

Detection and Typing of Herpes Simplex Virus (HSV) in Mucocutaneous Samples by TaqMan PCR Targeting a gB Segment Homologous for HSV Types 1 and 2

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Herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) are major causes of mucocutaneous lesions and severe infections of the central nervous system. Here a new semiautomated method for detecting and typing of HSV was used to analyze 479 mucocutaneous swab samples. After DNA extraction using a Magnapure LC robot, a 118-bp segment of the gB region was amplified by real-time PCR utilizing type-specific TaqMan probes to identify HSV-1 or HSV-2. HSV detection in a single well using probes labeled with carboxyfluorescein (FAM) for HSV-1 and JOE (6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein) for HSV-2 had a sensitivity similar to that seen in separate reactions. All but one of 217 samples (99.5%) that had been positive by virus culture were positive by TaqMan PCR, with a correct identification of type in all cases. Out of 262 samples negative by virus culture, 48 (18.3%) were positive by TaqMan PCR, with higher C_t values compared with culture positive samples ($P < 0.0001$). Overall, the C_t values for HSV-1 were lower than for HSV-2 (mean, 25.5 versus 27.9), but to some extent this could be due to weaker fluorescence by JOE. Lower C_t values for HSV-1 were seen also in the 202 genital samples (79 HSV-1, 122 HSV-2, 1 HSV-1 and HSV-2), indicating that HSV-1 replicates as well as HSV-2 in the genital area. HSV-1 constituted 40% of genital infections and was associated with lower mean age (29.2 versus 36.4 years), probably reflecting the fact that recurrent genital HSV-1 infections are rare.

Human herpes simplex virus (HSV) types 1 and 2 are common and important pathogens, which may cause severe disease in newborns and immunosuppressed patients. In immunocompetent subjects, both primary and reactivated infections are usually mild but may rarely spread to the central nervous system causing encephalitis, myelitis, or meningitis. HSV-1 typically causes orofacial blisters, keratitis, pneumonia, or encephalitis and has emerged as a common cause of genital herpes. HSV-2 typically causes genital lesions or meningitis (which may be recurrent). Both types may cause severe disease when transmitted perinatally.

Detection of HSV DNA by PCR has become an important method for early diagnosis of infections in the central nervous system (10, 19), and has also been described as an alternative to viral culture for identifying HSV in mucocutaneous lesions (2, 6, 8). Typing can be done in the enzyme immunoassay format using type-specific antibodies, or by PCR techniques that specifically amplify either genotype in separate reactions (20) or distinguishes the amplicons by probes or melting point analysis (20). Recently, methods based on real-time PCR have been used for quantitating HSV (1, 11, 19), but the clinical value of this is not yet established. Here we report a new real-time PCR method based on amplification of a homologous segment of the gB region and distinction of HSV-1 and HSV-2 by the use of TaqMan probes.

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MATERIALS AND METHODS

Samples. Cotton swabs were sent to the laboratory in buffered saline or viral transport medium. After viral culture inoculation the remaining volume was stored at -20°C until tested in this study. The majority (77%) of the samples were from genital lesions and were taken by gynecologists (the localization of the lesion was recorded for the 263 samples that turned out to be positive for HSV by PCR). Although sample handling and HSV diagnostics is well established in our city, a delay in sample transport and/or exposure to temperatures above 8°C could have occurred for a minority of the samples contributing to a negative virus culture.

Virus culture. Two hundred microliters of sample (cotton swab in transport medium) were transferred to tubes with Green Monkey kidney cell lines (GMK-AH1). The cells were cultured in Eagle's minimal essential medium supplemented with 2% calf serum and antibiotics. The cells were examined for cytopathogenic effect daily for 7 days and positive samples were subtyped by using type-specific monoclonal antibodies against HSV-1-glycoprotein C and HSV-2 glycoprotein G (14).

Extraction of HSV DNA. DNA was extracted in a Magnapure LC robot (Roche Diagnostics, Mannheim, Germany) using the Magnapure DNA Isolation Kit according to the manufacturer's instructions. The input and output volumes were set to 200 μl and 100 μl , respectively. In part of the study, freeze-thawing of the sample once was used as an alternative method for DNA preparation. In these cases 10 μl of the thawed sample was used in PCR without further procedures.

Real-time PCR. A 118-nucleotide segment of the gB region was amplified by the use of primers described in Table 1. The distinction between HSV-1 and HSV-2 is based on differences between the probes at five nucleotide positions. The reaction volume of 50 μl contained 25 μl universal master mix (UMM, Applied Biosystems, Foster City, CA), 10 μl of sample DNA, and primers and probes at concentrations described in Table 1. Amplification was done in a real-time PCR instrument ABI Prism 7000 (Applied Biosystems). After incubation for 2 min at 50°C (uracil-*N*-glycosylase digestion) and 10 min denaturation at 95°C , 45 cycles of two-step amplification (15 s at 95°C , 60 s at 58°C) were performed.

