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# New diagnostic test can detect chlamydia trachomatis in less than 20 minutes

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Researchers have developed a new assay for rapid and sensitive detection of *Chlamydia trachomatis*, the most common sexually transmitted infection (STI) in humans. This procedure takes less than 20 minutes and can be easily performed at the point of care (POC) during the patient's visit, reports *The Journal of Molecular Diagnostics*.

*C. trachomatis* affects 5% to 10% of the population and is particularly common in young adults under 25 years. It is a major public health concern due to its prevalence and potential severe long-term consequences. One of the main reasons it is so prevalent is that in the majority of cases (75% of women and 50% of men) there are minimal to no symptoms, and it therefore often goes undiagnosed. Infection is associated with non-gonococcal urethritis in men and several inflammatory reproductive tract syndromes in women such as inflammation of the uterine cervix and pelvic inflammatory disease. Untreated, the infection increases the risk of ectopic pregnancy and is one of the leading causes of female infertility worldwide.

The assay uses recombinase polymerase amplification (RPA), a nucleic acid amplification technique (NAAT), to detect *C. trachomatis* directly from urine samples. Because the assay's novel approach does not require the purification of total DNA from the urine sample, the need for specialized equipment is eliminated. The procedure is significantly less laborious, less time-consuming, and consequently less expensive. It is relatively simple to perform and could therefore be applied in numerous POC settings.

"The assay enables highly specific *C. trachomatis* detection with sensitivity levels significantly improved compared to currently available *C. trachomatis* POC assays," says Ülo Langel, PhD, Professor of Molecular Biotechnology, University of Tartu, Estonia, and Professor of Neurochemistry, Stockholm University, Sweden.

Existing polymerase chain reaction (PCR)-based techniques for testing *C. trachomatis* are widely applied but are only suitable for use in hospitals with trained staff and expensive machinery. Studies have shown that up to 50% of patients never return to get the diagnostic result or required treatment.

Although several rapid-diagnosis POC tests have already been developed, none offer a comparable sensitivity to hospital-based techniques. Recent independent studies have shown that currently available POC tests have a sensitivity of just 10% to 40%. Initial analysis of the new assay's performance indicated a specificity of 100% and a sensitivity of 83%, evidence of its potential reliability.

"The alarmingly poor performance of the available POC tests for *C. trachomatis* has limited their wider use, and there is a clear requirement for more sensitive and cost-effective diagnostic platforms. Hence, the need for an applicable on-site test that offers reasonably sensitive detection," concludes Prof. Langel.

## Technical details of the study

Recombinase polymerase amplification (RPA) is a nucleic acid amplification technique (NAAT) – a laboratory technique that involves the in vitro synthesis of many copies of DNA or RNA from one original template. These techniques have revolutionized diagnostic technology. Current technologies that allow the detection of amplification in real time are fast becoming diagnostic industry standards.

*C. trachomatis* cells contain plasmids (small DNA molecules that are separate from chromosomal DNA) that have a number of coding sequences. For identification and amplification by RPA, researchers selected a gene fragment within a gene (CDS2) that was conserved across sexually transmitted *C. trachomatis* strains. The assay does not require the purification of total DNA from the urine sample. Heating the sample for five minutes at 90°C is enough to release a sufficient amount of the amplification target to determine whether the pathogen is present. Urine contains polymerase chain reaction (PCR) inhibitors, but up to 5 µl of urine can be added without affecting sensitivity of the RPA, whereas the addition of 10 µl affects amplification efficiency significantly.

The *C. trachomatis* assay developed here was able to detect at least 50 copies of the CDS2 target. *C. trachomatis* harbors, on average, between four and ten copies of the plasmid per elementary body depending on the strain and development stage. The lowest detectable amount of the *C. trachomatis* RPA assay can therefore be translated to 5 to 12 pathogens per reaction and is in the same range as other nucleic acid amplification-based techniques.

The assay was tested on urine samples from 70 patients (51 females and 19 males) attending a sexual health clinic in Estonia. The samples were tested in parallel using RPA and Roche Cobas Amplicor *C. trachomatis* assays.

Fifty-eight samples tested negative in both assays. As no false negatives were detected, the clinical specificity of the *C. trachomatis* RPA assay can be estimated at 100%.

Twelve of the samples tested as positive using the Roche assay. Of these, 10 tested positive and two tested negative in the RPA reaction. Based on these results, the clinical sensitivity of the RPA assay can be estimated at

83%.

Of the 12 patients who tested positive, three complained of symptoms. The other nine patients were asymptomatic. Of the 58 *C. trachomatis*-negative patients, 15 (26%) complained of symptoms that could be associated with *C. trachomatis* infection. One of these tested positive for *N. gonorrhoeae* and *M. genitalium*. Others were diagnosed with bladder inflammation (two patients), bacterial vaginosis (five patients), yeast infection (four patients), or abdominal pain of non-gynecological origins (three patients).

**More information:** "Sensitive and rapid detection of Chlamydia trachomatis by recombinase polymerase amplification directly from urine samples," by Katrin Krõlov, Jekaterina Frolova, Oana Tudoran, Julia Suhorutsenko, Taavi Lehto, Hiljar Sibul, Imre Mäger, Made Laanpere, Indrek Tulp, and Ülo Langel, DOI: dx.doi.org/10.1016/j.jmoldx.2013.08.003. The *Journal of Molecular Diagnostics*, Volume 16, Issue 1 (January 2014)

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