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Use of Pooled Urine Samples and Automated DNA Isolation To Achieve Improved Sensitivity and Cost-Effectiveness of Large-Scale Testing for *Chlamydia trachomatis* in Pregnant Women

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The success of large-scale screening for *Chlamydia trachomatis* depends on the availability of noninvasive samples, low costs, and high-quality testing. To evaluate *C. trachomatis* testing with pregnant women, first-void urine specimens from 750 consecutive asymptomatic pregnant women from the Rotterdam area (The Netherlands) were collected. Initially, we investigated the performance of three different DNA isolation methods with 350 of these urines and 70 pools of 5 of the same subset of urine samples. The routinely used COBAS AMPLICOR test was compared to the COBAS AMPLICOR test with prior DNA isolation by use of the MagNA Pure large-volume kit and the MagNA Pure bacterial DNA isolation kit. The latter combination provided the best DNA test for pooled urines, with a sensitivity twice that of the other methods. Next, using all 750 urines, the COBAS AMPLICOR performance for individual testing was compared to pooled testing with the standard COBAS AMPLICOR procedure and subsequently to pooled testing with COBAS AMPLICOR in combination with the MagNA Pure bacterial DNA isolation kit. The sensitivity of COBAS AMPLICOR was 65% on individual and 42% on pooled urines but improved to 92% on pooled urines with the MagNA Pure bacterial DNA isolation kit, making this combination the best screening method. The *C. trachomatis* prevalence in this population appeared to be 6.4%. Additionally, the cost of the combined MagNA Pure bacterial DNA isolation kit and COBAS AMPLICOR method on pooled urines was only 56% of the cost of the standard COBAS AMPLICOR test applied to individual urines. Costs per positive case detected in the combined method were 39% of standard costs.

Chlamydia trachomatis is one of the major sexually transmitted pathogens, and high prevalences of chlamydial infection have been documented for asymptomatic women in many European countries (34). Asymptomatic carriers are of substantial importance in the transmission of *C. trachomatis* infection within a community. Asymptomatic chlamydial infection in pregnant women imposes an additional risk for acute and chronic consequences for the women themselves and their (unborn) offspring (2, 6, 8, 9, 16). In The Netherlands, *C. trachomatis* causes most sexually transmitted infections, with approximately 60,000 new cases estimated for a total population of 16 million in the year 2000. Studies in general practice have shown an increase in the incidence of chlamydial infections (26), but data covering other specific target groups outside of the sexually transmitted disease (STD) outpatient clinics are sparse (1). Dutch population-based screening for *C. trachomatis* is still under debate, with cost-effectiveness of screening, complexity of sampling, the reliability of test methods, and the nature of the target population as major issues of discussion (23).

In order to investigate the prevalence of chlamydial infection during pregnancy in Rotterdam, and the risk factors and consequences of chlamydial infection during pregnancy for women and newborns, a follow-up study was planned. We explored different methods for *C. trachomatis* testing with respect to sensitivity and cost-effectiveness. The preferred method for the detection of asymptomatic chlamydial infection with a low threshold should involve urine specimens in combination with nucleic acid amplification techniques (NAATs) (33). However, bacterial loads in urine are generally low, which has an adverse effect on the sensitivity of NAATs (25). Urines of asymptomatic women generate inferior NAAT results, sometimes 10% lower in sensitivity than attained for male urines (10, 30). To reduce the costs of chlamydial screening in low-prevalence populations, pooling of urine specimens has been suggested. Although some studies suggested 100% sensitivity of pooled testing compared to individual testing (7, 17), other studies showed a lower sensitivity (14, 21), which decreased most significantly when eight or more urines were pooled (13, 14). Another important aspect is that large-scale screening programs require automation of test procedures, which should simultaneously improve the quality of testing and reduce the costs.

To date limited data are available concerning NAAT performed on urines from (asymptomatic) pregnant women as well as for NAAT for pooled urines. We present a report of a study of 750 pregnant women in which the performance and

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costs of testing with both pooled urines and automated specimen preparation (using the MagNA Pure LC system and DNA amplification with the COBAS AMPLICOR system) for the detection of asymptomatic *C. trachomatis* infection were evaluated.

