MPs-PCR-CE method is highly sensitive, highly efficient and applicable for the detection and quantification of bacteria in authentic as well as experimental samples.

Listeria monocytogenes can cause serious invasive foodborne diseases. Sammarco et al. [57] developed amplified fragment length polymorphism (AFLP) with CE method for genotyping 50 strains of $L. monocytogenes$. Compared with AFLP coupled with agarose gel electrophoresis (AFLP-AGE), although a high congruency (94%) was observed between AFLP-CE and AFLP-AGE, they found that AFLP-CE required lower volumes of samples and significantly reduced analysis time. Chen et al. [58] developed MLVA-CE for sub-typing $L. monocytogenes$ isolates and found 34 characteristic DNA fingerprint profiles from 46 $L. monocytogenes$ isolates of 10 serotypes, achieving LODs of $10^3$–$10^4$ CFU/mL. Their results indicate that the MLVA-CE method is highly discriminating, easy to operate, and can produce portable numerical results.

Vibrio parahaemolyticus is an important foodborne pathogen in many maritime countries. Thus, a two-step PCR-CE genotyping approach was established by Xiao et al., combining 18 large variably-presented gene clusters (LVPCs) with VNTRs [59]. Stains with different potential pathogenicities were grouped into six distinct complexes using the frequency profiles of the LVPCs and virulence markers. These complexes were then analyzed with VNTRs to obtain more details for discriminating the strains. This study shows that a genetic fingerprint

![Figure 3: Schematic of MIm-MPs-PCR-CE [56]. MIm-MPs (50 mg) are dispersed in a conical flask containing 500 mL sample solution to concentrate $E. coli$ from a large volume of aqueous solution (1). The MIm-MPs-bacteria conjugates are isolated from aqueous samples using a magnet and transferred to a 15 mL centrifuge tube. Then, 10 mL 0.2 M citrate buffer (pH 7.0) is added, and the mixture is vigorously shaken for 60 min to elute $E. coli$ from the particles. An external magnet is employed for magnetic separation (2). The eluted bacteria are transferred to a 1.5 mL centrifuge tube. The bacteria DNA kit is used for the isolation of genomic DNA from the eluted $E. coli$ (3). Finally, the genomic DNA is obtained for PCR followed by CE analysis (4 and 5).]
that Dendrograms were constructed and the results indicated and Klebsiella oxytoca and GTG groups, and tentative subclusters were built, indicating potential for diagnosing bacteria-induced diseases [62].

Methods for screening a 16S rRNA gene marker have been developed to detect microbial pathogens rapidly and sensitively. PCR-CE-SSCP is one of such method. However, the peak was broadened by the interaction between the hydrophobic moieties in polymer matrices and DNA, deteriorating resolution. Thus, most 16S rRNA gene-specific markers cannot be successfully separated. Consequently, CE-SSCP analysis using conventional polymer matrices is not ideal. To address this problem, a high resolution CE-SSCP system using a polyethyleneoxide-polypropyleneoxide-polyethyleneoxide (PEO-PPO-PEO) triblock copolymer matrix displayed four-fold higher resolving power than linear polyacrylamide and commercial GeneScan can gel for the separation of same-sized DNA molecules. Five-fold proportionally increased corresponding amplicon peaks to quantitatively analyze the pathogen [64]. Using this PEO-PPO-PEO, this new PCR-CE-SSCP quantitatively detects 12 clinically important pathogens in a single experiment. With an LOD of 0.31–1.56 pg, this system shows great potential for diagnosing bacteria-induced diseases [62].

Although MLPA is suitable for amplifying specific markers to detect pathogens, using stuffers to ensure size-based separation with CE obstructs the detection of foodborne pathogens because internal hybridization and large variations of hybridization efficiency among probes should be avoided, greatly increasing the difficulty of designing stuffer sequences. Long stuffer sequences affect the quantitative consistency because the amplification efficiency fluctuates between short and long products. Thus, Chung et al. [65] developed stuffer-free MLPA-CE-SSCP to sensitively detect 10 foodborne pathogens. All 10 pathogens were reliably identified, with LODs of 0.5–5 pg of genomic DNA. These results demonstrate that stuffer-free MLPA-CE-SSCP is a sensitive and reliable technique for detecting pathogens. Chung et al. [66] developed antibody-conjugated magnetic nanoparticles (Ab-MNPs) pathogen capture-stuffer-free MLPA-CE-SSCP to detect nine pathogens. The detection limit of 10 cfu/ml was achieved and high concentrations of the other eight bacterial species mixed with target pathogen did not interrupt the detection of target pathogen, demonstrating this method is sensitive, specific and accurate for detecting bacterial pathogen. Furthermore, they found that stuffer-free MLPA-CE-SSCP and Ab-MNPs together facilitated robust pathogen detection in various applications. To develop MLPA on a microchip platform, Shin et al. [67] established MLPA-MCE-SSCP using a stuffer-free probe set for multiplex analysis of five foodborne pathogens and validated their method with DNA samples from infected milk (Figure 4).

Diagnosing bacterial infection rapidly is critical for choosing the appropriate therapeutic regimen during early phase of bacterial-induced disease. Due to its excellent sensitivity, resolution, and reproducibility, CE-SSCP combined with 16S rRNA gene-specific PCR is frequently used for identifying bacterial infections. However, even though CE-SSCP separates PCR products with high resolution, the generation of primer-dimers and non-specific amplification makes multiplex detection and quantification complicated. Thus, Shin et al. [68] developed a novel PCR-CE-SSCP using template-tagging followed by multiplex asymmetric PCR and subsequent CE-SSCP detection for multiplex detection and quantification of pathogens (Figure 5). They successfully used 16S rRNAs-PCR-CE-SSCP analysis for amplifying and quantifying seven septicemia-inducing pathogens, the LOQs and LODs were 1.05–83.2 pg and 0.58–19.65 pg, respectively, demonstrating the potential of this system for diagnosing infectious disease.

The non-specific amplification fluorescent products interferes with the proximity ligation assay (PLA) and
that PLA-MCE is a sensitive and specific tool for detecting bacteria [69].

Using rapid antimicrobial susceptibility tests to assess clinical isolates can provide useful information for commencing effective early therapy. However, traditional antimicrobial susceptibility testing is troublesome and time consuming. Therefore, Chung et al. [70] employed stuffer-free MLPA-CE-SSCP to develop a novel multiplex antibiotic susceptibility test. The values obtained following parallel multiple analyses are consistent with those estimated using a traditional broth dilution approach, but the handling time was reduced to <40% of that of traditional method and avoided false results from subjective observation, indicating that the stuffer-free MLPA-CE-SSCP method is a feasible alternative to the traditional approach.

