# 4
# DNA Microarrays for Pathogen Detection

*Holger Schulze, Maya Rubtsova, and Till T. Bachmann*

## 4.1
## Introduction

Since its invention at the end of the last century [1, 2], the DNA microarray technology has created a huge interest among people interested in infectious diseases, especially for diagnostics. The technology itself was repeatedly and extensively described in many textbooks and reviews and will not further be described here. For a thorough coverage of the technology, its applications, and the bioinformatic aspect of data analysis, see, for example, the three Chipping Forecast Supplements to *Nature Genetics* (Chipping Forecast Editorial, 1999; Chipping Forecast II Editorial, 2002; Chipping Forecast III Editorial, 2005). Basically, DNA microarrays are used to detect nucleic acid targets using nucleic acid probes which are bound in an array format to solid supports, making them easy to follow. The binding of nucleic acid targets, which are usually amplified by nucleic acid amplification strategies such as PCR (polymerase chain reaction), to these probes is conventionally followed by fluorescence signals and used to identify the organism they are originating from. For infectious diseases, these organisms are usually pathogens and therefore of greatest importance to healthcare. The application of DNA microarrays for pathogen detection has become a widespread field of research which was repeatedly reviewed, and a number of commercial products have been developed and launched. For some reviews on this topic, see [3–9]. The main driver for molecular diagnostics in infectious diseases is that culture-based testing, although still being the gold standard, is too slow because it can take 2–15 days until a result is received. In contrast to methods based on culture, molecular methods, and especially DNA microarrays, enable fast identification of the etiologic agent in infectious diseases. In addition, these methods allow the user to detect additional information relevant to the treatment decision which may be related to antibiotic resistance or virulence factors. DNA microarray technology appears to be an ideal choice for this purpose, as it enables a highly parallelized testing to a great information depth.

Although the traditional disciplines in infectious diseases following pathogen classes become increasingly less obvious, we decided to review the available

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literature separated in diagnostic microarrays for bacteria, viruses, fungi, and parasites because the body of DNA microarray literature on infectious disease diagnostics is very large (e.g., a narrow search in PubMed for "DNA microarray infecti* diagnostic" reveals 1490 publications (October 2013)). For the same reason, we mostly limited the search to the last 5 years. These publications are discussed in the sections which are accompanied by tables summarizing the key features of the described systems. A main aspect of this review is the consideration of commercially available DNA microarray-based diagnostics. We discuss these products in the sections for the respective pathogen class but summarize the commercial systems at the end of this overview in a table organized by company offering the product.

## 4.2
## DNA Microarrays for the Detection of Bacterial Pathogens

In recent years, a large number of DNA microarray tests for bacterial species identification have been developed for several clinical applications such as sepsis, pneumonia, and infectious diarrhea diagnostics or for the identification of certain pathogens such as *Mycobacterium* species [10], *Escherichia coli* [11–14], chlamydiae [15], and several other bacteria [16–21], as shown in Table 4.1. In the following Section 4.2.1 some examples of clinically validated microarray tests will be described in more detail.

### 4.2.1
### Major Bacterial Diseases

#### 4.2.1.1 Blood Stream Infections/Sepsis

Sepsis and pneumonia in adults and children are life-threatening diseases with high rates of morbidity and mortality worldwide. Of 7.6 million children who died in the first 5 years of their life in 2010, 14% died of pneumonia and 5% died of sepsis or meningitis [37]. Consequently, blood stream infections are a major target researchers aimed to address using DNA microarray-based pathogen detection. Wiesinger-Mayr *et al.* developed a DNA microarray test for the detection of 25 bacteria and fungi relevant for blood stream infection. The test for sepsis-causing organisms combined multiplex PCR with universal 16S rDNA and 18S rDNA specific primer pairs with hybridization of the fluorescence-labeled PCR products on a microarray consisting of 76 species-specific DNA probes. The time to result from pathogen isolation from blood samples to species identification was 6 h. The low sensitivity toward some bacteria, such as, for example, *Staphylococcus aureus* (limit of detection (LOD) = $10^5$ cfu/ml), represents a barrier for clinical application. In contrast, the LOD for *E. coli* detection in blood samples was 10 cfu/ml. Palka-Santini *et al.* developed a DNA microarray combined with a large-scale multiplex PCR with primer pairs to amplify up to 800 different gene segments from nine pathogenic species. The PCR amplicons corresponded to

**Table 4.1** DNA microarrays for bacterial species identification.

| References | CC | Target species | Sample | Preanalytics | Detection | TTR |
|---|---|---|---|---|---|---|
| [11] | JP | *S. aureus*, *E. coli*, *S. enterica*, *P. aeruginosa* | Reference strains | None | On-chip PCR directly from bacteria without DNA extraction, 23S rDNA amplification followed by on-chip primer extension, enzymatic precipitation reaction (alkaline phosphatase + BCIP-NTB) | 5–6 h |
| [22] | IT | *A. baumannii*, *B. fragilis*, *B. thetaiotaomicron*, *B. vulgatus*, *C. koseri*, *C. rodentium*, *C. difficile*, *C. kluyveri*, *C. perfringens*, *C. diphtheriae*, *C. efficiens*, *C. jeikeium*, *E. faecalis*, *E. coli*, *E. fergusonii*, *H. influenza*, *K. pneumonia*, *L. innocua*, *L. monocytogenes*, *M. luteus*, *O. anthropic*, *P. acnes*, *P. mirabilis*, *P. aeruginosa*, *P. fluorescens*, *P. mendocina*, *P. putida*, *S. enterica*, *S. aureus*, *S. carnosus*, *S.epidermidis*, *S. haemolyticus*, *S. lugdunensis*, *S. saprophyticus*, *S. maltophilia*, *S. equi*, *S. pyogenes* | Reference strains, clinical isolates, and two clinical saliva samples | DNA extraction, gDNA fragmentation (95 °C for 3 min), chemical labeling with Cy3 | Microarray hybridization, fluorescence detection | n/a |

(*continued overleaf*)

**Table 4.1** (*Continued*)

| References | CC | Target species | Sample | Preanalytics | Detection | TTR |
|---|---|---|---|---|---|---|
| [15] | DE | *Chlamydia* spp., *Chlamydophila* spp. | 282 animal and 11 human samples (nasal and conjunctival swabs, formalin-fixed, paraffin-embedded and fresh organ tissue, milk, feces, and cell culture) | DNA extraction, PCR (biotinylated primer) | Hybridization on Alere ArrayTube platform, enzymatic precipitation reaction (HRP/TMB), Alere ArrayTube Reader (transmission scanner) | 24 h |
| [23] | BE | *E. faecium*, *S. aureus*, *S. pneumoniae*, *A. calcoaceticus*, *B. cepacia*, *C. freundii*, *E. aerogenes*, *E. cloacae*, *E. coli*, *H. influenza*, *K. pneumoniae*, *L. pneumophila*, *M. catarrhalis*, *M. tuberculosis*, *P. aeruginosa*, *P. mirabilis*, *P. stuartii*, *P. vulgaris*, *S. maltophilia*, *S. marcescens*, *A. flavus*, *A. fumigatus*, *C. albicans* | 200 clinical isolates, 25 endotracheal aspirates, and sputum samples | DNA extraction, multiplex-PCR (fus/rps gene complex, gyrA gene) | Hybridization, Silverquant labeling (Eppendorf) | n/a |
| [24] | DE | *S. aureus*, *E. coli*, *P. aeruginosa* | 43 clinical isolates, 13 positive blood cultures | DNA extraction, no PCR, genomic DNA chemically labeled with fluorophores | Hybridization with Tecan HS400 hybridization station (17 h) on microarrays with 200–800 bp long probes | >24 h |

| | | | | | | |
|---|---|---|---|---|---|---|
| [25] | FR | *A. caviae*, *A. butzleri*, *C. coli*, *C. fetus*, *C. jejuni*, *C. upsaliensis*, *E. coli (EHEC, EIEC, EPEC*, and *STEC)*, *G. hollisae*, *K. oxytoca*, *L. monocytogenes*, *S. enterica*, *S. sonnei*, *T. whipplei*, *V. alginolyticus*, *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, *Y. enterocolitica*, *G. obscuriglobus*, *P. staleyi*, *P. brasiliensis/maris*, *P. limnophilus*, *R. baltica* + 7 enteropathogenic viruses | 40 pathological stool specimens | Diarrheal stools were lyophilized, vortexed with glass beads, specimen were ground using the FastPrep (MP Bio-medicals) apparatus, followed by lysis with the Nucleospin Tissue kit (Macherey Nagel) and DNA extraction using the Qiagen EZ1 DNA Tissue kit | Extracted genomic DNA was Cy3-labeled with the ULS Labeling Kit and hybridized on the microarray for 40 h | >48 h |
| [26] | CN | *Y. enterocolitica*, *S.* spp., *S. typhi*, *Brucella* spp., *V. cholerae*, and *E. coli O157:H7* | 362 clinical specimens (rectal swabs) | DNA extraction, multiplex PCR with biotinylated primer | DNA microarray hybridization + combined with tyramide signal amplification | No clear data: sample prep., 1 h hybridization, washing, readout, data analysis |
| [26] | CN | *Y. enterocolitica*, *Salmonella* spp., *S. typhi*, *Brucella* spp., *V. cholerae*, *E. coli* | 92 reference strains, 362 clinical samples (swabs) | DNA extraction, multiplex PCR | Low density DNA microarray, hybridization (1 h), enzymatic signal amplification (MRP + tyramide-signal-amplification), fluorescence detection | No clear data: sample prep., 1 h hybridization, washing, readout, data analysis |

(*continued overleaf*)

**Table 4.1** (*Continued*)

| References | CC | Target species | Sample | Preanalytics | Detection | TTR |
|---|---|---|---|---|---|---|
| [27] | KR | *Enterococcus* spp., *Listeria* spp., *Staphylococcus* spp., *Streptococcus* spp., *Bacteroides* spp., *E. coli*, *Enterobacter/Klebsiella* spp., *Hemophilus* spp., *Pseudomonas* spp., *S.* spp., *E. faecalis*, *E. faecium*, *E. avium*, *L. monocytogenes*, *L. ivanovii*, *L. grayi*, *L. welshimeri*, *S. aureus*, *S. epidermidis*, *S. saprophyticus*, *S. agalactiae*, *S. pneumoniae*, *S. pyogenes*, *S. bovis*, *B. fragilis*, *B. thetaiotaomicron*, *B. ovatus*, *E. cloacae*, *E. aerogenes*, *E. agglomerans*, *K. pneumoniae*, *K. oxytoca*, *H. influenzae*, *H. ducreyi*, *P. aeruginosa*, *P. stutzeri*, *S. marcescens*, *S. grimesii*, *S. entomophila* | 825 positive blood cultures | DNA extraction (InstaGene Matrix Kit), PCR with universal, biotinylated primer | 30 min hybridization in the presence of Cy5-streptavidin on microarrays with genus and species-specific probes targeting the bacterial ITS of the 16S-23S rRNA | 24 h |
| [28] | KR | *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, *S. enterica serovar Typhimurium*, *S. enterica serovar Enteritidis*, *S. flexneri*, *S. sonnei*, *enterotoxigenic E. coli (ETEC)*, *enterohemorrhagic E. coli (EHEC)*, *Y. enterocolitica* | 82 stool samples from patients with food-borne enteritis | DNA extraction (AccuPower Stool DNA extraction kit, Bioneer) | Multiplexed PCR of bacterial virulence-factor genes, chemically labeling (BioPrime Array CGH Genomic Labeling System, Invitrogen) + hybridization (4 h) | 12 h |

| | | | | | | |
|---|---|---|---|---|---|---|
| [29] | CN | *C. difficile*, *C. jejuni*, *C. perfringens*, *V. parahaemolyticus*, *P.* spp., *L. monocytogenes*, *B. cereus*, *Y. enterocolitica*, *E. faecalis*, *P. aeruginosa*, *S. aureus*, *E. coli*, *Shigella* spp., and *Salmonella* spp. | 1500 diarrheal stool samples + 200 stool samples of healthy individuals | DNA extraction (Qiagen QIAamp DNA Stool extraction kit), PCR with 16S rRNA primer + 2 virulence gene specific primer pairs | 1 h hybridization of Cy3-labeled PCR products in humidification chamber without mixing | 5 h |
| [30] | JP | *S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. intermedius*, *S. saprophyticus*, *S. warneri*, *S. pneumonia*, *S. pyogenes*, *S. agalactiae*, *S. bovis*, *S. canis*, *S. equisimilis*, *S. anginosus*, *S. constellatus*, *S. intermedius*, *S. mitis*, *S. oralis*, *S. sanguinis*, *A. viridans*, *G. morbillorum*, *M. luteus*, *B. cereus*, *B. subtilis*, *C. jeikeium*, *C. aquaticum*, *L. monocytogenes*, *E. coli*, *K. pneumonia*, *K. oxytoca*, *S. marcescens*, *S. Typhi Paratyphi A*, *E. cloacae*, *E. aerogenes*, *P. agglomerans*, *C. freundii*, *C. koseri*, *M. morganii*, *P. mirabilis*, *P. stuartii*, *P. aeruginosa*, *B. cepacia*, *S. maltophilia*, *A. calcoaceticus*, *A. faecalis*, *A. xylosoxidans*, *C. indologenes*, *C. meningosepticum*, *C. acidovorans*, *H. influenza*, *M. catarrhalis*, *N. meningitides*, *A. hydrophila*, *E. corrodens*, *A. actinomycetemcomitans*, *L. pneumophila*, *B. fragilis*, *P. melaninogenica*, *F. nucleatum*, *V. parvula*, *C. perfringens*, *E. lentum*, *P. acnes*, *L. acidophilus*, *P. magnus*, *E. faecalis*, *E. faecium E. avium*, *E. casseliflavus*, *E. gallinarum*, *M. gordonae*, *M. kansasii* | 335 cultures from patients with hematological diseases | Enrichment culture, DNA extraction, PCR with universal 16S rDNA primer (Cy5-labeled) | Hybridization for 1 h | 24 h |

(*continued overleaf*)

**Table 4.1** (*Continued*)

| References | CC | Target species | Sample | Preanalytics | Detection | TTR |
|---|---|---|---|---|---|---|
| [31] | DE | *Acinetobacter* spp., *E. cloacae*, *P. aeruginosa*, *P. mirabilis*, *K. pneumonia*, *K. oxytoca*, *E. coli*, *S. agalactiae*, *S. epidermidis*, *S. aureus*, *E. faecium*, *E. faecalis*, *Stenotrophomonas* spp., *C. albicans*, *S. pyrogenes*, *S. pneumoniae*, *S. viridans* | 9 reference strains + 3 swab samples from superficial wounds | DNA extraction, multiplex PCR (800 primer pairs), labeling PCR | Hybridization (18 h) in HS400 TECAN automated hybridization station, fluorescence detection | >24 h |
| [10] | KR | *M. tuberculosis*, *M. avium-intracellulare complex (MAC)*, *M. fortuitum*, *M. chelonae*, *M. abscessus*, *M. kansasii*, *M. gordonae*, *M. scrofulaceum*, *M. szulgai*, *M. vaccae*, *M. xenopi*, *M. terrae*, *M. flavescens*, *M. smegmatis*, *M. malmoense*, *M. simiae*, *M. marinum*, *M. ulcerans*, *M. gastri*, and *M. leprae* | 46 reference strains, 149 clinical isolates, 155 clinical sputum samples | DNA extraction, ITS PCR | Hybridization (30 min), fluorescence detection | 4.5 h |
| [32] | DE | *B. mallei*, *B. pseudomallei* | Reference strains | DNA extraction, multiplex-PCR (biotinylated primer) | Hybridization on Alere ArrayTube platform, enzymatic precipitation reaction (HRP/TMB), Alere ArrayTube Reader (transmission scanner) | n/a |
| [33] | CN | *E. coli O157:H7*, *V. cholerae*, *V. parahaemolyticus*, *S.* spp., *S. aureus*, *Rotavirus Norwalk virus* | 86 samples isolated from patients, fishery products, or environment | Enrichment culture, DNA extraction, multiplex PCR | Hybridization with biotinylated PCR products, streptavidin-coated magnetic bead incubation, readout by eye, CCD camera | 13 h |

| | | | | | | |
|---|---|---|---|---|---|---|
| [34] | AT | *A. baumannii*, *A. johnsonii*, *A. Iwoffii*, *A. radioresistens*, *C. freundii*, *C. koseri*, *E. cloacae*, *E. aerogenes*, *K. pneumoniae*, *K. oxytoca*, *E. coli*, *M. morganii*, *P. mirabilis*, *P. vulgaris*, *P. aerogenes*, *S. marcescens*, *S. maltophilia*, *S. pneumoniae*, *S. pyrogenes*, *E. faecium*, *Stapylococcus* spp., *S. aureus*, *S. epidermidis*, *E. faecalis*, *C. albicans*, *C. parapsilosis* | Reference strains and clinical isolates (blood) | DNA extraction, multiplex PCR (16S rRNA), fluorescence labeling via primer extension | Hybridization (1 h), fluorescence detection | 6 h |
| [35] | KR | *A. baumannii*, *A. succiniproducens*, *B. cepacia*, *B. catarrhalis*, *B. ovatus*, *B. vulgatus*, *B. fragilis*, *C. hominis*, *E. meningoseptica*, *C. acidovorans*, *E. cloacae*, *K. oxytoca*, *C. freundii*, *E. aerogenes*, *M. morganii*, *K. pneumoniae*, *E. coli*, *S. paratyphimurium*, *K. kingae*, *E. faecalis*, *E. faecium*, *O. anthropi*, *N. meningitides*, *N. gonorrhoeae*, *F. necrophorum*, *A. actinomycetemcomitans*, *L. monocytogenes*, *S. mutans*, *L. pneumophila*, *P. aeruginosa*, *P. anaerobius*, *P. mirabilis*, *P. vulgaris*, *S. maltophilia*, *S. marcescens*, *S. epidermidis*, *S. aureus*, *S. agalactiae*, *S. bovis*, *S. sanguis*, *S. anginosus*, *S. pneumoniae*, *V. cholerae*, *V. vulnificus*, *C. neoformans*, *C. glabrata*, *C. krusei*, *C. albicans*, *C. parapsilosis*, *C. tropicalis* | Reference strains + positive blood culture samples | DNA extraction, PCR with universal primer in two separate tubes, lambda exonuclease treatment to generate single stranded products | 6 h hybridization on microarrays with probes targeting the bacterial 23S rRNA, 16S–23S rRNA ITS, and the fungal 18S rRNA | 10 h (after positive blood culture) |
| [36] | CN | *E. coli*, *V. cholerae*, *V. parahaemolyticus*, *S. enterica*, *C. jejuni*, *Shigella* spp., *Y. enterocolitica*, *L. monocytogenes* | 34 stool specimens | DNA extraction, multiplex-PCR with Cy5-dUTP | 12 h hybridization with GenTAC hybridization station (Genomic Solutions) | 15 h |

ITS: internal transcribed spacer region; CC: country code.

200–800 bp long PCR product probes on a medium-density microarray. This approach enabled the detection of sepsis-causing pathogens as well as several virulence factors and antibiotic resistance genes down to 1 ng of target DNA [31]. Previously, this research group from the University of Cologne used the same type of microarray for direct PCR-free detection of *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa* from positive blood cultures [24, 38]. Yoo *et al.* developed a DNA microarray for the detection of 50 bacterial and 7 fungal species [35]. The probes were directed against the bacterial 23S rRNA, the 16S–23S rRNA ITS (internal transcribed spacer), and the fungal 18S rRNA. The authors tested 112 blood cultures with a sensitivity of 93% and an LOD of 10 cfu. A DNA microarray for the detection 30 bacteria associated with sepsis contained 1 universal, 3 Gram stain-specific, 9 genus-specific, and 30 species-specific probes directed against the 16S–23S rRNA ITS region. The microarray was validated with 825 positive blood culture samples from which 708 (85.8%) were identified correctly by the oligonucleotide chip [27]. Negoro *et al.* [30] developed a DNA microarray which covered 72 bacteria which are often identified in positive blood cultures in combination with a PCR using universal PCR primers to amplify a variable region of the 16S rDNA. The microarray method was validated with 335 blood specimens from 99 patients with hematological diseases who had febrile illness. The sensitivity and specificity of the microarray test compared with conventional blood culture testing were 92.7 and 99.3%, respectively.

The "Prove-it Sepsis" assay from Mobidiag (Helsinki, Finland) combined amplification and detection of the *gyrB*, *parE*, and *mecA* genes for the identification of 50 Gram-positive and Gram-negative bacteria in positive blood cultures [39]. The assay uses the Array Strip platform developed by Clondiag in Jena. The company was acquired by Alere in 2006. The microarray detection principle is based on the formation of a precipitating product from a horseradish peroxidase-catalyzed substrate reaction. The enzyme is bound to the hybridized PCR product via streptavidin–biotin interaction. The arrays are spotted on the bottom of microtiter plate wells. A large-scale study of 2107 positive blood-culture samples of 3318 blood samples from patients with clinically suspected sepsis investigated by the conventional culture method and the "Prove-it Sepsis" assay revealed a sensitivity of 95% and a specificity of 98% of the microarray method. This study also compared the mean turnaround time of the microarray method and the conventional culture method, revealing an average time advantage of 18 h for the microarray. The disadvantage of this sepsis test was that it still required an enrichment of blood culture and was not sensitive enough to detect bacteria directly from patient blood samples.

#### 4.2.1.2 **Pneumonia**

Pneumonia is a serious life-threatening condition which may be conceived in the community or in the healthcare environment. As the etiology of pneumonia is difficult to identify and rapid therapy initiation is of prime importance, DNA microarrays are a promising platform to develop commercial diagnostic assays. As an example, Eppendorf Array Technologies in Namur (Belgium) developed

a real-time Array-PCR platform for infectious disease diagnostics which combined the characteristics of real-time PCR with the multiplexing capability of microarray detection. A chip for the diagnosis of ventilator-associated pneumonia (VAP)chip covered 13 Gram-positive and Gram-negative bacteria and 24 antibiotic resistance genes that mediate resistance to beta-lactam agents, including extended-spectrum cephalosporins and carbapenems and *mecA* [40]. The VAPChip was validated with 292 reference strains and clinical isolates, showing 98.6% sensitivity and 100% specificity for species identification. Regarding the detection of the resistance genes, the system presented a global sensitivity and specificity of 98.7% and 97.7%, respectively. The LOD of the test was between 10 and 100 genome copies/PCR. The VAPChip does not appear to be commercially available. In contrast, Curetis AG developed and launched a diagnostic platform, the Unyvero System, which is aimed at decentralized diagnostics (see Table 4.2). The Unyvero System comprises sampling tubes, a lysator device, a cartridge with preloaded reagents, and a reader. The cartridge contains special departments for all analytical steps (DNA purification, amplification, and detection by hybridization on a membrane microarray with colorimetric detection). Once the cartridge is loaded into the analyzer, all sample processing happens automatically without any further operator interaction. The cartridge is a closed system, and any possibility of spilling sample or reagents as well as contamination risks are minimized. One of the products developed is the Pneumonia Cartridge, which analyzes the antibiotic resistance markers simultaneously with the pathogen detection. The Unyvero Pneumonia Cartridge determines 39 targets involving beta-lactam-resistance, including ESBL (extended-spectrum beta-lactamase) and KPC (*Klebsiella pneumoniae* carbapenemase), macrolide resistance, quinolone resistance, multidrug resistance, and the most clinically relevant pathogens in approximately 4 h.