MATERIALS AND METHODS

Patient population. Pregnant women and their offspring were enrolled in the Generation R study, a prospective multicenter, population-based cohort trial that includes 10,000 children and their mothers in Rotterdam. The study focuses on growth, development, and health of children from intrauterine fetal life to adolescence (11). Pregnant women before 24 weeks of gestational age who were Dutch residents and expected to deliver in the Rotterdam area were approached to take part in the study. After informed consent was obtained, women were asked for a fresh first-void urine specimen, preferably at a gestational age of 12 weeks. For the current study, 750 urine specimens were tested anonymously.

DNA amplification. Throughout the study, the automated *C. trachomatis* COBAS AMPLICOR PCR system (10) (Roche Diagnostics, Almere, The Netherlands) was used according to the manufacturer's instructions to detect chlamydial DNA in specimens processed by any of the methods described below. Positive specimens were subjected to quantitative LightCycler PCR (version 3.5) to assess the bacterial load (35). For this purpose, DNA was isolated from each specimen according to method IIIB (see below). The PCR protocol was based on the use of the FastStart DNA MasterPLUS SYBR Green I kit (Roche), the primers 5′-GGACAAATCGTATCTCGG-3′ and 5′-GAAACCAACTCTACGCTG-3′, and 40 amplification cycles. The same dilution range of *C. trachomatis* serovar E (10^0, 10^{-2}, and 10^{-4} [relative *C. trachomatis* concentrations]) was included in each run and used to calculate the concentration of target DNA relative to the initial copy number in the undiluted control. Since this control was not subjected to titration, the absolute number of bacteria could not be determined.

Processing of specimens. The 750 samples were analyzed in two separate batches. Initially, a group of 350 samples was tested according to six different protocols as outlined below and in Fig. 1 (methods IA to IIIB). Afterwards, all 750 samples were tested individually using COBAS AMPLICOR, tested in pools of five according to the COBAS AMPLICOR procedure, and tested in pools of five with preceding DNA purification by use of MagNA Pure bacterial DNA isolation kit III.

Method IA: COBAS AMPLICOR procedure on individual urines. Single urine specimens were processed according to the instructions of the COBAS AMPLICOR manufacturer (Roche Diagnostics). In short, a 500-μl urine specimen was diluted with 500 μl of washing buffer and centrifuged at 14,000 rpm. The pellet was resuspended in 250 μl of lysis buffer and centrifuged again after addition of 250 μl of diluent. The supernatant (50 μl) was used for PCR. The results were reported as negative or positive.

Method IB: COBAS AMPLICOR procedure on pooled urines. Pools for the COBAS AMPLICOR were made by adding 100 μl of five different urines into one tube. The 500-μl urine specimen was further processed as described above, and 50 μl of the supernatant was used for PCR. The urines from negative pools were reported as negative. Urines from positive pools were individually retested and reported as described for method IA.

Method IIA: MagNA Pure large-volume kit on individual urines. The (MagNA Pure LC DNA Isolation Kit—Large Volume; Roche Diagnostics) was used to isolate DNA from urines according to the manufacturer's instructions. From individual urines a 1,000-μl specimen was used. DNA was isolated in the automated MagNA Pure LC instrument using an elution volume of 100 μl, of which 25 μl was used for PCR. The results were reported as negative or positive.

Method IIB: MagNA Pure large-volume kit on pooled urines. The MagNA Pure large-volume kit (Roche Diagnostics) was used according to the manufacturer's instructions. Pools were made of five urines by adding 200 μl of each of the five urines into one tube. From these pools the full 1,000-μl specimen was taken and used without further processing. DNA was isolated in the automated MagNA Pure LC instrument using an elution volume of 100 μl, of which 25 μl was used for PCR. The urines from negative pools were reported as negative. Urines from positive pools were individually retested and reported as described for method IIA.

Method IIIA: MagNA Pure bacterial DNA isolation kit on individual urines. The MagNA Pure bacterial DNA isolation kit (MagNA Pure LC DNA Isolation Kit III; Roche Diagnostics) was used to isolate DNA from individual urines. From single urines 500 μl was taken and centrifuged for 10 min at 14,000 rpm. Subsequently 400 μl was removed and the pellet was resuspended in 100 μl of the remaining supernatant, mixed with 130 μl lysis buffer and 20 μl proteinase K, incubated for 10 min at 65°C, and denatured for 10 min at 95°C. Finally, DNA was isolated in the automated MagNA Pure LC instrument using a sample volume of 250 μl and an elution volume of 100 μl. Again, 25 μl was used for PCR. The results were reported as negative or positive.

