Rapid detection of glycoprotein G gene for the diagnosis and typing of herpes simplex virus infection in genital herpes

X F Fang, B Song, Y Y Tu, J Z Tong, J L Faul, H Bai

Objective: To develop a new, rapid, and convenient technique for the diagnosis and typing of herpes simplex virus (HSV) in genital herpes (GH).

Methods: Using samples from skin vesicle fluid and urogenital mucosal swabs of subjects with GH, conventional polymerase chain reaction (PCR) (directed to polymerase gene: PCRpG) were compared with a newly developed PCR (directed to HSV glycoprotein gene: PCRgG). Both PCR methods were compared with virus isolation culture (VI) with indirect immunofluorescent staining (IIF).

Results: 80 samples from 40 GH patients (25 males) were tested. Positive results were seen in 52.5% (42/80) using PCRpG compared with 40% (32/80) by VI. Most of PCRpG positive samples (95.1%) were caused by HSV-2 infection. In samples from healing lesions, HSV was detected more often by PCRpG than by VI. The results of typing by PCRpG and IIF were highly consistent.

Conclusion: PCRpG is more sensitive than VI and PCRpG in detecting HSV in urogenital samples from subjects with GH. PCRpG is a convenient technique for the rapid detection and typing of GH.

(Sex Transm Inf 1999;75:396–397)

Keywords: herpes simplex virus; genital herpes; polymerase chain reaction

Introduction
The current standard methods of detecting herpes simplex virus (HSV) infection (by virus isolation (VI) and enzyme immunoassay (EIA)) are relatively slow and the results may be non-specific.1-3 We wanted to investigate the effectiveness and convenience of using polymerase chain reaction (PCR) gG to detect HSV infection in subjects with GH. PCR is a popular research method in detecting HSV infection because of its high sensitivity and specificity.3 The HSV glycoprotein G gene has the least homology between HSV-1 and HSV-245 and we hypothesised that PCRpG might provide a sensitive and accurate method for the identification of HSV species in genital herpes (GH).

Materials and methods
Forty subjects (25 males) were recruited from the sexually transmitted diseases (STD) clinic at the affiliated hospital of SUMS, China. All subjects were diagnosed with GH by standard criteria: STD contact history and the grouping condition: primer 1 and primer 2 10 pmol, 5'-AGACATACGTAACGCACGCT). PCR HSV-2 HindIII L sequence 4901–4922 bp, 5' (HSV-1 US sequence, 4818–4837 bp or primer 1 (HSV-2 HindIII L sequence, 4707–4726 bp, 5' CCCCCATGCCAAGTATTGGGA); primer 2 (HSV-2 HindIII L sequence, 4707–4726 bp, 5' AGCTCCCGCTAAGGACATG), and primer 3 (HSV-1 US sequence, 4818–4837 bp or HSV-2 HindIII L sequence 4901–4922 bp, 5'-AGACATACGTAAGGACGCT). PCR condition: primer 1 and primer 2 10 pmol, primer 1 and primer 2 10 pmol, dNTP 200 µm, PCR buffer 6 µl, tempelim 2 µl, adding distilled water to 30 µl. The solution mix was heated to 94°C for 3 minutes and Taq polymerase 1.5 µl was added. The PCR reaction was cycled at 94°C (45 seconds), 58°C (45 seconds), and 72°C (1 minute) (for 35 cycles) and completed with 72°C for 5 minutes. PCR products were run on 2% agarose gel. Six gradient diluted samples (0.2 ml each) and positive controls (HSV-1 F and HSV-2 333) were used to plaque assay for minimum HSV detected concentration test.
Rapid detection of glycoprotein G gene for the diagnosis and typing of HSV infection in genital herpes

Results
CPE was observed in 32/80 samples, and infection by HSV was verified by IIF. In each of 30 samples CPE appeared within 1–3 days and in two samples CPE appeared on the sixth day. The infected HSV-1 cells formed a scattered pattern and most demonstrated ballooned degeneration, while cells infected by HSV-2 were fused into multinucleate giant cells as observed by inversion microscopy and IIF.

The minimum detected sample concentration was 0.75 pfu in HSV-1 and 1.25 pfu in HSV-2. The appearance of HSV-1gG product formation was 0.75 pfu in HSV-1 and 1.25 pfu in HSV-2. The appearance of HSV-1gG product formation was 0.75 pfu in HSV-1 and 1.25 pfu in HSV-2.

Table 2 Results according to type of lesion and site of sample

<table>
<thead>
<tr>
<th>HSV-1</th>
<th>HSV-2</th>
<th>+HSV-2</th>
<th>Total positive</th>
<th>Total negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td>2</td>
<td>39</td>
<td>42</td>
<td>38</td>
</tr>
<tr>
<td>IIF</td>
<td>2</td>
<td>28</td>
<td>32</td>
<td>48</td>
</tr>
</tbody>
</table>

Discussion
Virus isolation is currently the gold standard method for HSV detection in vesicle fluid. However, it is slow because of the need for cell culture techniques. In addition, patients may not attend a physician at the early stage of the disease. Our data show that PCrG promises to enhance the diagnostic efficiency of GH. Moreover, simultaneous quick and accurate typing is also possible. In contrast with PCrG products, which are of similar size (229 bp and 241 bp for HSV-1 and HSV-2), the PCrG products of HSV-1 and HSV-2 differ in size by more than 256 bp (216 bp and 496 bp respectively) and are therefore readily identifiable by standard agarose gel electrophoresis. Typing of HSV is important for our understanding of GH, because previous work has demonstrated genetic differences between HSV-1 and HSV-2 that may account for their distinct epidemiology, clinical features, and prognosis.

The result that HSV was detectable in swabs of urogenital mucosa has three important implications. (i) HSV may infect skin and mucosa simultaneously and co-infection by different HSV types remains to be demonstrated genetic differences between HSV-1 and HSV-2 that may account for their distinct epidemiology, clinical features, and prognosis.

The work was supported by the Chinese Natural Science Fund (39570195).

Contributors: XFR, BS, YXT, and JZT conducted the PCR, VI and IIF, and collected the samples; JLF wrote the paper and performed the statistical analysis. The authors wish to thank Dr AC Manson (Department of Pathology, Cambridge University, UK) for supplying HSVGg monoclonal antibodies.

Rapid detection of glycoprotein G gene for the diagnosis and typing of herpes simplex virus infection in genital herpes.

X F Fang, B Song, Y Y Tu, J Z Tong, J L Faul and H Bai

Sex Transm Infect 1999 75: 396-397
doi: 10.1136/sti.75.6.396

Updated information and services can be found at:
http://sti.bmj.com/content/75/6/396

These include:

Email alerting service

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/