#### 4.2.1.3 Infectious Diarrhea

Infectious diarrhea is one of the major public health problems worldwide. It accounted for nearly 10% of the 7.6 million deaths in children younger than 5 years in 2010 [37]. Several research groups developed DNA microarray tests for the detection of enteropathogenic bacteria directly from human diarrheal stool samples as an alternative to traditional culture-based methods. Donatin *et al.* [25] developed a DNA microarray for the detection of 33 enteropathogenic bacteria and 7 enteropathogenic viruses. Genomic DNA extracted from stool samples was fluorescence-labeled and hybridized on the microarray without prior amplification, but with a rather long hybridization time of 40 h. The microarray was tested with 40 pathological stool specimens, showing an overall specificity of 100% and an overall sensitivity of 97.5%. Jin *et al.* [26] developed an assay using DNA microarray coupled with multiplex PCR and TSA-Cy3 labeling for the simultaneous identification of six species of human diarrheal bacteria with an LOD of $10^3$ cfu/ml [26]. Tests of 362 anus swab samples collected from an epidemic of *C. cholera* revealed a specificity of 98.6%. You *et al.* combined a multiplex PCR of virulence gene targets of eight enteropathogenic bacteria with

**Table 4.2** Commercial DNA microarray assays for pathogen detection[a)] .

| Company | Product | CC | Target | Sample | Integration[b)] | Test components | Time to result[c)] | Status[d)] |
|---|---|---|---|---|---|---|---|---|
| Alere Technologies GmbH<br><br>www.alere-technologies.com | *S. aureus* Genotyping Kit 2.0 | DE | *S. aureus* (MRSA/MSSA, 336 genetic markers) | Colonies (pure and clonal) | No | Microarrays, reagents, positive control | 7–8 h (hands-on-time 80 min) | RUO |
| Alere Technologies GmbH | Influenza A Genotyping Kit 96 | DE | Influenza A virus (200 markers for H- and N-typing) | Field samples (e.g., swabs) | No | Microarrays, reagents | 7–8 h (hands-on-time 80 min) | RUO |
| Alere Technologies GmbH | *E. coli* Genotyping Kit | DE | 92 virulence genes/alleles | Colonies (pure and clonal) | No | Microarrays, reagents | 7–8 h (hands-on-time 110 min) | RUO |
| Alere Technologies GmbH | AMR-ve Genotyping Kit | DE | 54 resistance genes of gram-bacteria | Colonies (pure and clonal) | No | Microarrays, reagents, positive control | 7–8 h (hands-on-time 2 h) | RUO |
| Alere Technologies GmbH | *P. aeruginosa* 2 Genotyping Kit | DE | 99 probes for different genetic markers | Colonies (pure and clonal) | No | Microarrays, reagents | 7–8 h (hands-on-time 2 h) | RUO |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Alere Technologies GmbH | Salmonella SeroGenotyping Kit | DE | 132 different *Salmonella* serovars, including genetic markers for 28 O-antigens, 86 H-antigens and 62 antibiotic resistance genes | Colonies (pure and clonal) | No | Modular: microarrays and reagents | 7–8 h (hands-on-time 2 h) | RUO |
| Alere Technologies GmbH | *E. coli* stx (Shiga-Toxin) | DE | 70 genetic markers to identify all subtypes of Shiga-Toxin 1 and 2 | Colonies (pure and clonal) | No | Modular: microarrays and reagents | 7–8 h (hands-on-time 2 h) | DEV |
| Alere Technologies GmbH | *E. coli* H&O typing | DE | 210 genetic markers to identify 50 O-antigens and 59 H-antigens | Colonies (pure and clonal) | No | Modular: microarrays and reagents | 7–8 h (hands-on-time 2 h) | DEV |
| Alere Technologies GmbH | Carbapenemase typing | DE | 80 genetic markers to identify resistance genes for ESBLs and Carbapenemases, 15 species markers for Enterobacteriaceae *K. pneumoniae*, *P. aeruginosa*, *E. coli*, *A. baumannii*, *C. freundii* | Colonies (pure and clonal) | No | Modular: microarrays and reagents | 7–8 h (hands-on-time 2 h) | DEV |

( *continued overleaf* )

**Table 4.2** (*Continued*)

| Company | Product | CC | Target | Sample | Integration[b)] | Test components | Time to result[c)] | Status[d)] |
|---|---|---|---|---|---|---|---|---|
| AutoGenomics | INFINITI Respiratory Viral Plus | US | Influenza (A/Swine H1N1 and B), human parainfluenza virus (HPIV 1, 2, 3, 4), rhinovirus A and B, enterovirus (A, B, C, D), coronavirus (HKU1, OC43, NL63, 229E), human metapneumovirus (HMPV A and B), human respiratory syncytial virus (HRSV A and B), adenovirus A, B, C, E | 8 µl of purified RNA/ reaction | No, auto-mated workflow | BioFilmChip microarray and Intellipac Reagent Module | 3 h+ | CE |
| www.auto-genomics.com | | | | | | | | |
| AutoGenomics | INFINITI FLU A-sH1N1 | US | Influenza A-swine H1N1 | 8 µl of purified RNA/ reaction | No, auto-mated workflow | BioFilmChip microarray and Intellipac Reagent Module | 3 h+ | CE |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| AutoGenomics | • INFINITI HPV Genotyping<br>• INFINITI HPV-HR QUAD<br>• INFINITI HPV-QUAD | US | Human papillomavirus, subtypes | 50 ng DNA/ reaction | No, auto-mated workflow | BioFilmChip microarray, Intellipac Reagent Module and Amp Mix | 3 h+ | CE |
| AutoGenomics | • INFINITI STD-6 Panel<br>• INFINITI STD-6 QUAD | US | *C. trachomatis*, *N. gonorrhoeae*, *U. urealyticum*, *M. genitalium*, *M. hominis*, *T. vaginalis* | 50 ng DNA/ reaction | No, auto-mated workflow | BioFilmChip microarray, Intellipac Reagent Module and Amp Mix | 3 h+ | RUO |
| AutoGenomics | INFINITI CT/NG QUAD | US | *C. trachomatis*, *N. gonorrhoeae* | 50 ng DNA/ reaction | No, auto-mated workflow | BioFilmChip microarray, Intellipac Reagent Module and Amp Mix | 3 h+ | RUO |
| AutoGenomics | INFINITI Leuko QUAD | US | *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis* | 50 ng DNA/ reaction | No, auto-mated workflow | BioFilmChip microarray, Intellipac Reagent Module and Amp Mix | 3 h+ | RUO |
| AutoGenomics | INFINITI UroGen QUAD | US | *U. urealyticum*, *M. genitalium*, *M. hominis* | 50 ng DNA/ reaction | No, auto-mated workflow | BioFilmChip microarray, Intellipac Reagent Module and Amp Mix | 3 h+ | CE |
| AutoGenomics | INFINITI UroGen Plus QUAD | US | *U. urealyticu*, *U. parvum*, *M. genitalium*, *M. hominis* | 50 ng DNA/ reaction | No, auto-mated workflow | BioFilmChip microarray, Intellipac Reagent Module and Amp Mix | 3 h+ | RUO |

( *continued overleaf* )

**Table 4.2** (*Continued*)

| Company | Product | CC | Target | Sample | Integration[b)] | Test components | Time to result[c)] | Status[d)] |
|---|---|---|---|---|---|---|---|---|
| AutoGenomics | INFINITI Candida Vaginitis QUAD | US | *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis* | 50 ng DNA/ reaction | No, auto-mated workflow | BioFilmChip microarray, Intellipac Reagent Module and Amp Mix | 3 h+ | CE |
| AutoGenomics | INFINITI MDR-TB | US | *M. tuberculosis rpoB*, *katG*, *inhA*, *pncA*, *M. bovis* | Decont-aminated cell lysate (direct from sediment without DNA extraction) | No, auto-mated workflow | BioFilmChip microarray, Intellipac Reagent Module and Amp Mix | 3 h+ | RUO |
| AutoGenomics | INFINITI NTM Panel | US | *M. genus*, *M. avium complex (M. avium*, *M. Intracellulares)*, *M. kansasii*, *M. gordonae*, *M. scrofulaceum*, *M. xenopi*, *M. marinum/M. ulcerans*, *M. haemophilum*, *M. fortuitum*, *M. chelonae*, *M. abscessus/M. mucogenicum*, *M. smegmatis* | n/a | No, auto-mated workflow | BioFilmChip microarray, Intellipac Reagent Module and Amp Mix | 3 h+ | RUO |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| AutoGenomics | INFINITI Resolve QUAD | US | *G. vaginalis*, *C. albicans*,*C. glabrata*, *T. vaginalis*, *Mobiluncus* spp. *(mulieris* and *curtisii)* | 50 ng DNA/ reaction | No, auto-mated workflow | BioFilmChip microarray, Intellipac Reagent Module and Amp Mix | 3 h+ | CE |
| Capitalbio | Mycobacteria Resistance Array Kit | CN | 14 mutations in three genes associated with resistance to rifampicin and isoniazid | Clinical isolates or clinical sputum samples | No | Sample preparation kit, PCR reagents, microarray slides | na | CE |
| www.capi-talbio.com | | | | | | | | |
| Capitalbio | Mycobacteria Identification Array Kit | CN | *M. tuberculosis*, *M. Kansassi*, *M. chelonae*, *M. smegmatis*, *M. scrofulaceum*, *M. fortuitum*, *M. gordonae*, *M. avium*, *M. phlei*, *M. xenopi*, *M. marion* | Clinical isolates or clinical sputum samples | No | Sample preparation kit, PCR reagents, microarray slides | na | CE |
| Check Points | Check-MDR CT101 | NL | Carbapenemases (KPC, NDM), ESBLs (CTX-M, TEM, SHV), AmpCs (CMY, DHA, FOX, MOX, ACC, MIR, ACT) | Colonies (bacterial culture) | No | Ligation-mediated PCR reagents, Check-Points ArrayTube microarrays, detection buffer, blocking buffer, cunjugate solution, staining solution | 6.5 h (excluding extrac-tion) | CE |

( *continued overleaf* )

**Table 4.2** (*Continued*)

| Company | Product | CC | Target | Sample | Integration[b)] | Test components | Time to result[c)] | Status[d)] |
|---|---|---|---|---|---|---|---|---|
| www.check-points.nl | | | | | | | | RUO (US) |
| Check Points | Check-MDR CT102 | NL | Carbapenemases (KPC, NDM, VIM, IMP, OXA-48), ESBLs (CTX-M, TEM, SHV) | Colonies (bacterial culture) | No | Ligation-mediated PCR reagents, Check-Points ArrayTube microarrays, detection buffer, blocking buffer, cunjugate solution, staining solution | 6.5 h (excluding extraction) | CE<br>RUO (US) |
| Check Points | Check-MDR CT103 | NL | Carbapenemases (KPC, NDM, VIM, IMP, OXA-48), ESBLs (CTX-M, TEM, SHV), AmpCs (CMY, DHA, FOX, MOX, ACC, MIR, ACT) | Colonies (bacterial culture) | No | Ligation-mediated PCR reagents, Check-Points ArrayTube microarrays, detection buffer, blocking buffer, cunjugate solution, staining solution | 6.5 h (excluding extraction) | CE<br>RUO (US) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Chipron GmbH<br>www.chipron.com | BAC Dent 2.4 | DE | *P. gingivalis*, *A. actinomycetemcomitans*, *P. intermedia*, *T. denticola*, *T. forsythensis*, *F.* sp., *E. corrodens*, *C. ginivalis*, *P. micros*, *E. nodatum*, *C. rectus* | n/a | No | PCR-primer, LCD-arrays, hybridization buffer, label, dilution buffer, modulator, stain, wash buffer, pattern file, manual, MSDS | 2.5 h (excluding extraction) | CE |
| Chipron GmbH | HPV 3.5 | DE | Detection and typing of 32 HPV types (low and high risk): HPV 06, 11, 16, 18, 31, 33, 35, 39, 42, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 70, 72, 73, 81, 82, 83, 84, 90, 91 | n/a | No | PCR-primer, LCD-arrays, hybridization buffer, label, dilution buffer, modulator, stain, wash buffer, pattern file, manual, MSDS | 3.5 h (excluding extraction) | RUO |
| Chipron GmbH | Fungi 2.1 | DE | *A. flavus*, *A. fumigatus*, *A. versicolor*, *A. nidulans*, *A. niger*, *A. terreus*, *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. guilliermondii*, *C. kefyr*, *C. krusei*, *C. lambica*, *C. lusitaniae*, *C. parapsilosis*, *C. pelliculosa*, *C. tropicalis*, *Mucor* spp., *Rhizomucor* spp., *C. neoformans*, *P. variotii*, *S. prolificans*, *A. corymbifera* | n/a | No | PCR-primer, LCD-arrays, hybridization buffer, label, dilution buffer, modulator, stain, wash buffer, pattern file, manual, MSDS | 3 h (excluding extraction) | RUO |

( *continued overleaf* )

**Table 4.2** (*Continued*)

| Company | Product | CC | Target | Sample | Integration[b)] | Test components | Time to result[c)] | Status[d)] |
|---|---|---|---|---|---|---|---|---|
| Chipron GmbH | Dengue 1.4 | DE | Detection and typing of dengue virus 1, 2, 3, 4 | n/a | No | PCR-primer, LCD-arrays, hybridization buffer, label, dilution buffer, modulator, stain, wash buffer, pattern file, manual, MSDS | 3.5 h (excluding extraction) | RUO |
| Chipron GmbH | MYCO Direct 1.7 | DE | *M. tuberculosis* (complex), *M. avium*, *M. intracellulare*, *M. gordonae*, *M. kansasii*, *M. xenopi*, *M. malmoense*, *M. szulgai*, *M. celatum*, *M. haemophilum*, *M. heckeshornense*, *M. abscessus*, *M. chelonae*, *M. fortuitum* (complex), *M. peregrinum* | n/a | No | PCR-primer, LCD-arrays, hybridization buffer, label, dilution buffer, modulator, stain, wash buffer, pattern file, manual, MSDS | 2.5 h (excluding extraction) | RUO |
| Clondiag/Alere | ArrayTube/ Arraystrip | DE | OEM manufacturer for companies using the ArrayTube platform | n/a | No | ArrayTube microarray | n/a | n/a |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| www.clondiag.com | | | | | | | | |
| Curetis | Unyvero P50 | DE | Species: *A. baumannii*, *C. pneumoniae*, *Enterobacter* sp., *E. coli*, *H. influenzae*, *K. pneumoniae*, *K. oxytoca*, *L. pneumophila*, *M. catarrhalis*, *M. morganii*, *P. jirovecii*, *P.* sp., *P. aeruginosa*, *S. marcescens*, *S. aureus*, *S. maltophilia*, *S. pneumoniae* | Unprocessed primary respiratory samples: sputum, tracheal aspirate, tracheal lavage, bronchial aspirate, bronchial lavage | Yes (Unyvero Lysator + Unyvero Cartridge) | P50 Cartridge, Sample Handling Tool, Sample Tube, Sample Tube Cap, Mastermix | Approximately 4.5 h | CE |
| www.curetis.com | | | Antibiotic resistance: *ctx-M*, *dha*, *ebc*, *ermA*, *ermB*, *ermC*, *gyrA83*, *gyrA87*, *int1*, *kpc*, *mecA*, *mefA/E*, *msrA*, *oxa51 like*, *parC*, *shv*, *sul1*, *tem* | | | | | |
| Curetis | Unyvero i60 ITI | DE | >80 pathogen species and >20 antibiotic resistance markers relevant in implant and tissue infections | Primary orthopedic and chirurgic samples: sonification fluid, synovial fluid, pus, swabs, biopsy | Yes (Unyvero Lysator plus Unyvero Cartridge) | i60 Cartridge, Sample Tube, Sample Tube Cap, Master mix | <5 h | DEV, launch end 2013 |

( *continued overleaf* )

**Table 4.2** (*Continued*)

| Company | Product | CC | Target | Sample | Integration[b)] | Test components | Time to result[c)] | Status[d)] |
|---|---|---|---|---|---|---|---|---|
| Curetis | Blood Stream Infection Cartridge | DE | Not specified | — | n/a | — | n/a | Planned |
| Curetis | Tuberculosis cartridge | DE | Not specified | — | n/a | — | n/a | Planned |
| Dr Chip | DR. EV IVD Kit | TW | Pan-enterovirus, enterovirus 71, coxsackie A16 | Throat swab, rectal swab, feces, nasopharyngeal aspirate, serum, and cerebral spinal fluid | No | Reagents for RNA extraction, reverse transcription (RT), cDNA amplification, polymer chip hybridization, colorimetric detection | 6 h | RUO |
| www.bio-drchip.com.tw | | | | | | | | |
| Dr Chip | DR. RV IVD Kit | TW | Severe acute respiratory syndrome (SARS coronavirus), influenza A, influenza B, parainfluenza I, parainfluenza II, parainfluenza III, RSV, adenovirus | Throat swab in transport medium or nasopharyngeal aspirate | No | Reagents for viral RNA/DNA extraction, reverse transcription (RT), PCR, polymer chip hybridization, colorimetric detection | 6 h | RUO |
| Dr Chip | DR. Food Kit | TW | *S. aureus*, *E. coli*, *Y. enterocolitica*, *B. cereus*, *C. perfringens*, *L. monocytogenes*, *S.* spp., *S.* spp., *Vibrio* spp. | Food samples | No | n/a | 6 h | n/a |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Dr Chip | Dr. Milk Kit | TW | *Streptococcus* spp., *S. agalactiae*, *S. bovis*, *S. dysgalactiae*, *S. uberis*, *E. coli*, *S. aureus* | Milk samples | No | n/a | 6 h | n/a |
| Gene In | CombiChip Mycobacteria Genotyping DNA Chip | KR | Panmycobacteria, *M. vaccae*, *M. tuberculosis complex*, *M. xenopi*, *M. avium-intracellulare complex*, *M. terrae*, *M. fortuitum*, *M. flavescens*, *M. chelonae*, *M. smegmatis*, *M. abscessus*, *M. malmoense*, *M. kansasii*, *M. simiae*, *M. gordonae*, *M. marinum-ulcerance*, *M. scrofulaceum*, *M. gastri*, *M. szulgai*, *M. leprae* | n/a | No | n/a | n/a | n/a |
| www.genein.com | | | | | | | | |
| Genomica | CLART HPV2 | ES | Human papillomavirus, (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 68, 70, 71, 72, 73, 81, 82, 83, 84, 85 y 89) | Liquid cytology, swabs, and paraffin embedded tissues | No | Reagents for multiplex PCR to generate biotin-labeled products; detection on low density microarrays via streptavidin–peroxidase catalyzed preceipitaion reaction | 8 h | CE |

( *continued overleaf* )

**Table 4.2** (*Continued*)

| Company | Product | CC | Target | Sample | Integration[b)] | Test components | Time to result[c)] | Status[d)] |
|---|---|---|---|---|---|---|---|---|
| www.genomica.es | | | | | | | | |
| Genomica | CLART PneumoVir | ES | Adenovirus; bocavirus; coronavirus; enterovirus (echovirus); influenza virus A (subtypes H3N2 human, H1N1 human, B, C, and H1N1/2009); metapneumovirus (subtypes A and B); parainfluenza virus 1, 2, 3, and 4 (subtypes A y B); rhinovirus; respiratory syncitial virus type A (VSR-A); respiratory syncitial virus type B (VSR-B) | Nasopharyngeal wash, nasopharyngeal exudate, bronchoalveolar lavage | No | Extraction–purification reagents, amplification reagents (RT, multiplex-PCR), and visualization reagents (including microarrays) | Hours | CE |
| Genomica | CLART ENTHERPEX | ES | HSV-1, HSV-2, VZV, CMV, EBV, HHV-6, HHV-7, and HHV-8; poliovirus, echovirus, coxsackievirus | Swabs, serum, plasma, cerebrospinal fluidi, biopsies | No | Viral genome-specific fragment amplification via multiple RT-PCR, subsequent detection via hybridization with microorganism-specific binding probe arrays | 8 h | n/a |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Genomica | CLART EnteroBac | ES | *Salmonella* spp. (all the described species), *Shigella* spp. (*S. dysenteriae*, *S. sonnei*, *S. boydii*, and *S. flexneri*), *Y. enterocolitica*, *Y.* spp. (*Y. pestis*, *Y. pseudotuberculosis*, *Y. enterocolitica*), *C.* spp. (*C. lari*, *C. laridis*, *C. upsaliensis*, *C. jejuni*, *C. coli*), *C. jejuni*, *C. coli*, *E. coli enteropathogenic EPEC*: (*E. coli enterohemoragic*, *E. coli*, *enteroinvasive*, *E. coli*, *enterotoxigenic and E. coli enteropathogenic*), *C. difficile B*, *Aeromonas* spp., producers of aerolisin | Stool samples | No | Extraction–purification reagents (including extraction control), amplification reagents (multiplex-PCR), and visualization reagents (including microarrays) | Hours | CE |
| Genomica | CLART STIs A | ES | *C. trachomatis*, *subtypes*, *N. gonorrhoeae*, *M. genitalium*, *T. vaginalis* | Urine, swabs (cervical, vaginal, urethral, and rectal) | No | Amplification and visualization reagents (including CLART eight-well strip microarrays) | Hours | CE |
| Genomica | CLART STIs B | ES | *U. urealyticum/parvum*, *M. hominis C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, *C. tropicalis*, *C. guilliermondii*, *C. dubliniensis*, *Treponema pallidum*, *H. ducreyi*, herpes virus types I and II | Urine, swabs (cervical, vaginal, urethral, and rectal) | No | Amplification and visualization reagents (including CLART eight-well strip microarrays) | Hours | CE |

( *continued overleaf* )

**Table 4.2** (*Continued*)

| Company | Product | CC | Target | Sample | Integration[b)] | Test components | Time to result[c)] | Status[d)] |
|---|---|---|---|---|---|---|---|---|
| Genomica | CLART SeptiBac | ES | *S. aureus, S. epidermidis S. hominis, S. haemolyticus, S. pyogenes/dysgalactiae, S. pneumoniae, S. mitis, S. agalactiae, S. sanguinis/parasanguinis, Streptococcus de grupo milleri (including S. constellatus y S. anginosus), Detección genérica para Streptococcus* spp., *E. faecium, E. faecalis, Gram positive cocci (CGPs), L. monocytogenes, C. perfringens, E. coli, K. pneumoniae, K. oxytoca, K.* spp. *(K. pneumoniae, K, oxytoca), S. enteric, E. cloacae, E. aerogenes, Enterobacter* spp. *(E. cloacae, E. aerogenes), C. freundii, C.* spp. *(C. freundii, C. koserii), S. marcescens/plimutica P. vulgaris/penneri, P. mirabilis,* | Positive blood cultures | No | Amplification and visualization reagents (including CLART eight-well strip microarrays) | Hours | CE |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *P.* spp. *(P. vulgaris, P. mirabilis, P. penneri), H. influenza, H.* spp. *(H. influenzae, H. parainfluenzae), A. baumannii, B. fragilis, B.* spp. *(B. fragilis, B. faecis), Pseudomonas* spp. *(P. aeruginosa, P. putida, P. stuartii), S. maltophilia, C. albicans, C. glabrata, C. krusei* | | | | | |
| Great Basin | Staph ID/R | US | *S. aureus, Staphylococcus* species, *mecA* | Positive blood cultures | Yes | Catridge containing reagents for multiplex helicase-dependet amplification, microarray, detection based on HRP catalyzed precipitation reaction | 1.5 h | DEV |
| www.gbscience.com | | | | | | | | |
| Great Basin | *C. difficile* | US | *C. difficile, tcdA, tcdB* | — | Yes | Catridge containing reagents for multiplex helicase-dependet amplification, microarray, detection based on HRP catalyzed precipitation reaction | n/a | 510(k)/CE |