Method IIIB: MagNA Pure bacterial DNA isolation kit on pooled urines. The MagNA Pure LC Bacterial DNA Isolation Kit III (Roche Diagnostics) was used to isolate DNA from pooled urines. Pools were made of five urines by adding 200 μl of each of the five urines into one tube. From each pool the full 1,000 μl was taken and centrifuged for 10 min at 14,000 rpm. Subsequently 900 μl was removed, and the pellet was resuspended in 100 μl of the remaining supernatant, mixed with 130 μl lysis buffer and 20 μl proteinase K, incubated for 10 min at 65°C, and thereafter denatured for 10 min at 95°C. Finally, DNA was isolated in the automated MagNA Pure LC instrument using a sample volume of 250 μl and an elution volume of 100 μl. Again, 25 μl was used for PCR. The urines from negative pools were reported as negative. Urines from positive pools were individually retested and reported as described for method IIIA.

Figure 1 summarizes the various volumes used in each test method. In methods IIA, IIB, IIIA, and IIIB, the elution buffer did not contain $MgCl_2$ and consequently could not be used directly in the PCR. Therefore, the eluate for amplification was mixed 1:1 with $MgCl_2$-containing diluent from the COBAS AMPLICOR system. In the PCR 50 μl of this mixture was used.

Discrepancy analysis. A specimen was considered to be truly positive if one or more of the test methods described above gave results that were positive for individual samples. When a pool was positive, all individual samples were retested according to the same procedure as used for the pool in order to identify the positive specimen(s). A positive pool result was considered to be truly positive when one or more individual samples within the pool appeared to be positive by either method. A positive pool result was considered to be a false positive when none of the individual samples within the pool turned out positive. A negative pool result was considered a true negative in the presence of a positive internal inhibition control as included in the commercial COBAS AMPLICOR kit. All individual samples and pooled samples were retested when results were discrepant. When the internal control was negative, the sample contained inhibitors. Retesting was performed after diluting the specimen 10-fold and heating the sample for 10 min at 95°C.

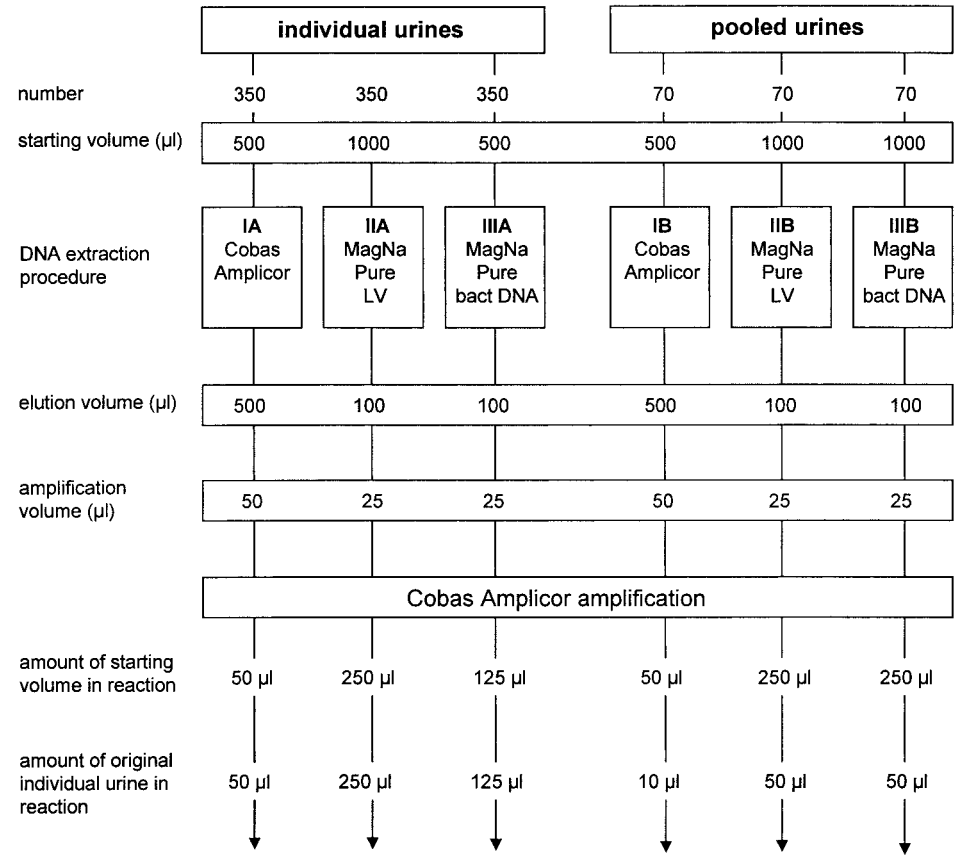
Costs. We calculated the costs of materials and reagents for individual and pooled testing by the standard COBAS AMPLICOR method and by COBAS AMPLICOR in combination with the MagNA Pure bacterial DNA isolation kit. We used list prices available at the time of the study. We assumed full runs for each test method, which consist of 20 specimens plus a positive and a negative control per run for the COBAS AMPLICOR and 32 MagNA Pure specimens. We calculated total costs and costs per positive case detected. We also calculated the costs per positive case using standard COBAS AMPLICOR for individual urines versus the combination of COBAS AMPLICOR with the MagNA Pure bacterial DNA isolation kit for pooled urines. This was done for hypothetical prevalences in a population ranging between 1% and 10%. Calculations were based on full runs and pools of five urines and the sensitivity determined for the COBAS AMPLICOR method with individual urines and for the combined method with pooled urines.

