Screening for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* among high-school participants using the Versant CT/GC DNA 1.0 assay (kinetic PCR)

Adolescents and young adults are at higher risk than the general population for acquiring sexually transmitted diseases (STDs) because they are more likely to engage in risky sexual behaviours such as unprotected sexual intercourse and to have multiple sexual partners. Interventions that promote STD testing among this risk group are therefore important. *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are the most common bacterial sexually transmitted infections (Gavin *et al*., 2009). Research has demonstrated that school-based screening is useful in identifying adolescents who are positive for chlamydia and gonorrhoea, and that the prevalence of STDs in the high-school student population is high enough to justify school-based screening (Centers for Disease Control and Prevention, 2010). This type of screening allows the capture of adolescents and young adults who are reluctant to seek medical care and, in addition, identifies those without symptoms who would otherwise not have been tested.

Despite the importance of STD testing in this group there have not, to our knowledge, been any screening studies of chlamydia and gonorrhoea infections among adolescents and young adults in Italy.

Brescia is the second largest city in Lombardy, with a population of around 197 000, and is the administrative capital of the Province of Brescia, one of the largest provinces in Italy with about 1.2 million inhabitants. Because of the large population and in order to fill the knowledge gap regarding STD screening among adolescents and young adults in Italy, Brescia was chosen as the basis for a large epidemiological study to analyse the prevalence of *C. trachomatis* and *N. gonorrhoeae* infections among adolescents and young adults in Italy.

The first analysis of the results by kPCR was conducted from November 2012 to April 2013 with the aim of analysing the prevalence of *C. trachomatis* and *N. gonorrhoeae* infections in the high-school student population. The schools were located in six geographical areas of Brescia, Italy. A total of 4368 18-year-old students were recruited from 34 schools that were included in the Clamigon Study, which was conducted from November 2012 to April 2013 with the aim of analysing the prevalence of *C. trachomatis* and *N. gonorrhoeae* infections in the high-school student population. The schools were located in six geographical areas of Brescia, Italy. Overall, 2718 of these young adults were recruited and contacted. Overall, 2718 of these young adults (62.2 %) consented to urine chlamydia and gonorrhoea screening, of whom 1112 (40.9 %) were men and 1606 (59.1 %) were women.

Participants were recruited from 34 schools that were included in the study population. This kinetic (k)PCR system is an automated system that joins the extraction of nucleic acids from 96 wells with subsequent real-time PCR. All positive results were confirmed using the Gen-Probe Aptima Combo 2 (AC2) assay and by sequencing for strain genotyping.

Data were stored and managed according to Italian privacy rules (Italian Data Protection Authority, 2009). The Versant CT/GC assay is run on the Versant kPCR molecular system. Urine samples were analysed by kPCR immediately after their arrival at the laboratory, with the kPCR assay performed according to the manufacturer’s instructions. Positive samples (2 ml) were inserted into the AC2 urine collection kit and sequentially subjected to analysis with the AC2. This was used as a comparative method because it is a test with high sensitivity and specificity, and is able to simultaneously detect *C. trachomatis* and *N. gonorrhoeae* in a single specimen. The AC2 assay was performed according to the manufacturer’s instructions. Strain typing was performed using ompA sequence determination according to a previously described method (Harding *et al*., 2010). Strains were categorized in genovars D–K. Multilocus sequence typing analysis was performed as already described (Klint *et al*., 2007). DNA was extracted from positive urine specimens using the NucliSENS easyMAG (bioMérieux), a platform specifically optimized for total nucleic acid extraction from biological samples, according to the manufacturer’s instructions.

Specimens that tested positive by the Versant CT/GC assay, the AC2 assay and strain typing were considered as true positives. Specimens that were positive with the Versant CT/GC assay and negative with the AC2 assay and strain typing were retested on the Versant assay to confirm the positive or negative result. The performance of the kPCR test compared with the AC2 assay in detecting CT and GC infections was calculated using conventional contingency tables to evaluate the positive per cent agreement, with 95 % confidence intervals (CIs).

The first analysis of the results by kPCR showed that 46 samples were positive for *C. trachomatis*, while all urine samples

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were negative for *N. gonorrhoeae*. There are no data about the prevalence of *N. gonorrhoeae* infections in adolescents and young adults in Italy, but it is known that this infection is less prevalent than chlamydia and, in the surveillance report of the European Centre for Disease Prevention and Control (2011), it was reported that age-specific rates of reported cases (also from Italy) are highest among 20–24-year-olds. Furthermore, rates have decreased for all age groups since 2000, with the largest drops seen among 15–19- and 20–24-year-olds. This might, in part, explain the results obtained in the segment of the population included in the current study.

The Versant CT/GC assay includes an internal control that was amplified in all samples, making it unlikely that inhibition occurred and excluding false-negative results. For this reason, we confirmed only CT-positive samples. Of the 46 samples that were positive with the Versant CT/GC assay, 36 were confirmed as positive with the AC2 assay (Table 1). All of the 10 samples with discrepant results had high Cq (quantification cycle; the cycle at which the target is quantified) values above 34–35 cycles, while the range of Cq values for all other kPCR-positive samples was 20–33 cycles. All of these samples were retested by kPCR. At retesting, all of the 10 discrepant samples were negative on the kPCR test. The finding of false-positive results at Cq values of more than 35 cycles has been reported in two previous studies (Bongaerts et al., 2011; Kerndt et al., 2011).

In accordance with the suggestion of Bongaerts et al. (2011), we recommend modifying the manufacturer’s instructions to establish a ‘grey zone’ in the case of Cq values of more than 35 cycles, alerting clinicians to the importance of retesting these samples to avoid false positivity. This is very important in particular settings, such as when screening adolescents and young adults, as a false-positive result can cause distress for individuals and create an unfavourable environment for discussing STD prevention and promoting healthy sexual behaviours.

Maria Antonia De Francesco,1 Franco Gargiulo,1 Alberto Matteelli,2 Paola Stefanelli,3 Cinzia Giagulli,1 Francesca Caccuri,1 Giorgio Piccinelli1 and Arnaldo Caruso1

1Institute of Microbiology, Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy
2Institute of Infectious and Tropical Diseases, University of Brescia, Brescia, Italy
3Department of Infectious, Parasitic & Immune-mediated Diseases, Istituto Superiore di Sanità, Rome, Italy

Correspondence
Maria Antonia De Francesco
maria.defrancesco@unibs.it


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