Different approaches have been described to diagnose LGV infections (Figure). The first 3 approaches have serious disadvantages: cell culture is rarely available in routine diagnostic settings, polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) analysis (usually nested PCR approaches are used) needs post-PCR restriction enzyme profiling, and sequencing requires additional analyses of sequence data to identify the Chlamydia trachomatis serovar responsible for infection. In addition, all 3 techniques are time consuming (at least 1–4 days to get a result), laborious, and require specially trained personnel in a sophisticated laboratory setting. Therefore, we developed a real-time PCR approach (TaqMan and Rotorgene) that can easily identify LGV strains in 2 hours with equipment that is available in almost all diagnostic settings.

We used the polymorphic membrane protein H gene (pmp gene) as a PCR target because it has a unique gap in LGV strains of C. trachomatis, compared to other serovars, which makes it highly specific. The following primers and probes were selected: LGV-F 5′ CTG TGC CAA CCT CAT CAT CAA 3′, LGV-R 5′ AGA CCC TTT CCG AGC ATC ACT 3′, and LGV MGB-probe 6-FAM-CCT GCA ACA GT. Real-time PCR conditions (20-µL format) for TaqMan were as follows: 2× TaqMan Universal Mastermix (Applied Biosystems, Foster City, CA, USA), 18 pmol each primer, 0.2 µmol/L probe, and 2 µL (LGV L2) DNA or clinical sample; 2 min at 50°C, 10 min at 95°C, and 40 cycles of 15 sec at 95°C and 1 min at 60°C. Conditions for Rotorgene were as follows: 10× buffer (Hoffman-La Roche Ltd, Basel, Switzerland), 10 pmol each primer, 0.04 µmol/L probe, 2 µL (LGV L2) DNA or clinical sample; 2 min at 50°C, 10 min at 95°C, and 45 cycles of 15 sec at 95°C and 1 min at 60°C. By using a previously described serial dilution of LGV L2 (7), sensitivity was assessed as 0.01 inclusion-forming units for both real-time PCR assays.

To determine specificity, we tested different C. trachomatis serovars and serovariants A, B, Ba, C, D, Da, D−, E, F, G, Ga, H, I, Ia, I−, J, Jv, K, L1, L2, L2b, L3, C. muridarum (MoPn), C. pneumoniae, C. pecorum, C. psittaci, and 32 other microorganisms that normally reside in the human perianal and urogenital region and in the oropharynx. These organisms included gram-positive and gram-negative bacteria and yeast: Acinetobacter baumannii, Campylobacter jejuni, Candida albicans, other yeast, Enterobacter agglomerans, Enterococcus faecalis, Escherichia coli, Streptococcus spp., Haemophilus influenzae, Klebsiella pneumoniae, Mycoplasma spp., Neisseria meningitidis, Pasteurella spp., Pseudomonas aeruginosa, Salmonella enteritidis, Shigella sonnei, Staphylococcus aureus, and others. Only LGV strains L1, L2, L2b, and L3 tested positive in both the TaqMan and Rotorgene assays, which shows the analytical specificity of real-time PCR.

Figure. Diagnosis of lymphogranuloma venereum. MIF, microimmunofluorescence; STI, sexually transmitted infection; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; PAA, poly acrylamide; BLAST, basic local alignment search tool.
Subsequently, we determined in a blinded setting the presence of LGV in a selected group of patients (clinical spectrum and epidemiology described elsewhere [8]) according to C. trachomatis–positive rectal swab (Chlamydia 2SP Collection & Transport Kit [Quelab] by commercially available PCR (COBAS AMPLICOR, Hoffman-La Roche Ltd). By using the 2 reference standard techniques to type C. trachomatis serovars (PCR-based RFLP of the omp1 gene or sequencing the variable segment 2 [VS-2] of the omp1 gene) (9,10) with DNA isolated from rectal swab specimens (standard isopropanol DNA isolation method), we identified 28 of 125 men as LGV-positive. These 28 samples were also positive in both the TaqMan and Rotorgene assays. We also identified 2 additional LGV infections, which were initially typed and then retested as single-strain infections with serovars E and D by both PCR-based RFLP analysis and VS-2 sequencing. This discrepancy is most likely due to a double infection, which will, in most cases, result in the preferential amplification of 1 strain in the omp1 PCR and PCR-based sequencing methods; in the TaqMan and Rotorgene assays, only LGV strains can be amplified. Whether this outbreak is partially technically driven must be assessed in the future by retrospectively investigating the presence of these LGV infections in men who have sex with men and the presence of the L2b strain in the past, since at present only LGV infections from 2003 to 2005 have been investigated.

Servaas A. Morré,†* Joke Spaargaren,‡+ Johannes S.A. Fennema,† Henry J.C. de Vries,‡++, Roel A. Coutinho,‡++, and A. Salvador Peña*

†These authors contributed equally to this study.

References


Address for correspondence: Servaas A. Morré, VU University Medical Center, Faculty of Medicine, Laboratory of Immunogenetics, Van der Boechorststraat 7, 1081 BT, Amsterdam, the Netherlands; fax: 31-20-44-48418; email: samorretravel@yahoo.co.uk

---

SARS Vaccine Protective in Mice

To the Editor: Less than a year after the identification of the severe acute respiratory syndrome coronavirus (SARS-CoV) (1), 3 independent laboratories reported protection from SARS-CoV challenge in animal models using a DNA vaccine or recombinant forms of the modified vaccinia Ankara or a parainfluenza virus, encoding the spike gene (2–4). Their protective efficacies are encouraging because they provide proof that a SARS-CoV vaccine is feasible. However, vaccines based on those technologies are not licensed for human use, and recommendation and licensing will likely take many years. We have developed an inactivated virus vaccine that induces neutralizing antibodies and protects against SARS-CoV challenge.

The vaccine was produced as described elsewhere (5). Briefly, the SARS-CoV (strain FRA, GenBank accession no. AY310120) was grown in Vero cells, inactivated with β-propiolactone (BPL), and complete inactivation was confirmed by 2 consecutive passages on Vero cells. Inactivated virus was purified by column chromatography followed by sucrose gradient centrifugation. The fraction containing virus was dialyzed.