Statistical analysis. Binomial 95% confidence intervals (CI) were calculated for the prevalences and sensitivities of the different DNA isolation methods. McNemar's test was used to compare the two methods. The nonparametric Kruskal-Wallis H test was used to compare median results.

RESULTS

Comparison of three different DNA isolation methods. Figure 1 summarizes the results of the analysis of the initial 350 urine specimens. Individual urines processed according to method IA, IIA, or IIIA scored positive in 15, 14, and 27 cases, respectively. This equals sensitivities of 51.7, 48.3, and 93.1% calculated on the basis of the number of true positives (n = 29). The specificity was 100% for all tests.

Nine pools were positive with the standard COBAS AMPLICOR test method (method IB). The use of the MagNA Pure large-volume kit also yielded 9 positive pools and the use of the MagNA Pure bacterial DNA isolation kit resulted in 19 positive pools, which included the 9 pools that were positive by the standard COBAS AMPLICOR test as well as by the MagNA Pure large-volume kit. Including the MagNA Pure



Results

method	IA	IIA	IIIA	IB	IIB	IIIB
pools positive (%)	NR	NR	NR	9 (12.9)	9 (12.9)	19 (27.1)
individual positives	15	14	27	12	11	27
prevalence estimate (%)	4.3	4.0	7.7	3.4	3.1	7.7
sensitivity (%)	51.7	48.3	93.1*	41.4	37.9	93.1*
specificity (%)	100	100	100	100	100	100

FIG. 1. Methods and results of individual and pooled testing by different DNA isolation methods. Sensitivity values marked with an asterisk indicate that the MagNA Pure bacterial DNA isolation kit provided the best method for DNA processing ($P < 0.01$ [McNemar's test]), with equal levels of sensitivity and specificity for pooled urines and individual urines. NR, not relevant.

bacterial DNA isolation kit clearly provided the most sensitive test method (McNemar's test; $P < 0.01$), with equal sensitivities when testing pooled urines compared to individual urines.

Comparison of the COBAS AMPLICOR method for individual urines versus pooled urines. Pooling of urines was compared to individual testing with the COBAS AMPLICOR method on all 750 urines; results are summarized in Table 1. Testing individual urines by the COBAS AMPLICOR method yielded 31 positive test results out of 750 specimens, resulting in an estimated prevalence for *C. trachomatis* of 4.1% among these pregnant women. Testing of pooled urines by the COBAS AMPLICOR method resulted in 15 positive pools out of 150 pools. Subsequent individual testing of the 75 urines from these 15 pools by the COBAS AMPLICOR yielded 20 positive tests, which with a total of 750 urines resulted in an estimated prevalence of 2.7%. Eleven specimens would have been reported falsely negative when using the COBAS AMPLICOR test only on pooled urines (11/730 = 1.5%), which proved the sensitivity of standard processing of pooled urines by the COBAS AMPLICOR method to be 65% compared to individual testing of urines by the COBAS AMPLICOR method. The number of truly positive samples was 48.

