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article

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

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**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 PCRgG compared with 40% (32/80) by VI. Most of PCRgG positive samples (95.1%) were caused by HSV-2 infection. In samples from healing lesions, HSV was detected more often by PCRgG, than by VI. The results of typing by PCRgG and IIF were highly consistent.

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

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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.<sup>1,2</sup> 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.<sup>3</sup> The HSV glycoprotein G gene has the least homology between HSV-1 and HSV-2<sup>4,5</sup> and we hypothesised that PCRgG 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 of vesicular lesions; and/or erosion, ulceration and crusting of healing lesions (Chinese Health ministry).<sup>6</sup> In each subject one sample was taken from genital skin lesions and one from the urethra (cervix and urethra in females). Vesicle fluid was withdrawn by syringe. Cotton swabs were used to sample healing lesions and the urogenital mucosa. Both samples were obtained at one time from each subject (a total of 80 samples).

Vero cells ( $1 \times 10^6$ /ml) (ATCC) were placed in 10% FBS-1640 medium in 24 well plastic plates with a cover slip and incubated in 5% carbon dioxide, 37°C for 24 hours. Samples

(0.2 ml) were added separately into four wells (two duplicates) and further incubated for another 24–48 hours and observed for cytopathogenic effect.

Thirty two positive cover slips were tested by indirect immunofluorescence (IIF) (monoclonal antibodies: anti-HSVgG1 and mouse anti-HSVgG2, gifts of Dr A C Minson, Department of pathology, division of virology, Cambridge University, were 1:1500 dilution) and negative ones would be continuously observed for a further 14 days.

Samples of viral transport medium (300 µl) were tested with 100 µl proteinase K-split solution, denatured in 95°C for 10 minutes, and stored at -20°C until use. The following primers were used for PCRgG reaction: primer 1 (HSV-1 US sequence, 4351–4370 bp: 5' CCCCATGCCAAGTATTGGA); 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'-AGACATACGTAACGCACGCT). PCR condition: primer 1 and primer 2 10 pmol, primer 3 20 pmol, dNTP 200 µm, PCR buffer 6 µl, templet 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.

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## Results

CPE was observed in 32/80 samples, and infection by HSV was verified by IIF. In each of 30 samples CPE appeared within 2–3 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 (216 bp), and HSV-2gG (490 bp) were compared with positive controls from standard strains.

Forty two samples were positive by PCRgG, 30 by both methods. PCR had a higher positive rate than VI in this cohort ( $\chi^2$  test,  $p < 0.025$ ). The sensitivity, specificity, positive and negative predictability of PCR were: 93.8%, 75%, 71.4%, 94.7% respectively. If the 12 cases of PCR positive, VI negative samples were assumed to be true positives, these values would be 95.5%, 100%, 100%, 94.7% respectively. There were 14 inconsistent samples between the two methods. Twelve PCRgG positive, VI negative were also positive by PCRpG. In two samples with VI positive PCRpG negative, one was negative and the other is HSV-2 positive by the PCRpG.

The results of typing by PCR and IIF are presented in table 1. Two of 42 samples (4.7%) were HSV-1 positive. The results of typing were consistent across all methods. Exceptions were HSV-1 and HSV-2 mixed positive samples. Two HSV-1 and HSV-2 positive VI samples were tested by both PCR methods. One was negative by both PCR techniques. The other was only HSV-2 positive by the PCRpG. One sample was positive by both PCRgGs, but was negative by PCRpG.

PCR and VI results in different duration and region are shown in table 2. No significant difference in vesicle stage or urogenital sampling was found between PCR and VI (Fisher's exact test,  $p > 0.05$ ). In contrast, there was a significant difference between PCRgG and VI in the detection of HSV in the healing stage ( $p = 0.03$ , Fisher's exact test). The positive rate for the detection of HSV by combining the two methods for skin lesions (47/80) was significantly higher than the positive rate for urogenital swabs (27/80), ( $p < 0.01$ , Fisher's exact test).

Table 1 Serotypes of detected HSV by PCR and IIF

	HSV-1	HSV-2	HSV-1 +HSV-2	Total positive	Total negative
PCR	2	39	1	42	38
IIF	2	28	2	32	48

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

	Numbers	VI (%)	PCR (%)	p Value
Vesicle lesions	17	16 (92.4)	14 (82.4)	0.3
Healing lesions	23	5 (21.7)	12 (52.2)	0.033
Urogenital swabs	40	11 (27.5)	16 (40)	0.17

## Discussion

Virus isolation is currently the gold standard method for HSV detection in vesicle fluid. However it is slow because it depends on viral culture techniques.<sup>1–7</sup> In addition, patients may not attend a physician at the early stage of the disease. Our data show that PCRgG has a higher detection rate in all GH samples, including healing lesions, compared with VI (at least using Vero cells). Our new PCRpG method is more rapid (< 4 hours) than VI and promises to enhance the diagnostic efficiency of GH. Moreover, simultaneous quick and accurate typing is also possible. In contrast with PCRpG products, which are of similar size (229 bp and 241 bp for HSV-1 and HSV-2),<sup>8</sup> the PCRpG 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<sup>4,5</sup> that may account for their distinct epidemiology, clinical features, and prognosis.<sup>9</sup>

The result that HSV was detectable in swabs of urogenital mucosa has three important implications. (i) HSV may infect skin and mucosa simultaneously; (ii) the urogenital mucosa could be a useful sampling site for HSV detection; and (iii) the urogenital mucosa may be an important route for the transmission of HSV. Whether HSV infection makes an individual more susceptible to infection by the same or different HSV types remains to be investigated. Local and generalised susceptibility to co-infection by different strains of HSV may warrant further investigation.

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Contributors: XFF, BS, YYT, and JZT conducted the PCR, VI and IIF, and collected the samples; JLF wrote the paper and performed the statistics; HB designed the PCR primers, conducted and supervised the study, wrote the paper, and performed the statistical analysis.

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