Author's response to reviews

Title: Clinical Utility of a Nested Nucleic Acid Amplification Format in Comparison to Viral Culture for the Diagnosis of Mucosal Herpes Simplex Infection

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PDF covering letter
BioMed Central Editorial Team,

Re: Clinical Utility of a Nested Nucleic Acid Amplification Format in Comparison to Viral Culture for the Diagnosis of Mucosal Herpes Simplex Infection

Thank you for your e-mail of 2.8.01 concerning this BMC manuscript. I have read and I am in agreement with the comments of the 2 reviewers. Accordingly I have made some minor modifications to allow their incorporation into the manuscript. These I have listed below with the comments of Dr Yam highlighted in red and those of Dr Slomka in blue.

All of the authors have read the full text of the article and are in agreement with its content. I am thus uploading the amended manuscript for your consideration.

Yours sincerely,

Dr Peter V Coyle MD FRCPath
Consultant Virologist.

(1) Reviewer Dr W Yam – Additions to the manuscript are highlighted in red.

Multiplex HSV-1 and HSV-2 nPCR  This was undertaken as previously described [13] using primers recognising the HSV-1 gpD gene and the HSV-2 gpG gene in a multiplex nPCR; HSV-1 and HSV-2 outer products were 221 and 184 base pairs (bp) while the inner products were 138 and 101 bp respectively.

Patients and Specimens  Twenty-six of the 90 patients seen were thought on clinical grounds to have mucosal herpes while the remaining 64 were assessed as having non-specific lesions inconsistent with herpetic involvement.

Virus Culture and nPCR  Taking the clinical diagnosis as the indicator of HSV infection, nPCR and culture had respective sensitivities of 19/26 (73%) and 12/26 (46%) (\(\chi^2\) p=0.02) and specificities of 62/64 (97%) and 63/64 (98%) (\(\chi^2\) p=1.0); all PCR positive specimens were repeatedly reactive.

Discussion  The potential for amplicon contamination associated with NAT assay use is well recognised and makes mandatory the use of (a) separate, designated work stations and (b) a uni-directional work-flow from specimen preparation to amplified product analysis; this model of operation is standard in our laboratory . The increased sensitivity and additional amplification step of nested NAT assays is thought especially to be coupled with compromised specificity resulting from false positive results.

While confirming the expected improvement in sensitivity ((19/26 (73%) and 12/26 (46%) (\(\chi^2\) p=0.02)) of nPCR, the overall poor performance of both the nPCR and virus culture assays was unexpected. Since both have performed optimally in external quality assessment programs (NEQAS, run by the Public Health Laboratory Service for viral culture and the QCCA program run by the European Society for Clinical Virology for HSV PCR) we do not believe assay under-performance to be
responsible. Additionally we have never encountered a specimen that yielded a virus on culture that was PCR negative, even without specimen extraction, indicating that PCR inhibitors are not involved or are uncommon.

(2) Reviewer Dr M Slomka – Additions to the manuscript are highlighted in blue.

**Specimens** Swabs were taken from appropriate oral (2) and ano-genital sites (88) including penile, vulval, vaginal and anal specimens.

**Patients** Over a 3 month period 90 patients who presented to the Department of Genitourinary Medicine, Royal Victoria Hospital, Belfast, were investigated for evidence of HSV infection. In addition to their routine assessment they were also selected for additional clinical categorisation by the attending consultants into those patients thought to (a) have, and (b) not have, clinical evidence in keeping with herpetic infection. Following a detailed history and examination patients with suspected herpetic infection were recorded as having: (i) primary HSV; (ii) first clinical episode of non-primary HSV; (iii) recurrent HSV.

The results of this study indicated that a nested format of NAT assay was not only suitable for routine application but essential for achieving adequate sensitivity levels. The sensitivity levels of nested NAT formats therefore warrant their consideration by laboratories offering diagnostic facilities for herpetic infection. The other major advantage of this multiplex nested format is that it removes the necessity for pre-test extraction and automatically types the virus identified. Nesting with these primers has proven sufficient to overcome any inhibitors which would be expected in specimens of this nature and to date we have been unable through audit to provide evidence to support the need for specimen extraction, which for our other routine NAT assays is essential.