( *continued overleaf* )

**Table 4.2** (*Continued*)

| Company | Product | CC | Target | Sample | Integration[b)] | Test components | Time to result[c)] | Status[d)] |
|---|---|---|---|---|---|---|---|---|
| Great Basin | Nasal MRSA | US | *S. aureus*, *mecA* | Nasal or perianal swabs | Yes | Transfer solution for nasal or perianal swabs + Catridge containing reagents for multiplex helicase-dependet amplification, microarray, detection based on HRP catalyzed precipitation reaction | n/a | DEV |
| Great Basin | Fungal pathogens | US | *Candida* species | Positive blood culture | Yes | Catridge containing reagents for multiplex helicase-dependet amplification, microarray, detection based on HRP catalyzed precipitation reaction | | DEV |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Greiner | CytoInspect | DE | *A. axanthum*, *A. laidlawii*, *A. modicum*, *A. morum*, *A. oculi*, *A. vituli*, *M. arginini*, *M. alkalescens*, *M. arthritidis*, *M. bovigenitalium*, *M. bovirhinis*, *M. bovis*, *M. bovoculi*, *M. buccale*, *M. californicum*, *M. canadense*, *M. canis*, *M. quirhinis*, *M. faucium*, *M. fermentans*, *M. flocculare*, *M. gallinaceum*, *M. gallinarum*, *M. gallisepticum*, *M. genitalium*, *M. glycophilum*, *M. hominis*, *M. hyopharyngis*, *M. hyopneumoniae*, *M. hyorhinis*, *M. Hyosynoviae*, *M. orale*, *M. pirum*, *M. pneumoniae*, *M. ulmonis*, *M. salivarium*, *M. synoviae*, *S. citri*, *S. kunkelii*, *U. diversum* | Cell cultures and other biological materials | No | DNA extraction kit and test kit containing PCR reagents, internal control, and microarrays | 5 h | n/a |

( *continued overleaf* )

**Table 4.2** (*Continued*)

| Company | Product | CC | Target | Sample | Integration[b)] | Test components | Time to result[c)] | Status[d)] |
|---|---|---|---|---|---|---|---|---|
| www.greinerbioone.com | | | | | | | | |
| Greiner | ParoCheck | DE | *P. gingivalis*, *A. actinomycetemcomitans*, *P. intermedia*, *F. nucleatum*, *E. corrodens*, *T. denticola*, *A. viscosus*, *T. forsythensis*, *C. rectus*, *P. micros*, *S. mitis*, *S. gordonii*, *P. nigrescens*, *S. constellatus*, *V. parvula*, *C. ginivalis*, *A. odontolyticus*, *C. concisus*, *E. nodatum*, *C. gracilis* | Gingival swab samples | No | Specimen collection kit + PCR mastermix, microarray slides, hybridization buffer, washing buffer | 2.5 h | CE |
| Greiner | PapilloCheck | DE | 24 Human papillomavirus types (6, 1, 40, 42, 43, 44; 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82) | Human cervical samples | No | PapilloCheck collection kit + detection kit containing PCR reagents and microarrays | 2.5 h | CE |
| Greiner/Amplex | GENSPEED MRSA | DE | *S. aureus* (MRSA/LA – MRSA (*mecC*)) | Nasal and throat swabs | No | Sampling solution, PCR mastermix, denaturation solution, microarray, localized enzymatic light generation (detected with photodiode arrays) | 1 h 15 min | CE |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| www.greinerbioone.com<br>www.hyplex.de | | | | | | | | |
| Hain Lifescience | 23 products based on DNA STRIP technology (line probe assays) and GenoQuick technology | DE | • GenoType MRSA Direct<br>• GenoType Staphylococcus<br>• GenoType MRSA<br>• GenoType Mycobacteria Direct<br>• GenoType MTBC<br>• GenoType M. CM<br>• GenoType M. AS<br>• GenoType MTBDRplus<br>•GenoType MTBDRsl<br>•GenoType LepraeDR<br>•GenoType HelicoDR<br>•GenoType CDiff<br>•GenoType EHEC<br>•GenoType Enterococcus<br>•GenoType Bac-IDent<br>•GenoType BC grampositive<br>•GenoType BC gramnegative<br>•micro-IDent<br>•micro-IDentplus<br>•GenoQuick MTB<br>•GenoQuick MRSA<br>•GenoQuick CT<br>•GenoQuick Bordetella | Patient specimens (blood, urine, swabs, stool, pulmonary, and extrapulmonary samples) | No | Extraction kits for DNA isolation, PCR, DNA STRIPs | GenoType – 4–6 h, GenoQuick – 2.5–3 h | CE |

( *continued overleaf* )

**Table 4.2** (*Continued*)

| Company | Product | CC | Target | Sample | Integration[b)] | Test components | Time to result[c)] | Status[d)] |
|---|---|---|---|---|---|---|---|---|
| www.hain-lifescience.de | | | | | | | | |
| Fujirebio Europe | INNO-LIPA | BE | HPV genotypes (high risk: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, low-risk: 6, 11, 40, 43, 44, 54, 70, other: 69, 71, 74), HBV genotyping | Cervical scrapes, potential use for biopsies, and paraffin-embedded material | No | Fully automated processing of strips (Auto-LiPA 48) Automated interpretation of the strips is provided with LiRAS® for LiPA HPV (article number: 81085, CE) | 3 h | CE |
| www.fujirebio-europe.com (Innogenetics www.innogenetics.com) | | | | | | | | |
| LG Lifescience | AdvanSure Mycobacteria GenoBlot Assay | KR | 25 mycobacteria species | Respiratory samples | No | Extraction buffer, pre-treatment solutions, 2 PCR mixtures, primer mix, strips, buffers, wash solutions, enzyme, and substrate solutions | 4 h after DNA isolation | n/a |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| www.lgls.com LG Lifescience | AdvanSure MDR-TB GenoBlot Assay | KR | *M. tuberculosis*, *rpoB*, *katG*, *inhA*, *ahpC* | Respiratory samples | No | Extraction buffer, pre-treatment solutions, 2 PCR mixtures, primer mix, strips, buffers, wash solutions, enzyme, and substrate solutions | 4 h after DNA isolation | n/a |
| Luminex | xTAG Respiratory Viral Panel (RVP) (xTAG RVPv1/xTAG RVP FAST) | US | Respiratory syncytial virus (RSV), RSV A, RSV B, influenza A, nonspecific influenza A, H1 subtype, H3 subtype, H5 subtype, Influenza B, parainfluenza 1, parainfluenza 2, parainfluenza 3, parainfluenza 4, metapneumovirus (hMPV), adenovirus, entero-rhinovirus, corona NL63, corona HKU1, corona 229E, corona OC43, corona SARS, bocavirus | Nasopharyngeal swabs | No, automated workflow | PCR master mix, reagents for primer extension step, universal tag system, color-coded beads | 8 h | IVD |

( *continued overleaf* )

**Table 4.2** (*Continued*)

| Company | Product | CC | Target | Sample | Integration[b)] | Test components | Time to result[c)] | Status[d)] |
|---|---|---|---|---|---|---|---|---|
| www.luminexcorp.com | | | | | | | | |
| Luminex | xTAG Gastrointestinal Pathogen Panel | US | Bacteria and bacterial toxins (*C.*, *C. difficile*, Toxin A/B, *E. coli* O157, enterotoxigenic *E. coli* (ETEC) LT/ST, Shiga-like Toxin producing *E. coli* (STEC) *stx1/stx2*, *S.*, *S.*, *V. cholerae*, *Y. enterocolitica*), viruses (adenovirus 40/41, norovirus GI/GII, rotavirus A), parasites (*Giardia*, *Cryptosporidium*, *E. histolytica*) | Stool specimens | No, automated workflow | PCR master mix, reagents for primer extension step, universal tag system, color-coded beads | 5 h | IVD |
| Mobidiag | Prove-it Bone and Joint | FI | Gram + (*mecA* meticillin resistance marker, *vanA* vancomycin resistance marker, *vanB* vancomycin resistance marker, *C. perfringens*, *E. casseliflavus*, *E. faecalis*, *E. faecium*, | Synovial fluid, bone biopsy, or tissue samples | No | PCR reagents, Prove-it strip arrays, reagents for HRP catalyzed precipitation reaction | 3.5 h (from extracted DNA) | CE |

| | |
|---|---|
| | *E. gallinarum*, *L. monocytogenes*, *S. aureus*, *S. epidermidis*, *S. agalactiae*, *S. dysgalactiae*, subspecies *equisimilis*, *S. pneumoniae*, *S. pyogenes*, Coagulase negative staphylococcus); Gram-(*A. baumannii*, *E. aerogenes*, *E. cloacae*, *E. coli*, *F. necrophorum*, *H. in_uenzae*, *K. kingae*, *K. oxytoca*, *K. pneumoniae*, *M. catarrhalis*, *N. meningitidis*, *P. mirabilis*, *P. vulgaris*, *P. aeruginosa*, *S. enterica* subspecies *enteric*, *S. marcescens*, *S. maltophilia*, *B. fragilis* group, *C. jejuni/coli*, *Enterobacteriaceae*, *N.* sp. non-*meningitidis)* |

( *continued overleaf* )

**Table 4.2** (*Continued*)

| Company | Product | CC | Target | Sample | Integration[b)] | Test components | Time to result[c)] | Status[d)] |
|---|---|---|---|---|---|---|---|---|
| www.mobidiag.com | | | | | | | | |
| Mobidiag | Prove-it Sepsis | FI | *mecA*, *E. faecalis*, *E. faecium*, *L. monocytogenes*, *S. aureus*, *S. agalactiae*, *S. dysgalactiae subspec equisimilis*, *S. epidermidis*, *S. pneumonia*, *S. pyogenes*, *C. perfringens*, *CNS*, *A. baumannii*, *E. aerogenes*, *E. cloacae*, *E. coli*, *H. influenzae*, *K. oxytoca*, *K. pneumoniae*, *N. meningitides*, *P. mirabilis*, *P. aeruginosa*, *S. enterica subs enterica*, *S. marcescens*, *S. maltophilia*, *Bacteroides fragilis group*, *C. jejuni/coli*, *Enterobacteriaceae* | Positive blood culture | No | PCR reagents, Prove-it strip arrays, reagents for HRP catalyzed precipitation reaction | 3.5 h | CE |
| Mobidiag | Prove-it Herpes | FI | *Herpes simplex virus 1*, *herpes simplex virus 2*, *herpes simplex virus 2*, *varicella-zoster virus*, *Epstein-Barr virus*, *cytomegalovirus*, *human herpes virus 6*, *human herpes virus 7* | Cerebral spinal fluid (CSF) samples | No | PCR reagents, Prove-it tube arrays, reagents for HRP catalyzed precipitation reaction | 2 h | CE |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Nanosphere | Respiratory Virus Plus | US | Influenza A, influenza B, RSV A, RSV B, flu A – 2009 H1N1, flu A – H3, flu A – H1, H275Y (oseltamivir resistance) | Nasopharyngeal swab samples | Yes (extraction tray, pipette tip cartridge, utility tray, test cartridge) | Single use, self-contained cartridge with reagent pack and microarray glass slide for fragmentation of viral RNA via on-board sonication, hybridization to capture probes, washing, hybridization of gold nanoparticle-labeled detection probes, and silver signal amplification | 2.5 h | IVD |
| www.nanosphere.us | | | | | | | | |
| Nanosphere | Gram-Positive Blood Culture | US | Species (*S. aureus*, *S. epidermidis*, *S. lugdunensis*, *S. anginosus* group, *S. agalactiae*, *S. pneumoniae*, *S. pyogenes*, *E. faecalis*, *E. faecium)* genus (*Staphylococcus* spp., *Streptococcus* spp., *M.* spp., *L.* spp.) resistance (*mecA*, *vanA*, *vanB)* | Positive blood culture | Yes (extraction tray, pipette tip cartridge, utility tray, test cartridge) | Single use, self-contained cartridge with reagent pack and microarray glass slide for fragmentation of genomic DNA via on-board sonication, hybridization to capture probes, washing, hybridization of gold nanoparticle-labeled detection probes, and silver signal amplification | 2.5 h | IVD |

( *continued overleaf* )

**Table 4.2** (*Continued*)

| Company | Product | CC | Target | Sample | Integration[b)] | Test components | Time to result[c)] | Status[d)] |
|---|---|---|---|---|---|---|---|---|
| Nanosphere | Gram-Negative Blood Culture | US | Species (*E. coli/S.* spp., *K. pneumoniae*, *K. oxytoca*, *P. aeruginosa*, *S. marcescens)*, genus (*A.* spp., *P.* spp., *C.* spp., *Enterobacter* spp.), resistance (KPC, NDM, CTX-M, VIM, IMP, OXA) | Positive blood culture | Yes (extraction tray, pipette tip cartridge, utility tray, test cartridge) | Single use, self-contained cartridge with reagent pack and microarray glass slide for fragmentation of genomic DNA via on-board sonication, hybridization to capture probes, washing, hybridization of gold nanoparticle-labeled detection probes, and silver signal amplification | 2 h | RUO |
| Nanosphere | *C. difficile* | US | *tcdA*, *tcdB* | Stool samples | Yes (extraction tray, pipette tip cartridge, utility tray, test cartridge) | Single use, self-contained cartridge with reagent pack and microarray glass slide for fragmentation of genomic DNA via on-board sonication, hybridization to capture probes, washing, | 2 h | RUO |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | hybridization of gold nanoparticle-labeled detection probes, and silver signal amplification | | |
| Nanosphere | Enteric Pathogens | US | Bacteria (*C.* spp., *S.* spp., *S.* spp., *Vibrio* spp., *Y. enterocolitica*) | Stool samples | Yes (extraction tray, pipette tip cartridge, utility tray, test cartridge) | Single use, self-contained cartridge with reagent pack and microarray glass slide for fragmentation of genomic DNA via on-board sonication, hybridization to capture probes, washing, hybridization of gold nanoparticle-labeled detection probes, and silver signal amplification | 2 h | RUO |
| | | | Targets, STEC, Shiga toxin gene 1 (*stx1*), Shiga toxin gene 2 (*stx2*), viruses (adenovirus, norovirus, rotavirus) | | | | | |

( *continued overleaf* )

**Table 4.2** (*Continued*)

| Company | Product | CC | Target | Sample | Integration[b)] | Test components | Time to result[c)] | Status[d)] |
|---|---|---|---|---|---|---|---|---|
| Nanosphere | Respiratory Pathogens | US | Influenza A, flu A – H1, flu A – H3, influenza B, RSV A, RSV B, parainfluenza 1–4, human metapneumovirus, adenovirus, rhinovirus (species A–C)/enterovirus (species A–D), *B. pertussis*, *Bordetella* spp. | Nasopharyngeal swab samples | Yes (extraction tray, pipette tip cartridge, utility tray, test cartridge) | Single use, self-contained cartridge with reagent pack and microarray glass slide for fragmentation of genomic DNA via on-board sonication, hybridization to capture probes, washing, hybridization of gold nanoparticle-labeled detection probes, and silver signal amplification | 2 h | RUO |
| Rheonix | HPV | US | 20 different clinically relevant HPV sub-types | Vaginal swabs | Yes (Rheonix CARD Consumable) | PCR reagents, microarray, reagents for HRP catalyzed precipitation reaction | 3.5 h | DEV |
| www.rheonix.com | | | | | | | | |
| Rheonix | Sepsis | US | 150 sepsis agents, including C. albicans, *E. coli,* and enterococcus | Whole blood | Yes (Rheonix CARD Consumable) | n/a | 3 h | DEV |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Rheonix | STI | US | Sexually transmitted infection agents (*N. gonorrhoeae*, *C. trachomatis*, *T. pallidum*, and *T. vaginalis*) | Urine samples | Yes (Rheonix CARD Consumable) | PCR reagents, microarray, reagents for HRP catalyzed precipitation reaction | 3.5 h | DEV |
| Rheonix | HIV | US | HIV screen and confirmatory assay | Saliva samples | Yes (Rheonix CARD Consumable) | n/a | n/a | DEV |
| Rheonix | UTI | US | Urinary tract infections | Urine samples | Yes (Rheonix CARD Consumable) | n/a | 3 h | DEV |
| Roche | LINEAR ARRAY® HPV Genotyping Test | CH | HPV genotypes 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39, CP6108 | Cervical cells collected in PreservCyt® solution | No | PCR reagents, microarray (linear array) | n/a | Canada-IVD<br>CE |
| Tessarae | RPM-Flu | US | >35 different bacterial genera and virus, types of upper respiratory pathogens, incl. influenza, including avian influenza | Throat swabs, nasal washes | No | RPM-Flu 3.1 Kit containing PCR reagents and array, RPM Wash-Stain-Hybridization Kit, additional reagents | 10 h | RUO |
| www.tessarae.com | | | | | | | | |
| Tessarae | RPM-TEI | US | >40 different bacterial genera and virus types of tropical and emerging pathogens | Throat swabs, nasal washes | No | RPM-TEI 1.0 Kit containing PCR reagents and array, RPM wash-stain-hybridization kit, additional reagents | 10 h | RUO |

( *continued overleaf* )

**Table 4.2** (*Continued*)

| Company | Product | CC | Target | Sample | Integration[b)] | Test components | Time to result[c)] | Status[d)] |
|---|---|---|---|---|---|---|---|---|
| Tessarae | RPM-HFV | US | Hemorrhagic fever viruses | Throat swabs, nasal washes, blood | No | RPM-HFV 1.0 Array, additional reagents | 10 h | RUO |
| Veredus | VereFlu | SG | Influenza A: H1, H3, H5, H7, H9 with subtype IDs of H1N1, H1N1/2009, H3N2, H5N1 and H9N2, and influenza B | Blood, serum and respiratory swabs (nasopharyngeal, throat, nasal, or throat aspirates) | Partially VereFlu™ chip combines multiplex PCR and Microarray on a single platform | Plastic slide | 3 h | IVD |
| www.vereduslabs.com | | | | | | | | |
| Veredus | VereMTB | SG | 10 different mycobacterium strains with special emphasis on *Mycobacterium tuberculosis* complex (MTBC) and its resistance to rifampicin and/or isoniazid | Pulmonary clinical samples, cultured samples | Partially (VereChip) | Plastic slide | 3 h | RUO |

a) All details are without guarantee.
b) Integration into a sample to answer system.
c) Time to result: Time from adding sample to detection system/assay until result is received.
d) Regulatory status: RUO – research use only; CE – CE-mark (CE-IVD) declaration of conformity EU directive 98/79/EC; DEV – in development; IVD – other regulatory approval for IVD; and n/a – no information found/does not apply.

DNA microarray detection with a comparable LOD of 58 cfu/ml [36]. A DNA microarray for the detection of 10 bacterial pathogens was developed by Kim *et al.* [28]. Here, the microarray detection was combined with multiplex PCR of bacterial specific virulence factors. Results obtained in a validation study with 82 stool samples from patients with foodborne enteritis fully coincided with standard cultivation and PCR approaches. Mao *et al.* [29] developed a DNA microarray for the detection of 14 bacterial pathogens based on universal 16S rDNA primers and species-specific probes [29]. *Shigella* and *Salmonella* could not be identified with this approach because their 16S rDNA sequences were too similar to the *E. coli* sequence. Therefore, additional pathogen-specific probes and primer sets were designed for the detection of *Shigella* and *Salmonella.* This assay was validated with 1500 diarrheal stool samples and 200 samples from healthy individuals and showed a sensitivity of 100% and specificity of 95.2% with an LOD of $10^3$ cfu/ml. A magnetic bead-based DNA microarray test for the simultaneous screening of five enteric bacteria and two viruses, which can be read out by the naked eye or a digital camera, was developed by Sun *et al.* [33]. The microarray detection was combined with two sets of asymmetric multiplex PCR systems. The hybridized biotinylated PCR products were detected by streptavidin-coated magnetic beads of diameter 1.05 μm. Tests with 86 samples isolated from patients, fishery products, or the environment revealed both specificity and sensitivity of 100% and an LOD of 50 ng DNA template or $10^3$ cfu/ml.

