

## EVALUATION OF COMMERCIALY AVAILABLE TESTS FOR *CHLAMYDIA* NUCLEIC ACID DETECTION IN SYNOVIAL FLUID OF PATIENTS

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### SUMMARY

Since the presence of *Chlamydia* nucleic acids has been shown in synovial fluid (SF) from some patients with *Chlamydia* reactive arthritis, we investigated whether commercially available tests, developed to detect *Chlamydia* nucleic acids in urogenital samples, could also be used for their detection in SF samples. We therefore tested SF samples, found positive with at least two different systems of DNA amplification in a previous study, with three commercially available kits. No positive results were obtained. It is concluded that the commercially available tests Gen-Probe PACE 2, Amplicor (developed by Roche Molecular Systems) and LCx (developed by Abbott Laboratories) do not have sufficient sensitivity to detect reliably *Chlamydia* RNA or DNA in SF.

KEY WORDS: *Chlamydia*, Synovial fluid, rRNA, DNA.

SINCE it has been shown that chlamydial rRNA and DNA are present in synovial fluid (SF) and tissue [1–4] of some patients with reactive arthritis and undifferentiated seronegative oligoarthritis, it could be tempting to use the different commercially available kits, developed to detect *Chlamydia* nucleic acids in urogenital samples, to look for *Chlamydia* nucleic acids in SF samples. These tests are much easier to perform than nested polymerase chain reaction (PCR) followed by gel electrophoresis, blotting and hybridization.

The first kit developed was a DNA–RNA hybridization system with a chemiluminescent-labelled, single-stranded DNA probe complementary to the *Chlamydia trachomatis* rRNA (PACE 2 developed by Gen-Probe). Later, a PCR test (Amplicor, developed by Roche Molecular Systems) became available, using a single set of biotinylated oligonucleotide primers directed against a 207 bp fragment of the *C. trachomatis* plasmid. Biotinylated amplicons are detected colorimetrically after specific hybridization with an immobilized oligonucleotide capture probe bound to the bottom of wells of a 96 microwell plate [5]. The last one is a ligase chain reaction (LCR)-based assay (LCx, developed by Abbott Laboratories). This uses two complementary pairs of probes targeted at a 48 bp fragment of the plasmid, which, when the correct template is available, hybridize next to each other. Polymerase acts to fill in the gap and then ligase can covalently join the pair of probes. Amplified products are detected with a microparticle sandwich immunoassay based on covalent labelling of the four probes with two different immunoreactive chemical groups [6]. These tests are aimed at use with urogenital samples, and the PCR- and LCR-based assays with urine samples as well. The

last two give excellent specificity and sensitivity when compared to culture and antigen detection [6, 7].

### PATIENTS AND METHODS

#### Patients

Thirteen to 16 SF samples found positive with at least two different systems of DNA amplification and 5–23 samples found negative in a previous study [1] were used to evaluate the commercially available tests.

#### Quantitation of *Chlamydia trachomatis* elementary bodies (EB)

A dilution panel of *C. trachomatis* serovar D (American Type Culture Collection, Rockville, MD, USA) was prepared from stock grown in McCoy cells. A fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody (MicroTrak, Syva, Palo Alto, CA, USA) was added as specified in the manufacturer's instructions. The FITC-antibody-stained EB were counted by epifluorescent microscopy.

#### Treatment of samples

SF pellets were prepared from aliquots of 1 ml as previously described [1]. Aliquots of 100  $\mu$ l were also prepared for each sample at the time of collection of the SF and stored at  $-80^{\circ}\text{C}$  until used.

#### Detection of *Chlamydia trachomatis* rRNA by Gen-Probe PACE 2 system (Gen-Probe Inc., San Diego, CA, USA)

SF pellets and 100  $\mu$ l aliquots were thawed, set into Gen-Probe transport tubes and vortexed to ensure suspension in the transport medium. They were otherwise processed according to the instructions of the kit manufacturer.

