

SEXUALLY TRANSMITTED INFECTIONS **A HIDDEN MENACE**

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Sexually transmitted infections (STIs) are a major and growing health problem in all parts of the world. According to the World Health Organisation (WHO) more than one million STIs are acquired worldwide every day. Untreated, they can lead to serious long-term sequelae, especially infertility. They also pose a risk during pregnancy, causing intrauterine death, premature birth or foetal damage. Moreover, they can be transmitted to the newborn during birth, causing severe postnatal infections.

Diagnosing STIs can be challenging. STIs are in many cases asymptomatic, and may only be identified when irreversible damage has already occurred. Infections with multiple pathogens are also possible, adding to the complexity of diagnosis. Thus, laboratory tests play a key role in identifying infections. PCR-based direct detection is one of the most important methods, particularly for pathogens that cannot be effectively cultivated. PCR-based detection allows the identification of both manifest and silent infections, and is thus suitable for diagnosis of symptomatic patients as well as for general screening. Further, due to amplification of the pathogen DNA, infections with a reduced pathogen number can also be reliably identified.

A broad screening for STIs is important, especially in asymptomatic or clinically ambiguous cases. Microarray technology provides an ideal platform for simultaneous direct detection of a wide range of relevant pathogens, for example, *Chlamydia trachomatis*, *Neisseria gonorrhoea*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Haemophilus ducreyi*, *Treponema pallidum*, *Trichomonas vaginalis* and herpes simplex viruses 1 and 2.

Chlamydia Trachomatis

Chlamydia trachomatis is a very small intracellular energy-parasitic bacterium and comprises different serotypes, which cause different diseases. Serotypes D to K cause urogenital infections and represent one of the most common STIs, with 131 million new infections worldwide per year according to the WHO. Infections are often asymptomatic, but can nevertheless lead to sterility. If symptoms develop they are urethritis, epididymitis and prostatitis in men and urethritis, and cervicitis and salpingitis/adnexitis in women. Perinatally transmitted

infections manifest with conjunctivitis and pneumonia in the newborn and can lead to life-long health problems. Serotypes L1, L2 and L3 cause lymphogranuloma venereum, a sexually transmitted disease, which mainly occurs in tropical regions. Serotypes A, B and C infect the conjunctiva and cornea of the eye causing trachoma which can lead to blindness. These serotypes are transmitted by infectious eye secretions.

Neisseria Gonorrhoeae

Neisseria gonorrhoeae is the causative agent of gonorrhoea, a worldwide distributed infectious disease which occurs exclusively in humans. According to the WHO, around 78 million new infections occur per year. The bacterium infects predominantly the epithelial cells of the female and male urethra, the cervical canal, the rectum and the conjunctiva. An ascending infection can lead to prostatitis, funiculitis, vesiculitis or epididymitis in men and pelvic inflammatory disease in women.

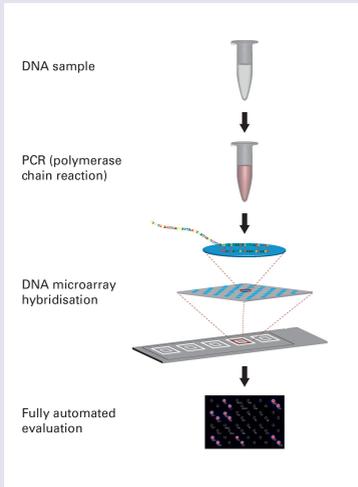
Treponema Pallidum

Treponema pallidum is the pathogenic agent of venereal syphilis, a widely distributed, chronic cyclic infectious disease. Humans are the only reservoir for the bacterial pathogen. The disease is divided into different stages encompassing localised symptoms (primary stage), generalised systemic manifestation (secondary stage), attacks on different organs (tertiary stage), and neurosyphilis with brain damage (quaternary stage). The stages do not always occur successively and some stages may be absent or recurrent. According to the WHO there are 5.6 million new infections worldwide per year. The main transmission route is sexual intercourse, while diaplacental transmission is also significant. In pregnancy, untreated early syphilis is responsible for one in four stillbirths and 14% of newborn deaths worldwide.

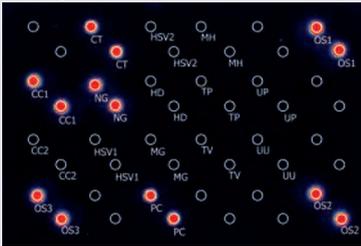
Mycoplasma and Ureaplasma

Mycoplasma and *Ureaplasma* are genera of very small, independently reproducing bacteria, which are distributed worldwide. They are mostly transmitted by droplet infection and sexual intercourse. To date, twelve species of *Mycoplasma* and two species of *Ureaplasma* have been described. 40–80% of women and 5–20% of men of sexually active age have a *Ureaplasma urealyticum* or *Ureaplasma* ►

▼ **FIGURE 1:** EUROArray procedure



▼ **FIGURE 2:** EUROArray results for STI pathogen detection



parvum infection of the lower genital tract. *Mycoplasma hominis* has a prevalence of 20–50% and *Mycoplasma genitalium* of 1–3% in sexually active adults. Since the infections are often asymptomatic, they frequently remain unnoticed and untreated.