## 4.3 Antibiotic Resistance Detection

The resistance of widespread Gram-positive and Gram-negative bacteria against antibiotics is a severe and growing issue in healthcare. Infections caused by resistant microorganisms often fail to respond to conventional treatment, resulting in prolonged illness, greater risk of death, and higher costs. A high percentage of hospital-acquired infections are caused by highly resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) or multidrug-resistant Gram-negative bacteria. In the European Union, about 25 000 patients die each year from infections caused by selected multidrug-resistant bacteria and the associated costs are estimated at about 1.5 billion Euros per year [41]. In the United States of America, infections with pathogens resistant to antimicrobials cost the healthcare system in excess of US$ 20 billion annually and generate more than 8 million additional hospital days. The annual societal costs exceed US$ 35 billion [42]. In order to tackle these challenges, a large number of DNA microarray tests have been developed in recent years (Tables 4.3 and 4.4). A few microarrays were developed that cover both Gram-positive and Gram-negative organisms. A broad, high-density microarray, which enabled the identification of 775 genes encoding resistance to aminoglycosides, beta-lactams, chloramphenicols, glycopeptides, heavy metals, lincosamides, macrolides, metronidazoles, polyketides, quaternary ammonium compounds, streptogramins, sulfonamides, tetracyclines,

**Table 4.3** DNA microarrays for antibiotic resistance detection in Gram-positive bacteria.

| References | CC | Target species | Sample | Preanalytics | Detection | TTR |
|---|---|---|---|---|---|---|
| [45] | ES | Rifampicin- and isoniazid-resistance in *M. tuberculosis*; Chipron LCD Array (Berlin, DE) mutations in *rpoB*, *katG* and *mabA-inhA*-region | 46 resistant clinical isolates | Multiplex PCR with biotinylated primer | Hybridization (30 min), enzymatic precipitation reaction (HRP), Chipron transmission scanner | 5 h |
| [46] | UK | 47 resistance genes in Gram-negative bacteria | 50 *E. coli* from human samples; 37 *S.* from human and veterinary samples | DNA extraction, primer elongation with biotinylated dUTP | Hybridization with 55 probes (20–30-mer), Alere ArrayTube, enzymatic precipitation reaction (HRP/tetramethyl benzidine derivate), Alere ArrayTube Reader (transmission scanner) | n/a |
| [40] | CA | *A. baumannii*, *P. aeruginosa*, *S. maltophilia*, *E. aerogenes*, *E. cloacae*, *E. coli*, *K. pneumoniae*, *K. oxytoca*, *S. marcescens*, *H. influenzae*, *L. pneumophila*, *S. aureus*, *S. pneumoniae*, *mecA*, carbapenem resistance genes *(OXA*, *KPC*, *VIM*, *IMP)*, beta-lactam resistance genes *(CTX-M*, *SHV*, *TEM)* | 292 reference strains and clinical isolates | Bacterial culture, DNA extraction | Real-time PCR with fluorescence-labeled primer + microarray hybridization in one tube, fluorescence readout after certain cycles | 24 h |
| [47] | CA | 189 virulence and 30 antibiotic resistance genes of *E. coli* | 3 reference strains, 73 characterized strains from previous studies | Bacterial culture, DNA extraction, no PCR, enzymatic fluorescence labeling (random primer + Klenow-polymerase + Cy5-dCTP), denaturation | Hybridization (overnight) with 348 70-mer probes, fluorescence detection | n/a |

| | | | | | | |
|---|---|---|---|---|---|---|
| [48] | US | 12 Gram-positive bacterial gene targets + 3 resistance genes (*mecA*, *vanA*, *vanB*) | 1252 positive blood culture samples | Verigene Gram-positive blood culture test (Nanosphere, USA) sample-to-answer system, magnetic bead-based DNA extraction, no PCR | Microarray hybridization, binding of nanoparticle-conjugated detection probes, readout with Verigene Reader | 15 h |
| [49] | IT | Glycopeptide resistance in *Enterococcus*, 42 genes (vanA, vanB, vanC1, vanC2/C3, vanD, vanE, vanG) | 13 reference strains, 24 *Enterococci* isolates (human and poultry) | Culture, DNA extraction, no PCR, enzymatic fluorescence labeling (random primer + Klenow-polymerase + Cy5-dCTP), denaturation | Hybridization (1 h) with 105 oligonucleotide probes (approximately 45-mer), fluorescence detection | No clear data: sample prep., 1 h hybridization, washing, readout, data analysis |
| [44] | US | 94 antibiotic resistance genes in Gram-positive and Gram-negative bacteria | 51 *E. coli*, *S.*, *Enterococcus* spp., *C. jejuni* | Culture, DNA extraction, no PCR, enzymatic fluorescence labeling (random primer + Klenow-polymerase + Cy5-dCTP) | Hybridization (overnight) with 70-mer probes, fluorescence detection | >24 h |
| [43] | US | 775 antibiotic resistance genes in Gram-positive and Gram-negative bacteria | 2 *S. enterica* strains, *MDR S. enterica serovars*, *E. coli*, *C.* spp., *Enterococcus* spp., *methicillin-resistant S. aureus*, *L.* spp., and *C. difficile* reference strains | Culture, DNA extraction, no PCR, enzymatic fluorescence labeling (random primer + Klenow-polymerase + Cy5-dCTP) | Hybridization (overnight) with 70-mer probes, fluorescence detection | >24 h* |

(*continued overleaf*)

**Table 4.3** (*Continued*)

| References | CC | Target species | Sample | Preanalytics | Detection | TTR |
|---|---|---|---|---|---|---|
| [50] | RU | Rifampicin and isoniazid resistance of *M. tuberculosis*; TB-Chip, 27 mutations in *rpoB*, 11 mutations in *katG*, 5 mutations in *inhA*, and mutations in *ahpC* | 220 clinical *M. tuberculosis* isolates and 113 sputum samples | DNA extraction, asymmetric PCR with fluorescence-labeled primer | Hybridization (6 h), fluorescence detection with portable reader (Biochip-IMB) | 12 h |
| [51] | JP | Rifampicin, ofloxacin, and dapsone resistance in *M. leprae*, mutations in *rpoB*, *gyrA*, and *folP1* | 139 clinical isolates | Multiplex PCR (biotinylated primer), denaturation | Hybridization (1 h), enzymatic precipitation reaction (HRP/TMB), transmission scanner | No clear data: sample prep., 1 h hybridization, washing, HRP reaction, readout, data analysis |
| [52] | DE | 23 resistance genes + 10 toxin genes in *S. aureus* | 100 MRSA clinical isolates | DNA extraction, linear multiplex amplification with biotin-labeled dUTP | Hybridization on Alere ArrayTube platform, enzymatic precipitation reaction (HRP/TMB), Alere ArrayTube Reader (transmission scanner) | n/a |
| [53] | DE | 185 *S. aureus* genes + 300 alleles thereof including virulence factors and resistance genes | 100 clinical isolates and reference strains | DNA extraction, linear multiplex amplification with biotin-labeled dUTP | Hybridization on Alere ArrayTube platform, enzymatic precipitation reaction (HRP/TMB), Alere ArrayTube Reader (transmission scanner) | n/a |
| [54] | DE | *mecA* alleles in *Staphylococci* | 78 clincal and veterinary *Staphylococcus* spp. | DNA extraction, linear multiplex amplification with biotin-labeled dUTP | Hybridization on Alere ArrayTube platform, enzymatic precipitation reaction, Alere ArrayTube Reader | n/a |

| | | | | | | |
|---|---|---|---|---|---|---|
| [55] | KR | Rifampicin and isoniazid resistance of *M. tuberculosis*; Combichip Mycobacteria (GeneIn Co., Ltd, Busan KR), mutations in *rpoB*, *katG* and *inhA* region | 243 clinical isolates (115 RIF resistant strains, 119 isoniazid resistant strains) | Culture, DNA extraction, PCR with biotinylated primer | Hybridization (30 min) on microarray with 25 probes (14–20-mer), streptavidin–Cy5, fluorescence detection | No clear data: sample prep., 0.5 h hybridization, washing, readout, data analysis |
| [56] | JP | Mutations in seven genes (*inh*, *KatG*, *rpoB*, *rrs*, *rpsL*, *embB)* which are related to *M. tuberculosis* drug-resistance | 48 sputum samples from tuberculosis patients | DNA extraction, multiplex PCR | Hybridization (1 h), enzymatic precipitation reaction | No clear data: sample prep., 1 h hybridization, washing, HRP reaction, readout, data analysis |
| [57] | UK | 84 *S. aureus* gene targets including species-specific, antibiotic resistance, toxin, and other virulence-associated genes | 43 *S. aureus* isolates | DNA extraction, no PCR | Hybridization with Cy3-labeled gDNA for 16 h with gentle agitation | 24 h |
| [58] | DE | 9 *S. aureus* resistance genes (*mecA*, *aacA-aphD*, *tetK*, *tetM*, *vat(A)*, *vat(B)*, *vat(C)*, *erm(A)*, *erm(C)*) and mutation in *grlA* | Reference strains and 13 clinical *S. aureus* isolates | 9-plex PCR + *grlA* PCR, labeling by random priming with fluorescence. HighPrime or by photochemical labeling with Psoralen-PEO-Biotin, denaturat. | Hybridization (4 h), fluorescence detection | No clear data: sample prep., 4 h hybridization, washing, readout, data analysis |
| [59] | CN | 8 mutations in the *rpoB* gene of *M. tuberculosis* | 102 rifampin-resistance strains | DNA extraction, *rpoB* PCR with fluorescence-labeled primer | Hybridization (1 h), fluorescence detection | No clear data: sample prep., 1 h hybridization, washing, readout, data analysis |

(*continued overleaf*)

**Table 4.3** (*Continued*)

| References | CC | Target species | Sample | Preanalytics | Detection | TTR |
|---|---|---|---|---|---|---|
| [60] | CN | 6 *Staphylococcal* resistance genes (*mecA*, *blaZ*, *aac*(6′)-*Ie-aph*(2″), *ermA*, *ermC*, *msrA*) und a variable 16S rRNA region for differentiation between *S. aureus* and coagulase-negative *Staphylococcus* (CoNS) species | 178 clinical *S. aureus* isolates and 237 CoNS isolates | DNA extraction, multiplex asymmetric PCR (8-plex), denaturation | Hybridization (2 h), fluorescence detection | 5 h |
| [61] | RU | 16 and 23 mutations within *gyrA* and *gyrB* gene, respectively (fluoroquinolone resistance), 4 mutations within the *rrs* gene and 5 mutations within the *eis* promoter region (aminoglycoside, and cpreomycin resistance) of *M. tuberculosis* | 65 *M. tuberculosis* clinical isolates + 61 clinical specimen (e.g., sputum, bronchioalveolar) | DNA extraction, multiplex PCR with fluorescence-labeled dUTP | Hybridization for 10–16 h, fluorescence detection | 24 h |
| [62] | US | *mecA* (MRSA), SCCmec junction sequence + *S. aureus* specific probe (*tuf*) | 87 bacterial isolates + 246 nasal swab samples | DNA extraction, multiplex asymmetric PCR with Cy3-labeled primer to generate single stranded products | Hybridization on Akonni gel element arrays (20–30-mer probes) for 3 h at room temperature without shaking, fluorescence detection | 8 h |

HRP/TMB: horseradish peroxidase–streptavidin complex/tetramethyl benzidine derivative.

**Table 4.4** DNA microarrays for antibiotic resistance detection in Gram-negative bacteria.

| References | CC | Target species | Sample | Preanalytics | Detection | TTR |
|---|---|---|---|---|---|---|
| [63] | AT | Gram-negative bacteria, 33 genes of beta-lactamases | 70 clinical strains | Genomic DNA extraction, amplification of only circular DNA molecules | 100-plex assay with padlock probes, microarrays on aldehyde-functionalized glass slides, hybridization (1 h), fluorescence detection | No clear data: sample prep., 1 h hybridization, washing, readout, data analysis |
| [64] | BE | Gram-negative bacteria, ESBL/AmpC/KPC/NDM-1 assay | 207 clinical isolates | DNA extraction, ligation, multiplex PCR | Check-MDR CT101 (Check-Points) | 7 h |
| [65] | DE | *S. enterica*, 77 antimicrobial resistance genes | 168 reference strains | Genomic DNA extraction, multiplex linear DNA amplification | Hybridization on Alere ArrayTube platform, enzymatic precipitation reaction (HRP/TMB), Alere ArrayTube Reader (transmission scanner) | 2 h (after sample prep.) |
| [66] | UK | Gram-negative bacteria, resistance to 75 clinically relevant antibiotics | 132 bacterial isolates | DNA extraction, multiplex linear DNA amplification | Hybridization on Alere ArrayTube platform, enzymatic precipitation reaction (HRP/TMB), Alere ArrayTube Reader (transmission scanner) | 5 h (after bacterial culture) |
| [67] | FR | Gram-negative bacteria, beta-lactamase genes of ESBLs (TEM, SHV, and CTX-M), pAmpC (CMY-2-like, DHA, FOX, ACC-1, ACT/MIR, and CMY-1-like/MOX) and of carbapenemases (KPC, OXA-48, VIM, IMP, NDM) | 187 Gram-negative bacilli isolates | Total bacterial DNA extraction, ligation, multiplex PCR | Check-MDR CT103 array (Check-Points) | 6.5 h (after DNA isolation) |

(*continued overleaf*)

**Table 4.4** (*Continued*)

| References | CC | Target species | Sample | Preanalytics | Detection | TTR |
|---|---|---|---|---|---|---|
| [68] | DE | *A. baumannii*, 91 target sequences associated with 26 antibiotic resistances | Bacterial strains | DNA extraction, three multiplex PCRs | Epoxy-functionalized glass slides, fluorescence detection | 4 h (after bacterial culture) |
| [69] | US | Gram-negative bacteria, important Gram-negative resistance determinants, virulence genes, and replicon markers, *Salmonella* specific markers, and *E. coli* pathogen type markers | *E. coli* and *S. enteric* strains | Total bacterial DNA extraction, biotinylation using a nick-translation kit | Glass slides coated with teflon/epoxy-silane, tyramide signal amplification, hybridization (16–18 h), fluorescence detection | No clear data: sample prep., 16–18 h hybridization, washing, readout, data analysis |
| [70] | CN | Gram-negative bacteria, SHV genotyping, 54 mutations in the $bla_{SHV}$ gene | 1320 clinical isolates | Plasmid DNA extraction, PCR | Microarrays on aldehyde-functionalized glass slides, hybridization (2 h), fluorescence detection | No clear data: sample prep., 2 h hybridization, washing, readout, data analysis |
| [71] | US | Gram-negative bacteria, clinically important class A beta-lactamase genes and $bla_{KPC}$ gene | 102 *Enterobacteriaceae* and 4 *A. baumannii* isolates | Genomic DNA extraction, ligation-mediated amplification | The Check KPC/ESBL (Check-Points) | n/a |
| [72] | US | *Enterobacteriaceae*, the $bla_{ESBL}$ and $bla_{KPC}$ genes | 40 blood samples, 20 non-lactose-fermenting organisms | DNA extraction, ligation-mediated amplification | The Check KPC/ESBL (Check-Points) | 18–20 h |
| [73] | CN | 17 categories of drug-resistant genes | *E.coli* reference strains, 42 clinical isolates | Entire bacterial DNA extraction, random amplification, extension with Klenow fragment | Microarrays on aldehyde-functionalized glass slides, hybridization (4 h), fluorescence detection | No clear data: sample prep., 4 h hybridization, washing, readout, data analysis |

| | | | | | | |
|---|---|---|---|---|---|---|
| [74] | US | *Enterobacteriaceae*, the $bla_{ESBL}$ and $bla_{KPC}$ genes | 1152 isolates collected from intraabdominal infections | Whole-genomic DNA, ligation-mediated amplification | The Check-KPC ESBL (Check-Points) | n/a |
| [75] | DE | Gram-negative bacteria, genotyping of $bla_{TEM}$, $bla_{SHV}$, $bla_{CTX\text{-}M}$ genes | 60 clinical isolates from respiratory samples, swabs, urine | Plasmid DNA extraction, PCRs | Epoxy-functionalized glass slides, fluorescence detection | 1.5 h (after sample preparation) |
| [76] | FR | Gram-negative bacteria, most prevalent carbapenemases (NDM, VIM, KPC, OXA-48, and IMP) and extended-spectrum beta-lactamase (ESBL) gene families (SHV, TEM, and CTX-M) | 144 Gram-negative strains | Total bacterial DNA extraction, ligation-mediated amplification | Check-MDR CT102 ESBL-Carbapenemase microarray (Check-Points) | 6.5 h (after DNA isolation) |
| [77] | UK | *K. pneumonia*, genotyping of the $bla_{KPC}$ genes | 12 reference strains, urine samples | Plasmid DNA extraction, PCR | Epoxy-functionalized glass slides, fluorescence detection ion | 5 h |
| [78] | NL | *Enterobacteriaceae*, the $bla_{ESBL}$ and $bla_{KPC}$ genes | 346 clinical isolates | DNA extraction, ligation-mediated amplification | The Check KPC/ESBL (Check-Points) | n/a |
| [79] | RU | *Enterobacteriaceae*, beta-lactamase genes of class A (TEM, SHV, and CTX-M), | 90 clinical isolates | DNA extraction, multiplex PCR | Microarray on porous membranes, biotin as a label, colorimetric detection | 4.5 h (after DNA isolation) |
| [80] | NL | *Enterobacteriaceae*, most prevalent class A ESBL genes | 212 *Enterobacteriaceae* isolates | DNA extraction, ligation-mediated amplification | The ESBL Array (Check-Points) | n/a |

(*continued overleaf*)

**Table 4.4** (*Continued*)

| References | CC | Target species | Sample | Preanalytics | Detection | TTR |
|---|---|---|---|---|---|---|
| [81] | NL | Gram-negative bacteria, most prevalent carbapenemases (NDM, VIM, KPC, OXA-48, and IMP) and extended-spectrum beta-lactamase (ESBL) gene families (SHV, TEM, and CTX-M) | 95 *Enterobacteriaceae* isolates | DNA extraction, ligation-mediated amplification | Check-MDR CT102 ESBL-Carbapenemase microarray (Check-Points) | 6.5 h (after DNA isolation) |
| [82] | RU | *A. baumannii*, *P. aeruginosa*, *Enterobacteriaceae*, 7 types of carbapenemase genes | 28 clinical isolates | DNA extraction, multiplex PCR | Microarray on porous membranes, biotin as a label, colorimetric detection | 4.5 h (after DNA isolation) |
| [83] | NL | *Enterobacteriaceae*, the $bla_{ESBL}$ and $bla_{KPC}$ genes | 638 *Enterobacteriaceae* isolates | DNA extraction, ligation-mediated amplification | The Check-KPC ESBL (Check-Points) | n/a |
| [84] | UK | *Enterobacteriaceae*, carbapenemases encoding genes | 57 clinical isolates | Total bacterial DNA extraction, ligation of two multi-component probes, PCR | Check-MDR CT102 ESBL-carbapenemase microarray (Check-Points) | 6.5 h (after DNA isolation) |

and trimethoprims as well as resistance transfer genes of Gram-negative and Gram-positive bacteria, was developed by Frye *et al.* [43]. This microarray covered virtually all antibiotic resistance genes found in the National Centre for Biotechnology Information (NCBI) database, thus reducing the subsequent assays necessary to identify specific resistance gene alleles. This microarray was intended to be used as a screening technique in studies of prevalence, epidemiology, and spread of resistance genes. It was built on an earlier version of a microarray with long 70-mer probes in order to cover all allelic variations of the corresponding resistance gene. This initial version, which was developed by the same group in 2006, covered 94 resistance genes [44].

### 4.3.1
### Gram-Negative Bacteria

The global dissemination of antibiotic resistance in Gram-negative bacteria has received much attention, particularly over the last decades [85]. Transmission of resistance genes among bacteria is highly diverse, involving mutations and horizontal acquisition via plasmids, transposons, and other mobile elements. The *Enterobacteriaceae* are among the most important causes of serious hospital-acquired and community-onset bacterial infections in humans, and resistance to antimicrobial agents in these bacteria has become an increasingly relevant problem [86]. International travel and tourism are important ways for the acquisition and fast spread of antimicrobial-resistant *Enterobacteriaceae* [87]. The variety and heterogeneity of antibiotic resistance markers in Gram-negative bacteria make the development of multiplex systems especially significant. High diversity of certain genetic elements, causing resistance, requires the detection of point mutations together with identification, and this is a feature of resistance in Gram-negative microorganisms. From this standpoint, DNA microarrays are a promising technology, enabling simultaneous detection of hundreds of genetic elements and their point mutations in a single test. It also has the potential for identification of genes playing a role in the transmission of resistance and virulence genes. There are several DNA microarrays described in the literature dealing with the multiplex detection of a large number of resistance genes and virulence factors of Gram-negative bacteria. A microarray developed by Bruant *et al.* [47] for the characterization of *E. coli* isolates contained long 70-mer probes for the detection of 189 virulence factors and 30 resistance genes. The production of bacterial enzymes beta-lactamases was shown to be the most common mechanism of antibiotic resistance in clinically important Gram-negative bacteria [88]. Currently, the term *beta-lactamase* is functional, and the family includes over 900 enzymes, which hydrolyze the beta-lactam ring of the antibiotic. According to the variability in the structure, beta-lactamases are divided into four molecular classes [89]. These enzymes are highly heterogeneous within each class, and certain clinically important types represent the groups of dozens of enzymes, having from one to five point mutations from the parent sequence. Certain mutations can change and expand the substrate activity profile of the enzyme,

and these enzymes are called *extended-spectrum beta-lactamases* (*ESBLs*). At present, ESBL-producing *Enterobacteriaceae* are the most widespread resistant bacteria worldwide, and they represent a major concern in both hospital and community settings. The last threat in Gram-negative bacteria is associated with the growing distribution of a variety of carbapenemases – beta-lactamases capable of hydrolyzing carbapenem antibiotics – and some of these enzymes were found to be multidrug resistant [90, 91]. Fu *et al.* [73] developed a high-throughput DNA microarray for the detection 17 categories of drug-resistant genes. One-hundred and fifteen oligonucleotide probes were of 42–45 nt length, and they were spotted on aldehyde-functionalized glass slides. Sample preparation involved bacterial DNA extraction, labeling with Cy3-dCTP by a random priming, and extension with Klenow fragment. The $3' \rightarrow 5'$ exonuclease activity of the Klenow fragment ensured the accuracy of the labeled DNA and eliminated the need for primers. The microarrays based on a traditional combination of PCR amplification and fluorescent labeling with following hybridization of glass slides were developed by Dally *et al.* [68] for the determination of 91 resistance determinants in *Acinetobacter baumannii*. The array contained 324 probes for the detection of certain beta-lactamases, aminoglycoside modifying enzymes, efflux systems, 10 resistance-relevant point mutations, and some others. The time for the whole analysis was about 4 h. The method was validated with 60 multidrug-resistant strains, and the results were in complete accordance with the automated susceptibility testing and single PCR results.

The potential of the microarray technology on glass slides with fluorescent detection was successfully demonstrated for the simultaneous determination of the set of point mutations in genes. Leinberger *et al.* [75] developed an integrated microarray for the simultaneous genotyping of the most clinically relevant beta-lactamases of class A (TEM, SHV, and CTX-M types). The microarray included 618 probes for 156 amino acid substitutions, which cover 99% of all TEM variants, 89% of SHV variants, and 50–83% of all CTX-M subgroups, published by the date of study (Figure 4.1). All positions known at that point to be associated with ESBL activity and inhibitor resistance were included in the array. The total assay time was about 5 h after DNA isolation. The sensitivity of ESBL detection was 93%, and genotyping results were 100% concordant with sequencing data. Later, a similar approach was applied for the determination of 54 mutations in SHV type beta-lactamases [70]. The method distinguished 67 variant alleles of the $bla_{\mathrm{SHV}}$ gene. It was validated on a collection of 1320 clinical isolates. The results were consistent with the genotypes confirmed by bidirectional sequencing. One of the advantages of microarrays is their ability to distinguish different alleles in one isolate in a single assay. The same technology was successfully applied for the genotyping of KPCs directly from urine specimens [77]. This method combined commercial sample preparation techniques straight from the clinical specimen with allele-specific hybridization on a microarray. The probes were designed to identify single nucleotide polymorphism (SNP) at the four positions of the $bla_{\mathrm{KPC}}$ gene. The LOD for target DNA was found to be 10–20 ng per assay depending on target treatment method with DNase and in the 100 cfu/ml range for the SNP

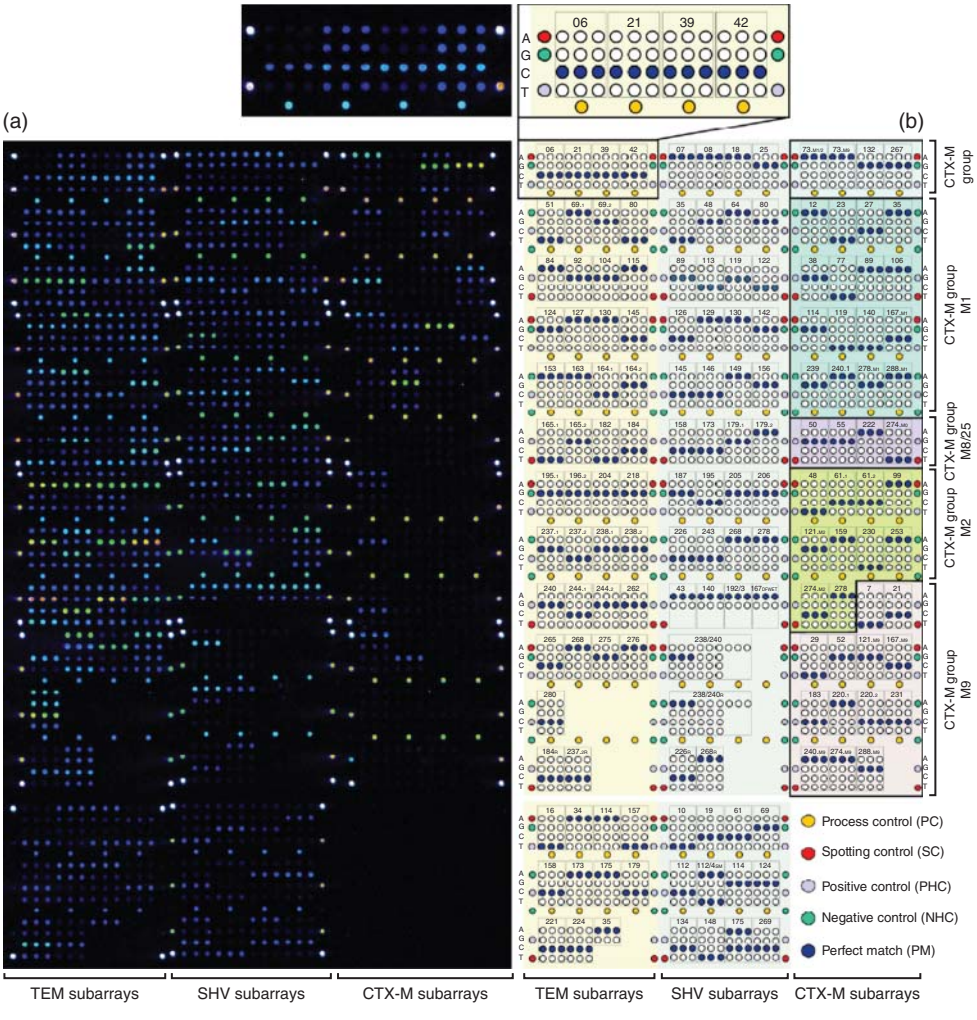


**Figure 4.1** DNA microarray for the detection of extended spectrum beta-lactamase genes *bla*TEM, *bla*SHV, or *bla*CTX-M. (a) Fluorescence image after hybridization and (b) oligonucleotide microarray layout [75]. Permissions: http://jcm.asm.org/content/48/2/460.long.