For each positive sample the C_t (threshold cycle) value was recorded. The C_t is the cycle when the fluorescence has become detectable and is in the exponen-

TABLE 1. Primers and probes used for Taqman detection of the gB region^a

| Primer or probe | Sequence | Label | T_m (°C) | μ M |
|-----------------|-----------------------------|------------------------|------------|---------|
| HSV1-F | GCAGTTTACGTACAACCACATACAGC | | 59.9 | 0.9 |
| HSV2-F | TGCAGTTTACGTATAACCACATACAGC | | 59.5 | 0.9 |
| HSV1&2-R | AGCTTGC GGGCCTCGTT | | 60.5 | 0.9 |
| HSV1-probe | CGGCCCAACATATCGTTGACATGGC | 5'FAM, 3'TAMRA | 70.3 | 0.2 |
| HSV2-probe | CGCCCCAGCATGTCGTTCCAGT | 5'FAM or JOE, 3' TAMRA | 70.0 | 0.2 |

^a FAM, 6-carboxyfluorescein; JOE, 6-carboxy-4', 5'-dichloro-2', 7'-dimethoxyfluorescein, TAMRA, 6-carboxytetramethyl-rhodamine. Nucleotides that differ from HSV-1 are underlined.

tial phase of amplification, and the C_t value is inversely proportional to the log concentration of target DNA.

In the first part of this evaluation each sample was run in parallel reactions with FAM (6-carboxyfluorescein) labeled probes; one for HSV-1 using HSV1-F, HSV1&2-R and HSV1-probe and one for HSV-2 using HSV-2-F, HSV1&2-R, and HSV-2-probe. In the second part we used a reaction mixture that contained primers and probes for both HSV-1 and HSV-2, then using a HSV-2 probe labeled with JOE (6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein). In some experiments the samples were diluted prior to PCR to evaluate amplification efficiency or the impact of inhibitory substances.

In additional experiments we evaluated the possibility to run the PCR (using the same reagents and setup) on a traditional PCR thermocycler (ABI 9700, Applied Biosystems), and use the real-time PCR instrument only for fluorescence reading, so-called post-PCR plate read. In this case, the PCR plate was transferred to the ABI Prism 7000 instrument directly after PCR, and fluorescence reading was carried out, using the "allelic discrimination" function in the SDS 7000 software.

Sample testing. Three sets of samples were tested. First, 41 consecutive samples that were positive in viral culture (20 HSV-1 and 21 HSV-2) were analyzed. Each of these samples was tested in 16 positions: four duplicate reactions with the HSV-1 probe and the HSV-2 probe. These four reactions evaluated different extraction protocols: Magnapure extraction without or with a subsequent 1:10 dilution, and freeze-thawing without or with a subsequent 1:10 or 1:100 dilution.

The second set consisted of 73 consecutive samples that were negative by viral culture. These samples were analyzed in duplicates for each of two sample preparations: Magnapure extraction (without subsequent dilution) or freeze-thawing with a subsequent 1:10 dilution. Finally, a third group was analyzed comprising 364 consecutive samples either positive ($n = 205$) or negative ($n = 159$) by virus culture. In this third group the reaction was run in a single well with a reaction mixture containing both a HSV-1 probe labeled with FAM and an HSV-2 probe labeled with JOE, and each sample was run without or with 1:4 predilution in water.

RESULTS

PCR efficiency and sensitivity. The overall sensitivity of a PCR test depends not only on the amplification efficiency of the PCR, but also on the capacity of the sample preparation procedure to extract DNA and remove inhibitory substances. In real-time PCR the amplification efficiency can be assessed by analyzing a given sample in serial dilution. Plotting the results should then yield a slope around 3.4, because in an optimal amplification, a tenfold dilution should correspond to 3.33 cycles.

The amplification efficiency of the PCR was estimated by analyzing purified HSV DNA diluted in five 1:10 steps. The C_t values then ranged from 9.1 to 22.6 with an R^2 of 0.998 and slope of 3.40 for HSV-1, and from 21.8 to 36.1 with an R^2 of 0.996 and a slope of 3.55 for HSV-2, indicating good PCR efficiencies for both assays.

To establish that the test had an acceptable sensitivity its performance was compared with the nested qualitative PCR that we use in clinical diagnostics (21). Two samples (representing HSV-1 or HSV-2) were diluted 1:2 in 5 steps and

analyzed in duplicate by both the TaqMan assay and nested PCR. The end-point titer by the TaqMan assay was the same for HSV-1 and one 1:2 dilution step better than the nested PCR for HSV-2, indicating that the real-time PCR had a similar or better sensitivity than the nested PCR.