Haemophilus Ducreyi

Haemophilus ducreyi is the causative agent of *ulcus molle*, also known as chancroid, which occurs predominantly in tropical regions in Africa, South-East Asia and Latin America and is exclusive to humans. It is transmitted by sexual intercourse, and spreads particularly in population groups with poor personal hygiene. The disease manifests with single or multiple soft genital ulcers, which are usually painful.

Trichomonas Vaginalis

Trichomonas vaginalis is a parasitic protozoan, which causes trichomoniasis. The parasite can survive for a long time in the vagina and urethra of women, particularly when the vaginal flora is disrupted. In men it infects the urethra and prostate gland. According to the WHO around

143 million people worldwide become infected per year. Infections can proceed symptomatically, with symptoms resembling other urinary tract infections, or asymptomatically.

Herpes Simplex Viruses 1 and 2

Herpes simplex virus 1 (HSV-1) and herpes simplex virus 2 (HSV-2) are worldwide distributed human pathogenic viruses of the family Herpesviridae. It is assumed that 70–90% of the population becomes infected with HSV-1 and 7–20% with HSV-2 during their lifetime. Facial and lip herpes is generally caused by HSV-1, with infection often occurring already in childhood through close bodily contact with infected persons. Genital herpes is caused predominantly by HSV-2, but also by HSV-1, and is generally acquired at a later time point via sexual contact. According to the WHO, more than 500 million people worldwide are living with genital herpes infection. Primary infections manifest with ulcers or inflammatory skin blisters. Complications of herpes include encephalitis, meningitis and disseminated HSV infection in immunodeficient patients.

STI Detection by Microarray

The eleven STI pathogens described above can be directly detected in parallel in one reaction by microarray based on established EUROArray technology. The detection is highly specific and sensitive and offers a huge time advantage over pathogen cultivation. In the procedure (Figure 1), specific sections of the pathogen DNA from patient samples are amplified by one multiplex PCR using highly specific primers. During this process, generated PCR products are labelled with a fluorescent dye. The PCR reaction is then incubated with a biochip microarray slide containing immobilised complementary probes. The PCR products hybridise with their complementary probes and are subsequently detected by means of their fluorescence signals (Figure 2). Integrated controls verify correct performance of the test and absence of PCR contamination. The evaluation, interpretation and archiving of the results is fully automated and thus highly standardised and objective. A detailed result report is produced for each patient. A human papillomavirus (HPV) microarray based on the same technology can be performed in parallel if required.

Detection Specifications and Evaluation

The lower detection limit of the microarray analysis amounts to 10–100 DNA copies per reaction, depending on the pathogen. Template DNA in concentrations ranging from the lower detection limit to two million copies can be used in the PCR without any cross reactions with probes from other pathogens. Moreover, cross reactivity with 28 further anogenital microorganisms has been excluded experimentally.

In an evaluation study, 325 swab samples and 134 urine samples were analysed with the microarray. There was a very good agreement with the precharacterisation. Additionally, in many cases pathogens that were not included in the precharacterisation were detected. The additional findings were verified by further independent tests. Thus, the microarray provides reliable results and exceptionally broad screening capabilities.

Perspectives

Timely diagnosis of STIs is critical for preventing life-changing consequences such as infertility, miscarriage and foetal disorders. The risk of acquiring and transmitting HIV is, moreover, increased with STIs such as syphilis, chancroid and herpes. Once identified, the most common STIs such as chlamydia, gonorrhoea, syphilis and trichomoniasis can be cured with antibiotics, while HSV infections can be modulated through antiviral therapy. Due to the increasing spread of STIs, screening programmes, e.g. for chlamydia and gonorrhoea, have been introduced in some countries, especially for young people, in whom infections are most prevalent. STI testing is, moreover, an important preventative medical examination in pregnant women and immunosuppressed patients, for example HIV patients. Sexual partners of infected persons should always be tested and treated at the same time. Microarrays with a broad range of parameters are a simple and highly effective technique to screen for the many different STIs circulating in the population, especially in cases with multiple asymptomatic infections. The extensive information obtained from the microarray analysis can help to limit the sequelae and curtail the spread of these highly prevalent infections. 