discrimination depending on the target gene and host organism. The reported total assay time was 3.5–5 h after DNA extraction. The need for rapid determination of resistance markers in clinical practice has promotes the development of effective and low-cost identification methods. Therefore, many efforts have been directed toward the improvement of technology and the replacement of its most costly components. A common approach is the replacement of the fluorescent label with biotin, which then is detected with conjugates of streptavidin with enzymes, mostly horseradish peroxidase. The enzyme is detected by an optical method by means of accumulation of a color product on a support. Colorimetric

microarrays were developed for determining the clinically important ESBLs of TEM, SHV, and CTX-M types in *Enterobacteriaceae* [79] and seven types of carbapenemases in *A. baumannii* and *P. aeruginosa* [82]. The arrays contain several oligonucleotide probes for type and subgroup determination as well as for the detection of important SNPs. Combination of inexpensive membrane supports with inexpensive colorimetric detection makes the method suitable for clinical application. Properties of biotin as a label are attractive because of the small size of its molecule, which has a small effect on the conformation of the target molecule. In addition, the technique of tyramide signal amplification was developed and realized in the amplification kit by Perkin Elmer (Waltham, MA). Biotin as a label, amplified and then coupled with the fluorescent label Alexa 555, was used by Davis *et al.* [69] in the method for the determination of resistance in *E. coli* and *Salmonella enterica*. The array included 203 60-mer oligonucleotide probes, including 117 for resistance genes, 16 for virulence genes, 25 for replicon markers, and 45 other markers. In this work, an important study of inter and intralaboratory validity of the method was carried out. The intralaboratory validity was shown to be high enough in contrast to the interlaboratory reliability. The conclusion was made about the technical complexity of microarray technologies. Each step of the array, including extraction, labeling, hybridization, detection, and data analysis, has a potential for errors and even in the most experienced hands may need troubleshooting to achieve reproducible results. An approach to solve the variability issue lies in reducing the manual process steps and carrying out the complete process in a single tube. It was realized in technology of the ArrayTube by Clondiag, now part of Alere Technologies (Jena, Germany). The ArrayTube platform consists of an array integrated into a micro reaction vial. Labeling by biotin occurs in the course of the multiplex primer extension reaction and is highly specific. The detection is based on colorimetric development of enzymatic reaction. Batchelor *et al.* [46] used the ArrayTube platform for the detection of antibiotic resistance in Gram-negative bacteria. The array contained 55 20–30-mer probes for the identification of genes encoding resistance to aminoglycosides, trimethoprim, sulfonamides, tetracyclines, and beta-lactams, including ESBLs. The assay has been validated in two studies with 50 *E. coli* and 85 *Salmonella* isolates from human and veterinary origin [46, 92]. At present, several arrays for detecting resistance markers are commercially available (see Table 4.2). The AMR-ve Genotyping Kit for the determination of 54 resistance genes in Gram-negative bacteria includes several ESBL markers. The Carbapenemase Typing Kit includes 15 species markers for *Enterobacteriaceae* and 80 genetic markers to identify resistance genes for ESBLs and carbapenemases. Newer developments focused on the ArrayStrip, which combines three individual assays on one microarray. At present, *E. coli* Genotyping is available, which represents a combined assay and includes several hundred markers for the detection of resistance genes, virulence genes, and for H/O serogenotyping. The properties of the ArrayTube and ArrayStrip were recently shown in detecting of Gram-negative bacteria resistant to 75 clinically relevant antibiotics [66] and serogenotyping of *Salmonella* [65]. The method is meant to be easy to use, as all

main steps are performed in a single tube or strip, which aims to decrease manual work and minimize handling errors. The advantage of using microarrays in the strip format is that it is fabricated as a microarray chip at the bottom of each well of a 96-well plate, allowing a large number of genes and up to 96 samples to be tested simultaneously. The same technology platform is used by Check-Points (the Netherlands), which offers the test systems for identification of different beta-lactamase genes: ESBLs, carbapenemases, and AmpCs in various combinations (Check-MDR CT101, 102, 103). The method involves DNA extraction, ligation-mediated PCR, amplification of the ligated probes, and hybridization on the microarray placed at the bottom of the ArrayTube. The hybridization results are developed by colorimetric peroxidase-based detection. Increased attention has recently been focused on the validation of Check-Points commercial systems for the identification of ESBLs and carbapenemases. Eleven publications devoted to the evaluation of Check-MDRs and other kits have been published during last 3 years [64, 67, 72, 74, 76, 78, 80, 81, 83, 84]. This reflects both the special relevance of this area and the prospects of using this technology. Large surveys on Check MDR assays revealed high specificity and sensitivity close to 100%. The method gave good results on most of *Enterobacteriaceae, Pseudomonas* spp., and nonfermenter isolates, but several important for nonfermenter genes were missing. Multiple resistance genes were correctly identified, whatever the complex genetic background of the isolates tested. The method was shown to discriminate effectively between ESBL and non-ESBL beta-lactamases of the same family in one strain, which is difficult to detect with the other methods. At the same time, one of the key mutations of the SHV E240K was reported which was not detected in certain isolates. Some failures in detection of carbapenemases in *K. pneumoniae* isolates and *Enterobacter cloacae* isolate were observed. One of the technique's limitations discussed was that it identified only known genes. Despite some drawbacks, investigators came to the conclusion that these commercially available DNA microarrays presented a practical, rapid and a highly accurate tool for the detection of clinically important beta-lactamase genes. They were recommended as confirmation tests in a routine clinical setting.

The latest development in the field of beta-lactamase gene detection by microarrays was based on a 100-plex assay with padlock probes [63]. The padlock probes with the average nucleotide length of 83 nt were designed for the 33 clinically most common beta-lactamases, including CTX-M, TEM, SHV, KPC, IMP, SME, GES, IMI, PER, RTG, SFO, and VEB. Every padlock probe contains a universal sequence for C2CA (circle-to-circle amplification) and a unique barcode sequence for specific detection on a microarray. The C2CA sequence contained a restriction site, which was necessary for the amplification step. The 5′ and 3′ target recognition arms anneal specifically to a target DNA and are subsequently ligated. These ligated padlock probes are then amplified using C2CA, which consists of two linked rolling cycles. A microarray of 299 oligonucleotides was used for the ligated padlock probe detection. Every sequence-tagged padlock probe was complementary to only one microarray oligonucleotide. The method was shown to provide a very high degree of multiplexing and throughput.

### 4.3.2 Gram-Positive Bacteria

The most prevalent causes of bacterial bloodstream infections are Gram-positive bacteria, which account for 52–77% of bacterial sepsis [93]. Bloodstream infections with Gram-positive bacteria are often complicated by antimicrobial resistance. Rapid identification of the bacterial infection and the determination of its antibiotic susceptibility are essential for the correct treatment decision making. The Verigene Gram-Positive Blood Culture test (Nanosphere, Northbrook, USA) enables the identification of 12 Gram-positive bacteria and of three resistance genes (*mecA*, *vanA,* and *vanB*) directly from positive blood cultures (see Table 4.2). The integrated sample-to-answer microarray test has been successfully validated in several multicenter studies with a large number of positive blood cultures [48, 94]. The assay relies on integrated magnetic bead-based DNA extraction and direct target hybridization without PCR amplification, followed by binding of a second nanoparticle-conjugated detection probe. The time-to-result of the test was 2.5 h which was on average 42 h faster compared to the routine culture methods as shown in the validation studies. Another microarray for vancomycin resistance detection was developed by Cassone *et al.* [49]. The microarray contained 105 probes targeting 42 genes within the glycopeptide resistance clusters in *Enterococcus* (*vanA*, *vanB*, *vanC1*, *vanC2/vanC3*, *vanD*, *vanE*, and *vanG*).

#### 4.3.2.1 *Staphylococcus aureus*

MRSA is responsible for a significant proportion of hospital- and community-acquired infections. MRSA strains are resistant to all beta-lactam antibiotics in current clinical use. The resistance is caused by an alternate penicillin-binding protein (PBP2a) which is encoded by the *mecA* gene. There are several *S. aureus* genotyping microarrays [95] which enable the detection of a large number of resistance genes and virulence-associated toxin genes in parallel [52, 53, 57] as well as further DNA microarrays which focus on the detection of *S. aureus* resistance genes [54, 58, 60, 62]. A broad range *S. aureus* genotyping DNA microarray has been designed by Monecke *et al.* [53] based on the ArrayTube platform (see Table 4.2). The microarray allowed the assignment of isolates to strains and clonal complexes in addition to the assessment of virulence and antibiotic resistance. This genotyping microarray has been shown to be a useful tool for rapid strain identification and epidemiological surveillance and outbreak tracing as has been used, for example, to characterize a collection of MRSA isolates from hospitals in the German region of Eastern Saxony [96]. A microarray for the discrimination of *mecA* alleles containing 29 different *mecA* probes was designed by Monecke *et al.* [54] using the Alere ArrayTube platform. A DNA microarray targeting 84 genes targets including species-specific, antibiotic resistance, toxin, and other virulence-associated genes was developed at the University of Nottingham [57]. The microarray allowed the identification of 13 *S. aureus* isolates simultaneously on one chip based on detection of fluorescence-labeled genomic DNA without

prior PCR amplification. A microarray for rapid identification of *S. aureus* antibiotic resistance profiles has been developed by Strommenger *et al.* [58]. The microarray covered 10 clinically relevant antibiotic resistance genes (*mecA*, *aacA-aphD, tetK, tetM, vat*(A), *vat*(B), *vat*(C), *erm*(A), *erm*(C)) including one point mutation in the *grlA* gene and was combined with a previously developed multiplex PCR [97]. Zhu *et al.* developed a DNA microarray for the detection of six antibiotic resistance genes leading to penicillin, methicillin, aminoglycoside, macrolide, lincosamide, and streptogramin B (MLSB) resistance in staphylococci. The array also included probes directed against a variable region in the 16S rRNA gene to discriminate between *S. aureus* and coagulase-negative staphylococci [60].

An example for commercial developments for MRSA detection was developed by Akonni Biosystems (Frederick, USA), which comprises probes directed against the *mecA* gene, the SCC*mec* junction, and the *tuf* gene for *S. aureus* species identification [62]. Three-dimensional gel-based polymer microarrays with integrated probes [98] were combined with multiplex PCR amplification of the extracted genomic DNA. The assay contained seven SCC*mec*-specific primers and three probes to cover different SCC*mec* types and had an LOD of 250 fg genomic DNA, which is equal to approximately 80 cfu. A validation study with 246 nasal swab samples revealed a sensitivity of 80.5% and a specificity of 96.6%. Besides the TruArray MRSA Test, the company's website lists the TruArray MDR-TB Test (*Mycobacterium tuberculosis*, *Mycobacterium avium*, rifampin, and isoniazid resistance) and the TruArray HE Test (*Herpes simplex*, *Varicella zoster*). The Akonni tests are predicated on gel element microarray technology, with increased probe immobilization per unit area compared to conventional planar arrays. This three-dimensional structure is described to both enhance the sensitivity and decrease the hybridization time [99]. The gel element arrays have been shown to have high thermomechanical stability and can be reproducibly manufactured [98]. These attributes translate into sensitive and low-cost consumables and instruments. Akonni's assay development efforts are focused primarily on simplifying the overall microarray workflow for the end user by combining amplification and microarray detection into a single, closed amplicon consumable [100–102]. Example assays demonstrated using this simplified workflow include influenza subtyping with oseltamivir resistance typing, an encephalitis panel of DNA and RNA viruses, and multidrug resistant tuberculosis (TB) [100]. A companion device that Akonni developed for sample preparation is the TruTip, which consists of a binding matrix integrated into a pipette tip [103, 104]. The form factor is both field-portable and amenable to automation. The large porosity of the silica matrix allows easy processing of challenging clinical samples. Simplifying microarray work flow and consumables in this manner leads to lower cost consumables and systems that are better aligned for use at the point of need or in the field (Figure 4.2).

#### 4.3.2.2 **Mycobacteria**

In 2011, there were an estimated 8.7 million new cases of TB and 1.4 million people died from TB [105]. Multidrug-resistant strains of *Mycobacterium*

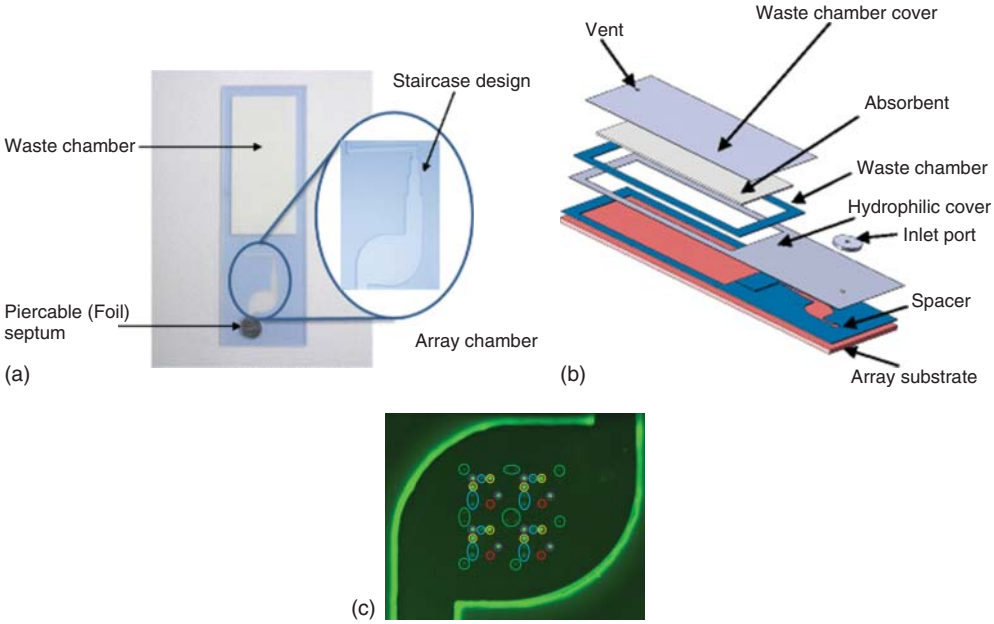


**Figure 4.2** Integrated DNA microarray assay. (a) Flow cell, (b) components of the flow cell assembly, and (c) MRSA v4.0 array following on-chip amplification of 105 genomic copies of MRSA [102]. Permissions: http://link.springer.com/article/10.1007%2Fs10544-011-9584-9/fulltext.html.

*tuberculosis* (MDR-TB) accounted for approximately 5% of the 6.2 million TB cases notified in 2011. Out of these MDR-TB cases, 9% were extensively drug-resistant tuberculosis (XDR-TB), defined as MDR-TB plus resistance to a fluoroquinolone and at least one of three injectable second-line drugs (amikacin, kanamycin, or capreomycin). The development of resistance in *M. tuberculosis* results from mutations in drug resistance genes (*rpoB*, *inhA*, *katG*, *pncA*, *embB*, *rrs*, *gyrA*, or *gyrB*). The Engelhardt Institute of Molecular Biology in Moscow (Russia) developed a commercially available microarray test (TB-Biochip) for MDR-TB detection [50], which has been successfully validated in several studies [106, 107]. In 2013, the same group published a new DNA microarray test for XDR-TB diagnosis [61]. Both tests combine multiplex PCR with microarray detection. The XDR-TB assay could detect down to 300 genome copies per assay. The microarray test allowed the diagnosis of XDR-TB within one working day compared to several weeks to months required for traditional culture-based TB drug resistance tests. There are a number of commercially available tests for *M. tuberculosis* available, and they are listed in Table 4.2. Shimizu *et al.* [56] developed a DNA microarray that covered mutations in seven genes (*inh*, *KatG*, *rpoB*, *rrs*, *rpsL*, *embB*) which are responsible for antibiotic resistance against five anti-TB drugs (rifampicin, isoniazid, streptomycin, kanamycin, an ethambutol). The DNA microarray results were compared with standard

culture-based sensitivity testing which took 2 months from sample collection. The DNA microarray showed a high sensitivity (>90%) for all five drug resistances and a high specificity for rifampicin and ethambutol resistance, but a reduced specificity for the detection of isoniazid and kanamycin resistance. A microarray to detect the dominant site mutations at different positions within the *rpoB* gene was developed by Sun *et al.* [59] containing four wild-type and eight mutant probes. Matsuoka *et al.* [51] developed a DNA microarray for the detection of mutations in the *Mycobacterium leprae* genome which confer resistance to key drugs for leprosy treatment. The microarray contained 19 different probes to detect relevant mutations in the *folP1*, *rpoB*, and *gyrA* genes which are responsible for resistance against dapsone, rifampicin, and ofloxacin.

## 4.4 DNA Microarrays for Virus Diagnostics

During last decades, the field of viral infection diagnostics has developed from the use of immunological tests to wider use of molecular technologies based on genetic identification. DNA chips and microarrays offer effective methods for the rapid detection of genetic variations of viruses and permit a conceptually new approach for viruses testing. The advantages of microarray technology, including a significant increase in productivity and multiplexing, led to the development of methods for the identification of multiple viruses and bacteria in a single assay. The development of a molecular genetic assay for the simultaneous detection of multiple viruses is highly challenging because of the significant genetic diversity even in a single viral species. Viral genomes do not have universal genes such as the 16S or 18S rRNA, which serve as probe targets for bacterial and fungal pathogens. The molecular targets for viruses differ, depending on the conserved genes of each viral group/species. Thus, success in probe design is largely dependent on the amount of sequence data available for the given species. A summary of different DNA-arrays developed during the last 5 years is presented in Table 4.6.

A first microarray designed for detection of a wide range of viruses as pathogens was the ViroChip [108]. The first array version contained 1600 probes derived from the 140 complete viral genomes available in GenBank when the array was designed. The last published version of the array developed contains over 36 000 probes and allow the identification of over 1500 viruses from GenBank submitted by December 2009 [109]. The ViroChip was fabricated by spotting synthesized oligonucleotides on the surface of glass slides. The oligos were 70-mer and matched the sequences common to a taxonomic family, but not found in other families. For some families, the oligos were selected at the genus level. The main advantage of the ViroChip was its ability to identify novel viruses within a known family as the probes were designed against conserved sequences. Different additional probes can be added or deleted if necessary to get the desired spectrum of targets, which is not limited to viruses and may include, for instance, probes for the detection of bacteria and/or fungi. To overcome the limitation

of spotting tens of thousands of commercially synthesized oligos on the slides, which leads to high cost for each array design, the latest version of the ViroChip utilizes the Agilent ink-jet array platform. Another DNA microarray representing a wide-spectrum approach for pathogen detection is the GreeneChipPm and its part GreeneChipVr [110]. They are high-density oligonucleotide arrays, fabricated using the same Agilent platform as the ViroChip. GreeneChipPm contains a total of about 29 500 60-mer oligonucleotide probes including around 9500 probes for vertebrate viruses together with the probes for pathogenic bacteria, fungi, and protozoa. GreeneChipVr contains the probes for viral infections only. Viral probes were designed to target a minimum of three genomic regions for each family or genus of a virus. In most cases, one highly conserved region was chosen, along with two or more variable regions. Bacterial, fungal, and protozoan probes were selected by a similar strategy, except that the target sequences were chosen only from the 16S ribosomal RNA genes of bacteria and 18S rRNAs of fungi and protozoa. Correct identification of the viruses at the species level was performed when virus-infected cell cultures and clinical samples were tested. Bacterial identification was poorer because of the choice of 16S rRNA as the target gene; and certain bacteria could only be identified at family or class level. The sensitivity of this chip was comparable to that of the ViroChip because of the use of long oligos of the 60-mer.

One of the most comprehensive pathogen detection arrays, which combines identification of bacteria and all known viruses, was designed at the Lawrence Livermore National Laboratory [111]. The Lawrence Livermore Microbial Detection Array (LLMDA) contained target probes for ~900 bacterial and ~2200 viral species. Similar to the GreeneChip, probes from 50 to 66 bases long were selected by two strategies. "Discovery" probes match genome regions that are unique to a taxonomic family or subfamily, but were shared by the species within that family. These probes may also target novel species within a known family. "Census" probes target highly variable regions that are unique to an individual species or strain. They were designed to identify the specific strain of an organism. Bacterial probes were selected from full genome sequences, rather than from 16S rRNA genes as in the GreeneChip, enabling the discrimination at the strain level for most bacteria. The arrays were fabricated by the NimbleGen platform based on photocatalytic synthesis, using a digital micromirror device to control the addition of nucleotides. This technology provides higher probe densities than any spotted oligo arrays or the Agilent inkjet platform, with up to 4.2 million features per array. The advantages of the LLMDA were the broad coverage of bacteria and viruses, as well as of eukaryotic pathogens. Its disadvantages include its cost (compared to spotted arrays such as the ViroChip) and the need (unlike with "universal" arrays) to design new probes periodically to incorporate new genomes deposited in the GenBank. The same array principle was used by Erlandsson *et al.* [112] for the identification of different DNA and RNA viruses. The changes concerned the protocols for sample retreatment and amplification. It was suggested to use multiple displacement amplification (MDA)-based whole genome isothermal amplification by the Phi29 polymerase. The advantage of the method is that both DNA and RNA

viruses could be run through the same protocol. Although subtype identification was not a goal for the probe design, the method discriminated between different virus subtypes. In several samples analyzed, more than one virus was found, including DNA and RNA viruses in some cases. The microarray was proposed for the simultaneous detection of viruses as well as bacteria and could be useful in the diagnosis of diseases with combined viral and bacterial components or those with symptoms that clinically are very similar, making it hard to conclude about the diagnosis. The presented microarray may also be a suitable research tool for finding new pathogens.

Currently, there are certain approaches to the determination of several viruses in a single assay implemented in commercial systems. A resequencing microarray PATHOGENID v2.0 based on technology for high-density oligonucleotide microarrays was developed in collaboration of Affymetrix and the Pasteur Institute. It was designed for the identification of 949 genes, including 126 different viral sequences, 18 of which correspond to highly pathogenic viral agents [113]. The design strategy was based on an alignment covering the whole gene in a search for consensus sequences specific for one gene. The sample preparation involved the following steps: RNA extraction, reverse transcription of cDNA using random primers, and isothermal amplification by Phi29 polymerase. Then the amplicons were fragmented, labeled with fluorescent dyes, and hybridized on the microarrays. The overnight hybridization resulted in relatively long assay time. The method does not require the design of specific primers for all potential targets and the setting up of the corresponding PCRs. This technology was applied for the identification of influenza viruses of different host origins (humans, pigs, horses, and birds) [114], highly pathogenic viruses causing hemorrhagic fever [115], and viruses causing arboviral diseases [116]. The microarray was shown to identify sequences having 13–14% of divergence with the tiled sequence, or stretches of 20 consecutive nucleotides identical to the tiled sequence. The approach allows not only the detection of the currently known isolates but also the discovery of new ones with reliable sequence information.