Cross-reactivity and genotype mixtures. Ten culture-positive mucocutaneous samples (five HSV-1 and five HSV-2) were analyzed by PCR using a master mix for the nonmatching HSV type. Despite a very high virus concentrations in some samples (C_t range, 20 to 35 for HSV-1, 18 to 29 for HSV-2) no cross-reactions were seen. Cross-reactivity was also absent in the subsequent testing of clinical samples, even in the 20 samples with C_t values below 20.

Mixtures representing different proportions of two samples (HSV-1 and HSV-2) which both had shown a C_t around 30 were tested. The minority strain could be detected at expected C_t values when diluted 1/9 or 1/81 (Table 2).

Samples positive by viral culture tested in separate PCRs for HSV-1 and HSV-2. The samples were analyzed by TaqMan PCR after different pretreatments and dilutions. Firstly, we wanted to compare sample preparation by Magnapure with a simple method (freeze-thawing) we knew was used by others. The latter approach, which releases viral DNA by lysis, might have a reduced sensitivity due to inhibition or incomplete DNA yield. Secondly, we analyzed the samples with or without predilution as a means to estimate the amplification efficiency and degree of inhibition.

Only one culture-positive sample (HSV-2) was negative by PCR. The remaining samples were positive after Magnapure extraction with mean C_t values of 22.5 ± 1.0 standard error of the mean; range, 14.6 to 35.1 ($n = 20$) for HSV-1 and 23.5 ± 0.7 (range, 18.1 to 29.2; $n = 21$) for HSV-2. After dilution 1:100 the mean C_t increased by 7.7 cycles indicating absence of inhibitors.

Conversely, strong inhibition was observed in samples analyzed after only freeze-thawing. The majority (32/41) of these samples were negative by TaqMan PCR when analyzed with-

TABLE 2. Detection of HSV types 1 and 2 in artificial mixtures of two samples

| HSV-1:HSV-2 mixture | HSV-1 (C_t) | HSV-2 (C_t) |
|---------------------|-----------------|-----------------|
| 1:1 | 31.0 | 29.3 |
| 1:1/9 | 30.3 | 32.3 |
| 1:1/81 | 30.5 | 38.0 |
| 1/9:1 | 35.9 | 28.5 |
| 1/81:1 | 41.0 | 28.6 |

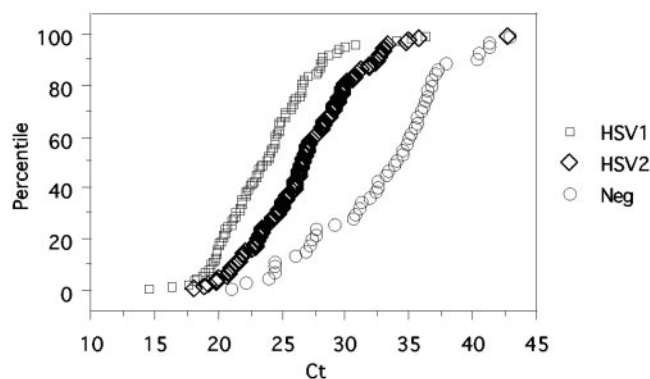


FIG. 1. Percentile plot showing C_t values for the 264 samples that were positive by TaqMan PCR. The samples are grouped on the basis of the result of virus culture (94 HSV-1, 123 HSV-2, and 48 negative).

out predilution, despite the very high viral loads documented by PCR after Magnapure extraction. However, after predilution 1:10 or 1:100 all the samples were reactive by PCR with mean C_t values around 30. The C_t values in freeze-thawed samples diluted 1:100 were only 1.5 cycles higher than in those extracted by Magnapure and diluted 1:100, indicating that most of the inhibition was lost at this dilution. In freeze-thawed samples diluted 1:10 inhibition was more pronounced and corresponded to 4.2 cycles (compared to extraction by Magnapure). Therefore, the overall sensitivity was only slightly lower in freeze-thawed samples diluted 1:100 compared to 1:10. Altogether, detection after Magnapure extraction without dilution before PCR had a considerably higher sensitivity than with any of the freeze-thawing strategies, with mean, C_t values being seven to eight cycles lower.

Samples negative by viral culture tested in separate PCRs.

In the initial testing of 73 culture-negative samples by TaqMan PCR for HSV-1 and HSV-2 in parallel reactions, 18 (24.7%) were positive by PCR after Magnapure extraction. The mean, C_t value was 31.6 for HSV-1 and 34.2 for HSV-2, i.e., overall significantly higher than for the 41 culture-positive samples described above (mean, 32.7 versus 23.0, $P < 0.0001$). TaqMan detection after freeze-thawing was positive in 8 samples, 4 HSV-1 (C_t 25 to 34), 4 HSV-2 (C_t 31 to 40). Thus, 10 samples were only positive by TaqMan PCR after Magnapure extraction.