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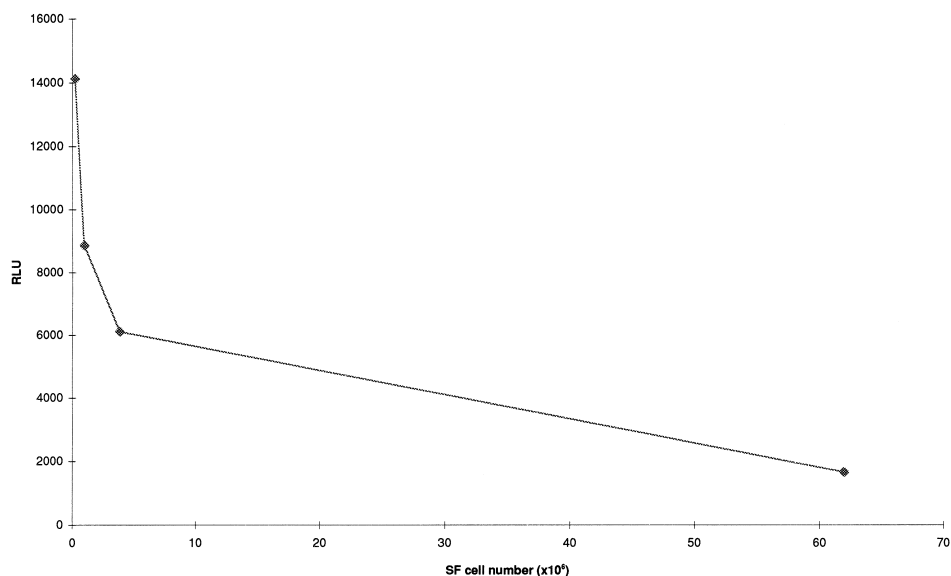


FIG. 1.—Gen-Probe PACE 2 system: response for 10 000 *Chlamydia trachomatis* elementary bodies; influence of the SF cell number on the relative light unit (RLU) values.

*Detection of Chlamydia trachomatis plasmid DNA by AmpliCor (Roche Molecular Systems, Basel, Switzerland)*

Different experimental conditions were tested. SF pellets and 100  $\mu$ l aliquots were either treated with hyaluronidase and proteinase K as previously described [1], or processed as urine samples according to the manufacturer's instructions.

*Detection of Chlamydia trachomatis plasmid DNA by LCx (Abbott Laboratories, North Chicago, IL, USA)*

Different experimental conditions were tested. Hundred microlitre aliquots of SF were either diluted 1/2 or 1/10 (with or without proteinase K treatment [1] for dilution 1/2). After heating to 97°C for 15 min,

100  $\mu$ l of the mixture were used for the amplification procedure.

*Statistics*

Differences between groups were estimated using the two-tail *t*-test (Student).

RESULTS

*Sensitivity of the Gen-Probe PACE 2 system*

A positive result was always obtained when 10 000 EB were added to four different pellets prepared from 1 ml of control SF ( $2.4 \times 10^5$ – $6.2 \times 10^7$  cells) (Fig. 1). However, as mentioned previously, the dissolution of the SF pellets was not always complete. With 100  $\mu$ l aliquots, a positive result (close to the

TABLE I  
Results of testing SF for the presence of *Chlamydia trachomatis* (CT) rRNA by the Gen-Probe PACE 2 system

Sensitivity						
	Number of CT elementary bodies present in the test	Number of SF cells present in the test	Relative light units (RLU)	Cut-off	RLU sample/RLU cut-off	Results
SF pellet	2000	$12 \times 10^6$	1655	403	4.1	+
	200	$12 \times 10^6$	174	403	0.43	–
	20	$12 \times 10^6$	144	403	0.36	–
100 $\mu$ l aliquot	2000	$1.2 \times 10^6$	444	403	1.1	+
	200	$1.2 \times 10^6$	133	403	0.33	–
	20	$1.2 \times 10^6$	60	403	0.15	–
Results obtained with samples tested						
Type of samples	Number of SF cells present in the test (minimum–maximum)	Relative light units (RLU) (minimum–maximum)	Cut-off	RLU sample/RLU cut-off (mean $\pm$ S.D.)	Results	
SF pellets previously found positive <i>n</i> = 15	$0.05 \times 10^6$ – $44 \times 10^6$	33–239	403 or 455	$0.24 \pm 0.12$	–	
SF pellets previously found negative <i>n</i> = 23	$1 \times 10^6$ – $18 \times 10^6$	27–191	403 or 455	$0.18 \pm 0.1$	–	
<i>t</i> -test				ns		

TABLE II

Results of testing SF for the presence of *Chlamydia trachomatis* (CT) plasmid DNA by Amplicor (Roche Molecular Systems)