Another array was developed using technology by Chipron (Berlin, Germany), which sells low-density arrays on transparent, prestructured polymer supports containing eight identical arrays in well-separated, individually addressable hybridization fields. It was used for the determination of the Crimean–Congo hemorrhagic fever virus [117] and the Lassa virus [118]. Colorimetric detection is based on the use of biotin as label of target DNA, which is then developed with a conjugate of streptavidin with horseradish peroxidase. The method is fast and takes less than 1 h after target DNA preparation. It is also inexpensive and simple, and the detection may be performed with the naked eye, and it is proposed for both clinical diagnostic and outbreak investigations (Figure 4.3).

Various commercial vendors are developing or offer DNA microarray-based assays for virus detection (Table 4.2). The technology of Clondiag (Jena, Germany) uses oligonucleotide microarrays on pieces of glass or porous membranes which are placed into a modified standard lab reaction vessel (ArrayTube) (Figure 4.1).

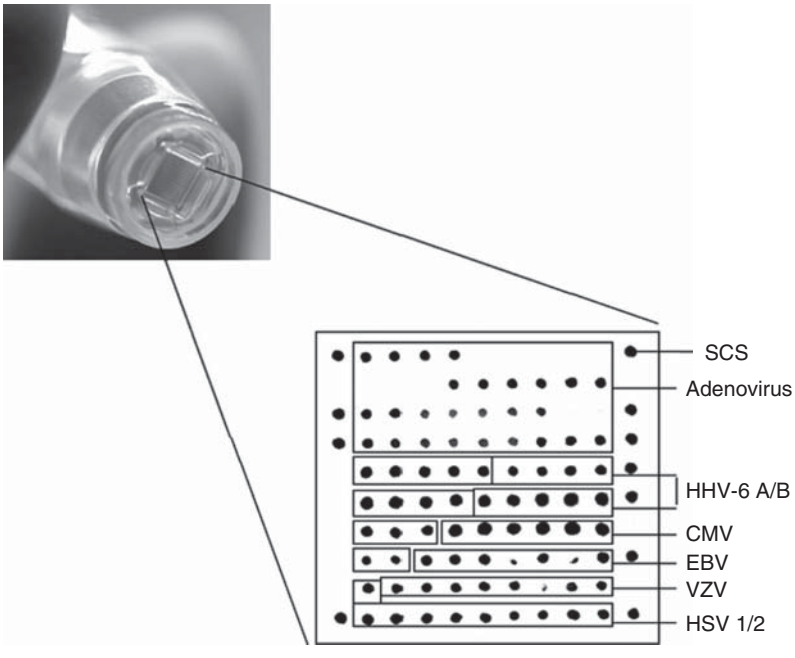


**Figure 4.3** VINAray for the detection of human herpesvirus types 1 or 2 (HSV 1/2), varicella zoster virus (VZV), Epstein–Barr virus (EBV), cytomegalovirus (CMV), human herpesvirus-6 types A or B (HHV-6 A/B), and adenovirus genera A–F (adenovirus). The DNA array was based on the ArrayTube platform (Alere, Jena) [119]. Permissions: http://www.journals.elsevier.com/journal-of-virological-methods/.

This makes the performance of hybridization, washing, and detection significantly simpler. Colorimetric detection is based on enzymatic amplification of the signal as in the previous technique. It is performed on a specially designed portable scanner. This technique was applied for the development of VINAray (VIrus DNAmicroarray) to determine herpes simplex virus types 1 and 2, varicella zoster virus (VZV), Epstein–Barr virus (EBV), cytomegalovirus (CMV), and human herpesvirus-6 types A and B [119]. The method was shown to be easy to handle and inexpensive, and allows processing of a large number of samples. The additional advantage of the technique is the compatibility with standard laboratory equipment. Akonni Biosystems (Frederick, USA) developed TruArray HE, a qualitative test for the rapid detection of herpes simplex virus 1, herpes simplex virus 2, and VZV in the cerebrospinal fluid. The test involves workflow-integrated amplification, hybridization, detection, and data analysis. The determination takes 3 h. The TruArray HE is the first array-based test for rapidly detecting and differentiating between three of the most important viral cerebrospinal fluid infections. In the next section, microarrays experimentally developed for identifying individual types of viruses will be described.

### 4.4.1
### Human Papillomaviruses

Human papillomaviruses (HPVs) are causal agents involved in the development of carcinomas in the cervix uteri as well as pathological changes of the skin. Papillomaviruses are small, double-stranded DNA viruses with an icosahedral capsid, and infect the stratified epithelium of mucosal or cutaneous surfaces. The entire HPV class consists of over 120 viruses. The oncogenic activity of HPV is dependent on its genotype. The virus types are classified into high-risk types associated with cervical cancer and recognized as playing a role also in other cancers (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) and low-risk types known as *causative pathogens of condyloma acuminatum* (types 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and 89) [120]. To ensure correct diagnostics, adequate treatment, monitoring of HPV vaccine implementation, and monitoring of coinfections with multiple HPVs, it is desirable not only to detect HPV but also perform the genotyping assay. Currently, testing and genotyping of HPV is seen as a complement or substitute for analysis of cytology for cervical cancer screening. Various experimental approaches combine the consecutive studies of microarray technique steps: extraction of viral DNA, amplification by multiplex PCR, followed by hybridization on the array. Most established HPV typing assays are based on consensus PCR to amplify the relatively conserved L1 gene region. One of the recently custom-designed microarrays was aimed at the detection of all 120 known HPV genotypes [121]. The microarray was fabricated by Agilent inkjet printing technology for *in situ* synthesis of oligonucleotide probes on a slide. The probe sequences were located on the L1 gene segments and targeted the amplicon sequences of MY of 450 bp and FAP of 480 bp. The final microarray contained in total more than 4300 probes 60-mer in length in six replicates distributed in a random layout. Hybridization of two labeled amplicons took 36 h. Combination of two targeted amplicons improved the assay specificity. It was pointed out that a probable limitation of the array design was lack of target-specific probes for E6/E7 genes. Simultaneous detection of L1, E6, and E7 genes will widen the detection for low- and high-grade dysplasia and cancer. In 2011, Gheit and colleagues demonstrated the APEX (arrayed-primer extension) chip for the genotyping of 19 HPV types [122]. The method involves the hybridization of PCR amplicons with oligonucleotide probes arrayed onto silanized slides. Then oligonucleotides were extended with labeled cyanine 5-ddUTP and detected. The method had been developed previously and repeatedly published [119, 123–125]. With the aim to improve hybridization efficiency and, thus, the analytical performance of the technique, a method with peptide nucleic acids (PNAs) as probes was developed for genotyping of 32 medically important HPVs [126]. PNAs contain uncharged *N*-(2-aminoethyl)-glycine scaffold instead of a negatively charged ribose–phosphate nucleic acid backbone, and nucleotide bases are attached via a methylene carbonyl linker. PNAs can complementarily hybridize with DNA, which makes their application very promising. PNA–DNA duplexes reveal higher stability than their DNA–DNA or DNA–RNA analogs. The synthetic backbone

of PNAs also means that they are not degraded by nucleases or proteases. This results in a high level of resistance to enzyme degradation and extended lifetime of PNA probes. The total time of PNA arrays starting from DNA extraction, was 4.5 h.

#### 4.4.1.1 Commercial HPV Tests

At present one of the most common tests used to detect genital HPV in cervical samples is the Hybrid Capture II (HCII) (Digene HPV test; Qiagen) assay [127]. The HCII assay is based on hybridization in a solution with a mixture of nonisotopic, single-stranded, full-length RNA probes against 13 high-risk types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68). The assay detects several high-risk types in one reaction and uses a convenient format. However, it does not provide any genotype-specific information. Recently, a new commercially available CE-marked genotyping assay, the CLART HPV 2 (CLART) (Genomica, Madrid, Spain), was developed for the detection and genotyping of 35 HPV types (20 high-risk and 15 low-risk types) in single or multiple infections (Table 4.2). The assay is based on PCR and hybridization on a low-density array. The method was evaluated with 425 cervical samples [128], and the data were compared with the HCII test, which has a defined clinical cutoff. For the detection of 13 high-risk HPV types, both the CLART-13 HR-HPV and the HCII assays showed identical clinical performance, with a clinical sensitivity of 96%. For 17 HR-HPV types, the clinical sensitivity of the CLART assay was 96.9%. The specificity of the CLART and the HCII assays was also very similar. The CLART assay has the advantage in the amount of HPV types, detected in a single test, which is important for the screening and studies required for vaccination trials and for monitoring the efficacy of HPV vaccines.

Another commercially available assay for the detection and genotyping of HPV in routine clinical specimens is CAPH (Clinical Arrays Papillomavirus Humano) (now CLART® HPV2, Table 4.2), Genomica, Spain). In studies, it detected simple or mixed-type infections with 35 HPV types (20 high-risk and 15 low-risk types). The method was validated with 408 clinical samples [129]. A fairly good agreement was found between the results obtained by CAPH assays and HCII. However, in 20.1% of the HCII-positive specimens, the CAPH assay identified LR- and HR-HPV types that were not included in the first assay. These results could point to unspecific cross-reactivity between the probes used for the 13 HR-HPV types included in the HCII kit and other types (i.e., types 6, 11, 53, 61, 66, 70, 72, 81, and 85). False-positive results are less likely in the CAPH assay, since it can detect the 35 most common HPV genotypes. By the CAPH assay, 9.5% of HCII-positive specimens were negative, and 44% of them had a negative Papanicolaou smear test. It was found that screening with HCII tests, followed by HPV detection and genotyping with the CAPH assay, allowed optimal identification of women at high risk for the development of cervical cancer. Furthermore, a better prediction of disease progression was achieved.

The INFINITI HPV-QUAD (AutoGenomics, Carlsbad, CA) assay is a commercially available genotyping platform for the detection and identification of HPV,

in which multiplex PCR is followed by automated processing for allele-specific primer extension and, then, hybridization. The probes target the E1 gene of the HPV genome. The analytical performance of the HPV-QUAD assay was evaluated using liquid cervical cytology specimens, and the results were compared with those obtained using the HCII test (Digene HPV test; Qiagen) [130]. The comparison performed on 197 cervical specimens showed an overall agreement of 83%. Also, it was found possible to use for HPV QUAD different types of specimens including denatured material after the HC2 assay. This may improve the workflow by allowing the testing directly from HC2 assay.

Simultaneously with microarrays on solid supports, high-throughput arrays of another type have been developed. A novel DNA detection assay incorporating the Luminex xMAP suspension array based on using 5.6 mm polystyrene microspheres was applied to the genotyping of HPV in cervical samples [131]. The method was developed for the detection of 26 HPVs (16 high-risk types and 10 low-risk types). To evaluate clinical performance of the liquid bead array, 624 cervical samples were tested, and the HPV genotyping results were compared with those from commercially available systems based on hybridization on solid chips and the DNA sequencing method. The sensitivity for detection of high-risk HPV was 99.0% (liquid bead array), 94.7% (HPV DNA chip (MyGene)), and 85.3% (HCII (Digene Diagnostic)). The suspension array had proven to be as accurate as sequencing in identifying individual HPV types, even in cases with multiple HPV infections. Mistyping between high- and low-risk types was 5.4 and 1.1% for the DNA chip and the liquid bead array methods, respectively. Most of mistyping occurred in samples from multiple infections. The high-level mismatches with DNA chip technology might be explained by the sequence similarity among the 24 probes designed against the 150 bp L1 fragment. As a result, the liquid bead array system demonstrated better performance because of the favorable hybridization kinetics, which was similar to the kinetics of solution-phase hybridization. The accuracy of genotyping in the liquid bead array was comparable to that of the sequencing method. This technique has advantages to be automated or deployed on a high-throughput platform and applied for large-scale epidemiological and vaccination studies.

The use of premanufactured array limits the possibility of identifying new variants of a target or novel pathogen strains. Some approaches use a platform with the ability to synthesize *in situ* the oligonucleotides with unique probe sequences, designed by the researcher. The method of application of the Combimatrix® B3 CustomArray™ technology (Combimatrix, Seattle, USA) for HPV strains detection has been reported [132]. The technology of the CustomArray™ is based on a semiconductor chip bearing up to 95 000 electrodes that can be individually activated digitally, enabling the synthesis of oligonucleotides of different lengths (up to 40 bases) using standard phosphoramidite chemistry. The hybridization of fluorescently labeled DNA takes 16.5 h, which is followed by the fluorescent detection. Different studies were aimed at evaluating the microarray technology useful for the simultaneous detection and typing of HPV in routine clinical diagnostics. From demands of routine clinical requirements, faster methods

and those that do not need sophisticated equipment and data management are preferable. Several low- to medium-density arrays, based on the hybridization of labeled target DNA with capture probe with fluorescent [133, 134] or colorimetric detection [135, 136], have been developed for HPV genotyping. Another method was based on multiplex amplification of the viral L1 region with improved primers and subsequent ligation reaction of two adjacently hybridized ssDNA probes [137]. The probes constitute a target–specific probe pair, which becomes detectable only if the probes are ligated together in the presence of a complementary target. One of the specific ssDNA probes contained a fluorescent label. After ligation, the mixture is hybridized on the array. Additional ligation step improves the selectivity of the method.

An electrochemical genosensor array method was used for the simultaneous detection of three high-risk human HPVs (16, 18, and 45) found in invasive cervical cancer [138]. The method uses hybridization in a sandwich format on an array consisting of 16 gold working electrodes. Electrochemical detection utilizes horseradish peroxidase as a reporter for second revealing probes. Hybridization time was 1 h. The LODs were 220, 170, and 110 pM for HPV 16, 18, and 45, respectively. To maintain the desired balance of high sensitivity of the assay and the low cost of analysis, many improvements have been made for signal detection and amplification techniques. New developments in HPV testing on microarrays involve the investigation of nanoparticles as labels. A method for introducing luminescent silica nanoparticles into DNA via the classical linkage system of biotin–streptavidin was described by Riccò *et al.* [139]. It was shown that the incorporation of dyes into silica spheres instead of free dyes improves signal intensity and sensitivity of the assay. These nanostructures were found to be suitable labels for the microarrays on glass slides. The principle of light scattering by silica nanoparticles was used to develop a simple DNA chip with visual detection [140]. Silica particles were used as labels for the probes, which, during sandwich hybridization, form a dendrimer-like network of particles which increase markedly the aggregation degree of the particles. The spots with positive hybridization appeared brightly white and visible. Then they may be detected with a flatbed scanner or even by the naked eye. When applied to a target HPV DNA, the LOD was 1 nM by using a flatbed scanner. The total time used for the two-step hybridization analysis, including sample preparation and amplification, was 5–6 h, which is comparable to that of commercial HPV DNA chips with fluorescent detection.

### 4.4.2
### Respiratory Viruses

Detection of acute respiratory infections of viral etiology remains one of the most urgent tasks in clinical virology. In humans, these viruses cause recurrent annual epidemics and have a significant effect on health systems worldwide. Their impact increases when they affect immunocompromised adults or those with chronic deceases, children, and older people. This group of viruses involves respiratory

viruses, influenza viruses, adenoviruses, enteroviruses, and others. The early diagnosis of these viruses is crucial in order to avoid unnecessary antibiotic use, to establish the appropriate treatment, and to prevent infection transmission. Recent advantages in diagnostics have aimed at improving the molecular-genetic methods, their main limitation being caused by genetic variability as many respiratory viruses are RNA viruses. Currently, various commercial molecular assays, which are based on a combination of PCR and hybridization using DNA arrays, have been developed for the identification of respiratory viruses. The Luminex xTAG® Respiratory Viral Panel (RVP) (Luminex Molecular Diagnostics) was the first panel that received FDA approval. It detects influenza A and B, including seasonal H1 and H3, adenovirus, parainfluenza 1–3, respiratory syncytial virus A and B, human metapneumovirus, and human rhinovirus. xTAG RVP showed a sensitivity and specificity of 100 and 91%, respectively, and also had the ability to differentiate human from nonhuman influenza A H1. Its version RVP Fast takes 8–10 h to accomplish the whole procedure [141].

CLART Pneumovir DNA array assay (Genomica, Spain) was developed to detect 11 genera of respiratory viruses simultaneously [142]. This is a low-density microarray with colorimetric detection based on biotin as a label which is introduced in DNA during amplification reaction and is revealed after hybridization with a streptavidin–peroxidase conjugate. The method takes 8 h after nucleic acid extraction and is characterized by high sensitivity and broad detection of respiratory viruses. Its latter version CLART 1 Pneumovir DNA assay allows simultaneous detection and identification of 17 types and subtypes of human respiratory viruses. The results of the array were compared with those of conventional virological methods (direct immunofluorescence and virus isolation) and real-time PCRs for the detection of respiratory viruses [143]. The microarray showed high sensitivity, ranging from 10 to 100 copies of viral genomes per amplification tube, and higher detection rates (91% vs 70%), even for common respiratory viruses (78% vs 68%). The DNA microarray identified 67% of mixed respiratory tract infections, whereas none was detected by the conventional respiratory virus detection assays.

Different microarrays were developed for the subtyping of influenza A viruses. Influenza A virus consists of eight negative single-stranded RNA segments and can be classified into various subtypes based on structure differences of two surface glycoproteins: hemagglutinin (HA) and neuraminidase (NA). A total of 16 HA genotypes (H1–H16) and 9 NA genotypes (N1–N9) have been identified. Seasonal epidemics require rapid typing methods for the determination of these alleles. Several microarrays based on classical method of hybridization analysis on glass slides with fluorescent detection were developed, which differed in their approaches for probes design and selection [144–146]. Perhaps the most promising area in applying microarray technology in clinical microbiology is the use of low- or middle-density microarrays. Such arrays may be used for both diagnostic testing and epidemiological investigations. Examples of such arrays for subtyping of influenza A viruses were described by Cannon *et al.* [16] and Heil *et al.* [147]. Several assays were transferred to a microtiterstrip or 96-well plate

format allowing simultaneous testing of 8–96 samples [148, 149]. In the assays adapted for use in clinical practice, the main improvements concern replacing expensive instrumentation with low-cost detection technology and simplification of the assay performance. Several methods use colorimetric detection based on biotin as a label for target, which is then revealed with streptavidin conjugated to peroxidase [148], a photoinitiator label [150], or gold nanoparticles enhanced by silver staining [151]. The latter method identifies H5N1 viral nucleic acid and simultaneously provides subtype identification of influenza A virus in the absence of target amplification. The target, which is whole viral genome RNA, hybridizes with immobilized capture probes. Then the intermediate oligos with poly A tail form a sandwich complex, which can bind another poly-dT, modified with gold nanoparticles. Twenty samples can be tested in approximately 2.5 h after RNA extraction. A silicon-based microfluidic system was recently developed for the specific detection of viral strains (influenza A and H1N1 2009) [152]. The system was composed of two functional modules: a multiplexed PCR module, and a multiplexed silicon nanowire module for DNA determination. The modules were fabricated by a top-down, standard CMOS technology. The sensitivity achieved was of 20–30 fg $\mu l^{-1}$ for H1N1 and seasonal FluA in 10 μl of a sample.

### 4.4.3
### Enteric Viruses

An microarray based on the Combimatrix platform was developed for the identification of enteric virus pathogens (norovirus, rotavirus, adenovirus, and astrovirus) [153]. These viruses are important foodborne and waterborne pathogens and frequently cause nonbacterial gastroenteritis worldwide. The probes were of standard types, of discrete tiling types, and of overlapping types. A total of 551 oligonucleotide probes were spotted onto the array. The fluorescently labeled DNA samples were generated by nested PCR. It was demonstrated that the multiplex RT-PCR assay specifically amplified partial sequences of four enteric viruses and the subsequent microarray assay was capable of sensitive and simultaneous detection of those viruses.

### 4.4.4
### Hepatitis Viruses

The hepatitis viruses, named A–E, cause acute and chronic liver disease depending on the virus. Laboratory-based diagnosis usually relies on the detection of specific markers of infection for each virus by the use of immunological tests. Molecular tests for viral nucleic acids began to be used for laboratory diagnostics and in monitoring antiviral treatment efficacy. A DNA microarray on the Affymetrix *in situ* synthesis technology combined with fluorescent detection was developed to identify hepatitis B virus (HBV) genotypes and to detect the mutations in the S, Pol, Core, and X genes [154]. Microarray contained the probes of 20-mer or the detection of 994 mutants located at 298 genomic positions in the HBV

genome. Amplification of whole virus genome makes the assay perspective for the intended clinical use. It allows full analysis of most specimens with more than 5000 genome copies/ml. For samples with lower viral loads, down to approximately 400 genome copies/ml, genotyping and detection of most resistance mutations is still possible. Another microarray for the detection of point mutations in hepatitis B virus associated with antiviral resistance used PNAs to improve the discrimination [155]. The PNA array correctly identified viral mutants and has high concordance (98.3%) with direct sequencing in detecting antiviral-resistant mutations.

A technology of 3D DNA microarrays was applied for the genotyping of hepatitis C virus (HCV) [156]. In this method, oligonucleotide probes are immobilized on the microarray surface in hemispherical hydrogel cells. Immobilization is carried out with the formation of covalent bonds upon ultraviolet irradiation. Assymmetric PCR was used to obtain predominantly single-stranded, fluorescently labeled product. Amplicons were hybridized on the microarray for 14–16 h. The results were 100% concordant for the genotype and 99.7% concordant for the subtype with the results obtained by direct sequencing of the NS5B segment. Several microarrays on the determination of hepatitis viruses used biotin as a label for the target DNA. One of the arrays uses further development with a conjugate of streptavidin with a fluorescent label [157]. This method ensures fast determination because of co-hybridization of target DNA and a conjugate at one stage. In another approach for simultaneous determination of HCV and human immunodeficiency virus type-1 (HIV-1), the development of biotin was performed by a conjugate of streptavidin–gold nanoparticles with following silver staining [158]. In one of the latest developments for the identification of HCV, a single platform was proposed for the determination of virus gene and anti-HCV antibodies [159]. A universal probe of 59 bases was designed, which covered all different HCV genotypes. Thiol-modified oligonucelotides were immobilized onto epoxy-modified glass slides. Fluorescent label Cy 3 was introduced into target DNA in the course of reverse transcription PCR. The efficiency of hybridization at short-time incubations was compared for different reaction formats: direct hybridization of labeled target DNA and indirect hybridization with several labeled detection probes having certain degeneracies for targeting different virus genotypes. Protein probes contained HCV NS4 and core antigen. Serum samples were used as a target, and sheep anti-mouse IgG–Cy3 conjugate developed the immune complexes formed on a protein microarray. Optimization of the assay performance made it possible to reduce significantly the assay time for both antibodies and DNA to approximately 15 min without any loss of sensitivity. This approach is considered a prerequisite for a point-of-care test for the rapid detection of HCV infection in clinical samples.