It is reported that microbial dysbiosis and pouchitis have a strong relationship. To study the relationship, Lim et al. [71] applied T-RFLP-CE to analyze patients with and without pouchitis. They found that although the total bacterial diversity in patients with pouchitis was similar to that in healthy subjects, the majority of these organisms in the patient population were novel. These results indicated that T-RFLP-CE is useful for identifying candidate microorganisms that may be correlated with pouchitis.

The influences on periodontopathic biofilm by sugar alcohols are still not clear. To evaluate the effect of sugar alcohols for suppressing the formation of periodontopathic biofilm, Hashino et al. [72] constructed a heterotypic biofilm model consisted of *Streptococcus gordonii* and *Porphyromonas gingivalis*, and used CE-MS to analyze the metabolomic profiles of erythritol-treated *P. gingivalis* and *S. gordonii* cells. Their results indicate that erythritol...
has suppressive effects on the development of dual species biofilm through several pathways, including growth inhibition causing by DNA and RNA depletion, reduced extracellular matrix production, and altered dipeptide acquisition and amino acid metabolism.

Melanin interacts with numerous molecules including DNA. Geng et al. [73] used the CE-spectroscopic technique of circular dichroism to study how bacterial melanin interacts with dsDNA. They discover that melanin intercalates between the base pairs of DNA, and this indicates that the cytoplasmic localization of melanin may offer a new strategy in using microcalorimetry to inhibit cellular metabolism.

In fluorescence in situ hybridization (FISH) for detecting bacteria, cells surface situation has a great impact on the binding of single-stranded DNA (ssDNA) and the selection of aptamers. Lactobacillus acidophilus, E. coli, their protoplasts, and their interactions with an ssDNA library were studied using capillary zone electrophoresis (CZE) and affinity capillary electrophoresis (ACE). The results showed that protoplasts without cell walls had apparently stronger interactions with the ssDNA library than did bacteria with intact cell walls. Thus, the cell surface situation determined the binding affinity with ssDNA, which should be considered in the selection of whole cell aptamers and further aptamer applications [74].

**Detecting and diagnosing fungi**

The rapid identification of candidemia and invasive candidiasis at the species level is critical for the timely monitoring of infected patients and choosing appropriate antifungal treatment. Thus, a commercially available multiplex PCR-CGE (Seeplex) was used to identify Candida species. The Seeplex assay was found to be specific and rapid for identifying six clinically important Candida species [75] and 80 clinical strains of Candida spp. [76]. Gago et al. [77] developed PCR-high resolution melting (HRM) analysis to genotype Candida albicans strains and compared the resulting data with those from microsatellite length polymorphism (MLP)-CE analysis. They found that MLP-CE has higher discrimination power (0.92 vs. 0.77) but PCR-HRM provided a more cost-effective alternative for genotyping C. albicans in a clinical experiment. Lantz et al. [78] developed a FISH-CE for detecting C. albicans in whole blood. Contaminating C. albicans cells were detected at a concentration of approximately $7.0 \times 10^9$ red blood cells/mL, and a single injected C. albicans cell was detected by bright Candida-specific fluorescence using FISH, indicating that FISH-CE is advantageous in applications of human diagnostics.

Knowing which transcription factors binds in which region of a gene during induction or suppression is critical for understanding the mechanisms of transcription.
mediated PCR-CGE were analyzed with their newly developed sulphate methylation, HCl DNA cleavage, and ligation-mediated PCR-CGE were analyzed with their newly developed in vivo footprinting analysis software tool (ivFAST) (Figure 7). Using an internal standard, this new method compares in vivo footprinting results from different conditions to one another. These authors also used this method to determine the mechanisms associated with the condition-dependent regulation of the cellobiohydrolase 2 and endoxygenase 2 genes. Based on their results, the authors suggest that this method would be useful for large scale investigating and examining regulatory factors in filamentous fungi as well as all other organisms.

_Nocardia_ species are ubiquitous Gram-positive bacilli causing various clinical syndromes, such as localized cutaneous infections, systemic infections, with a preference to infect the central nervous system. Thus, Wehrhahn et al. [80] established a method based on PCR-CGE. After amplifying the _Nocardia_ 16S-23S rRNA IGS region, PCR products were analyzed using CGE for identifying _Nocardia_ species. Fragment sizes showing ≥ 0.5-bp difference could be discriminated, and the costs was less than one third of that of 16S rRNA sequencing, with _Nocardia_ isolates (145 with 19 species) and non-Nocardia aerobic actinomycetes (4) were successfully identified, indicating that PCR-IGS-CGE genotyping is easy operation, reliable, cost-effective and a useful alternative to 16S rRNA sequencing for identifying and subtyping _Nocardia_ isolates.

The trichothecene mycotoxin deoxynivalenol produced by _Fusarium_ has adverse health effects on the human immune system and induces rRNA cleavage. He et al. [81] applied CE combined with northern blot, etc. techniques to study targets and intra-cellular signaling mechanisms of rRNA cleavage induced by deoxynivalenol. They found that deoxynivalenol-induced rRNA cleavage may be closely associated with apoptosis activation and the serial activation of PKR/Hck → p38 → p53 → caspase 8/9 → caspase 3.

Pneumonia caused by the fungus _Pneumocystis jirovecii_ causes respiratory diseases in patients with AIDS. Esteves et al. [82] applied multiplex-PCR (MPCR)-single-base extension (SBE)-CE to identify multiple SNPs at three different _P. jirovecii_ loci that encode dihydrofolate reductase, mitochondrial large-subunit rRNA, and superoxide dismutase. They compared the results of MPCR-SBE-CE with those from MPCR-direct sequencing and those from MPCR-SBE-acrylamide gel electrophoresis techniques and found that the polymorphisms studied were potentially suitable genetic targets associated with clinical data regarding _P. jirovecii_-induced pneumonia, and could be used as prognostic factors of outcomes to help manage pneumonia caused by _P. jirovecii_. They also found that CE had a better concordance (98.4%) with direct sequencing than acrylamide gel electrophoresis (96.9%). These results indicated that MPCR-SBE-CE is a fast, reliable tool for genotyping _P. jirovecii_ SNPs encoded by different loci that are valuable for epidemiological research and prognostic diagnosis of pneumonia caused by _P. jirovecii_.

_Rhizopus oryzae_ frequently causes zygomycosis. Thus, Baghela et al. [83] developed a multi locus microsatellite genotyping (MLMT)-CE approach to genotype _R. oryzae_. They found three polymorphic microsatellite loci that were stable with good discrimination power for seven subcultures, indicating that MLMT-CE is a valuable method for genotyping _R. oryzae_.

Human cryptococcal infection is life threatening opportunistic infection for people with AIDS, and fecal matter from birds is a likely source of the infection. Thus, Illnait-Zaragoti et al. [84] applied PCR-CE to study this relationship. The genotypic analysis from pigeon guano samples suggested that additional sources contribute to human cryptococcal infections, and PCR-CE analysis based on the selected microsatellite markers distinguished 104 genotypes with the discriminatory power was 0.993, indicating that this PCR-CE was an effective method for the epidemiologic study of _Cryptococcus neoformans var. grubii_.

