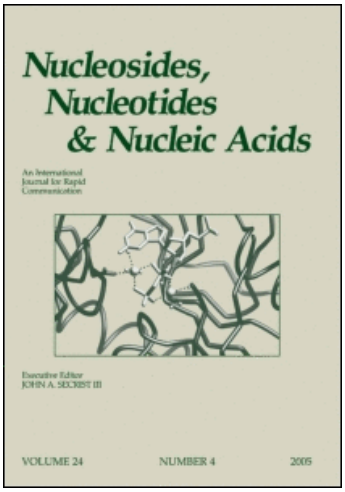

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

LABORATORY DIAGNOSIS OF RESPIRATORY DISEASES: PCR VERSUS SEROLOGY

H. Haaheim[a]; L. Vorland[a]; T. J. Gutteberg[a]
[a] Department of Microbiology, University Hospital of Tromsø, Norway

Online publication date: 31 March 2001

To cite this Article Haaheim, H. , Vorland, L. and Gutteberg, T. J.(2001) 'LABORATORY DIAGNOSIS OF RESPIRATORY DISEASES: PCR VERSUS SEROLOGY', Nucleosides, Nucleotides and Nucleic Acids, 20: 4, 1255 — 1258
To link to this Article: DOI: 10.1081/NCN-100002530
URL: http://dx.doi.org/10.1081/NCN-100002530

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(4–7), 1255–1258 (2001)

LABORATORY DIAGNOSIS OF RESPIRATORY DISEASES: PCR VERSUS SEROLOGY

H. Haaheim,[1,*] L. Vorland,[1,2] and T. J. Gutteberg[1,2]

[1]Department of Microbiology, University Hospital of Tromsø, N-9038, Norway
[2]Department of Medical Microbiology, University of Tromsø, Norway

ABSTRACT

We present a comparison between serology and genetic detection of three bacterial pathogens causing lower respiratory tract infection (LRI). We evaluated serology and PCR for the detection of *Mycoplasma pneumoniae*, *Bordetella pertussis* and *Chlamydia pneumoniae* from 1712 nasopharyngeal and serum samples. For 856 nasopharyngeal samples, average PCR time was 7.2 days, the parallel serum samples was 13 days. Automated extraction of nucleic acids reduces average PCR time to 6.7 days.

Rapid diagnosis of a disease is the basis for effective treatment and cure of a patient. In the case of LRI caused by *C. pneumoniae* (CP), *M. pneumoniae* (MP) and *B. pertussis* (BP), serological assays have been the traditional choice of laboratory diagnosis. PCR has become an integrated part in analyzing these clinical specimens the later years (1–3). This study aims to briefly compare and evaluate different factors when applying these two techniques in the diagnostic laboratory.

MATERIALS AND METHODS

1. *Clinical specimens*: Nasopharyngeal and serum samples were drawn upon symptoms of LRI from the patient within one week. Serum samples were stored

*Corresponding author.

at −20°C until processing for antibody detection. Nasopharyngeal samples were stored at +4°C until extraction for PCR.

Sample preparation: Each nasopharyngeal sample was divided in two aliquots. One aliquot was processed as native sample, and the other was spiked with 2000 copies/ml internal control upon extraction. Extraction was performed using either the manual QIAamp DNA mini kit or the automatic QIAamp 96 DNA Blood BioRobot9604 Kit (Qiagen Inc. Valencia, California) according to the manufacturer's instruction.

PCR: The genetic detection of CP, MP, and BP was carried out mixing 5 μl extracted sample with respective master mixes containing nucleotides, primers and 1, 0 U of Taq Polymerase GOLD (Perkin-Elmer Corp., Foster City, California) (1–3). Internal and external controls such as buffer, ddH2O, and DNA of interest were included. PCR products (15 μl) were separated by 3% agarose-gel electrophoresis at 70 mA for 1h in buffer 0.75 × PBS and visualized with etidium bromide (0.5 μg/ml).

Serology: Antibody detection was performed using the ELISA classic Mycoplasma pneumoniae IgG, IgA and IgM test kit (Virion-Serion GmbH, Würtzburg, Germany), Pertusscan 2 + 2TM (Euro-Diagnostica, Arnheim, the Netherlands), and the Chlamydia pneumoniae IgG, IgM, and IgA micro IF test (Labsystems Oy, Helsinki, Finland) according to manufacturer's instructions.

