A NEW ompA-BASED DNA MICROARRAY ASSAY FOR RAPID GENOTYPING OF CHLAMYDIA TRACHOMATIS STRAINS

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Background information

• Chlamydia (C.) trachomatis infection is the most prevalent sexually transmitted bacterial disease.
• C. trachomatis strains are known to be divergent at both genome and proteome levels.
• Typing is an important tool to reveal transmission pathways.
• Currently, 17 human serovars have been generally accepted. Serovars A to C are commonly associated with trachoma, serovars D to K primarily cause urogenital infections, and L1 to L3 serovars are the agents of lymphogranuloma venereum.
• MOMP represents one of the immunodominant antigens and is encoded by the ompA gene.
• This gene consists of four variable domains (VD1-4) that are flanked and separated by five conserved domains (see Fig. 1).
• Genotyping is generally done using PCR-restriction fragment length polymorphism (PCR-RFLP) and/or ompA sequencing. Although accurate, both methods lack high sensitivity and rapidity and are difficult to conduct in a routine diagnostic environment.

Micorarray platforms

• ArrayTube™ and ArrayStrip™ platforms work with routine diagnostic applications.
  • Key components: 3x3-mm or 4x4-mm microarrays implanted on the bottom of a 1.5-ml standard plastic reaction vial or a standard microtiter plate strip of 8 wells, respectively.
  • Advantages: completed within a working day, affordable cost, easy handling, use of common lab equipment, small reaction volumes, hybridization and staining reaction controls included.
  • Use in routine diagnosis is well documented.

Genotyping assay development

Comprehensive in silico analysis of all available ompA sequences of C. trachomatis was conducted in order to assess the diversity among naturally occurring strains. A GenBank search revealed a total of 381 entries with 54 unique sequences. The basic principles for probe selection included i) avoidance of cross-hybridization, ii) use of multiple probes per genotype, and iii) harmonization of biophysical parameters (melting temperature, G+C content, self annealing capacity). Analysis of the global alignment revealed both highly conserved and highly diverse sequence windows. The latter were located in VDs 1, 2 and 4 and served as foci for probe design. In an iterative process, a panel of 61 oligonucleotide probes binding to these segments was selected.

Subsequently, we designed theoretical patterns of all genotype reference strains, where perfect target-probe matches were assumed to produce the strongest signals (100%), a single mismatch was assigned to a 60% signal, two mismatches to 30%, three to 10%, and more mismatches to the absence of a signal. Close similarity between theoretically expected and experimentally obtained hybridization patterns was the central criterion in the optimization process of the microarray hybridization protocol.

Conclusions & outlook

We have been able to demonstrate the excellent performance of the newly developed C. trachomatis genotyping test. The present microarray platform is open and flexible, thus allowing extension beyond the currently covered 17 serotypes, for instance in the case of newly emerging types. In addition, the use of the pan-Chlamydia microarray will enable the diagnostician to simultaneously identify the Chlamydiaceae spp. involved (based on the 23S rRNA gene) and conduct genotyping of C. trachomatis (and C. psittaci, if applicable) based on ompA genes.

Acknowledgements

• Steffen Kube (Clondiag): development of the PatternMatch algorithm.
• Technical assistance: Christine Greiplm, Simone Bettermann, Sabine Schiel, Jens Engelmann, Elke Miller, and Jana Sachtschal.
• Grants: German Federal Ministry for Education and Research (BMBF, 01 KI 0720 ); European Commission within the Sixth Framework Programme (European EpigeneChlamydia Consortium: Network of Excellence European Virtual Institute for Functional Genomics of Bacterial Pathogens).

Further reading

• Borel et al. 2008. Mol Cell Probes 22:55-64 (DNA microarray)
• Steffen Kube (Clondiag): development of the PatternMatch algorithm
• Sachse et al. 2008. BMC Microbiol 8:63 (genotyping C. psittaci)
• www.fli.bund.de, www.clondiag.com