Sitthinamsuwon et al. [85] also applied PCR-CE to study the T-cell receptor gene rearrangement in a 51-year-old woman showing cutaneous involvement caused by extranodal natural killer /T-cell lymphoma of the colon that microscopically mimicked mycosis fungoides. Their results showed that except for the histomorphologic findings, clinical information and molecular genetic studies including CE-based T-cell receptor gene rearrangement analysis are helpful for diagnosing cutaneous lymphoma.

_Aspergillus_ is a threat to public health because it produces allergens that easily infect immunocompromised patients or people with cystic fibrosis or asthma. Collagen-like genes are reported to show species-specific conservation across the non-collagenous regions and strain-specific polymorphism in the collagen-like regions. Thus, Tuntevski et al. [86] applied PCR-CE based on detecting the conserved regions of the _Aspergillus_ collagen-like genes to identify _Aspergillus_. They demonstrated that the _Aspergillus_ _Aspergillus_ collagen-like genes was a useful PCR targets for discriminating _Aspergillus_ species that are clinical relevant, and that PCR-CE is sensitive and specific for identifying _Aspergillus_ colonization and invasive aspergillosis in immunocompromised patients.
Simian varicella zoster virus (SVV) infection in non-human primates is used as a model to study varicella zoster virus (VZV) infection and pathogenesis in humans. However, the sensitivity of macroarray analysis is too low to detect the low-abundance SVV transcripts. Thus, Nagel et al. [87] developed RT-PCR-CGE to sensitively analyze the SVV transcriptome in SVV-infected monkey cells. They found transcripts corresponding to all 69 predicted SVV...
open reading frames (ORFs). Several to several hundred copies for ORFs could be detected. Virus gene transcription, not only during primary infection, but also during latency and reactivation, could also be analyzed. These results indicate that RT-PCR-CGE is sufficiently sensitive for the detection of SVV transcripts.

Array technology is unable to detect all VZV gene transcripts in human ganglia because of the low abundance of the ganglionic RNA and highly variable VZV gene transcription. Thus, multiplex RT-PCR-CGE (GeXPS) was developed to detect transcripts corresponding to all 68 predicted unique VZV ORFs in VZV-infected MeWo cells [88] (Figure 8). As few as 20 copies of VZV gene-specific transcripts could be detected and only five multiplex RT-PCR analyses that 500 ng RNA is enough for analyzing all VZV gene transcripts were needed to analyze the whole VZV transcriptome, demonstrating that GeXPS is a rapid analysis method for detecting all VZV gene transcripts produced during latency.

The confirmation of virus targets using aptamers is critical for aptamer-based biosensor platforms and for studying protein function, but the binding constant of aptamers with their targets are hard to determine because the purification and quantification of targets are difficult. Therefore, Zhang et al. [89] developed a modified CE-based approach to determine the dissociation constant of aptamers whose targets were difficult to quantify. They validated their method by applying it to a set of DNA aptamers binding with the avian influenza virus H9N2. Their results indicate that this technique is useful for evaluating receptor-ligand interactions.

A fast and accurate MCE approach was established for separating small DNA molecules that are associated with infectious disease [90] (Figure 9). First, PCR amplified the spike glycoprotein gene of the feline infectious peritonitis virus, and then the PCR products were analyzed within 10 s using CGE-LIF, this PCR-CGE-LIF analysis was 50 times faster than that for conventional slab gel electrophoresis. When this PCR-CGE-LIF method was used with a multi-channel MCE for high throughput analysis, the RSDs of migration time and peak area were <1.0%. These results indicate that PCR-CGE-LIF is a fast and accurate tool for analyzing infectious disease-related DNA.

Figure 8: GeXPS multiplex RT-PCR-CGE strategy [88].
(1) Total RNA extracted from 2×10⁷ uninfected or VZV-infected MeWo cells was reverse transcribed using 19–22 chimeric reverse primers each containing 20 nucleotides complementary to the target gene (solid black) coupled with a 19-nucleotide universal reverse sequence (solid gray). (2) The cDNA was PCR-amplified in cycles 1–7 using the chimeric primers to synthesize the 19–22 gene-amplification products, each of which contained universal tags at both termini. (3) Subsequent PCR amplifications (cycles 7–35) using universal forward and reverse primers to yield D4-fluorescent-labeled amplification products corresponding to each of the 19–22 specific genes tested. (4) PCR products were analyzed using CGE.

Figure 9: Single- and multi-channel MCE with LIF detection systems [90].
(1) Physical layout and (2) schematic diagram of the single-channel MCE system. (3) Physical layout and (4) schematic diagram of the multi-channel MCE system. CCD, charge-coupled device; EL, excitation light OL, objective lens; PMT, photomultiplier tube; reservoir 1, buffer inlet; reservoir 2, sample outlet; reservoir 3, buffer waste; reservoir 4, sample inlet.
Residual host cell DNA is a potential threat for cell culture-derived vaccines. In addition to the quantity of residual DNA, the size distribution of the DNA is also crucial for determining the related risk factor. Thus, Shen et al. [91] developed a CGE-LIF approach for analyzing the size of residual DNA. They successfully applied this approach to analyze the size of the residual DNA in vaccine samples produced by cell culture. Using this approach, as low as 0.025 ng/mL per DNA fragment can be quantitatively analyzed. Their results indicate that the CGE-LIF method is highly sensitive, specific and high resolution for determining residual DNA size.

With the development of viral therapeutics, new analytical approaches for viral quantification that are high efficient, rapid and accurate are needed. Thus, Azizi et al. [92] developed a new viral quantitative CE (viral qCE) (Figure 10) to determine: 1) the amount of intact virus particles (IVP); 2) the number of DNA contamination; and 3) the level of viral degradation after sonicating, vortexing, and freeze-thaw cycles, completed in a few hours with a wide range of virus concentrations (10^4–10^13 ivp/mL). For this technique, CZE-LIF is used to separate IVPs from DNA and RNA impurities, and sample DNA and RNA is stained by YOYO-1 dye. After using proteinase K to digest the viral capsid and ribonucleoproteins, he vesicular stomatitis virus (VSV) is lysed and the RNA is released. Therefore, the initial concentration of the intact virus is determined according to the acquired nucleic acid peak. After treating the virus sample further with NaOH, RNA is hydrolyzed, leaving only residual DNA, which is quantified using a DNA standard curve.

Mironov et al. [93] also developed a viral qCE for quantifying and characterizing intact viruses. CE was used to separate IVPs and the residual DNA, and to determine the quantification of virus contaminated with DNA impurities (Figure 11). The encapsulated and free DNA were detected.
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with LIF using intercalating YOYO-1 dye. After using protease K to lyse VV, all encapsulated DNA was dissolved, leaving free DNA. The change of peak area and a DNA standard curve were used to determine the initial concentration of intact viruses. The authors found that this viral qCE has a wide quantitative range for the oncolytic vaccinia virus (10^6–10^{12} ivp/mL).

