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Letters to the Editor

Chlamydia pneumoniae in Peripheral Blood Mononuclear Cells Isolated from Individuals Younger than 20 Years or Older than 60 Years

Serological studies of *Chlamydia pneumoniae* in humans have shown a correlation between the age of a population and the number of individuals positive for *C. pneumoniae* antibodies. Grayston (1) has reported that individuals younger than 30 had 50% seropositivity and that individuals older than 60 had 80% seropositivity. We searched for *C. pneumoniae* DNA in peripheral blood mononuclear cells (PBMCs) isolated from two groups: those younger than 20 years of age and those older than 60 years of age.

PBMCs were prepared using either Sigma Histopaque-1077 (Sigma Chemical Company, St. Louis, MO) or traditional Ficoll-based methods. Isolated PBMCs were resuspended in 4 ml of RPMI medium purchased from Invitrogen (Carlsbad, CA). Aliquots (1 ml) were held at −80°C for later analysis.

Two 1-ml frozen aliquots were shipped to the Johns Hopkins University (JHU) on dry ice, and one was processed at the Providence Portland Medical Center (PPMC). The PPMC samples were extracted using the bacterial cell protocol in the QIAamp DNA blood mini kit (QIAGEN, Valencia, CA). The extracted DNA was resuspended in 50 μl extraction buffer. The JHU samples were extracted with a Roche MagNA Pure LC robot, with reagents and protocols determined by the manufacturer.

PPMC used a Cepheid SmartCycler (Cepheid, Sunnyvale, CA) with primers and probes that target the *C. pneumoniae* outer membrane protein gene sequence number 144566 from the National Center for Biological Information (www.ncbi.nlm.nih.gov) purchased from Applied Biosystems (Foster City, CA). Primer Express software and Visible OMP software were used to design and simulate the performance of the primers and the probe. BLAST was used to verify the specificities of the primers and probe.

The forward primer was 5′-AAG GGC TAT AAA GGC GTT GCT-3′.

The reverse primer was 5′-AGA CTT TGT TCC AGT AGC TGT TGC T-3′.

The probe was 5′-6-carboxyfluorecein TCC CCT TGC CAA CAG ACG CTG G 6-carboxytetramethylrhodamine-3′.

OmniMix bead reagents from Cepheid and 1 μl extract were used per assay. The assay was sensitive to 5 fg *C. pneumoniae* DNA determined by serial dilution of *C. pneumoniae* DNA purchased from Applied Biosystems. The positive extraction control was *C. pneumoniae* in cultured HeLa cells, a gift of Dr. J. Mahoney. The negative control was PCR-grade water. The cycling protocol was 95°C for 120 seconds, followed by 40 cycles of 95°C for 15 seconds and 60°C for 30 seconds. Optics were activated during the anneal/extend period.

The samples from the younger group were examined with real-time PCR, a traditional touchdown procedure (3), and a traditional nested PCR (4). Detection in these two methods was by polyacrylamide gel electrophoresis. The samples from the older group were examined with only the real-time PCR and inhibition detection by PCR with the human albumin gene.

The albumin forward primer was 5′-GCT GTC ATC TCT TGT GGG CTG T-3′.

The albumin reverse primer was 5′-AAA CTC ATG GGA GCT GCT GGT T-3′.

The albumin probe was 5′-/Iowa black/CCT GTC ATG CCC ACA CAA ATC TCT CC/CY-5/-3′.

At JHU, samples were analyzed with real-time PCR performed on a Roche LightCycler that targeted the 16S rRNA gene (2).

In the younger-than-20 group, two samples were positive for *C. pneumoniae* in duplicate real-time PCR assays, and one of the two was positive in both traditional assays at PPMC. At JHU, no sample was positive in duplicate real-time PCR assays. In the older-than-60 group, there were no samples positive for *C. pneumoniae* on duplicate assays at either site. No inhibition was noted.

Two different laboratories, using different extraction procedures and different real-time PCR targets, failed to find evidence of *Chlamydia pneumoniae* in the PBMCs of both a cohort of individuals younger than 20 and a cohort of individuals older than 60. At this time, we have no definite explanation for the discrepancy between PCR results and those expected due to serological studies. More work and more interlaboratory studies will be required to solve this discrepancy.

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S. J. Kohlhepp*
Infectious Diseases Laboratory
Chiles Research Institute
Providence Portland Medical Center
Portland, Oregon

J. Hardick
C. Gaydos
Johns Hopkins University
School of Medicine
Baltimore, Maryland

*Phone: (503) 215-6141
Fax: (503) 215-6052
E-mail: sue.kohlhepp@providence.org