Sensitivity			
Number of CT elementary bodies present in the test	Number of SF cells present in the test	OD (cut-off: 0.25)	Results
1000	$0.008 \times 10^5$	0.058	-
1000	$0.03 \times 10^5$	1.364	+
1000	$0.8 \times 10^5$	0.054	-
1000	$3.5 \times 10^5$	0.062	-
1000	$7.3 \times 10^5$	0.041	-

Results obtained with samples tested

Type of samples	Number of SF cells present in the test (minimum-maximum)	OD (cut-off: 0.25) (mean $\pm$ s.d.)	Results
SF previously found positive <i>n</i> = 16	$0.2 \times 10^5$ - $4.5 \times 10^5$	$0.088 \pm 0.040$	-
SF previously found negative <i>n</i> = 5	$0.008 \times 10^5$ - $7.3 \times 10^5$	$0.076 \pm 0.01$	-
<i>t</i> -test		ns	

detection limit) was also obtained when 10 000 EB were added (2000 EB present in the test) (Table I).

#### Sensitivity of the Amplicor system

When SF pellets were mixed with 1.25 ml of urine resuspension buffer and 1.25 ml of urine diluent, no dissolution was observed, except for one of the 34 pellets tested. This pellet contained the lowest number of SF cells ( $5 \times 10^4$ ) and a positive result was obtained when 20 EB were added.

However, since these experimental conditions did not allow the majority of the pellets to be dissolved, other experimental conditions were tested. With samples previously found positive [1], the highest optical density values were obtained when 100  $\mu$ l aliquots of SF were mixed with 100  $\mu$ l of urine resuspension buffer and 100  $\mu$ l of urine diluent. In these conditions, 1000 EB were added to five different SF preparations and one positive result was obtained for one sample containing  $0.03 \times 10^5$  cells (in the test) (Table II).

#### Sensitivity of the LCx system

With samples previously found positive [1], the highest rates of fluorescence were obtained when aliquots of SF were mixed with an equal volume of urine specimen resuspension buffer. In these conditions, 60 EB were added to five different SF preparations and two positive results were obtained for the samples containing  $0.01 \times 10^6$  and  $1.1 \times 10^6$  cells (in the test) (Table III).

#### Comparison of the sensitivities of the different systems to detect *Chlamydia trachomatis* nucleic acids in SF

The sensitivities varied from 20 EB/ml with nested PCR targeting plasmid sequences [1] to 100 000 EB/ml of SF with the Gen-Probe PACE 2 system. The best sensitivity of the commercially available tests was

TABLE III

Results of testing SF for the presence of *Chlamydia trachomatis* (CT) plasmid DNA by LCx (Abbott Laboratories)

Sensitivity			
Number of CT elementary bodies present in the test	Number of SF cells present in the test	OD (cut-off: 1)	Results
60	$0.003 \times 10^6$	0.28	-
60	$0.01 \times 10^6$	3.17	+
60	$0.25 \times 10^6$	0.61	-
60	$1.1 \times 10^6$	2.97	+
60	$2.2 \times 10^6$	0.42	-

Results obtained with samples tested

Type of samples	Number of SF cells present in the test (minimum-maximum)	OD (cut-off: 0.25) (mean $\pm$ s.d.)	Results
SF previously found positive <i>n</i> = 13	$0.05 \times 10^6$ - $1.4 \times 10^6$	$0.33 \pm 0.22$	-
SF previously found negative <i>n</i> = 5	$0.003 \times 10^6$ - $2.2 \times 10^6$	$0.39 \pm 0.25$	-
<i>t</i> -test		ns	

obtained with the LCx system (1200 EB/ml of SF) (Table IV).

#### Detection of *Chlamydia trachomatis* rRNA and plasmid DNA by commercially available tests in SF samples

SF samples previously found positive with at least two different systems of DNA amplification [1] and SF samples previously found negative were tested. No positive results were obtained according to the instructions of the kit manufacturers and the values obtained for samples previously found positive were not significantly different from those obtained for samples previously found negative (Tables I-III).

With the Gen-Probe PACE 2 system, three SF aliquots of 100  $\mu$ l previously found positive and 12 previously found negative were also tested and found negative.