Virus identification is traditionally determined by culture or serological methods, but these procedures are time consuming and tedious. Although qPCR for detecting target viruses is sensitive and specific, it is not appropriate for identifying an unknown virus in a sample. Therefore, Wang et al. [94] developed a virus identification approach for rapidly revealing the identity of unknown viruses in samples, which composed of one-step RT-PCR, liquid hybridization, probe depletion, CE, and a signal processing algorithm. The mechanism is showed in Figure 12

Figure 11: Schematic diagram of viral qCE analysis [93].
(1) A mixture of intact virus particles with encapsulated DNA (a green curve in an oval) and the free contaminated DNA (a green curve without an oval) is injected into the capillary as a short plug.
(2) When an electric field is applied (E > 0 V/cm), viruses and the free DNA are separated into two fractions.
(3) After lysis, the virus fraction disappears and the free DNA peak increases. The gain shows the amount of encapsulated DNA before lysis and is used to count intact virus particles. DNA is stained with YOYO-1 dye.

Figure 12: Schematic diagram illustrating the strategy for rapid identification of multiple viruses [94].
in the ITR, indicating that they are valuable methods for developing rAAV gene therapy.

To detect H1N1 influenza virus infection, Chung et al. [96] developed stuffer-free MLPA-CE-SSCP to distinguish different types of influenza A including H1N1. The LOD was 32.5 pfu/mL virus samples and this method accurately detected five relevant gene markers for confirming infection. Furthermore, using this approach, all the infections could be confirmed in a day. Lim et al. [97] developed a Palm PCR-portable CE system for rapidly analyzing influenza A (H1N1) virus, in which the analytical time from PCR to CE detection was only 20 min. Two novel H1N1-related genes were successfully detected, together with LODs of 6.3–72 pg/μL for DNAs. These results indicate that the Palm PCR-portable CE is a rapid, on-site, sensitive approach for molecular diagnosis.

Higher RNA structure affects key functions in most coding and non-coding RNAs. Most technologies for determining RNA structure are unable to analyze RNA from a small number of biological samples. The highly sensitive selective 2′-hydroxyl acylation analyzed by primer extension (SHAPE) [98–101] combined with high resolution CE could resolve this problem and quantitatively analyze RNA structure to solve RNA structure-related function problems. Thus, Jacob et al. [102] developed SHAPE-CE to structurally analyze genomic RNA from the xenotropic murine leukemia virus-related virus (XMRV) (Figure 13). With zeptomole molecular for LODs and subfemtomolar sensitivity for complete SHAPE experiments, hundreds of individual RNA structures were determined. The dimeric structure of the XMRV packaging domain, dynamic interactions between the packaging domain RNA and viral nucleocapsid protein, and the packaging signal for this virus were all studied using SHAPE-CE. Despite extensive sequence differences between XMRV and the studied moloney murine leukemia virus, the structure in the regulatory domains was similar, and principles for γ retrovirus RNA genome packaging were revealed [102]. Although SHAPE-CE has become a leading technique for determining RNA structure, data processing is complicated and time consuming, restricting its application. Therefore, an improved approach for data collection and sequences of algorithms incorporated into a program called fast analysis of SHAPE traces (FAST) was developed to significantly reduce processing time [103]. SHAPE-CE-FAST was successfully applied to determine the secondary structure in approximate 900 nucleotides of HCV genome and to find the entry site where a small molecule inhibitor binds with the HCV internal ribosome. These results indicate that FAST has the ability to process the high throughput data, allowing SHAPE-CE to quickly determine the structures for the RNAs of interest.

Recently, researchers found that microRNAs (miRNAs) are associated with virus-induced diseases; thus, miRNAs have become important biomarkers for diagnosing and analyzing biological processes in a timely manner. A new approach was developed to simultaneously detect multiple miRNAs within a single capillary that was based on combining a tandem adenosine-tailed DNA bridge-assisted splinted ligation with denaturing CGE-LIF [104] (Figure 14). This approach significantly improved resolution and detected multiple miRNAs in a single capillary according to the length differences in the specified target bridge DNA. Five miRNAs of Epstein-Barr virus (EBV) were successfully detected with a linear range of 1.0 pM–1.0 nM and a LOD of 190 fM. Moreover, only one labeled probe is enough for the detection of all miRNAs with having a small sample requirement make this approach much more economical than other existing commercial products. These results indicate that the CE-based method for detecting multiple miRNAs is rapid, cost-effective, and has a great potential for screening vast numbers of isomiRs.
Hand, foot, and mouth disease (HFMD) is a contagious enteroviral disease that occurs in children and is caused primarily by enterovirus 71 (EV71), coxsackievirus A16 (CVA16), and other serotypes of coxsackievirus and echovirus. A multiplex reverse transcription (RT)-PCR-CE-LIF approach (GeXP) was developed to simultaneously detect nine serotypes of enterovirus-related HFMD [105] (Figure 15). As little as 0.03 of the tissue culture infective dose of EV71 and CVA16, 10 copies of panenterovirus, EV71, CVA16, CVB1, and CVB5, and 100 copies of 10 premixed RNA templates were detected, with 180 suspected stool specimens successfully evaluated, the sensitivities and specificities of GeXP assay were respectively, 98.79%–91.67% and 80.00%–100%. Moreover, this assay could be effectively carried out in routine testing environments to allow users analyzing more samples in less time than traditional analytical platforms. These results demonstrated that the GeXP is a sensitive, inexpensive, and high throughputs approach for genotyping nine serotypes of HFMD-related enteroviruses [105].

Real-time PCR is restricted to a few of targets within a single reaction. Thus, Hlousek et al. [106] developed an ICEPlex system using a scalable target analysis routine to solve this problem. ICEPlex combines PCR thermal cycling with high efficiency CE separation for two-color quantitative detection. This system enabled direct measurements of accumulated amplicons, three orders of magnitude for the range of optical detection and at least seven logs for the range of detectable target concentrations. The system could separate more than 50 DNA fragments in a single mixture, providing broad multiple abilities for analyzing a wide spectrum of nucleic acid amplification fragments. These results demonstrate that ICEPlex is a valuable method for analyzing viral DNA and RNA targets, detecting genetic variants, and conducting RT-PCR gene expression panels.

The regulatory protein trans-activator of transcription (Tat) is essential to human immunodeficiency virus 1 (HIV-1) viral replication and plays a key role in HIV-associated neurocognitive disorders [107]. Numerous strategies are proposed to deactivate Tat function, including small molecule inhibitors to block Tat–transactivation-responsive RNA (TAR) interactions. Thus, Fourtounis et al. [108] developed a new fluorescence-based multiwell CE platform for characterizing protein–RNA interactions. The binding and interaction of HIV-1 Tat to TAR were studied by this CE-based method and the authors discovered that neomycin inhibited Tat-TAR binding. Their results...
indicate this approach is also useful for studying other nucleic acid-protein interactions.