In recent years, great efforts are being made to develop of miniaturized multiplex microfluidic technology for rapid diagnosis suitable for clinical settings. It intergates the efficient sample handling by microfluidics with the high-throughput parallel analysis on microarrays. A novel microfluidic device with microbead array and quantum dot as labels was developed for HBV genotyping [160]. The integration of a polystyrene beads-based array into microfluidic technology

combines the rapid binding kinetics of homogeneous assays with improved liquid handling and high sensitivity of the fluorescence detection of quantum dots. The method showed the detection limit of 0.03 nM of synthetic DNA target using TAMRA as label, which corresponded to an absolute detection sensitivity of $1 \times 10^{-16}$ mol of target molecules in a sample volume of 10 μl. The assay time after sample preparation was less than 1 h. The latest microfluidic microbead-based system for the rapid diagnosis of the HIV-1 has integrated chambers for a sample pretreatment, nucleic acid amplification, cell lysis/hybridization, and an optical detection module [161]. Four primer sets were designed targeting the most conserved regions in vpr, tat, gag, and LTR. The system performed the purification and enrichment of target DNA from infected T cells as well as multiple detection of HIV-1 by the probe-coated beads in real time within 95 min. The microfluidic chips have obvious advantages for fast, accurate, and inexpensive diagnostics. The microarrays recently developed for the identification of some other viruses are listed in Table 4.5.

## 4.5
## DNA Microarrays for Detection of Fungal Pathogens

In recent decades, a clear tendency to increase the frequency and expand the infections caused by opportunistic fungi has been observed worldwide. This occurs as a result of advanced medical treatments and the increase in the number of immunosuppressed patients and patients with autoimmune disorders. These potential pathogens are widely distributed in nature and diverse in their morphological and biological characteristics, the fungi belonging to the genera *Candida, Aspergillus, Rhizopus, Mucor, Absidia, Penicillium*, and so on. They have a common property – the ability for long-term contact with the skin and mucous membranes of humans either without causing lesions or causing them only in the presence of additional factors that inhibit local and systemic host defense mechanisms. Numerous yeasts and molds contribute to clinical diseases, and *Candida* spp. and *Aspergillus* spp. were the most common so far with predominance of *Candida albicans*. But because of changes in treatment strategies, patterns of fungal species have shifted from yeast infections toward a higher proportion of non-albicans *Candida* and molds, including various species of *Aspergillus*, *Fusarium*, and *Mucorales*. An increase in rare mold infections, which are often resistant to antifungal drugs, was also recently observed.

In view of the growing incidence and high mortality rates of invasive mycoses, the effective detection of fungal pathogens in the clinical material and the proper assessment of their value are of great importance both for the individual patients and for understanding the pathology mechanism. Conventional approaches such as microscopy, cultivation, and histopathology are based on morphological and physiological tests, but the diagnosis remains difficult because of limited sensitivity and specificity; also they require several days or even weeks for analysis. Changes in fungal patterns make it necessary to identify a variety of pathogens in

**Table 4.5** DNA microarrays for human virus detection.

| References | CC | Target species | Sample | Preanalytics | Detection | TTR |
|---|---|---|---|---|---|---|
| [109] | US | Over ~1500 viruses, full spectrum of viruses associated with respiratory infections, novel viral pathogens, such as SARS coronavirus and 2009 pandemic H1N1 influenza virus | Nasal swab sample | NA extraction, RT of viral RNA, amplification by random PCR, a smear of 200–1000 bp | Virochip contains ~36 000 probes, fluorescent label Cy3, hybridization (overnight) | ~24 h |
| [110] | US | 1710 vertebrate virus species, 135 bacterial, 73 fungal, and 63 parasite genera | Nasopharyngeal aspirates, blood, urine, and tissue | NA extraction, random primed PCR amplification | GreeneChipPm, GreenChipVr, high-density arrays on glass slides, fluorescence detection | No clear data: sample prep., 16 h hybridization, washing, readout, data analysis |
| [111] | US | Bacteria and all known viruses (including phages), ~2200 virus species, ~900 bacterial species | Fecal, serum, and respiratory samples | RNA extraction. RT to DNA, PCR | Lawrence Livermore Microbial Detection Array (LLMDA), high density oligo microarray, ~38 000 probes for viruses, ~3500 for bacteria, hybridization (16 h), fluorescence label | >24 h |
| [112] | US | Various viruses and bacteria | Cerebrospinal fluid, urine, serum, cervical smear, feces, skin lesion, and tracheal aspirate | NA extraction, RT of viral RNA, multiple displacement amplification (MDA)-by the Phi29 polymerase | Lawrence Livermore Microbial Detection Array (LLMDA version 2), labeling using nick translation with Cy3-labeled random nanomer primers | >24 h |

(*continued overleaf*)

**Table 4.5** (*Continued*)

| References | CC | Target species | Sample | Preanalytics | Detection | TTR |
|---|---|---|---|---|---|---|
| [131] | KR | Human papillomavirus, 26 HPV types (16 high-risk and 10 low-risk types) | 624 cervical samples | DNA extraction, Amplification of the L1 gene by PCR | Luminex xMAP suspension array, biotin as a label, development with streptavidin-R-phycoery-thrin, fluorescence detection | n/a |
| [128] | PT | Human papillomavirus, 35HPV types (20 high-risk and 15 low-risk types) | 425 cervical samples | PCR with biotinylated primers, amplification of a fragment a 450 bp fragment within the HPV, L1 region | CLART human papillomavirus 2, low-density microarray, biotin as a label | n/a |
| [129] | ES | Human papillomavirus, 35 HPV types (20 high risk and 15 low risk) | 408 cervical specimens | DNA extraction, multiplex PCR | Microarray hybridization on Genomica HPV test, CAPH (clinical arrays papillomavirus humano) | 8 h |
| [132] | UK | Human papillomavirus | Virus strains | Labeled DNA | Combimatrix B3 CustomArray fluorescent label Cy3 or Cy5, 16.5 h hybridization | No clear data: sample prep., 16.5 h hybridization, washing, readout, data analysis |
| [121] | US | Human papillomavirus 120 HPV types | Cervicovaginal specimens | Genomic DNA extraction, PCR | Glass slides, fluorescence label Cy3 or Cy 5-dUTP, 36 h hybridization | No clear data: sample prep., 36 h hybridization, washing, readout, data analysis |

| | | | | | | |
|---|---|---|---|---|---|---|
| [138] | ES | Human papillomavirus | Cervical samples | DNA extraction, PCR | Electrochemical genosensor array, microfluidic cell, sandwich format, HRP-labeled secondary reporter probe | No clear data: sample prep., 2 × 20 min hybridization, HRP reaction, readout, data analysis |
| [139] | IT | Human papillomavirus | DNA strand | PCR | Glass slides, luminescent silica nanoparticles as a label, 3 h hybridization | No clear data: sample prep., 3 h hybridization, washing, readout, data analysis |
| [140] | KR | Human papillomavirus, synthetic HPV-16 target | The SiHa cell line | DNA extraction, PCR-amplified HPV-16 DNA target | Sandwich DNA hybridization, visual detection based on the light scattering of silica nanoparticles, dendrimer-like silica particle networks | 5–6 h |
| [130] | US | Human papillomavirus | 197 cervical specimens | DNA extraction, multiplex PCR, allele specific primer extension | The INFINITI HPV-QUAD assay, film-based chip array, fluorescence label, 1.5 h hybridization | No clear data: sample prep., 1.5 h hybridization, washing, readout, data analysis |
| [126] | KR | Human papillomavirus, 32 HPV types | 72 cervical scrapes | DNA extraction, single-tube nested PCR, one biotinylated primer | Peptide nucleic acid (PNA) probes, Cy5-streptavidin | 4.5 h |

(*continued overleaf*)

**Table 4.5** (*Continued*)

| References | CC | Target species | Sample | Preanalytics | Detection | TTR |
|---|---|---|---|---|---|---|
| [134] | KR | Human papillomavirus, 14 high-risk HPV types and 5 low risk types | Cervical specimens | DNA extraction, PCR | HPV 9G DNAChips, fluorescent label Cy 5 | 1 h (after PCR) |
| [135] | IT | Human papillomavirus, HPV 6, 16, 31, and 51 | — | Real-time PCR | A plastic modular chip, two sequential steps performed on the same device, development with streptavidin–alkaline phosphatase, colorimetric detection | No clear data: 0.5 h hybridization, 0.7 h detection |
| [133] | DE | Human papillomavirus, 12 HPV types | — | DNA extraction, PCR | 36 probes, Streptavidin-Cy5 conjugate, fluorescence detection with CCD camera | 3 h |
| [136] | DE | Human papillomavirus | 123 gynecological swab samples | DNA extraction, PCR with biotin labeling, exonuclease digestion | Hydrogel-based biochip assay, Streptavidin-Cy5, Streptavidin-alkaline phosphatase | 3 h |
| [137] | FI | Human papillomavirus, 15 high-risk HPV types and 2 low risk types | Cervical samples | DNA extraction, multiplex PCR, ligation, one of the probes is fluorescence-labeled | Low-density universal microarray | No clear data: sample prep., 2 h hybridization, washing, readout, data analysis |
| [122] | FR | Human papillomavirus, 19 HPV types | Cervical samples | DNA extraction, multiplex PCR | APEX chip based on primer extension, fluorescence detection (Cy5) | n/a |

| | | | | | | |
|---|---|---|---|---|---|---|
| [142] | FR | 11 respiratory viruses, RSV A and B; Flu virus A, B, C; PIV 1, 2, 3, 4A, 4B; hMPV A and B; ADV (33 subtypes), EV (50 subtypes), hRV (65 subtypes); HCoV (subtype 229E); HBoV | Nasopharyngeal or tracheo-bronchic aspirates | RNA extraction, two multiplex RT-PCR or PCR | CLART Pneumovir DNA array (Genomica, Spain), biotin as a label, colorimetric detection with streptavidin–peroxidase conjugate | 8 h (after nucleic acid extraction) |
| [143] | FR | 17 respiratory viruses (influenza A including seasonal A/H1N1 and A/H3N2 strains, influenza B, influenza C, parainfluenza 1, 2, 3, 4A, and 4B, hRSV A and B, human rhinoviruses, adenoviruses, EVs specieB, human bocavirus, coronavirus E-229, and the human metapneumovirus A and B | 138 nasopharyngeal aspirate samples | DNA/RNA extraction, RT-PCR, or PCR | CLART1 PneumoVir kit V16.3 (Genomica, Spain), biotin as a label, colorimetric detection with streptavidin–peroxidase conjugate | 8 h (after nucleic acid extraction) |
| [114] | FR | Influenza viruses from different host origins (humans, pigs, horses, and birds) | Virus strains | RNA extraction, WTA amplification | PathogenID v2.0 – a resequencing microarray, Affymetrix protocol | n/a |
| [144] | CN | Influenza A (H1N1) virus | Throat swab specimens | RNA extraction | Aldehyde-activated slides, fluorescence detection (Cy3 or Cy5) | No clear data: sample prep., 2.5 h hybridization, washing, readout, data analysis |
| [145] | CN | Influenza A virus of five subtypes: H1N1, H1N2, H3N2, H5N1, and H9N2 | Virus strains, throat swabs, and nasopharyngeal swabs | RNA extraction, RT-PCR | Aldehyde-modified glass fluorescent label Cy3 | 7 h |

(*continued overleaf*)

**Table 4.5** (*Continued*)

| References | CC | Target species | Sample | Preanalytics | Detection | TTR |
|---|---|---|---|---|---|---|
| [146] | RU | Influenza A virus | Tissue culture | Genomic RNA isolation, RT-PCR | Fluorescence detection | No clear data: sample prep., 2 h hybridization, washing, readout, data analysis |
| [150] | US | Influenza | — | RNA extraction, RT-PCR, lambda exonuclease treatment | The ampliPHOX colorimetric detection technology | 6 h |
| [151] | US | Major influenza A virus strains (H1N1, H3N2) | Virus isolates | RNA extraction, PCR-free | Hybridization in sandwich format, gold nanoparticle as a label, silver staining | 2.5 h (after RNA extraction) for 20 samples |
| [16] | IE | 16 viral and two atypical bacterial respiratory pathogens | Throat swabs | NA extraction, PCR | Low density oligonucleotide microarray, biotin as a label, development with streptavidin–horseradish peroxidize (15 min) | No clear data: sample prep., 1 h hybridization, washing, HRP reaction (15 min), readout, data analysis |
| [147] | US | Influenza A/H1N1 of various species origin (human H1N1, human H3N2, 2009 pandemic influenza A H1N1, swine H1N1, and a wide variety of avian, equine, canine, and swine influenza viruses) | — | RNA extraction, RT-PCR | MChip, a low density microarray, artificial neural network (ANN) | No clear data: sample prep., 1 h hybridization, washing, readout, data analysis |

| | | | | | | |
|---|---|---|---|---|---|---|
| [148] | DE | Influenza virus A | Throat swabs, bronchoalveolar lavage | RNA extraction, RT PCR | Microarray in 8-well microstrip format, Biotin as a label, colorimetric detection based on horseradish peroxidase | No clear data: sample prep., 1 h hybridization, washing, HRP reaction, readout, data analysis |
| [149] | FI | 10 common adenovirus genotypes causing human infections | Nasopharyngeal specimens | RT-PCR | Microarrays in 96-well microtiter plates, photoluminescence | No clear data: sample prep., 1 h hybridization, washing, readout, data analysis |
| [152] | SG | Influenza A (H1N1) | DNA templates from hemagglutinin gene and polymerase basic protein 2 | PCR | Silicon-based microfluidic system | No clear data: PCR, hybridization (0.25–0.33 h) |
| [162] | FI | Enteroviruses (*Picornaviridae* family) | Virus cell cultures | RNA extraction, RT-PCR, Cy5-labeled reverse primer | Microarrays in 96-well microtiter plates | No clear data: sample prep., 1 h hybridization, washing, readout, data analysis |
| [153] | KR | Enteroviruses: Adenoviruses (AdVs), astroviruses (AstVs), noroviruses (NoVs), and rotaviruses (RoVs) | Patients' stool samples | NA extraction, multiplex RT-PCR | The CombiMatrix CustomArray (Macrogen, KR), fluorescent label Cy3 | 19–20 h |

(*continued overleaf*)

**Table 4.5** (*Continued*)

| References | CC | Target species | Sample | Preanalytics | Detection | TTR |
|---|---|---|---|---|---|---|
| [159] | UK | Hepatitis C virus | Serum | Total RNA extraction, RT-PCR | DNA microarray test to detect HCV RNA and a protein microarray to detect human anti-HCV antibodies on a single platform, fluorescence detection (Cy3) | 0.25 h (after RT-PCR) |
| [156] | FR | Hepatitis C virus | 345 positive serum samples | RNA extraction, asymmetric PCR | Low-density hydrogel-based biochips, fluorescence detection | No clear data: sample prep., 14–16 h hybridization, washing, readout, data analysis |
| [154] | FR | Hepatitis B virus genotypes and mutations | Serum | DNA extraction | The Affymetrix in situ synthesis technology, fluorescent label | n/a |
| [155] | KR | Hepatitis B virus, antiviral resistance | 68 Serum samples | DNA extraction, the single-closed-tube nested PCR method | An array using peptide nucleic acid (PNA) biotin-labeled PCR fragments Cy5–streptavidin | n/a |
| [157] | KR | Hepatitis C virus | Serum | RT-assym PCR | Biotin-labeled PCR product and streptavidin–Cy3 conjugate were cohybridized simultaneously | <2 h (after amplification) |

| | | | | | | |
|---|---|---|---|---|---|---|
| [160] | CN | Hepatitis B virus | HBV DNA samples | PCR, T7 *in vitro* transcription | Microfluidic chip, SuperAvidinTM coated polystyrene beads, quantum dot as labels | <1 h (after sample prep.) |
| [163] | CN | Hepatitis C virus | 607 positive serum samples | RNA extraction | An oligonucleotide chip genotyping test, colorimetric detection | n/a |
| [158] | CN | Human immunodeficiency virus type-1 (HIV-1) and hepatitis C virus | Blood | NA extraction, Multiplex asymmetric PCR | Glass slides, biotin as a label, gold label silver staining, visual detection | No clear data: sample prep., 3 h hybridization, washing, silver staining |
| [161] | TW | Human immunodeficiency virus-1 (HIV-1) | HIV-1 cell line samples | — | Microfluidic system using magnetic beads, optical detection | 2 h |
| [164] | FR | 9 DNA and RNA neurotropic viruses: herpes simplex virus 1 (HSV-1), HSV-2, varicella-zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpesvirus 6 (HHV-6), HHV-7, HHV-8, and the human enteroviruses (HEVs) | Cerebrospinal fluid | Total NA extraction, Viral genome-specific fragment amplification by multiplex PCR | Arrays in eight-well strips, colorimetric detection | n/a |
| [119] | DE | Herpes simplex virus types 1 and 2, varicella zoster virus, cytomegalovirus, Epstein–Barr virus, human herpesvirus-6 types A and B, and adenovirus genera A–F | Plasma | DNA extraction, PCR | VINAray, CLONDIAG chip Technologies, ArrayTube, biotin as a label, horseradish peroxidase–streptavidin conjugate | No clear data: sample prep., 1 h hybridization, washing, HRP reaction, readout, data analysis |

(*continued overleaf*)

**Table 4.5** (*Continued*)

| References | CC | Target species | Sample | Preanalytics | Detection | TTR |
|---|---|---|---|---|---|---|
| [115] | FR | Highly pathogenic viruses Several hemorrhagic fevers Crimean–Congo hemorrhagic fever virus, five different families of BSL-4 viruses (*Arenaviridae*, *Bunyaviridae*, *Filoviridae*, *Flaviviridae*, and *Paramyxoviridae*) | Infected cell supernatant, organ, serum | RNA extraction, reverse transcription, Isothermal amplification by Phi29 polymerase | High-density resequencing microarray PATHOGENID v2.0 | 48 h |
| [117] | DE | Crimean-Congo hemorrhagic fever virus | 63 serum samples | RNA extraction, RT-PCR | Low-density macroarrays on polymer support, biotin as a label, colorimetric detection | No clear data: sample prep., 0.5 h hybridization, washing, readout, data analysis |
| [118] | DE | Lassa Virus | Serum | RNA extraction, RT PCR | Custom-made array by Chipron, colorimetric detection based on horseradish peroxidase | n/a |

**Table 4.6** DNA microarrays for human fungal pathogen detection.

| References | CC | Target species | Sample | Preanalytics | Detection | TTR |
|---|---|---|---|---|---|---|
| [165] | TW | 20 species of fungi *Acrophialophora Fusispora Aspergillus* spp., *Candida* spp., *Paecilomyces*, and other | 75 sputum samples | DNA extraction, PCR amplification of ITS regions with universal primers | Middle density arrays on nylon membrane, digoxigenin as a label, development with antidigoxigenin antibodies-alkaline phosphatase, colorimetric detection | 8 h (after DNA extraction) |
| [166] | IT | 24 species belonging to 10 genera, *Candida* spp. *Aspergillus* spp., *Trichophyton species*, *C. neoformans*, *T. cutaneum*, *E. floccosum*, *F. solani*, *M. canis*, *P. marneffei*, and *S. cerevisiae* | 20 clinical fungal isolates | DNA extraction, amplification of the regions of ITS1, ITS2, and the 5.8S rRNA gene with universal primers | Microarray based on arrayed-primer extension technique (APEX), fluorescence detection (Cy5) | 5–6 h (after DNA extraction) |
| [167] | TW | 64 fungal species | 57 clinical isolates | DNA extraction, amplification of the ITS regions and a part of the 5.8S ribosomal DNA with universal primers | Microarrays on a nylon membrane, digoxigenin as a label, development with antidigoxigenin antibodies-alkaline phosphatase, colorimetric detection | 24 h (starting from isolated colonies) |
| [168] | TW | 77 yeast species (16 genera) and 66 mold species (33 genera) *Candida* spp. *Aspergillus* spp. *Trichosporon* | 116 blood samples | DNA extraction, amplification of the ITS regions with universal primers | Two middle density arrays on nylon membrane, digoxigenin as a label, development with antidigoxigenin antibodies-alkaline phosphatase, colorimetric detection | 16–24 h (from the time at which growth was detected) |

(*continued overleaf*)

**Table 4.6** (*Continued*)

| References | CC | Target species | Sample | Preanalytics | Detection | TTR |
|---|---|---|---|---|---|---|
| [169] | DE | 12 target species *Candida* spp. *Aspergillus* spp. | 21 strains from clinical isolates | DNA extraction, amplification of the ITS regions with universal primers | Oligonucleotide microarray on glass slide, fluorescence detection (Cy3), data normalization | 4 h (after DNA extraction) |
| [170] | ZA | Potentially mycotoxigenic fungi:a total of 40 food-borne fungi including *Alternaria*, *Aspergillus*, *Bipolaris*, *Claviceps*, *Curvularia*, *Diplodia*, *Drechslera*, *Eurotium*, *Fusarium*, *Penicillium*, and *Pithomyces* | Fungal strains | Total genomic fungal DNA extraction, labeling without amplification | Microarrays on glass slides, fluorescence detection (Cy3 or Cy5) | No clear data: sample prep., 24 h hybridization, washing, readout, data analysis |
| [171] | TW | 23 medically important mold pathogens including *Aspergillus* spp.,*Fusarium* spp., *Mucor* spp.,*Rhizopus* spp., *R. pusillus*,*P. marneffei*, *S. vasiformis*, *A. elegans*, *L. corymbifer*, and *S. racemosum* | Blood samples | DNA extraction, PCR with universal primers | Microsphere-based suspension array, biotin as a label, development with streptavidin-R-phycoerythrin, fluorescence detection | 5 h |
| [110] | US | 1710 vertebrate virus species, 135 bacterial, 73 fungal, and 63 parasite genera | Nasopharyngeal aspirates, blood, urine, and tissue | NA extraction, random primed PCR amplification | GreeneChipPm, high-density array on glass slides, fluorescence detection | No clear data: sample prep., 16 h hybridization, washing, readout, data analysis |

| | | | | | | |
|---|---|---|---|---|---|---|
| [172] | JP | 26 fungal species *Candida* spp. *Aspergillus* spp. *Trichosporon* spp. | Nail specimens, fungal strains | DNA extraction, amplification of full ITS sequence | Microarrays on glass slides fabricated by Bubble Jet technology, fluorescence detection (Cy3) | No clear data: sample prep., 4 h hybridization, washing, readout, data analysis |
| [173] | DE | 14 fungal pathogens (*Aspergillus* spp. *Candida* spp., *F. oxysporum*, *F. solani*, *M. racemosus*, *R. microsporus*, *S. prolificans*, and *T. asahii*) | Blood, bronchoalveolar lavage, and tissue samples | DNA extraction, multiplex PCR for amplification parts of 18S, 5.8S, and ITS 1 regions of the fungal rRNA genes | Oligonucleotide microarray on glass slide, fluorescence detection (Cy3) | n/a |
| [35] | KR | 50 bacteria and 7 pathogens causing bloodstream infections (*Candida* spp., *Aspergillus* spp., *Cryptococcus* spp.) | Blood | Genomic DNA extraction, Two PCRs with universal primers | Glass slides, fluorescence detection | 10 h (from a positive blood culture) |

one test. The necessity of fast methods thus becomes more and more apparent. Molecular-based techniques are becoming attractive because of their sensitivity and specificity, as well as the arrays due to their multiplexing properties and reducing the analysis time. In the past few years, DNA array technology has been used to identify a variety of yeasts and molds. A summary of various arrays developed is presented in Table 4.6. In most cases, oligonucleotide capture probes were used for targeting sequences of the ITS regions of the rRNA gene cassette. A high-density microarray GreeneChipPm designed for detection of a wide range of pathogens involving bacteria, viral, parasite, and 73 fungal genera was developed on the Agilent platform [110]. This DNA microarray allowed the detection of the microbial pathogens causing bloodstream infections and clinically dominant fungi such as *Candida*, *Aspergillus*, and *Cryptococcus*, which account for more than 10% of the pathogens in septicemia. The identification was based on oligonucleotide probes of 60-mer, which were synthesized on glass slides by using an inkjet deposition system. The probes were targeted at the 18S rRNA gene. Another high-density microarray was developed for the identification of 50 bacteria and 7 fungi in blood [35]. As *C. albicans* is an important fungus responsible for more than 50% of fungal bloodstream infections and is associated with high mortality, it was identified at the genus level by selecting a highly conserved regions. *Candida tropicalis* and *Candida parapsilosis*, *Aspergillus*, and *Cryptococcus* were also detected at the genus level. *Candida glabrata* and *Candida krusei* were identified at the species level because of their resistance to fluconazole. The probes were designed on the basis of the ITS regions of the 18S rRNA. Together with the genus- and species-specific probes, the array includes two broad-ranged fungi probes to reduce amount of false-negative results which might be obtained as a result of the sequence diversity among clinical species.