Typing in single well. Finally, detection of HSV-1 and HSV-2 in a single well was evaluated on a further consecutive

TABLE 3. TaqMan PCR results in 479 mucocutaneous samples positive or negative by virus culture

| Cell culture | Taqman PCR result | | | | C_t (mean ±SEM) |
|-----------------------|--------------------|--------------------|----------------------------|-----------------------|-------------------------|
| | HSV-1 (n = 120) | HSV-2 (n = 143) | HSV-1/ HSV-2 (n = 1) | Negative (n = 215) | |
| HSV-1 | 93 | | | | 23.7 ± 0.4 |
| HSV-2 | | 122 | | | 26.8 ± 0.4 |
| HSV-1/HSV-2 | | | 1 | 1 | |
| Negative | 27 | 21 | | 214 | 32.8 ± 0.8 |
| C_t (mean ± SEM) | 25.5 ± 0.5 | 27.9 ± 0.4 | | | |

TABLE 4. Origin of 264 mucocutaneous samples that were positive by TaqMan PCR

| Location | HSV-1 (n = 120) | HSV-2 (n = 143) | HSV-1 and HSV-2 (n = 1) |
|-----------------|--------------------|--------------------|----------------------------|
| Head | 24 | 1 | |
| Mouth or lips | 16 | | |
| Chin or cheek | 6 | | |
| Eye | 1 | 1 | |
| Ear | 1 | | |
| Finger | 1 | 2 | |
| Genital/gluteal | 79 | 122 | 1 |
| Unknown | 16 | 17 | |

364 mucocutaneous samples, 189 (52%) of which were negative by virus culture. All the 175 samples that were positive by culture were also positive by TaqMan PCR; 73 HSV-1, 101 HSV-2, and 1 HSV-1/HSV-2 mixture. In the sample where TaqMan PCR showed coinfection the C_t was 24.1 for HSV-1 and 29.5 for HSV-2, and in this sample coinfection was identified also by virus culture. In addition, 30 of the 189 samples

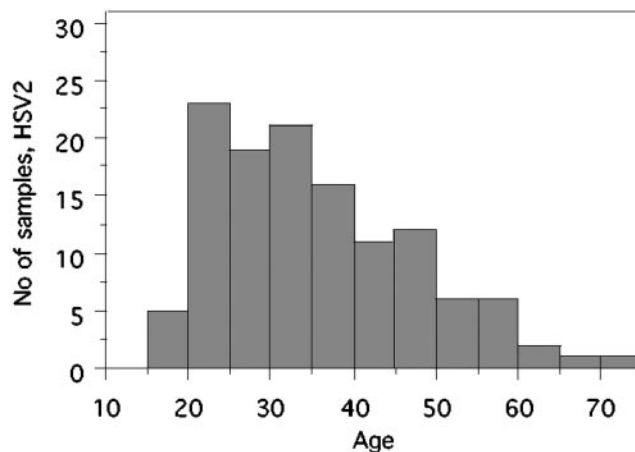
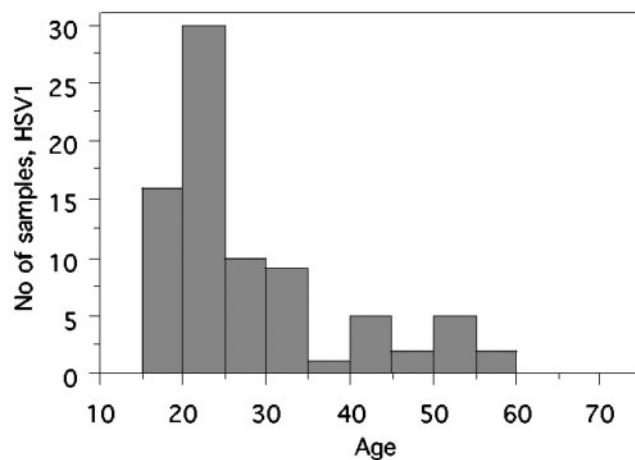


FIG. 2. Histograms showing the age (in years) of patients whose genital samples were TaqMan PCR positive for HSV-1 or HSV-2.