Wongnoi et al. [109] studied the effects of antiretroviral (ARV) drugs administered to HIV-infected pregnant women on hematological parameters and hemoglobin synthesis in their ARV-exposed newborns in the presence and absence of thalassemia trait and of ARV drugs in worsening anemia in newborns carrying thalassemia. The hematological parameters and hemoglobin typing were analyzed using an automated blood counter with CE. The results showed that ARV-exposed newborns who were thalassemia carriers had the lowest levels of hemoglobin and hematocrit. The ARV drugs used for preventing HIV-maternal-neonatal transmission altered hematological parameters but did not affect hemoglobin synthesis in newborns with and without thalassemia trait. However, thalassemia and ARV drugs might have synergistic effects in inducing severe anemia.

HIV-1 treatment is frequently limited by new viral drug resistance. Thus, Jakobsen et al. [110] developed a PCR-CE approach (HIV-SNaPshot) to detect mutations causing drug resistance to HIV-1 reverse transcriptase inhibitors that combined a multiplex primer extension with CE detection to discover changed nucleotides in nine important drug-resistant mutation positions. They detected mutations to the levels of 5% in viral quasispecies populations, with additional resistance mutations and most non-B subtype mutations also detected, concluding that the HIV-SNaPshot method is sensitive and effective for determining minority HIV-1 resistance mutations.

Due to the advantages of high sensitivity, speediness, and high throughput, multiplex PCR is used to detect several targets in a single run and is becoming an effective and attractive technique in molecular diagnostics. Thus, Stevenson et al. [111] developed a multiple-PCR-CE method (Seeplex) for detecting viral and bacterial respiratory pathogens, with using dual priming oligonucleotide technology to improve multiplex PCR efficiency (Figure 16). They found that 28 of the 30 collected respiratory samples previously testing positive were successfully confirmed. Their results indicate that Seeplex is a potential alternative for detecting multiplex respiratory pathogens. Furthermore, Thaitrong et al. [112] developed a two-layer, four-channel PCR-CE microchip that integrates nucleic acid amplification, sample cleaning and concentration, CE separation and detection for analyzing four human respiratory viral pathogens (Figure 17). This microchip successfully analyzed samples from nasopharyngeal swabs, achieving LODs of 25–100 copies/reactor for ribonucleic acid analogs of all four viral targets. These results indicate that this portable microchip is valuable for automatically and sensitively detecting multiple pathogens.

To study how the load and multiple genotypes of the John Cunningham virus affected the immune system in patients with transplants, Yin et al. [113] used qPCR to quantify the viral load and CE to genotype the virus. They found that both viral load and genotypes are critical parameters for understanding the immune milieu in patients to prevent subsequent complications.

The polymorphism of genes encoding the cytomegalovirus envelope protein is used to classify the strain and associatedopathogenesis. Thus, Grosjean et al. [114] developed PCR-RFLP-CE to analyze cytomegalovirus strain diversity and detect multiple-strain infection in a large number of toddlers. This approach simultaneously determined gB, gH, and gN genotypes and provided accurate classification of cytomegalovirus strains, indicating that this method may facilitate large epidemiologic studies.

To develop a novel detection method for human papillomavirus (HPV) infection, Hong et al. [115] developed HPV4 auto-capillary electrophoresis (ACE) and compared the resulting data with those from the hybrid capture (HC) 2 assay and PCR-HPV typing. The results of the HPV 4 ACE test and the HC 2 assay were in agreement for detecting high risk HPVs (85.4%, κ=0.71) and also agreed with the
PCR-HPV typing in the detection of HPV 16 and HPV 18 genotypes (89.9%, $\kappa=0.65$), indicating that the HPV 4 ACE test is a highly effective and alternative tool for detecting high risk HPVs and genotyping HPV 16 and HPV 18.

HCV translation starts from the internal ribosome entry site (IRES). IRES-dependent translation can be inhibited by aptamers targeting two different binding sites in the HCV IRES domain. Anti-viral RNA inhibitors fusing these aptamers are likely to apply to multiple viral targets. Thus, Kikuchi et al. [116] applied MCE to analyze RNA-RNA and aptamer-IRES interactions. They found that conjugated molecules combined with aptamers for different target recognition sites potentiated the prohibitive activity by improving the domain-binding efficiency. Danilovic et al. [117] applied PCR-RFLP-CE to evaluate the frequency of the cytotoxic T lymphocyte-associated factor 4 (CTLA-4) gene polymorphisms in chronic HCV infected patients and correlated their results with clinical and histological results. They analyze 112 HCV-infected patients and 183 healthy controls and found that the CTLA-4 gene polymorphisms were correlated with HCV-infection, eight $AT$ repetitions were more frequent in HCV-infected patients, $-318C$ and $+49G$ alleles were related with genotype 1 and 3 infections, and increased $AT$ repetitions in the $3'$ untranslated region favored serious necroinflammatory activity in liver biopsies [117].

Detecting virus rapidly and sensitively facilitates effective and early response to various viral diseases. Thus, Vaculovicova et al. [118] developed a method that combined magnetic nanoparticles (MNPs) isolation of hepatitis B virus (HBV)-specific DNA fragments with instant microchip CGE analysis (Figure 18). This approach detected HBV with an LOD of 1 ng/mL.

Using a separation technique as an alternative approach for traditional virological methods may provide additional information for fundamental research. Thus, Oita et al. [119] developed a CE method combined with 12-experiment Plackett-Burman design for analyzing poliovirus. They reduced the detection concentration of poliovirus to 140 $\mu$g/mL.

To accurately measure acyclovir concentrations in pregnant women and their fetuses at delivery and study the correlations between acyclovir concentration, labor time, and time since last acyclovir dose, Leung et al. [120] measured acyclovir levels using CE and calculated the associations between the labor time, time since last acyclovir dose and the concentration of acyclovir in blood. They found that women orally administering acyclovir in late pregnancy might have concentrations at delivery insufficient to prevent viral shedding. The authors suggested that considering acyclovir treatment during labor to prevent viral shedding.

Traditional techniques for genotyping herpes simplex virus 1 (HSV-1) are unable to sub-genotype strains. Thus, Dean et al. [121] used the hypervariable reiterative repeat regions in US1 and US12 introns as targets for strain

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**Figure 17:** Schematic of the microdevice preparation and operation [112].