The middle-density microarray on glass slides with fluorescent detection was developed in 2005 [169]. Capture probes of 16–24 bases were complementary to the ITS1 and ITS2 regions of the fungal rRNA genes. The method allowed the discrimination between 12 most common pathogenic *Candida* and *Aspergillus* organisms at the species level. It was the first microarray with a special quantification technique by a normalization procedure to enhance the specificity of the results. DNA microarray based on the same technique was also developed for the determination of for the analysis of neutropenic patients with invasive fungal infections [173]. It uses longer capture probes of 22–70 bases, which were targeted for the 18S, 5.8S, and ITS 1 regions of the fungal rRNA genes. Although universal fungal primers are widely used for target amplification, the authors designed the primers for amplification of the ITS1 region to improve the hybridization efficiency by reducing the amplicon size. It allowed the investigation of noncultured clinical samples. Another array on glass slides with fluorescent detection was constructed for the identification of 26 fungal species [172]. Full ITS regions including the 5.8S rRNA gene were chosen as target nucleic acid to improve the identification at the species level. They were amplified by PCR with universal primers. Capture probes of 20–29 bases were designed for discrimination both at the genus and species level. This type of array was

fabricated using Bubble Jet technology, in which thiolated oligonucleotides were immobilized onto a glass surface modified with maleimide groups. It was found that there was a possibility for false-positive hybridization results for specimens from healthy controls. The reason was related to commensal fungi, which are unavoidable in the clinical setting. Hence, a positive result of fungal DNA by microarrays is the rationale for a more detailed diagnostics.

Further improvements in microarray technology allowed the development of an identification method for oligonucleotide microarray for the identification of potential mycotoxigenic fungi without amplification of target DNA [170]. It was fluorescently labeled using a random approach. This labeling strategy was applied to reduce the secondary structures of DNA. The probes of 14–25 bases were complementary to the sequence variations in the elongation factor 1-alpha and the ITS regions of the rRNA gene. The array contains different probes – genus and species-specific probes and those specific for genes leading to toxin production. A combination of multiple probes for each species improves the discriminatory power of the assay because of the decrease in cross-hybridization, which may be a problem in the identification of different fungal species. Another approach consisted in the introduction of LNAs (locked nucleic acids) into the probe structure. It was also shown to improve the hybridization stringency and decrease cross-hybridization. Microarrays on porous membrane supports with alternative colorimetric detection were developed for the determination of 64 clinically relevant molds [167]. The capture probes were designed against the ITS1 and ITS2 regions of the fungal rRNA genes of filamentous fungi. The detection procedure uses digoxigenin as a label for the target DNA. It is revealed with a conjugate of antidigoxigenin antibodies with alkaline phosphatase. The enzyme catalyzes then the formation of a colored product adsorbed on a surface of the membrane. Later, the same method was adopted for the determination of 20 fungal species in the sputum of patients with cystic fibrosis [165]. It was shown that the technique was relatively simple, time-saving, and more sensitive than determination in a culture. However, neither the array method nor cultures allow differentiation between colonization and a true respiratory infection. At the same time, early detection of colonization is of importance for the initiation of an appropriate antifungal therapy in order to eradicate it before chronic colonization can develop. The presence of fungal DNA was detected in several samples which were negative in culture, which was then confirmed cloning and resequencing of the ITS fragments. A similar colorimetric DNA array was developed for the identification of bloodstream fungal infections [168]. The method involves two arrays and allows identifying 77 yeast species and 66 mold species.

An oligonucleotide microarray based on the APEX technique was developed for the identification of 24 fungal species belonging to 10 genera frequently isolated from invasive and superficial infections [166]. Species-specific oligonucleotide probes were complementary to the ITS1 and ITS2 regions. The labeling of PCR products was not required. The method was based on complementary hybridization of target DNA with immobilized probes and specific extension of a single base at the 3′ side of the anchored probe. Negligible level of cross-hybridization

was detected. The feature of the array was its excellent discrimination power even for highly related species such as *C. parapsilosis*, *Candida orthopsilosis*, and *Candida metapsilosis*. One of the newest developments is a microsphere-based suspension array for the identification of 23 clinically relevant filamentous molds and dimorphic fungi [171]. The group- and species-specific probes were targeted at the ITS2 region. The probes were coupled to carboxylated beads. After hybridization with target DNA labeled by biotin, the beads were incubated with a conjugate of streptavidin-R-phycoerythrin, and then the fluorescence was analyzed by a special suspension array system. The method revealed high specificity, as all species- and groups-specific probes were correctly targeted to the strains. The achieved sensitivity showed that the method could be applied for the detection of fungal species in human blood.

## 4.6 DNA Microarrays for Parasite Diagnostics

### 4.6.1 Malaria

Parasite infections are among the most burdensome diseases for humans, especially in low-income developing countries. Malaria, caused by the *Plasmodium falciparum*, infects millions of people worldwide and is responsible for over a million deaths worldwide per year, and the treatment options are limited to a few antimalarial drugs such as sulfadoxine–pyrimethamine [174]. Unfortunately, antimalarial drug resistance is on the rise. Drug resistance is often caused by gene duplication or SNPs. The latter genetic markers are highly suitable targets for DNA microarray analysis, and thus several studies were conducted and published using DNA microarray-based SNP tying for the detection of antimalarial drug resistance. Andreas Crameri published a study, as part of the ResMalChip FP5 project and supported by further funders, on the development and application of a DNA microarray for the genotyping of 34 SNPs in five genes, that is, *pfdhfr*, *pfdhps*, *pfcrt*, *pfmdr1*, and *pfATPase* [175]. The assay comprised a PCR amplification of the target regions, followed by a nested PCR approach, two color primer extension, and DNA microarray hybridization. For the full four-base SNP typing, two separate reactions were required.

The authors rated the genotyping assay to be highly suitable for low-resource settings and calculated the assay costs of 0.27 euro per SNP and an assay time of 15 h for four 96-well plates. The limit of detection given was 10–100 parasites. They also described the assay as suitable to cope with the problem of multiplicity of infections and the determination of the most dominant haplotype representing the clinical malaria-causing clone. While the present study only worked with cultured organisms, the authors and collaborators conducted further successful field studies on the basis of the microarray system using patient samples in Tanzania [176], Niger [177], Papua New Guinea [178], and the Solomon Islands [179]. In an interesting study, the group around Hans-Peter Beck used the same

technological approach to genotype polymorphisms, that is, *CYP2A6*, *CYP2B6*, *CYP2C8*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A4*, *CYP3A5*, and *NAT2*, which are known to have an impact on human malarial drug metabolisms [180]. The authors concluded that microarrays were not the ideal tool for this purpose due to a lower robustness in their study and projected a stronger impact of sequencing for future epidemiological studies.

In a related publication, Steenkeste *et al.* [181] describe further development of the previous primer extension/microarray into a zip-code array, called *FlexiChip*. The system, which combined the four color primer extension reaction with a zip code array, was designed using a previously described protocol [182] to overcome the target limitation by the previous microarray assay [175] (Figure 4.4).

The monitoring of drug resistance SNPs in *Plasmodium falciparum* was also approached using the Luminex Xmap technology by creating liquid-phase arrays and ligase reactions for SNP genotyping [183, 184]. Comparative genome hybridization for was done using Affymetrix scrMalaria arrays (custom 25-mer oligonucleotide arrays, 5159 predicted genes, 260 596 probes) [185], spotted 70-mer DNA microarrays [186], and microarrays based on the Nimble Gen platform [187]. Genomic mapping technologies were reviewed by Su *et al.* [188]. While of high scientific value for the study of genome variation in *P. falciparum*, these studies have no direct relevance for diagnostic microbiology. In a study led by the Chinese Centre for Disease Control and Prevention [189], a DNA oligonucleotide microarray was designed to detect SNPs in the five resistance-conferring genes (chloroquine resistance transporter (*Pfcrt*), multidrug resistance 1 (*Pfmdr1*), dihydrofolate reductase (*Pfdhfr*), dihydropteroate synthetase (*Pfdhps*), ATPase 6 (*PfATPase6*) in *P. falciparum* in combination with a multiplex PCR preanalytical regimen. Cross-reactivity studies were successfully performed using genomic DNA isolated from *Plasmodium berghei*, *Plasmodium cynomolgi,* and *Plasmodium vivax.* As not all example isolates for all potential resistance genotype could be obtained, a series of 20 combinations of 37 synthetic oligonucleotides (50-mer) were tested. The array assay showed good performance and was considered a promising tool for resistance-conferring SNP detection in *P. falciparum.* It is not known whether the system was used in subsequent studies, as no related citing articles were found.

### 4.6.2
### Waterborne Parasites

Water supply is a major factor in managing risk by microbial contamination and human parasites. The traditional approach by which water quality is assessed, that is, by monitoring indicator organisms such as coliform bacteria derived from the human fecal flora, is increasingly considered insufficient and being replaced by the detection of specific pathogens such as *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium parvum* as leading examples for human eukaryotic parasites. Consequently, a number of studies focused on the molecular detection of these organisms, and DNA microarray-based publications

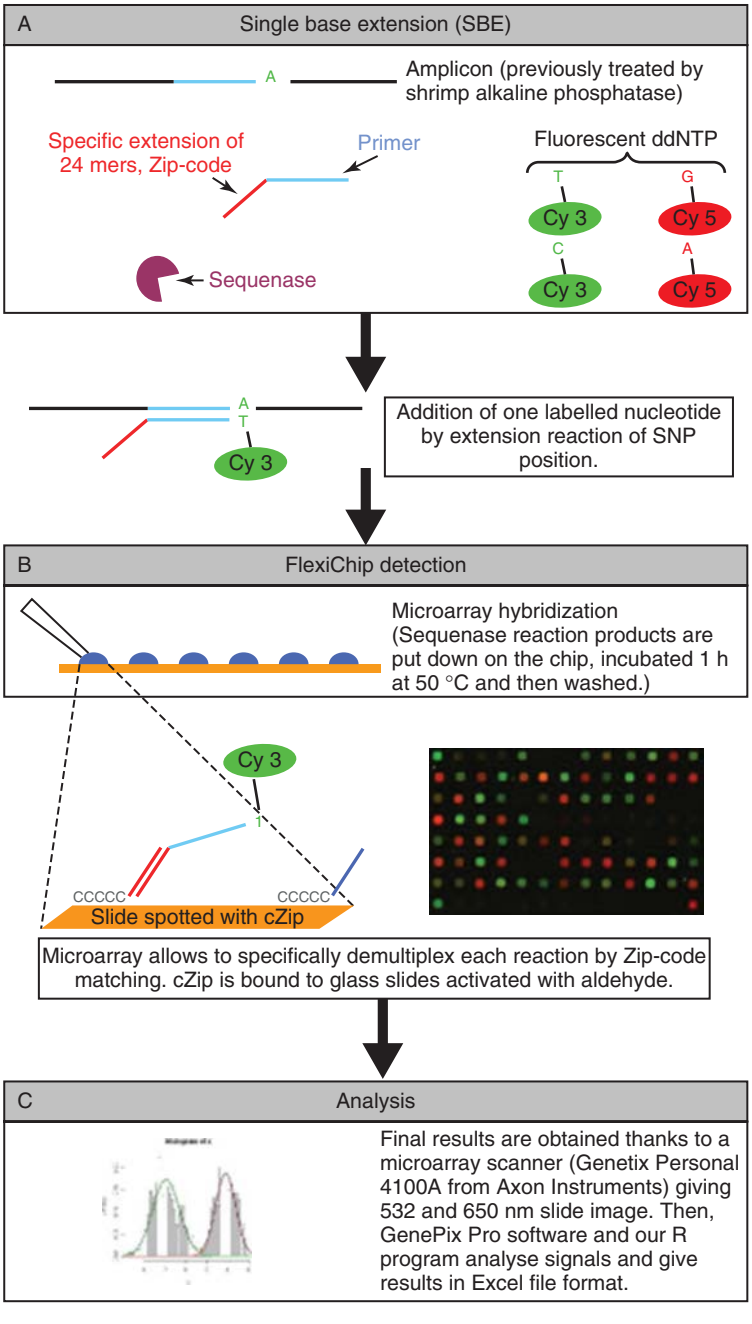


**Figure 4.4** Detection of antimalarial drug resistance in *P. falciparum* using an SNP typing DNA microarray (FlexiChip) [181]. Permissions: http://www.malariajournal.com/content/8/1/229/figure/F1.

are listed here (Table 4.7). Most recently, Brinkman *et al.* [190] described a DNA microarray-based on the CombiMatrix microarray platform for the detection of fecal indicator bacteria (*Enterococcus faecium*), waterborne pathogens (*C. parvum, Cryptosporidium hominis*), and biothreat agents (*Bacillus anthracis, Francisella tularensis*). The study aimed to develop a detection assay capable of detecting the pathogens without the need for target amplification. Having probes targeted against the ribosomal genes, the system managed to detect genomic DNA originating from cultures of $10^7$ organisms. To model real sample detection, the authors spiked 100 ng genomic DNA in purified and concentrated tap water samples, and showed successful detection. They mention the detection of 20 copies in of *F. tularensis* genomic DNA in experiments investigating serial dilutions but give no further data to qualify the claim. Lee *et al.* [191] took a more focused approach in the detection of six species and four genera of the most important protozoan parasites. They designed a low-density DNA oligonucleotide microarray with probes against the small subunit rRNA genes; the targets were amplified using a conventional PCR and post-labeling. Interestingly, the authors tested the array performance in simulated environmental samples in which DNA was mixed with genomic DNA from *C. parvum*. Here, the authors could achieve a detection limit of 1000 copies of the SSU rRNA gene. The authors give a positive outlook on the use of the system in environmental monitoring, but follow-on studies are not known. In 2004, Wang and colleagues described a DNA oligonucleotide microarray for the detection of *Entamoeba histolytica*, *Entamoeba dispar*, *Giardia lamblia*, and *C. parvum* [192]. Here, the target DNA was amplified in a multiplex PCR reaction targeting a variety of non-rRNA genes (except for *C. parvum*) and genotyped using the several oligo probe sets on the microarray. The PCR products were directly labeled by Cy3-dCTP. Very low limits of detection could be achieved down to a level of five *G. lamblia* trophozoites. Also, the authors claimed the capability of the array to specifically detect co-infections, as demonstrated for 10 : 1 mixtures of two *G. lamblia* strains. As an early example, Straub *et al.* [193] developed and used a DNA microarray to discriminate various *Cryptosporidium* strains by genotyping *hsp70* in environmental isolates. The DNA microarray performed well in typing *C. parvum* strains, *C. wrairi, C. meleagridis, C. felis,* and *C. baileyi* and was highly regarded regarding its impact on regulatory compliance and epidemiology.

### 4.6.3
### Other Parasites

Interestingly, the literature base for other human parasite testing using DNA microarrays is relatively limited. Two examples have been identified for human pathogenic microsporidia [195] and *Toxoplasma* [194]. Bahl *et al.* designed an oligonucleotide DNA microarray for genome-wide expression profiling for *Toxoplasma* and SNP genotyping of *Toxoplasma gondii* and *P. falciparum* to be used as a research tool by the *T. gondii* research community. Wang and colleagues used a conventional spotted DNA oligonucleotide microarray and PCR target

**Table 4.7** DNA microarrays for human parasite and protozoan pathogen detection.

| References | CC | Target species | Sample | Preanalytics | Detection | TTR |
|---|---|---|---|---|---|---|
| [194] | US | *T. gondii*, *P. falciparum* | Pure cultures | DNA isolation, fragmentation biotin-14-dCTP labeling | Affymetrix Toxoplasma gondii GeneChip | n/a |
| [179] | CH | *P. falciparum (drug resistance, pfdhfr, pfdhps, pfcrt, pfmdr1, pfATPase)* | Blood samples | gDNA extraction, PCR, fragmentation, PCR primer extension fluorescent labeling | Nested PCR, four color primer extension, spotted multiwell oligo array, fluorescence detection | No clear data: sample prep., 1.5 h + hybridization, 42 min washing, readout, data analysis |
| [190] | US | *C. parvum*,*C. hominis*, *E. faecium*, *B. anthracis*, *F. tularensis* | Pure cultures, spiked tap water concentrates | gDNA extraction (MasterPure DNA purification kit (Epicenter Biotechnologies, Madison, WI, USA) from $10^7$ cells. Labeling Cy3-dCTP using BioPrime DNA labeling system (Invitrogen) | CombiMatrix 12 and 4 × 2 K CustomArray | No clear data: sample prep., 13 h + hybridization, washing, readout, data analysis |
| [185] | UK | *P. falciparum* | Cultures | Whole genome amplification (multiple displacement amplification (MDA)), fragmentation, end labeling | Affymetrix scrMalaria arrays, microarray-based comparative genomic hybridization (CGH) | n/a |
| [175] | CH | *P. falciparum (drug resistance, pfdhfr, pfdhps, pfcrt, pfmdr1, pfATPase)* | Cultures | gDNA extraction, PCR, fragmentation, PCR primer extension fluorescent labeling | Nested PCR, four color primer extension, spotted multiwell oligo array, fluorescence detection | No clear data: sample prep., 1.5 h + hybridization, 42 min washing, readout, data analysis |

| | | | | | | |
|---|---|---|---|---|---|---|
| [177] | FR | *P. falciparum (drug resistance, pfdhfr, pfdhps, pfcrt, pfmdr1, pfATPase)* | Blood samples | gDNA extraction, PCR, fragmentation, PCR primer extension fluorescent labeling | Nested PCR, four color primer extension, spotted multiwell oligo array, fluorescence detection | No clear data: sample prep., 1.5 h + hybridization, 42 min washing, readout, data analysis |
| [191] | CA | *Acanthamoeba* spp. *B. hominis*, *C. parvum*, *E. histolytica*, *G. intestinalis*, *N. fowleri*, *N. gruberi* | gDNA, pure cultures | gDNA extraction, PCR, post PCR fluorescent labeling | Spotted oligo array, fluorescence detection | n/a |
| [178] | CH | *P. falciparum (drug resistance, pfdhfr, pfdhps, pfcrt, pfmdr1, pfATPase)* | Blood samples | gDNA extraction, PCR, fragmentation, PCR primer extension fluorescent labeling | Nested PCR, four color primer extension, spotted multiwell oligo array, fluorescence detection | No clear data: sample prep., 1.5 h + hybridization, 42 min washing, readout, data analysis |
| [176] | CH | *P. falciparum (drug resistance, pfdhfr, pfdhps, pfcrt, pfmdr1, pfATPase)* | Blood samples | gDNA extraction, PCR, fragmentation, PCR primer extension fluorescent labeling | Nested PCR, four color primer extension, spotted multiwell oligo array, fluorescence detection | No clear data: sample prep., 1.5 h + hybridization, 42 min washing, readout, data analysis |
| [181] | FR | *P. falciparum (drug resistance, pfdhfr, pfdhps, pfcrt, pfmdr1, pfATPase)* | Blood samples | QIAamp DNA Mini Kit | Spotted oligo array (zip code/FlexiChip), fluorescence detection | No clear data: sample prep., 1 h + hybridization, 45 min washing, readout, data analysis |

(*continued overleaf*)

**Table 4.7** (*Continued*)

| References | CC | Target species | Sample | Preanalytics | Detection | TTR |
|---|---|---|---|---|---|---|
| [193] | US | *Cryptosporidium* spp., *C. parvum* | Pure cultures (aiming for water) | gDNA extraction, fluorescent PCR labeling | Spotted oligo array, fluorescence detection | n/a |
| [195] | US | *E. bieneusi*, *E. cuniculi*, *E. hellem*, *E. intestinalis* | Pure cultures from culture-enriched isolates, urine, fecal specimens, and mucosal aspirates | gDNA extraction, fluorescent PCR labeling | Spotted oligo array, fluorescence detection | No clear data: sample prep., 1 h + hybridization, washing, readout, data analysis |
| [192] | US | *E. histolytica*, *G. lamblia*, *C. parvum*, | Pure cultures (aiming for water) | gDNA extraction, fluorescent PCR labeling | Spotted oligo array, fluorescence detection | No clear data: sample prep., 1 h + hybridization, washing, readout, data analysis |
| [189] | CN | *P. falciparum (drug resistance, pfdhfr, pfdhps, pfcrt, pfmdr1, pfATPase)* | Cultures, dried blood spot | gDNA extraction, PCR, fragmentation, post PCR fluorescent labeling | Spotted oligo array, fluorescence detection | n/a |

amplification to detect various Enterozytozoon species. With a demonstrated detection limit of 10 spores, the authors tested the microarray in a clinical setting. Here, from 20 fecal samples from AIDS patients, 12 were microsporidia positive with 11 having mixed infections. While the study is already from 2005, it clearly demonstrates the applicability of such assay to clinical samples and provides the ground for further investigations with a clinical or epidemiological focus.

## 4.7 Conclusion an Outlook

For almost 25 years, DNA microarray technology has undergone remarkable developments spawning a plethora of scientific studies and commercial developments focusing infectious diseases, pathogen detection, and well beyond. As the design and implementation of content for DNA microarrays, that is, the design of probes for molecular targets, as well as the readout technology became routine, it increasingly turned out that the technical bottleneck for diagnostic DNA microarray development lies in the preanalytical steps. Sampling, nucleic acid extraction, and possibly amplification are crucial requirements for any molecular assay and, of course, also including DNA microarrays. While the technologies for the individual process steps exist, the integration in sample to answer formats that allow routine clinical use remains a major challenge, which so far has been solved only in a few cases. Finally, clinical relevance, market adoption, and cost models for reimbursement are among the key factors for the success of diagnostic DNA microarray assays for infectious diseases. In addition, as integration of microarray assays is well under way in a number of cases, DNA microarray technology is tackled from various ends. With regard to integration and molecular assay design, for example, advanced electrochemical DNA biosensors are much simpler and are gaining ground in the commercial [196] and academic sector [197]. However, DNA microarrays can be understood as complex DNA biosensors to which they are superior because of their multiplexing capability for ultra parallel analysis. Nevertheless, to the top end of complexity and deep molecular analysis of a sample, next-generation sequencing is increasingly taking over from DNA microarrays. While developments for clinical microbiology and outbreak detection are taking off recently [198, 199], examples of applications in routine clinical diagnostics and point-of-care detection are yet to be demonstrated. Here, DNA microarrays still have an advantage and will certainly be further used to drive establishing molecular diagnostics in the clinical routine for improved diagnostics and better treatment of infectious diseases.

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