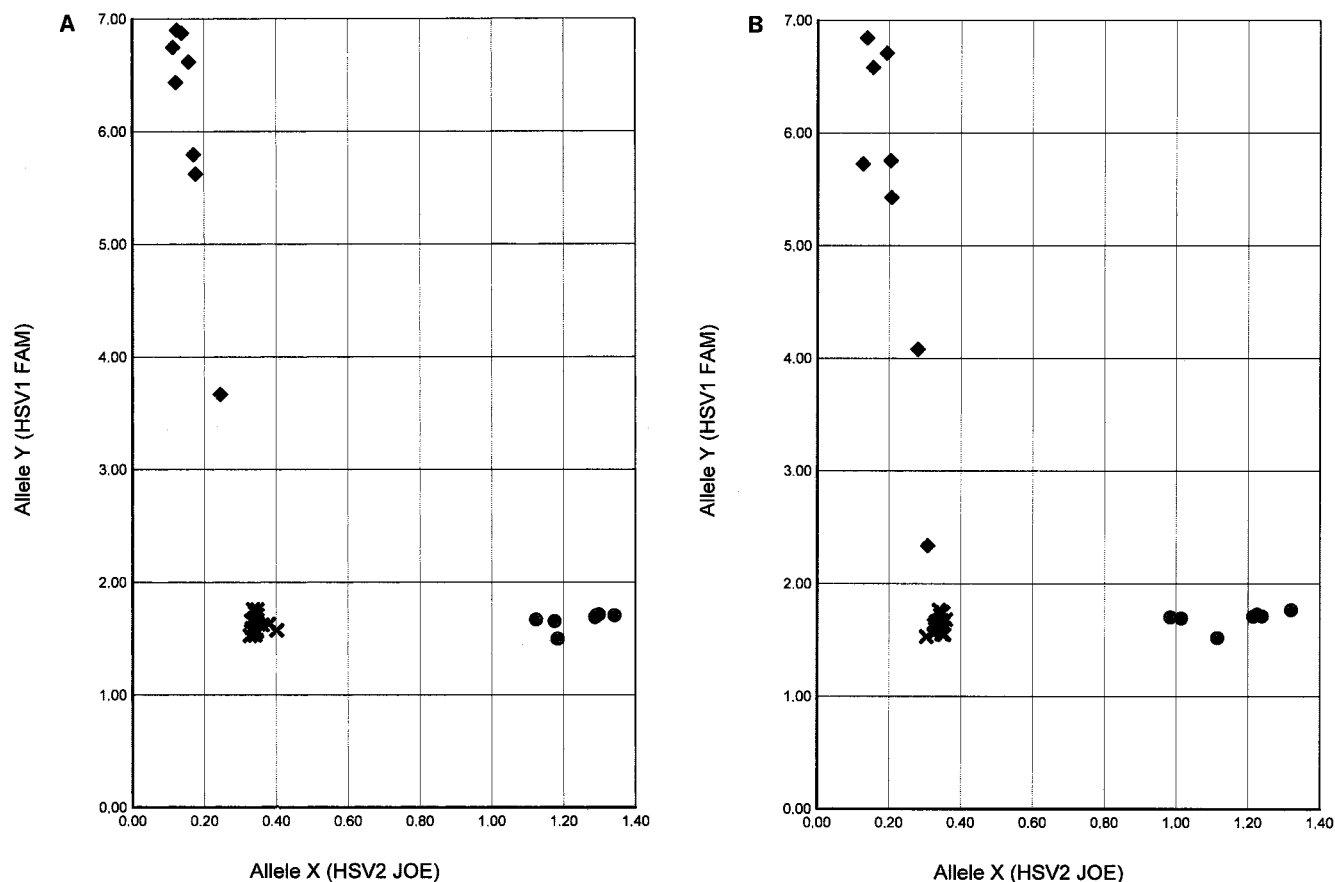


FIG. 3. Plots showing fluorescence during real-time PCR and from post-PCR plate read after PCR on ABI 7000 (A) and ABI 9700 (B) instruments in a run analyzing 32 samples, of which 8 were HSV-1, 7 were HSV-2, and 17 were negative. The x axis shows fluorescence (delta Rn) from JOE/HSV-2, and the x axis shows delta Rn for FAM/HSV-1.

(15.9%) that were culture negative were positive by TaqMan PCR (17 HSV-1 and 13 HSV-2).

The mean C_t values were 2.7 cycles lower for HSV-1 than for HSV-2 (mean, 25.5 ± 0.51 [\pm standard error of the mean] versus 28.2 ± 0.43). However, part of this difference was probably due to a weaker fluorescence by the JOE-marked HSV-2 probe (which increased the C_t by up to 1.8 cycles, not shown). As shown in Fig. 1, the C_t values were higher in culture-negative than in culture-positive samples (mean, 31.8 versus 24.0 for HSV-1, 33.9 versus 27.5 for HSV-2, $P < 0.0001$ for both).