(1) To avoid contamination of the reactor, an 8% linear polyacrylamide gel (gray) is loaded into the coinjector prior to introducing the bis/acrylamide monomer solution (brown). (2) A streptavidin-copoly-merized 5% bis/acrylamide gel plug 40 $\mu$m deep $\times$120 $\mu$m wide $\times$500 $\mu$m long is formed within the microchannel through in situ photopolymerization (gold). (3) The plug is sandwiched by a separation matrix manually loaded from both the anode and cathode. (4) The assay is started by filling the reactor with PCR (or RT-PCR) cocktail (blue), and immediately thermocycling. (5) After the amplification is completed, the sample is electrophoresed (red arrow) through the capture plug, where the biotinylated PCR products are captured. (6) The captured dsDNA is dehybridized at 67 °C, releasing the fluorescently labeled ss-DNA for size-based separation at 300 V/cm.
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comparison and developed a PCR-CE method to produce signatures for analysis. Primary clinical specimens were successfully analyzed for viral diagnosis. In addition, the signatures matched across multiple samples together with some additional signature matches were likely to be associated with epidemiology. Their results indicate that this PCR-CE approach has multiple potential clinical and public health uses.

RNA viruses show highly replication error rates and thus produce diverse viral quasispecies. However, the traditional methods for cloning and sequencing these variants in a quasispecies are expensive, troublesome, and time consuming. Thus, Gulija et al. [122] applied PCR-CE-SSCP and PCR-RFLP-CE methods to quantify two similar viral variants in a mumps virus strain. They found that although the LODs for both methods were 5% of the minor variant, the results using different operations were diverse. When PCR amplicons of the two variants were mixed, the quantitative results of both methods were similar. However, when variants were mixed prior to PCR, the quantitative results were different. These results indicate that caution should be exercised when only one method is used to quantitatively analyze complex samples.

To investigate the association between microsatellite instability in vulvar squamous cell carcinoma (VSCC) and HPV, DNA samples from primary VSCC and from metastatic lymph nodes were analyzed, and the microsatellite instability status was examined using PCR-CE. No microsatellite instability was found in any of the patients with VSCC. Thus, microsatellite instability is likely not correlated with the HPV occurrence in patients with this carcinoma [123].

**Detecting mycoplasma, chlamydia and rickettsia**

The spotted fever group (SFG) rickettsiae cause several tick-borne rickettsioses in humans worldwide. To determine the prevalence of SFG rickettsiae in *Amblyomma variegatum* from seven districts across Uganda, Nakao et al. [124] obtained hypervariable IGSs (dksA-xerC, mppA-purC, and

**Figure 18:** Scheme of the complete multistep process of MNPs isolation and analysis on a single CE chip [118]. The chip priming (1), addition of the MNPs into the wells (2), flushing of the particles with phosphate buffer (3), immobilization (4), removal of unbound compounds (5), flushing of the particles with phosphate buffer (6), elution (heating to 85 °C for 5 min) (7), addition of 5% loading buffer (8), addition of gel with fluorescent stain and ladder (9), CE analysis (10).
rpmE-tRNA) as targets and developed multiplex PCR-CE for identification of *Rickettsia* species. They successfully identified 136 samples. This study provided a start for developing *Rickettsia* species identification based on the sizes of IGSs, which might be particularly well suited for preliminary species identification in epidemiological investigations.

*Mycoplasma hominis* causes a variety of urogenital and extragenital infections in humans. To study their genetic diversities, Férandon et al. [125] applied MLVA-CE to analyze 210 urogenital and extragenital clinical isolates. Forty MLVA types were found, and a large scale study of *M. hominis* isolates analyzed using MLVA revealed high genotypic diversity. These results demonstrate that MLVA-CE is effective for genotyping *M. hominis*. However, this approach is too discriminating to be applied in a large epidemiological study, although it is useful for molecularly studying individual species [125, 126].

*Chlamydia trachomatis* most frequently causes sexually transmitted infections. Genotyping can reveal transmission patterns, determine whether the infections persistent or new, and investigate the emergence of specific clones. Therefore, Peuchant et al. [127] developed MLVA-CE (MLVA-5) for discriminating *C. trachomatis* genovar D to K isolates. They successfully analyzed 220 genovar E and 94 non-E genovar *C. trachomatis* isolates. Among all used methods, MLVA-5 showed the highest discriminatory ability, without using cultures or troublesome sequencing steps. These results demonstrate that MLVA-5 is a high resolution and high efficiency genotyping tool for examining genovars D to K *C. trachomatis* infections directly in samples.

Mutations in domain V of the 23S rRNA gene of *Mycoplasma pneumoniae* obstruct the binding of macrolides with rRNA, causing macrolide resistance. Thus, Lin et al. [128] developed an nPCR-CE-SSCP method to detect macrolide-resistant mutations. The A2063G, T2611C, and A2063T mutations were successful identified with macrolide-resistant mutants directly identified from clinical samples, indicating that nPCR-CE-SSCP is an effective analytical method for detecting genetic mutations relevant to antibiotic resistance and that this approach may offer rapid assistance to clinicians for choosing an appropriate therapy.

### Simultaneously detecting multiple types of pathogens

Pan et al. [129] developed an MCE that combined multichamber PCR with multichannel CE separation to analyze samples in parallel (Figure 19). The microchip consisted of three functional units: temperature control, multiple PCR, and multiple channel CE separation. A homogeneous temperature field was produced with a platinum and titanium microheater, and temperature monitoring was conducted with a platinum chip sensor. A separation channel was used to connect chip-PCR and chip separation, greatly simplifying the design. After chip-PCR, the PCR products were injected into parallel separation channels for automatic detection under an electric field. This MCE successfully detected multiple pathogens including HBV and *M. tuberculosis*, and genotyped the human leukocyte antigen-B27, the RSDs of migration time for peaks across the four channels in one operation were <4.40% and those in the same channel for three runs were <1.2%, with the entire PCR-CE analysis was completed within 1.5 h, demonstrating that MCE is reliable, rapid and effective for parallel genetic analyses.

A reliable and safe method to identify pathogens causing sexually transmitted diseases is necessary for reducing the transmission of infection. Thus, Samra et al. [130] applied a multiplex PCR-CE system (Seeplex®) to simultaneously detect six pathogens from genital and urinary specimens of 113 patients with sexually transmitted diseases. They found that Seeplex® has a sensitivity of 98%–100% and a specificity of 97%–100%, which indicates this system is highly sensitive and specific for

![Figure 19: Schematics of the integrated microfluidic device [129].](image-url)
rapidly detecting multiple pathogens. Lee et al. [131] also applied a multiple PCR-CE (Seeplex®) to simultaneously detect *C. trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *M. hominis*, and *Trichomonas vaginalis*. The Seeplex® system displayed sensitivity and specificity of 99.7%–100%, with further results indicated that this system is a sensitive, cost-effective, and rapid tool for simultaneously detecting multiple pathogens.

Coupland et al. [132] also used multiple PCR-CE (Seeplex®) to simultaneously detect four viruses and 10 bacteria associated with acute gastroenteritis because acute gastroenteritis involves bacterial, viral, and parasitical infections. Conventional diagnostic methods detected 98 pathogens in 96 samples while Seeplex® detected 81 pathogens in 75 samples, with the sensitivity and specificity were respective 84.21%–100% (except *C. difficile* was 50%) and 96.23%–100%. Moreover, their results showed that additional multiple infections could be diagnosed using the Seeplex® system, indicating that it is a sensitive and significant diagnostic tool for comprehensively diagnosing acute gastroenteritis based on a majority of the pathogens.