Performance of the MagNA Pure bacterial DNA isolation kit with pooled urines. Pooled urines were tested using the standard COBAS AMPLICOR method as described above, and results were compared to the performance of the combination of the COBAS AMPLICOR method with the

TABLE 1. Test results and costs of individual and pooled urines by different DNA isolation methods

Procedure	No. of positive tests/no. tested	No. of positive-testing women/ no. tested	% Estimated prevalence (95% CI)	% Estimated sensitivity (95% CI)	Total cost (euros)	Cost per case detected (euros)
COBAS AMPLICOR						
Individual urines	31/750	31/750	4.1 (2.8–5.8)	65 (49–78)	8,522	275
Pooled urines	15/150	20/750	2.7 (1.6–4.1)	42 (28–57)	2,562	128
MagNA Pure bacterial DNA isolation kit						
Pooled urines	34/150	44/750	5.9 (4.3–7.8)	92 (80–98)	4,770	108

MagNA Pure bacterial DNA isolation kit (Table 1). All 750 urines were tested in pools of five urines. A total of 34 pools tested positive by COBAS AMPLICOR after DNA isolation was done with the MagNA Pure bacterial DNA isolation kit. Subsequent testing of the 170 individual urine specimens yielded 44 positive urines compared to 20 urines by the standard COBAS AMPLICOR method (McNemar's test; $P < 0.001$).

Two pools which were positive after DNA isolation with the MagNA Pure bacterial DNA isolation kit could not be confirmed by individual testing of urines in either isolation method and were considered false positives. One other pool was positive after DNA isolation by the MagNA Pure bacterial DNA isolation kit, but the individual urines were negative. However, one urine from this pool was positive in the standard COBAS AMPLICOR assay for individual urines. Therefore, the pool/urine result was considered to be a true positive.

Altogether, 48 urines were positive for *C. trachomatis* after individual testing by the COBAS AMPLICOR method with or without the prior use of the MagNA Pure bacterial DNA isolation kit, revealing a prevalence of *C. trachomatis* infection of 6.4% in this population.

When positive individual testing in either method as the gold standard is considered, routine individual testing of urines with the COBAS AMPLICOR method proved to have a sensitivity of 65%. This sensitivity dropped to 42% when the COBAS AMPLICOR method on pooled urines was used. However, when using pooled urines with the combination of the COBAS AMPLICOR method after initial DNA isolation was done with the MagNA Pure bacterial DNA isolation kit, the sensitivity was 92% (see Table 1 for exact figures).

Inhibition. The COBAS AMPLICOR procedure showed inhibition for one (0.7%) of the pools and for 37 (4.9%) of the individually tested urines. After DNA isolation by MagNA Pure LC procedures, no (0%) inhibition was found among pooled urines and inhibition was found only once (0.6%) while testing individual urines.

Bacterial load in pools. Positive urine specimens were subjected to quantitative LightCycler PCR to assess the bacterial load. Figure 2 illustrates the relative *Chlamydia trachomatis* DNA concentrations of pooled urines observed with the use of the LightCycler PCR in relation to the standard COBAS AMPLICOR test results. True positive pools which tested negative by the standard COBAS AMPLICOR method had significantly lower relative *C. trachomatis* concentrations than positive pools (Kruskal-Wallis H test; $P < 0.001$), confirming that bacterial titers do contribute significantly to the sensitivity of testing. Figure 3 illustrates the relative frequency distributions of the bacterial loads established in the urine samples obtained from these essentially symptom-free females. Note that most of the loads are relatively low but that no correlation with the bacterial load in urine samples from symptomatic patients has been made.

Costs. The Dutch costs for a COBAS AMPLICOR test was 10.33 euros per sample (isolation, 0.82 euro; amplification, 4.71 euros; detection, 4.80 euros) and for the MagNA Pure isolation was 4.04 euros per sample with a full run. The cost of the combined method (isolation with the MagNA Pure and subsequent amplification plus detection by COBAS AMPLICOR) was 13.55 euros. The total costs for 750 specimens were, there-

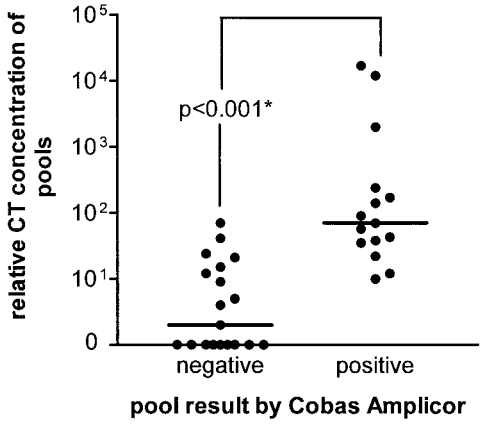


FIG. 2. Relative *Chlamydia trachomatis* (CT) concentrations of pooled urines observed via LightCycler in relation to standard COBAS AMPLICOR test results. Horizontal lines represent the medians of the relative *C. trachomatis* concentrations of pooled urines. *, *P* value (Kruskal-Wallis H test).