In parallel (i.e., in the same run) with detection in a single well with HSV-1-FAM/HSV-2-JOE probes, 15 of the culture-positive samples (5 HSV-1, 10 HSV-2) were analyzed also in separate wells with FAM-labeled probes for both HSV-1 and HSV-2 to compare sensitivity. Detection in a single well then produced slightly higher C_t values, with a C_t difference (one well versus separate reactions) of 0.94 ± 0.58 (\pm standard deviation) cycles for HSV-1, $P = 0.022$, and 0.54 ± 0.57 cycles for HSV-2, $P = 0.015$. This indicates that single-well detection has a marginally lower sensitivity (corresponding to less than one cycle) compared to detection in separate PCRs.

We also tested the potential impact of inhibition by analyzing 246 of the samples with and without diluting the sample 1:4 in H_2O after Magnapure extraction. The median C_t difference

then was 2.0 (interquartile range, 1.6 to 2.4) indicating that inhibition in general was absent. However, inhibition might be present in a minor fraction, because 3 samples were positive only after predilution (C_t 34.0 to 38.7). On the other hand 3 other samples were positive only without predilution (C_t 35.0 to 41.2).

Sample data and HSV type for all samples. Table 3 summarizes the TaqMan PCR results for all the 478 samples analyzed in this study. Overall, 18.3% (48/262) of culture-negative samples were positive by TaqMan PCR. Of the culture-negative samples, 81% of those from nearby clinics (<15 km) compared to 33% of those from distant clinics had a C_t value above 31. As shown in Table 4, the majority (76%) of the 264 that were PCR positive had been taken from genital lesions, and out of these 29.2% were HSV-1. As depicted in Fig. 2, the patients with HSV-1 were younger than those with HSV-2 (mean age, 36.4 versus 29.2 years), with the majority being between 15 and 25 years old.

Evaluation of plate read procedure. The possibility to run the amplification on a traditional PCR instrument and do the post-PCR plate read on the ABI Prism 7000 instrument was evaluated on a set of 32 samples, which were also run in parallel as a real-time PCR. The mean C_t was 29.3 ± 5.7 (\pm standard deviation) for the eight HSV-1 and 28.5 ± 4.0 for the seven HSV-2 samples (17 were PCR negative). The results

