Reviewer’s report

**Title:** Non-detection of Chlamydia species in carotid atheroma using generic primers by nested PCR in a population with a high prevalence of Chlamydia pneumoniae antibody

**Authors:**

Grace M Ong ( grace.ong@bll.n-i.nhs.uk )  
Peter V Coyle ( peter.coyle@bll.n-i.nhs.uk )  
Aires AB Barros D'Sa ( Aires.barrosd@royalhospitals.n-i.nhs.uk )  
W Glenn McCluggage ( Glenn.mccluggage@bll.n-i.nhs.uk )  
W Paul McCluggage ( p.mccluggage@qub.ac.uk )  
Hugh J O'Neill ( hugh.oneill@bll.n-i.nhs.uk )  
Dorothy E Wyatt ( dorothy.wyatt@bll.n-i.nhs.uk )  
Kathleen B Bamford ( k.bamford@ic.ac.uk )  
Barny O'Loughlin ( b.oloughlin@qub.ac.uk )  
Conall McCaughey ( conall.mccaughey@bll.n-i.nhs.uk )

**Version:** 1  **Date:** 10 Aug 2001

**Reviewer:** Prof Peter Timms

**Level of interest:** A paper whose findings are important to those with closely related research interests

**Advice on publication:** Accept after revision, which I do not need to see

MANUSCRIPT REVIEW : Non-detection of Chlamydia species in carotid atheroma using generic primers by nested PCR in a population with a high prevalence of Chlamydia pneumoniae antibody by Ong, et al.

1. **General comment:** This manuscript by Ong and collaborators entitled "Non-detection of Chlamydia species in carotid atheroma using generic primers by nested PCR in a population with a high prevalence of Chlamydia pneumoniae antibody" is an important observation in relation to the reliability of PCR methods to detect (or not detect in this case) the presence of Chlamydia in atherosclerotic plaque material. Eventhough many authors have reported the detection of Chlamydia pneumoniae in various atherosclerotic plaque tissues using various PCR methods, others have reported negative results. As such, this report is an important one in showing the difficulty in finding Chlamydia DNA under some circumstances.

2. **Major criticism:** Much of the validity of this report relies on the quality of the PCR assay used. Generally, the authors have presented a very thorough and well controlled PCR assay methodology. The sensitivity limits of the assay however are not clear. The authors mention that the assay was sensitive to 10-7 for C.trachomatis and 10-6 for C.pneumoniae but this is presumably based on serial dilutions on cell culture grown Chlamydia. They mention in the next sentence that this relates to 1 and 0.001 TCID50, but do not explain what TCID50 is or how it was estimated. I feel that this is a sufficiently important aspect of the work that more detail is needed here.

3. **Major criticism:** The other aspect of the assay methodology which could be improved (and which is critical if the negative data is to be totally accepted) is that a much better spiking control would have
been to have added whole chlamydial particles to the control samples, prior to the DNA extraction step, and to process this specimen in parallel to other plaque specimens. This would answer the critical question of whether the extraction step was the one in which the chlamydial DNA was possibly lost. This control is very different to either; (i) spiking by adding extracted chlamydial DNA after the main specimen extraction procedure and (ii) testing for the presence of host cell DNA by a HLA DRB gene PCR. Given the importance of this aspect of the test, then I believe that the authors should address these issues somewhere in the text of the manuscript. The other controls were excellent and strongly support the validity of the test procedure.

4. Final comment: Overall however, this is an important observation and should be published so that others who might also have negative results are encouraged to report what otherwise might be considered negative results.

Reviewer: Peter Timms 10 August 2001

Competing interests:

None declared.