An accurate expression map of functionally important genes is critical for the study of systems biology. Quantitative PCR is generally used for this purpose, but most commercial instruments cannot simultaneously detect more than four targets due to limitations imposed by fluorescence dyes with restricted distinct excitation and emission spectra. One alternative technology is MLPA; however, conventional MLPA separates PCR products according to size, which increases the difficulty of probe design and compromises analytical results. Therefore, Shin et al. [133] developed a modified MLPA-CE-SSCP and used this system to analyze 33 *E. coli* metabolic genes and 16 *Caenorhabditis elegans* longevity-associated genes (Figure 20). The relative expression of the genes was quantitatively determined using similar length probes. Moreover, compared

![Figure 20: CE-SSCP analysis of stuffer-free MLPA probes amplified using a ligation-dependent method [133].](image)

1. Assay procedures for conventional and stuffer-free methods are shown in parallel. (3) Electropherograms for probes of two separate sets of *E. coli* metabolic genes, one belonging to glycolysis/pentose phosphate pathways and the other belonging to the tricarboxylic acid cycle/glycolate pathway. (4) Electropherogram for probes of *C. elegans* longevity-associated genes. Any two assay products in these two examples are baseline-separated, even when they are the identical in size. The number above each peak represents the size of the final assay product. The x- and y-axes indicate migration time and relative fluorescence intensity (in arbitrary units), respectively.
to conventional MLPA that standard deviations were general within 10%, this modified MLPA-CE-SSCP produced <5% of standard deviations with the analysis was reproducible regardless of signal strength. This observed improved accuracy compared with that for conventional MLPA indicates that MLPA-CE-SSCP is a promising analytical method for use in a variety of biological systems.

The microbial content of air is associated with public health; yet, the data about airborne microflora in enclosed spaces is lacking. Therefore, Gaüzère et al. [134] applied CE-SSCP and other high throughput molecular methods to study the microorganism distribution of indoor air. They found that the bacterial diversity in indoor airborn is stable, whereas the corresponding eukaryote group is unstable. The core species belong primarily to the Proteobacteria and Actinobacteria, and to the genera Paracoccus spp, Acinetobacter sp., Pseudomonas sp., Enhydrobacter sp., Sphingomonas sp., Staphylococcus sp., and Streptococcus sp. [134].

The rapid identification of biological agents is crucial for preventing casualties during attacks with biological weapons. Thus, Cho et al. [6] developed an effective and sensitive method that combined a DNA biobarcode assay with MCE for detecting multiple biological agents. The principle is as follows. First, magnetic microparticle-pathogen-polystyrene microbead complexes are formed, and then the FAM-labeled single-stranded barcode DNA is released from the complexes upon denaturation. Barcode DNAs having different lengths represent different pathogens, so that the specific peak elution time in MCE accurately discriminates the target within 3 min (Figure 21). Using this DNA biobarcode-MCE, Bacillus anthracis, Francisella tularensis, Y. pestis, and vaccinia virus were successful confirmed with an LOD of 50 CFU/mL, and botulinum toxin A was detected at levels as low as 12.5 Ag/mL. These results demonstrate that DNA biobarcode-MCE analysis is a fast, sensitive, effective, multiplex, and precise approach for identifying biological agents.

**Limitations, advantages and future development of CE based on nucleic acid detection for infectious disease diagnosis**

Many researchers have been attracted to CE analysis since it was developed, especially for analyzing DNA
However, for more than 30 years, CE has been used primarily in laboratory research rather than in clinical practice. The application of CE to the analysis of nucleic acids appears to be hindered by six limitations. The first is that CE is a developing technique in analytical chemistry. Genetic diagnostics based on nucleic acid analysis is associated more with medicine and molecular biology. The combination of these two fields requires researchers knowledgeable in both subjects, but many scientists lack such a comprehensive background. The relatively low sensitivity of CE analysis is the second limitation in the field of DNA analysis [136–140]. The double bond formed by the base group in DNA has a low UV-visible absorption, and a normal UV detector has a low sensitivity. The intrinsic low detection sensitivity drawbacks of CE, which are the limited light absorption dimension and the restricted sample injection length resulting in small sample injection volumes, also greatly restrain the application of CE-UV to DNA analysis. Consequently, fluorescence detection becomes the solution for the sensitivity challenges of DNA analysis used by most researchers [26, 135, 141]. However, this solution demands labeling samples with a fluorescence marker or intercalating dyes [142]. Unfortunately, these procedures are time consuming and expensive, and most dyes are toxic (such as ethidium bromide). In addition, the data derived from fluorescence detection electropherograms require integration with those from the more widely used UV electropherograms. Consequently, fluorescence detection has not yet achieved extensive application in clinical diagnosis. Although CE assays have improved greatly over those in the preliminary stages of development, their stability, reliability, and reproducibility are not as good as those provided by traditional techniques [122, 143], and sometimes the accuracy of the assays requires additional corrections [32, 144]. Thus, the results derived from CE analyses continue to require verification by accepted methods, such as DGGE, direct sequencing, and real-time fluorescence quantitative PCR. This third limitation of CE, that the technique is still under development and validation, may be the reason that most of the diagnostic methods based on CE are not widely applied to clinical practice. The fourth and fifth limitations are that the equipment and reagents for CE are more expensive than other techniques [145, 146] and that necessary auxiliary equipment pre-existing in the clinic must be made compatibles with CE. This is especially a drawback when developing an automated identification system. The final limitation for the use of CE in clinical applications is that although MCE was developed many years ago, the high efficiency, high speed, microscale, automation and high throughput advantages of this technique are not prominently known because the theory behind MCE is not yet mature, and MCE has a relatively low automation.

However, the application of CE in clinical practice has many advantages. The traditional methods for separating DNA are slab gel electrophoresis and various types of affinity chromatography. Although these techniques enjoy widespread use in many laboratories, they suffer some intrinsic systematic disadvantages that have remained unsolved. First, preparation and separation is time consuming (requiring at least 2 h), labor-intensive, and has a low efficiency. Second, sample elution and post-processing are difficult and often rely on the use of toxic and mutagenic reagents (such as ethidium bromide and organic solvents) that may harm the health of operator. Third, the waste from these techniques causes severe pollution. Fourth, these methods are limited by the difficulties of accurate size estimation, digital-based data preparation, and data interpretation and transferability [147]. These issues are increasingly urgent when considering future applications in infectious disease diagnosis where speed, safety, and effectiveness are compulsory for clinical needs. By contrast, the aforementioned shortcomings for analysis using CE may be offset by its higher resolution [148, 149], better sensitivity and reproducibility [150], rapidity [151], ease of use on a microscale, and few harmful reagents. Moreover, CE can complete a single detection in as little as a few minutes to dozens of minutes, so that hundreds of detections can be finished in a single day. Even more detections can be acquired using MCE, meeting the need for rapidly analyzing a large number of clinical samples. Although the equipment for CE may be relatively expensive, the operational costs are not because the consumed reagents are mainly water, inorganic salt, and a small amount of gel. Thus, after the investment of fixed assets, CE becomes cost-effective and has a great potential for use in clinical diagnostics (Tables 1 and 2) [147].