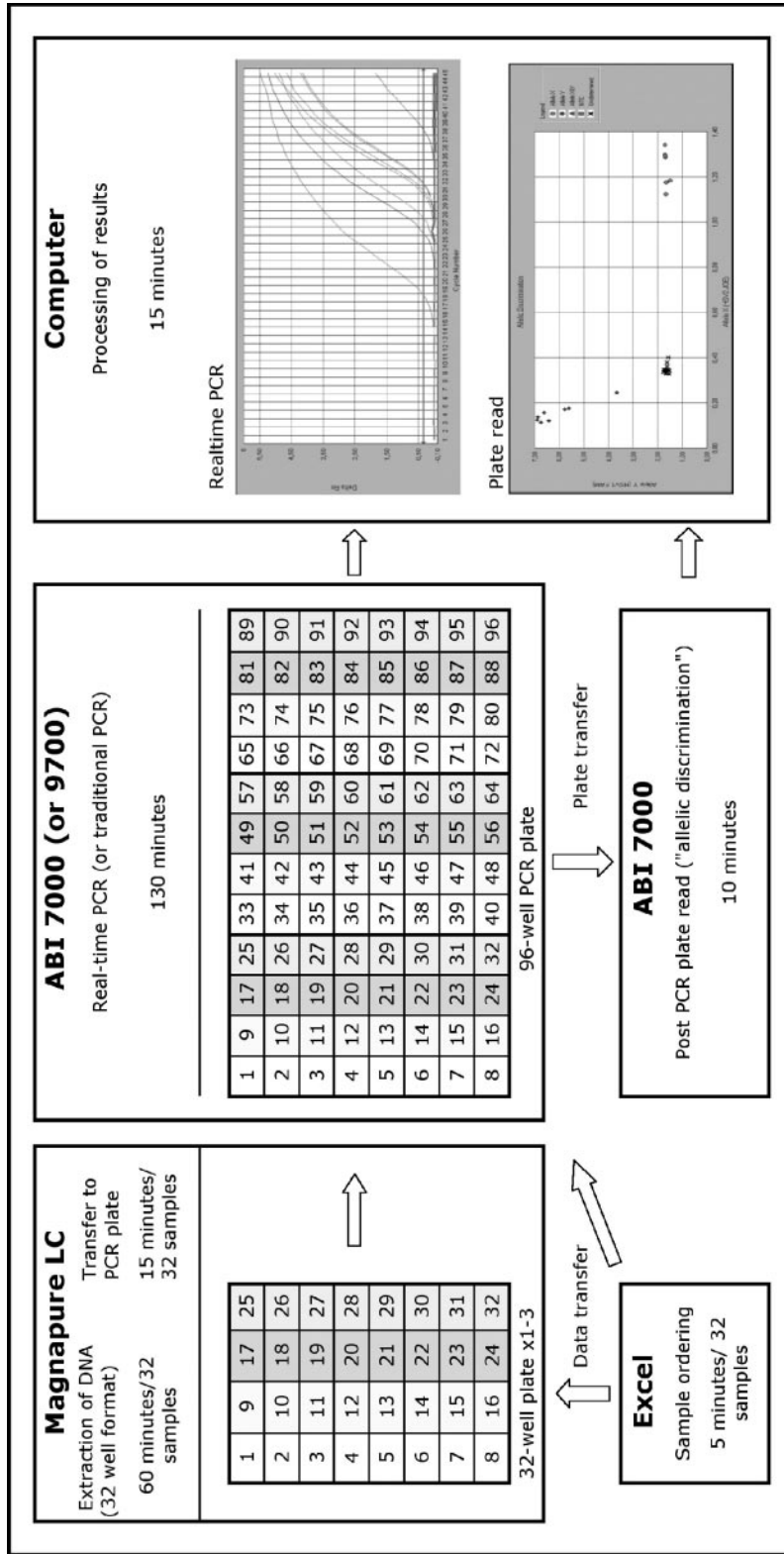


FIG. 4. Overview of the HSV typing process.

TABLE 5. Comparison of post-PCR fluorescence (plate read) after parallel PCR runs on the ABI 7000 and 9700

| Result | ABI 7000 | | | ABI 9700 | |
|----------|--------------------|-----------------|-----------------|-----------------|-----------------|
| | C_t mean (range) | Delta Rn FAM | Delta Rn JOE | Delta Rn FAM | Delta Rn JOE |
| HSV-1 | 28.5 (18.4–38.2) | 6.08 ± 1.09 | 0.16 ± 0.04 | 5.43 ± 1.54 | 0.20 ± 0.06 |
| HSV-2 | 29.4 (22.0–33.0) | 1.66 ± 0.08 | 1.24 ± 0.08 | 1.68 ± 0.08 | 1.05 ± 0.12 |
| Negative | | 1.64 ± 0.07 | 0.35 ± 0.02 | 1.65 ± 0.07 | 0.34 ± 0.01 |

from plate read after PCR on the 9700 instrument agreed well with plate read after real-time PCR on the 7000 instrument, (Fig. 3; Table 5), with similar fluorescence signals: The mean delta Rn ratio (ABI 7000/ABI 9700) was 1.17 ± 0.21 (\pm standard deviation) for HSV-1 and 1.08 ± 0.05 for HSV-2. However, one HSV-1 sample, which in real-time PCR had a C_t of 38.2 and was clearly identified as positive on plate read after PCR in the 7000 instrument (with a delta Rn of 3.67), showed a borderline reaction on plate read after PCR in the 9700 instrument (with a delta Rn of 2.34).

DISCUSSION

Here we present a real-time PCR method for detection and typing of herpes simplex virus in mucocutaneous lesions. The method comprises DNA extraction by a Magnapure LC robot, subsequent amplification of homologous segments of the gB genes of HSV-1 and HSV-2, and detection using TaqMan probes in a 96-well format on an ABI Prism 7000 real-time PCR instrument (Fig. 4).

The primer regions in the targeted segment of gB are almost identical, but the probe region differs by 5 nucleotides between HSV-1 and HSV-2. This difference allowed genotyping without cross-reactivity even at very high virus concentrations (i.e., C_t values below 20, probably corresponding to virus concentrations above 10^8 copies/ml). An advantage of targeting homologous gene segments is that C_t values for HSV-1 and HSV-2 are comparable and that mutual standards or control samples may be used. This would be of particular value if the assay is adapted to yield quantitative results, which by others has been done in separate reactions for HSV-1 and HSV-2 (19).

In our laboratory, a nested PCR targeting the gD and gG regions has been used for the past 10 years for identifying HSV-1 and HSV-2 (21). In comparison with these tests, the real-time PCR had an equal or even slightly higher sensitivity. Probably, this high sensitivity is due to several factors, such as an efficient extraction protocol using Magnapure (7), a small amplicon size and T_m values for primers and probes within recommended limits. Compared with nested PCR the processing time is shorter, the contamination risk is less, and specificity is enhanced by hybridization.

We also evaluated a very simple preparation of mucocutaneous samples, i.e., only freeze-thawing. We then noticed a marked inhibitory impact in mucocutaneous samples (5), which may originate either from substances in the sample or from the transport medium. Most of the inhibition disappeared when the sample was diluted 1:10 or 1:100 or when Magnapure extraction was used. The overall performance of freeze-thawing was acceptable and all but one of the culture-positive and some culture-negative samples were reactive by PCR after this preparation. Although we prefer the Magnapure extraction

because it demonstrated an even higher sensitivity, the simplicity and lower cost make freeze-thawing preparation an attractive alternative, in particular if equipment for automated DNA extraction is lacking.

