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Evidence of Labile Inhibitors in the Detection of *Chlamydia trachomatis* in Cervical Specimens by Polymerase Chain Reaction

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A total of 276 cervical swabs (241 from first visits and 35 from follow-up visits) from 241 women were tested for *Chlamydia trachomatis* by polymerase chain reaction (PCR) and enzyme immunoassay (EIA). Sixty-one smears (53 from first visits and 8 from follow-up visits) from 53 women were stained by direct fluorescent antibody (DFA). Twenty-one (8.7%) women had positive swabs in at least two different tests. All follow-up swabs (collected between 3 days and 3 weeks after the first clinical visit) were positive in at least one test when the woman had been positive at the first visit and no antibiotic treatment had been initiated. Including swabs from follow-up visits and DFA results, the respective sensitivities and specificities of the assays were as follows: PCR, 75.9% and 100%; EIA, 69% and 98.4%. The seven swabs that were false negative by PCR (tested initially after thawing from −20°C) were mailed nonrefrigerated to the assay manufacturer, where they tested true positive. These data point to labile inhibitors of the PCR, predominantly cervical mucus.

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Chlamydia trachomatis infection is the most common sexually transmitted bacterial disease worldwide (1) and is estimated to be responsible for two-thirds of tubal factor infertility (2). Genital chlamydial infections frequently persist for years, predominantly in an asymptomatic state with recurrent symptomatic episodes.

The sensitivities of *Chlamydia trachomatis* culture, direct immunofluorescence, and enzyme-linked immunosorbent assays (EIAs) in cervical specimens have been shown to be in the range of no more than 70 to 80% due to low-level infections (3, 4). Up to 40% of specimens from either male or female subjects contain fewer than ten elementary bodies (5). The polymerase chain reaction (PCR) should, in theory, be able to detect a single DNA molecule by a billion-fold amplification through 30 cycles. However, studies evaluating the Amplicor PCR (Hoffmann-La Roche, Switzerland) claim sensitivities in cervical swabs that are just 10 to 20% above culture (6, 7) or even lower than culture (8).

Observations pointing to PCR inhibition are mentioned in the clinical studies cited above, but the phenomenon is poorly characterized and the mechanism unknown. In this study, results from clinical follow-up visits as previously described (4, 9–11) were included in the evaluation of the Amplicor PCR. By including follow-up swabs, the number of positive specimens was increased, thereby allowing a more direct observation of possible PCR inhibitors.

Materials and Methods. A total of 276 cervical swabs (241 from first visits and 35 from follow-up visits) were collected at random swab order from 241 women experiencing lower abdominal pain who attended the gynecology outpatient clinic at the University Hospital of Freiburg, Germany. All swabs were tested by Amplicor PCR and Ideia EIA (Röhm Pharma, Germany). Fifty-three of the 241 women had cervicitis, irregular bleeding, mucopurulent discharge, or a positive cervical swab

Table 1: Sensitivities of polymerase chain reaction (PCR), enzyme immunoassay (EIA), and direct fluorescent antibody (DFA) at midcycle (day 10–15) compared with day 1–9, day 16–28, and post-partum.

	Sensitivity		
Day of menstrual cycle	PCR*	EIA*	DFA
10–15	20% (1/5)	80% (4/5)	100% (3/3)
1–9, 16–28, and postpartum	90% (19/21)	67% (14/21)	60% (9/15)

* Women using oral contraceptives were excluded, reducing the total number of PCR and EIA specimens from 29 to 26.

taken at an earlier visit. From these women, sixty-one additional cervical swabs (53 at first visits and 8 at follow-up visits) were collected at random swab order and stained by MicroTrak (Syva, USA) direct fluorescent antibody (DFA). After collection, the cervical specimens were refrigerated at 4°C and then stored at −20°C prior to processing. Before test performance all samples were thawed overnight at 4°C.

Polymerase chain reaction, EIA, and DFA were performed using original probe-collection materials and following each manufacturer's recommendations. Excessive cervical mucus was removed from the exocervix. Fifty μl of the 2 ml Amplicor cervical specimen solution were tested by PCR, and 200 μl of the 1 ml Ideia cervical specimen solution were tested by EIA. Direct fluorescent antibody smears containing more than five elementary bodies were considered positive.

Patients were considered true positive for *Chlamydia trachomatis* if they had positive cervical swabs in at least two different tests (PCR, EIA, or DFA) from first or follow-up visits (expanded gold standard). All patients with cervical swabs that were positive in only one test at one clinical visit were re-examined at a follow-up visit. The test was considered false positive if the initial positive result could not be confirmed by a different test at the follow-up visit.

Among other samples, all cervical specimens with discrepant results were mailed blinded and nonrefrigerated in 1 ml STM medium plus 1 ml specimen diluent to the PCR manufacturer.

Results and Discussion. Twenty-one (8.7%) of 241 women were *Chlamydia trachomatis* positive according to the expanded gold standard applied in this study. Including swabs from follow-up visits and DFA results, the respective sensitivities and specificities of the assays were as follows: PCR (retesting by the manufacturer not considered), 75.9% (22 of 29) and 100% (247 of 247); EIA, 69% (20 of 29) and 98.4% (243 of 247).