Conclusions and outlook

Overall, although the application of CE based on nucleic acid detection has its limitations, the many advantages over the traditional culture-based, phenotype and biochemical test identification still attract many researchers to this approach [33, 152]. The development of this technique has been and the future developments are likely to be as follows:

1. DNA pre-concentration methods were applied in CE to resolve the sensitivity problem [153, 154] (Figure 22).
2. MCE with the advantages of high speed, high throughput, high efficiency, and use on a microscale, was further developed [155], with special emphasis placed on meeting practical requirements and high automation. Such developments in MCE included: MCE integrated bacteria lysis, cleanup, PCR, and CE detection for diagnosis of bacteria [156]; Caliper LabChip 90 system performed strain genotyping in one sixth the time of other microfluidics systems (e.g. the Agilent 2100 bioanalyzer) [144]; large-size dsDNA was separated ultra rapidly and efficiently in a blended polymer matrix by MCE [157]; small DNA fragments were analyzed using MCE-AD without requiring any preconditioning or use of polyacrylamide gel in the microchannel [158]; MCE based on a short capillary and a slotted-vial array automated the sample introduction system for high speed separation of DNA fragments without increasing the separation electric field strength [159]; MCE applied to analysis of DNA with 500–5000 bp and for analysis of DNA ligation [160]; MCE employed embedded sub-micron pillar arrays to produce different retarding obstacles for the DNA molecules allowing for the separation of DNA in a free buffer solution [161].

3. CE combined with other favorable factors (polymerases, sequencing gels, etc.) to develop next-generation sequencing-based diagnostic technology [147, 162–165].

4. Consolidated criteria will need to be established for integrating and comparing diagnostic results from different laboratories using different detection methods [166].

5. Multiplex PCR-CE was given priority for development due to its high sensitivity, expeditiousness, high throughput, and cost-effectiveness while the drawbacks were eliminated, such as multiple primer sets.

Table 1: Performance characteristics of various genotyping methods for Clostridium difficile [147].

<table>
<thead>
<tr>
<th>Method</th>
<th>Target</th>
<th>Discriminatory power</th>
<th>Ability to type</th>
<th>Reproducibility</th>
<th>Ease of interpretation</th>
<th>Technical complexity</th>
<th>Transporability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Band-based</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>REA</td>
<td>Whole genome</td>
<td>Good</td>
<td>Fair</td>
<td>Fair</td>
<td>Poor</td>
<td>Moderate</td>
<td>Poor</td>
</tr>
<tr>
<td>PFGE</td>
<td>Whole genome</td>
<td>Moderate</td>
<td>Fair</td>
<td>Moderate</td>
<td>Fair</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td>PCR ribotyping</td>
<td>16S-23S ISR</td>
<td>Good</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Good</td>
<td>Moderate</td>
<td>Low</td>
</tr>
<tr>
<td>Capillary PCR</td>
<td>16S-23S ISR</td>
<td>Excellent</td>
<td>Poor</td>
<td>Excellent</td>
<td>Good</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td>MLVA</td>
<td>Whole genome tandem repeats</td>
<td>Excellent</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Excellent</td>
<td>Moderate</td>
<td>Excellent</td>
</tr>
<tr>
<td>Sequence-based</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLST 7HG</td>
<td>7 HG</td>
<td>Good</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Excellent</td>
<td>Moderate</td>
<td>Excellent</td>
</tr>
<tr>
<td>SNP typing</td>
<td>Whole genome SNPs</td>
<td>Good</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Good</td>
<td>Moderate</td>
<td>Good</td>
</tr>
</tbody>
</table>

HG, housekeeping genes; ISR, intergenic spacer region; MLST, multilocus sequence typing; MLVA, multilocus variable-number tandem repeat analysis; PCR, polymerase chain reaction; PFGE, pulsed-field gel electrophoresis; REA, restriction endonuclease analysis; SNP, single nucleotide polymorphism.

Table 2: Techniques, time and costs associated with various genotyping methods for Clostridium difficile [147].

<table>
<thead>
<tr>
<th>Genotyping method</th>
<th>Techniques</th>
<th>Turnaround time, days</th>
<th>Hands-on time, h</th>
<th>Costs</th>
</tr>
</thead>
<tbody>
<tr>
<td>REA</td>
<td>DI,ER,GE</td>
<td>2</td>
<td>2</td>
<td>Low</td>
</tr>
<tr>
<td>PFGE</td>
<td>DI,ER,GE</td>
<td>2–4</td>
<td>6</td>
<td>Moderate</td>
</tr>
<tr>
<td>PCR ribotyping</td>
<td>DI,PCR,GE</td>
<td>1–1.5</td>
<td>2</td>
<td>Low/moderate</td>
</tr>
<tr>
<td>Capillary ribotyping</td>
<td>DI,PCR,CE</td>
<td>1</td>
<td>2</td>
<td>Moderate/high</td>
</tr>
<tr>
<td>MLVA</td>
<td>DI,PCR,CE</td>
<td>2</td>
<td>8</td>
<td>Moderate/high</td>
</tr>
<tr>
<td>MLST</td>
<td>DI,PCR,PPP,SE</td>
<td>4</td>
<td>8</td>
<td>Moderate/high</td>
</tr>
<tr>
<td>SNP typing</td>
<td>DI,LP,TA,SE</td>
<td>5</td>
<td>3</td>
<td>High</td>
</tr>
</tbody>
</table>

CE, capillary electrophoresis; DI, DNA isolation; ER, enzyme restriction; GE, gel electrophoresis; LP, library preparation; MLST, multilocus sequence typing; MLVA, multilocus variable-number tandem repeat analysis; PCR, polymerase chain reaction; PFGE, pulsed-field gel electrophoresis; REA, restriction endonuclease analysis; SE, sequencing; SNP, single nucleotide polymorphism; TA, template amplification.

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resulting in complexity of reaction conditions and some primer sets generating preferentially PCR products resulting in false results.

6. CE combined with other techniques capitalizes on the advantages of the other techniques, such as CE combined with MS for the detection of PCR-amplified microbial nucleic acids [167, 168]. CE is a rapid and highly efficient separation technique, while MS has a higher specificity and sensitivity. Thus, CE-MS is a more powerful technique, providing detailed information about analytes for more accurate diagnoses.

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