**Diagnosis of Chlamydia trachomatis using self-collected non-invasive specimens – the Australian experience**

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_C. trachomatis_ are small, non-motile, obligate intracellular bacteria that typically infect human eukaryotic columnar epithelial cells. _C. trachomatis_ infections result in a number of diseases of worldwide public health concern, including trachoma, lymphogranuloma venereum (LGV) and urogenital infections. Chlamydia is the most common sexually transmitted bacterial pathogen worldwide and in Australia has exhibited a steady rise in prevalence. National notification rates of newly diagnosed chlamydia infections have increased nearly four-fold since 1994 and more than doubled since 1999 (Figure 1).

A plausible reason for the rise in Australian _C. trachomatis_ notifications may be an increase in the levels of testing, as a consequence of availability of more rapid, sensitive and non-invasive tests utilising nucleic acid amplification testing (NAAT). However, it is likely that this increase is not only due to increased testing, as the percentage increase in rates of chlamydia notifications in Australia far exceeds those of annual Medicare test numbers.

 Paramount in the control of _C. trachomatis_ infection is prompt recognition and appropriate treatment. Even when screening is incorporated into control strategies; lack of access to appropriate services (especially in rural and remote areas), reluctance of at-risk populations to attend for treatment, fear of invasive genital examinations, and lower sensitivities of conventional diagnostic assays reduces the effectiveness of such programmes.

Therefore, accurate, cost-effective, reliable diagnostic assays are needed to impact on the incidence of chlamydia. With the advent of NAAT, including target and signal amplification methods, the detection of _C. trachomatis_ and other sexually transmitted infections (STIs) has been revolutionised and has allowed the use of self-collected non-invasive sampling techniques, some of which are, for example, first-void urine, cervicovaginal lavage, sanitary napkins, low vaginal swabs, and tampons (Figure 2). Most studies evaluating self-sampling with molecular diagnostic techniques have demonstrated an equivalent or superior detection of chlamydia when compared to conventional sampling and detection methods. In this article, the utility of self-sampling collection devices for detection of _C. trachomatis_ is reviewed.

**Self-collected sampling and testing by NAAT**

Conventional diagnostic assays for detection of _C. trachomatis_ lack sensitivity, require viable organisms, fastidious transport conditions and usually a clinician to obtain samples from the site of infection. In Australia, a number of NAAT technologies, including polymerase chain reaction (PCR), transcription mediated amplification (TMA) and strand displacement amplification (SDA) are in use for detection of _C. trachomatis_. Generally, NAATs allow the use of self-collected samples from material collected further away from the original site of infection that may consequently contain fewer organisms than in conventional swab samples. In addition transport conditions are less critical for test performance. Therefore, these sample...
types can be taken in the privacy of the home and mailed directly to the diagnostic laboratory. In contrast to traditional internal examinations and genital swab collections at the physician’s office, such strategies for testing have led to improved partner tracing and universal screening, including difficult to reach populations \[16, 17\]. Several self-sampling methods such as first-void urine, cervicovaginal lavage, low vaginal swabs, mini menstrual pads, and tampons have been described \[4-15\].

Urine samples were among the first self-sampling methods to be utilised and adapted for molecular diagnosis of \textit{C. trachomatis} and have become an acceptable and widely applicable method of sampling in both men and women \[4, 5\].

Tampons and swabs provide a very convenient sampling method for detection of chlamydia in women \[8-15\]. Due to their larger surface area, tampons collect a better sample in cases where detection of multiple targets are desired, or where there are low amounts of pathogen. In addition, tampons generally have a much less stringent transport criteria than swabs or urine \[5\]. Recently, self-collected swabs have also been utilised in collecting anal samples from men who have sex with men (MSM) for successful detection of \textit{C. trachomatis} \[18\] and can provide a convenient sampling method for screening of LGV strains among this population.