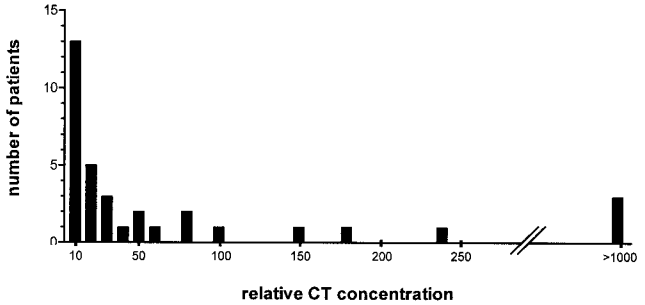


FIG. 3. Relative *Chlamydia trachomatis* (CT) concentrations versus numbers of patients falling in different titer classes. Note that most patients fall within the low-titer classes.

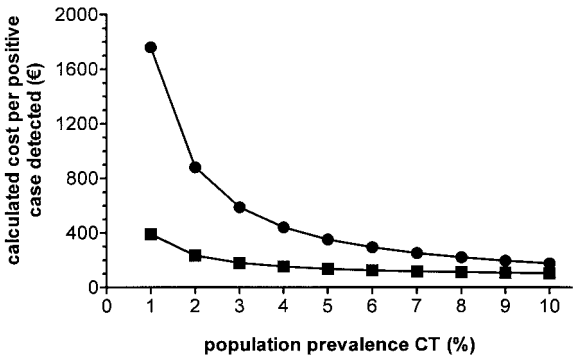


FIG. 4. Costs per positive *Chlamydia trachomatis* case detected in relation to population prevalences. ●, COBAS AMPLICOR results for individual urines; ■, pooled MagNA Pure plus COBAS AMPLICOR results.

fore, 8,522 euros for the COBAS AMPLICOR method with individual urines (750 tested with 75 controls) and 2,562 euros when pooled testing by COBAS AMPLICOR was followed by individual testing (150 pools with 15 controls plus 15 positive pools times 5 individual tests with 8 controls, making 248 tests). The costs for pooled and individual urines with the prior use of the MagNA Pure bacterial DNA isolation kit were 4,770 euros (150 pools with 15 controls plus 34 positive pools times 5 individual urines with 17 controls, making 352 tests). The calculation of screening cost per positive detected case of *C. trachomatis* infection incorporated the sensitivities found in this study: 65% for the COBAS AMPLICOR test for individual urines, 42% for the COBAS AMPLICOR for pooled urines, and 92% for the use of the MagNA Pure bacterial DNA isolation kit in combination with the COBAS AMPLICOR PCR test for pooled urines. Screening costs per positive detected case were lowest with the use of the latter combination (Table 1).

Figure 4 illustrates the difference in costs between standard COBAS AMPLICOR used for individual urines compared to the use of the MagNA Pure bacterial DNA isolation kit in combination with the COBAS AMPLICOR PCR test for pooled urines for hypothetical prevalences ranging from 1% to 10%.

DISCUSSION

Technological aspects. We analyzed 750 individual urine samples by several methods. Overall, 31 samples tested positive upon individual testing using the COBAS AMPLICOR platform. When pooled urines were used without prior DNA purification the sensitivity of the test dropped significantly; only 20 women tested positive. However, upon usage of the MagNA Pure DNA isolation system, an overall number of 48 women tested truly positive (see Table 1 for a summary). So this study shows that pooling of urines combined with prior DNA isolation by use of the MagNA Pure bacterial DNA isolation kit is a reliable and cost-effective way to both increase the sensitivity of testing (by 27%) and decrease the costs per detected case (by 62%) during large-scale testing for asymptomatic *C. trachomatis* infection among pregnant women. Furthermore, we show a 6.4% prevalence of *C. trachomatis* carriage in apparently healthy pregnant women in this Dutch area.