In the later part of the study we used Magnapure extraction and coamplification in a single well with an HSV-1-probe labeled with FAM and an HSV-2-probe labeled with JOE. We then analyzed 364 consecutive clinical samples, comparing this method with virus culture and subsequent typing by enzyme immunoassay. TaqMan detection proved superior, because in addition to correctly identifying all samples that were positive by culture, 16% (30/189) of samples that were culture-negative were positive by PCR.

Most of the culture-positive samples were reactive with low C_t values, indicating high virus concentrations. The mean C_t value was 2.5 cycles higher for HSV-2 than for HSV-1 indicating that virus secretion may be lower in HSV-2 than in HSV-1 lesions. However, part of this difference was calculated to be due to a weaker fluorescence from the JOE-marked HSV-2 probe. Still, the observation indicates that HSV-1 replicates similarly or at a slightly higher rate than HSV-2, also in the genital area from which the majority of the samples were collected. It should however be kept in mind that such a difference might be due to the fact that primary infections probably were more frequent among the HSV-1 positive samples. This could also be of relevance for the interpretation of the only case with dual (genital) infection in which the C_t value was 4.4 cycles lower for HSV-1 than for HSV-2. Our findings seem to contrast to data reported by van Doornum et al., who observed lower mean, C_t for HSV-2 than for HSV-1 (22). Therefore, further quantitation studies including samples from defined primary versus recurrent infection of the two subtypes are warranted.

In accordance with previous reports from the Göteborg area (17), we found that 40% of the genital samples harbored HSV-1. Although clinical information about previous episodes were lacking, most of the HSV-1 cases were probably primary infections (15), while recurrences most likely constituted a substantial proportion of those with HSV-2 (16). This would fit well with the finding that patients with HSV-2 were older ($P < 0.001$) than those with HSV-1. This difference was however confined to women, suggesting that young women more frequently acquire primary genital HSV-1 infection by oral sex (13) (or possibly that recurrent HSV-2 is more frequent in older females).

In accordance with previous findings (7, 22), the culture-positive samples in general had C_t values below 30, while a most of the culture-negative samples that were positive by TaqMan PCR had C_t above 30, indicating that a positive culture corresponds to a certain and relatively high concentration

of virus. Because a C_t of 30 typically reflects a DNA concentration around 100,000 copies/ml, and 200 μ l are used for virus culture, then in general more than 20,000 virions appears to be required for a positive culture. However, under favorable conditions one PFU of HSV corresponds to 10 to 100 virus particles (9), suggesting that only a small fraction (<1%) of the virions in the clinical samples were viable. In the cases where culture was negative despite C_t values below 30, the proportion of viable virions was probably even lower due to unfavorable transport conditions. This is supported by the fact that culture-negative samples with low C_t values were more often from more distant clinics.

Real-time PCR of HSV has previously been used by others. Ryncarz et al. (19) used TaqMan probes, targeting a conserved part of gB for quantification and a segment of gG for typing. Others have used TaqMan probes to identify different amplicons for HSV-1 and HSV-2. For example, Weidman et al. targeted gD1 and gG2 (23), while van Doornum et al. targeted gG1 and gD2 (22). Our assay amplifies homologous regions with few sequence differences between HSV-1 and HSV-2 apart from five positions in the probe region, allowing quantification and typing in one reaction. This strategy may also be applied on a LightCycler instrument, where the type can be identified by melting point analysis using SYBR green (2, 18) or hybridizing FRET probes (6, 8). Of these methods, the hybridizing probes have the advantage of a higher specificity, but the relatively large probing segment increases the risk of mismatches and unexpected melting temperatures (3).

At present, the LightCycler instrument also has a limited processing capacity, as only 32 samples can be analyzed in each run. In addition to the larger sample size itself, an advantage with the 96-well format is that the PCR may be run on a traditional thermocycler, followed by fluorescence detection using post-PCR plate read. This option should be of value for laboratories that have a limited real-time PCR capacity, as it reduces the demand for time on the instrument to around 10 min.

In summary, detection and typing of HSV by this new TaqMan PCR after Magnapure extraction of DNA had a sensitivity superior to virus culture and equivalent to that of a nested qualitative PCR. Detection of HSV-1 and HSV-2 in a single well had a sensitivity similar to detection in separate wells, an approach which is preferable because reagent costs are reduced and through-put may be increased to 96 samples in each run. The high sensitivity in combination with high specificity and capacity make it suitable for clinical diagnosis of HSV in mucocutaneous lesions.

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