RESULTS

Assay performance: Table 1 shows results for PCR and serology. In 1999 and 2000 we observed, respectively, 3 and 9 PCR and serology positive samples, 324 and 363 PCR and serology negative samples, and 50 and 108 discrepant PCR and serology results.

Table 1. Test Results Using Species Specific PCR or Serology for the Detection of *B. Pertussis* ($n = 196$), *C. Pneumoniae* ($n = 297$), and *M. Pneumoniae* ($n = 363$). Numbers in Boldface Represent Intra-Methodological Agreement with Test Results

		2000		1999	
		Ab pos	Ab neg	Ab pos	Ab neg
B. pertussis	PCR pos	2[1]	7	1	9
	PCR neg	21	47	11	98
C. pneumoniae	PCR pos	3	26	0	5
	PCR neg	18	142	3	100
M. pneumoniae	PCR pos	4	12	2	2
	PCR neg	24	173	20	126

[1]Numbers in boldface where derived when both tests where positive. Due to biological differences (i.e. the presence of pathogen prior to antibodies or antibodies present subsequent to removal of pathogen) no methodological Gold standard could be applied to determine degree of false positives and negatives.

Laboratory costs: Estimated costs for MIF and EIA range between US$ 8 and 11. PCR costs range between US$ 8 and 10 depending on whether the PCR is nested or conventional. All estimates include disposables and reagents, however; it does not include wages and payroll taxes.

On-hand workload: On-hand workload was calculated to be about three hours for EIA. MIF on-hand workload is six hours. PCR makes up for three to four hours depending on whether the PCR is nested or conventional.

Time frame: "Time frame" covers the registration of clinical sample to distribution of a written report to the medical practitioner. Average PCR time frame was 7.2 days. Parallel serum samples had an average time frame of 13 days. After the introduction of robotic extraction of nucleic acids in May 2000, average PCR time frame was reduced to 6.7 days.

DISCUSSION

Few samples have been found positive in both serological assay and PCR. More samples have been found positive in serological assays than in PCR. A positive PCR depends on the presence of the microbial DNA. Treatment, host defense, or nucleases may have removed the DNA prior to sample collection. Serological assays may in general have some false positive results, e.g. because of cross-reactivity with other species. PCR is highly specific, and more sensitive than culture and other non-cultural methods (4). Half or more of the samples with positive PCR are found negative in serological assays. IgM antibody characteristically appear 7–10 days after primary infection and reach maximum titers within 2–3 weeks (5). If the sample is taken in an early stage, the microbe will be present, giving a positive PCR result, while antibody will not yet have been produced in sufficient amount, giving a serological negative result.

The calculation of laboratory costs will be inaccurate because of different degrees of manual work and international fluctuations in the rate of exchange. Concerning EIA, the prize will increase when a few samples are analyzed in preference to processing a full sample plate. The same would probably be the case with a higher degree of automation with the PCR techniques. When including laboratory equipment, the serological assays will be the least expensive. However, taking into consideration the governmental reimbursement issued in Norway, PCR forcible exceeds earning ability compared to serology. Introducing automatic nucleic acid extraction from clinical material did reduce workload and time consumption, further reducing total cost. In addition to the high specificity and sensitivity in the acute stage of infection, this endorses investments in required equipment and qualified staff.

We aspire to perform PCR at the early stage of infection and to do a serology follow up 2 weeks later. For an accurate and fast diagnosis, PCR is our choice and recommendation when sample is drawn early after onset of disease. We also believe that the introduction of automated nucleic acid extraction and real-time qualitative detection of PCR products will further reduce average time consumption to <5 days and thereby offer the medical practitioner and his patients better services.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

REFERENCES

1. Tong, C. Y. W. and Sillis, M. *J. Clin. Pathol.*, **1993**, *46*, 313–317.
2. Abele-Horn, M., Busch, U., Nitscko, H., Jacobs, E., Bax, R., Pfaff, F., Schaffer, B., Heesemann, J. *J. Clin. Microbiol.*, **1998**, *36*(2), 548–551.
3. Wadowsky, R. M., Laus, S., Libert, T., States, S. J. and Ehrlich, G. D. *J. Clin. Microbiol.*, **1994**, *32*(4), 1054–1057.
4. Murray, P. R. (ed.), in *Manual of Clinical Microbiology*, **1999**, 620, 787, 800–801.
5. Noel R. Rose (ed.), in *Manual of Clinical Laboratory Immunology*, **1992**, 551.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Request Permission or Order Reprints Instantly!