We used the COBAS AMPLICOR test in our study because it is fully automated and its performance is good (10, 28, 30, 32), being less prone to experimental variation than the AMPLICOR test (19, 22). However, the sensitivity of COBAS AMPLICOR for female urines is in the range of 80 to 90%, as has been shown in STD outpatient populations (10, 30). A major problem with the use of urine specimens is inhibition. Urinalysis has shown that various substances are responsible for inhibition (15) and that between 2% and 4% of urine specimens contain inhibitors (4, 28). The sensitivity, however, could be improved by using a modified specimen-processing procedure (18). In our study the inhibition was slightly higher when using the COBAS AMPLICOR method for individual urines (4.9%) but much lower when using the same method for pooled urines (0.7%). However, automated DNA isolation from urines by use of the MagNA Pure Bacterial DNA Isolation Kit prior to COBAS AMPLICOR reduced the inhibition significantly in both individual and pooled testing. This significantly improved the sensitivity of *C. trachomatis* detection. In addition, the use of the MagNA Pure bacterial DNA isolation kit prior to COBAS AMPLICOR resulted in higher sensitivity than automated DNA isolation with the MagNA Pure large-volume kit, which may be explained by the additional use of proteinase K prior to DNA isolation in the bacterial DNA Kit.

Sample pooling and cost aspects. Pooling of urines is important to reduce the costs of screening. However, some describe a significant reduction of the sensitivity (7, 17), whereas others reported a similar sensitivity with pooling (13, 14, 21). In our study, pooling with the COBAS AMPLICOR method resulted in a significant reduction of the sensitivity, which is probably due to the dilution of positive specimens and—as shown—not to the introduction of inhibitors from other urines in a pool. However, the use of the MagNA Pure bacterial DNA isolation kit restored and even improved the sensitivity. The combined procedure was the only method producing acceptable results with pooled urines. Therefore, pooling of urines in large screening programs for the detection of asymptomatic *C. trachomatis* infections should only be used in conjunction with DNA isolation methods that yield highly purified DNA. It should be noted that the sensitivity of our procedure was 92% and not 100%. A low copy number of chlamydial targets in positive urine specimens in our population of asymptomatic women—as shown in Fig. 2—can explain this. Other variables influencing the sensitivity are the quality of specimens and the timing of sampling (5). The sensitivity of screening could be improved by testing multiple specimens obtained at various time points, but this would compromise the cost-benefit ratio of screening programs.

***C. trachomatis* screening among pregnant women.** Pregnant women could be a specific target group for *C. trachomatis* screening. Antenatal screening, as recommended by the Centers for Disease Control and Prevention (3), may be beneficial for decreasing morbidity among women themselves but also to prevent vertical (infant) and horizontal (partner) transmission (2, 6, 8, 9, 24). Screening of pregnant women usually yields prevalences similar to those obtained with nonpregnant women. In Europe, the prevalence of *C. trachomatis* infection

among asymptomatic women was recently estimated to range from 1.7% to 17%, depending on setting, context, and country (34). The prevalence of 6.4% for apparently healthy pregnant Dutch women is much higher than previously reported for asymptomatic women in general practices (2.9% and 4.9% in 1996 and 1997) or in a general obstetric and gynecological population (4.5% in 2002) (1, 27, 31) and approaches the chlamydial prevalence of 7.3% that was found in 1998 among women consulting the STD outpatient clinic in Rotterdam (29). However, these figures must be interpreted with caution since test format is clearly important. Testing of individual urines by the COBAS AMPLICOR method without prior DNA isolation by the MagNA Pure bacterial DNA isolation kit yielded a much lower prevalence of 4.1%.

Screening programs are considered to be cost-effective when the prevalence of *C. trachomatis* infection is higher than 3 to 6% (12, 20, 23, 33). The introduction of improved technology for screening may reveal higher prevalences, rendering screening programs cost-effective. It was shown that COBAS AMPLICOR testing was more cost-effective with pooled urines compared to individual urines, but pooling reduced sensitivity. However, usage of the MagNA Pure bacterial DNA isolation kit increased sensitivity and appeared to be more cost-effective: the calculated costs per detected case in the combined method with pooling were a mere 39% of the costs of individual testing with COBAS AMPLICOR.

In conclusion, we show that pooled testing for *C. trachomatis* infection in asymptomatic pregnant women can be developed for large-scale testing provided that the COBAS AMPLICOR method is used together with prior chlamydial DNA isolation by use of the MagNA Pure bacterial DNA isolation kit. This combination significantly improves sensitivity and decreases costs.

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