TABLE IV

Sensitivities of the different systems tested to detect *Chlamydia trachomatis* nucleic acids in SF

	Minimal number of <i>Chlamydia trachomatis</i> elementary bodies to obtain positivity	
	Present in the test	Present in 1 ml of SF
Nested PCR targeting plasmid sequences [1]	1-10	20-200
PACE 2 (Gen-Probe)	2000	10 000†-100 000‡
Amplicor (Roche Molecular Systems)	(20)*-1000	(1000)*-60 000
LCx (Abbott Laboratories)	60	1200

\*Result obtained only with the SF pellet containing the lowest number of cells ( $5 \times 10^4$ ).

†Result obtained with SF pellets (not always completely dissolved).

‡Result obtained with 100  $\mu$ l aliquots of SF.

For the commercially available PCR system (Amplicor), processing the specimens after heat treatment to 95°C and centrifugation decreased the values for both groups of samples.

### DISCUSSION

Three commercially available tests, developed to detect *Chlamydia* nucleic acids in urogenital samples, were tested with SF samples. We performed the assays either directly on SF pellets (with Gen-Probe PACE 2 and Amplicor) or on 100 µl aliquots, without nucleic acid extraction, in order to minimize sample handling and to see whether these kits could be used in a very simple way to detect *Chlamydia* nucleic acids in SF specimens. However, it is important to be aware that the sensitivity of these tests depends largely on the specimen processing. To be used with good chances of success, it is necessary to test a large amount of a sample, but this requirement is contraindicated with the presence of Taq polymerase in two of these kits. Indeed, this enzyme is sensitive to inhibitors present in SF samples and will be less inhibited if the specimens are more diluted. The 'ideal' experimental conditions are a balance between these two requirements.

The use of SF pellets could be an advantage since it allows a larger number of cells to be tested without inhibitor products susceptible to being present in SF. The comparison of the sensitivities obtained for the SF pellets and the 100 µl aliquots was performed with the Gen-Probe PACE 2 system and with Amplicor. For both systems, the best sensitivity is obtained with SF pellets. However, their use is limited by the difficulty of dissolving them and, if the pellets are not completely dissolved, it is difficult to assume an efficient detection of nucleic acids.

The sensitivity of the kits was tested by adding increasing amounts of *C. trachomatis* EB to SF samples before processing. With Amplicor, a sensitivity of 20 EB was obtained when a non-inflammatory SF containing  $5 \times 10^4$  cells/ml was used. However, it was not possible to test the other samples in the same experimental conditions since they were not dissolved. In conditions allowing the processing of all the samples, the best sensitivity of the three commercially available tests varied from 2000 (Gen-Probe PACE 2) to 60 EB (LCx), corresponding to 100 000 (Gen-Probe PACE 2) to 1200 EB (LCx) per millilitre of SF.

For the Gen-Probe PACE 2 system, we obtained a higher sensitivity than Kuipers *et al.* [8] who reported a sensitivity of  $1 \times 10^6$  EB/ml of SF. This discrepancy could be explained by differences in the specimen processing or by the number of cells present in the samples. Indeed, our results show that the higher the number of cells, the lower the sensitivity of this system.

For the PCR and LCR systems, the sensitivities also depended on the samples and not only on the cell number. The presence of more amplification inhibitors in some particular SF could explain the variable sensitivities observed.

Samples previously found positive with at least two different systems of DNA amplification [1] were tested with these commercially available tests and no positive results were obtained. This could be explained by the insensitivity of these tests. Indeed, the DNA amplification systems developed by Bas *et al.* [1] had a sensitivity between 1 and 50 EB (present in the test). However, the LCx system had a sensitivity close to 50 EB for 2/5 samples. It could be possible to find positive results in samples without a strong inhibition effect or in synovial tissue samples since Branigan *et al.* [9] found that chlamydial DNA is more reliably identified in tissues than in SF. It is also important to be aware of the difficulty of determining the sensitivity of these assays, the DNA template coming from added EB is probably not comparable in quality and quantity to the DNA coming from SF samples.

For each test, we compared the means obtained for samples previously found positive with the means obtained for samples previously found negative in order to see whether a difference could be observed in spite of the fact that the values were lower than the cut-off. No significant differences were observed.

In conclusion, in our experimental conditions, the commercially available kits Gen-Probe PACE 2, Amplicor and LCx cannot be reliably used to detect *Chlamydia* RNA or DNA in SF.

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