Modified menstrual pads have also been utilised in non-invasive collection of genital and urethral cells \[7\]. Similar to other self-collected samples, menstrual pads also have much less stringent transport criteria than urine or swabs. It has been suggested that menstrual pads are more sensitive in detection of urethral infections that could be missed by biological material collected only from the vagina \[7\].

Though not utilised to a great extent for the detection of chlamydia due to the availability and convenience of swab and urine sampling, vaginal lavage is another method for obtaining vaginal secretions, whereby the patient flushes the vagina using a pipette containing a sterile saline solution to collect the vaginal lavage \[6\].

In addition to urine, assessment of chlamydial infection in men has also been evaluated using semen. This type of specimen has been used for screening of sperm donors and in prevalence studies where obtaining samples is not possible by other means, ie assessing STI prevalence in clients of sex workers \[19, 20\].

**Acceptability, adequacy and convenience of self-collected samples**

Self-collected samples have the advantage that the patient can be tested without an invasive procedure requiring a speculum, and the necessity for specialist clinicians. For women this may increase their compliance with screening. This was evaluated in a study utilising tampon sampling in women in remote areas with limited clinical services in the Northern Territory (NT) in Australia \[13\] (NT covers 1,346,000 km\(^2\) and has a population of 174,000, with approximately 40,000 living in remote rural areas many hundreds of kilometres from the major population centres and with limited access to medical services). Studies were carried out on the applicability of the tampon sampling for detection of \textit{C. trachomatis} in Aboriginal and Torres Strait Islander women and in non-Aboriginal and Torres Strait Islander women attending STD and family planning clinics in the urban area, as well as community health centres in rural and remote areas of the NT “Top End” \[13\]. In this study, over 94% of all women approached agreed to provide a tampon specimen, demonstrating a high patient acceptance level. It is noteworthy that the tampon method was shown to be culturally acceptable and easily incorporated into health clinics.

**Comparison of self-collected samples**

From the time of menarche until menopause, most women routinely use tampons monthly throughout the year, and the need for instruction on self-collection of this sample is minimal. Interestingly, 25% of one study population preferred tampon sampling to urine sampling, indicating that the tampon specimen is more convenient \[14\]. Tampons also have less stringent transport criteria and can be transported long distances without the need for refrigeration. In a study conducted in a low prevalence setting for \textit{C. trachomatis} (2.4%), 6% of the physician-collected samples had no beta-globin amplified (61,932 endocervical swab
samples), whereas this housekeeping gene was detected and amplified from all tampon samples. This indicates that the tampon collects more human cells and is a more representative sample than the physician-collected swab.

In Central Australia, a study comparing self-administered samples (tampon, first-void urine and self-collected low vaginal swab) to physician-collected endocervical and vaginal swabs utilising PCR for detection of STIs including C. trachomatis, found that unassessable samples (where there was either insufficient sample collected or inhibition detected) varied depending on the sample type used. Only one tampon sample was unassessable whereas 21 urine, 32 low vaginal and 57 physician-collected endocervical samples were determined to be inadequate for testing. Another noteworthy point raised from this study was the importance of extraction of the sample for DNA prior to PCR testing – the likely reason for the unassessable differences. Tampons were tested for STIs by PCR, both pre- and post-DNA extraction; 36 would have been unassessable had this step not already been in place. Overall, tampons were found to be significantly superior to swabs and urine specimens. Although DNA extraction incorporated into the testing strategy would reduce inhibition rates, most commercial assays do not routinely incorporate this step due to cost implications.

Urine specimens are a convenient sampling method and have been shown to befavoured over self-collection. However, with recent changes to postal regulations, samples such as urine and lavage would need to be sent via specialised courier and this would increase the cost of testing for specimens mailed by patients.

Overall, self-collected sampling followed by detection by NAAIs can offer better compliance for screening patients for C. trachomatis. However, selection of self-collected sampling devices should be made according to the population, facilities and testing strategies available in the region.

References