All women with discrepant results in PCR and EIA were re-examined. Only 15 (52%) of 29 true-positive cervical specimens were both PCR and EIA positive at the follow-up visit. Five specimens (3 with positive follow-up swabs and 2 with positive DFA and follow-up swabs) were false negative by PCR and true positive by EIA. Nine specimens (7 with positive follow-up swabs and 2 with positive DFA and follow-up swabs) were false negative by EIA and true positive by PCR (7 at initial testing and 2 after repeat testing by the manufacturer).

In contrast to PCR and EIA, the sensitivity of DFA decreased significantly ($p < 0.01$) from first- to third-order swab; although the smaller number of specimens assayed and the resulting potential for selection bias must be noted here. The sensitivities for first-, second- and third-order swabs, respectively, were as follows: PCR, 75% (12/16), 78% (7/9), and 75% (3/4); EIA, 56% (5/9), 73% (8/11), and 78% (7/9); and DFA, 91% (10/11), 67% (2/3), and 33% (3/9).

In contrast to EIA and DFA, PCR was significantly less sensitive at midcycle ($p < 0.001$) than at the postovulatory, menstrual, and post-partum phases (Table 1), suggesting cervical mucus as a possible inhibiting factor. When no oral contraceptives are taken, the amount of cervical mucus increases considerably in the endocervix between days 10 and 14 of the menstrual cycle and disappears within hours after ovulation. Swabs collected during the preovulatory phase are inevitably (and sometimes heavily) contaminated with mucus. Cervical mucus was observed in up to 20% of all cervical specimens collected for PCR assay, making pipetting of the 50 μl sample for amplification difficult. The only specimen true positive by PCR at midcycle (day 10) was tested after five days' incubation at room temperature in 1 ml STM plus

Table 2: Optical densities (ODs) of cervical swabs false negative (OD<0.250) by polymerase chain reaction (PCR) at our laboratory (OD initial) and retested by the PCR manufacturer[a] (OD manufacturer). Enzyme immunosorbent assay (EIA) and direct fluorescent antibody (DFA) results are shown for comparison.

Sample no.	Day of menstrual cycle	OD initial	OD manufacturer	EIA	DFA	Swab order
363BP	11	0.088	2.188	neg.	n.d.	EIA / PCR
267CT	13	0.229	3.518	++	+	PCR / EIA / DFA
255KB	11	0.078	3.221	++	n.d.	PCR / EIA
370WN	14	0.076	>4.0	+++	+++	PCR / EIA / DFA
251MH	1	0.078	3.501	+++	n.d.	PCR / EIA
392SV[b]	27	0.061	1.257	++	neg.	EIA / DFA / PCR
400KC	18	0.054	1.828	neg.	neg.	EIA / DFA / PCR

[a] Samples were mailed nonrefrigerated to the manufacturer.
[b] Sample from a patient using an oral contraceptive.
n.d., not done; neg., negative; +, ++, +++, positive.

1 ml specimen diluent (a deviation from the manufacturer's recommendations). No more mucus was observed when the specimens were retested by the PCR manufacturer, and all of the initially false-negative specimens tested true positive there (Table 2). In contrast to cervical swabs, none of six false-negative urines tested true positive at the manufacturer (data not shown).

We observed that cervical mucus swells jelly-like in STM medium (making pipetting difficult) and remains swollen when the specimen is kept frozen at −20°C. At room temperature the mucus is transformed to a flocculent precipitate within several days after addition of 1 ml specimen diluent. Mucus precipitation takes only minutes when the sample is heated to 95°C.

Loeffelholz et al. (6) pointed out that heating to 95°C alleviated PCR inhibition in some but not all specimens, which correlates well with our observation of mucus precipitation at 95°C. Interestingly, EIA specimens are heated to 95°C before test performance whereas Amplicor PCR specimens are not. Phillips et al. (12) observed cervical mucus partially obscuring the DFA slide in 38% of all cervical swabs, but mucus did not preclude examination of any slide (methanol fixation).

Bauwens et al. (8) observed 11 cervical specimens false negative by Amplicor PCR, with up to 15,000 inclusions in the corresponding cell culture. Seven of these 11 turned true positive on repeat analysis two to five days after the initial run (storage conditions not mentioned), which corresponds with our data.

The optical densities of specimens 251MH, 392SV, and 400KC reported by the PCR manufacturer (Table 2) suggest some labile, possibly nonmucus, inhibitors.

The data of this study were evaluated retrospectively, and the circumstantial evidence pointing to cervical mucus as a major inhibitor of the Amplicor PCR is strong. For definite proof, a prospective study should be designed with documentation of menstrual cycle, mucus contamination of cervical specimens, and storage conditions. Aliquots of all samples should be spiked with the positive Amplicor control for indication of any inhibition.

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European Multicentre Evaluation of a Commercial System for Identification of Methicillin-Resistant *Staphylococcus aureus*

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A commercial system for the rapid detection of methicillin-resistant *Staphylococcus aureus*, the BBL Crystal MRSA test (C-MRSA ID; Becton Dickinson, USA), was evaluated prospectively and compared with a polymerase chain reaction test for the presence of the *mecA* gene. Ten European centres tested a total of 676 isolates of *Staphylococcus aureus* from blood cultures. The system correctly identified 661 (97.8%) isolates within 4 h. All but three *mecA* gene-negative isolates (99.4% specificity) yielded a negative C-MRSA ID reaction, and 158 of 170 *mecA* gene-positive isolates were accurately detected (92.9% sensitivity). After repeated testing of discrepant results, sensitivity and specificity increased to 99% and 100%, respectively.

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