There have been several important advances in the range of available diagnostic tests for genital herpes simplex virus (HSV) infection in recent years; polymerase chain reaction (PCR) is emerging in routine clinical use and the potential role of type-specific serological tests is currently under debate. Several large trials of prophylactic vaccines, subsequently proved to be ineffective, have expanded knowledge of the transmission and epidemiology of HSV infection. This article discusses optimal application of recent research evidence to clinical care, structured around the key issues for patients and their partners. These include acquisition and transmission of genital HSV-1 and HSV-2 infection, the natural history of genital herpes, and the role of partner notification.

Geneital infection with herpes simplex virus (HSV) is highly prevalent and the annual number of symptomatic cases continues to increase. Despite the substantial burden of disease created by this common viral infection, its management may not be as effective as it could be. Symptomatic infection remains underdiagnosed and anecdotal experience suggests that patients commonly receive conflicting information from healthcare professionals. All this appears surprising, given the rate at which clinical research into genital herpes has proliferated over the past two decades. Significant recent advances have been made in our understanding of the epidemiology and transmission of HSV, and new diagnostic methodologies have been intensively developed and commercially promoted. The challenge for specialists in sexual health (and colleagues in the other healthcare settings where patients with genital herpes frequently present), is consistent translation of all this research evidence into high quality clinical care.

This article reviews recent clinically important developments in diagnosis and information provision, structured around the key issues for patients and their partners.

**DIAGNOSIS: HOW BEST SHOULD WE USE THE AVAILABLE DIAGNOSTIC TESTS?**

**Viral detection and characterisation**

**Culture**

Virus isolation in cell culture has been the mainstay of HSV diagnosis over the past two decades, used by 97% of genitourinary medicine (GUM) clinics in the UK. Virus culture requires a laboratory with tissue culture facilities and is highly dependent on the clinical lesions stage. Although HSV can be isolated from over 90% of vesicular or pustular lesions, the isolation rate from ulcerative lesions is only 70% and falls to 27% at the crust-stage. Transport of live virus to the laboratory within a short period is mandatory, requiring maintenance of the cold chain at 4°C. The characteristic cytopathic effect of HSV generally appears within 24 to 72 hours, but may take up to five days. Virus isolation is therefore slow and labour intensive, but has the advantage of demonstrating active infection within a clinical lesion and also allows virus typing and antiviral sensitivity testing.

**PCR**

Polymerase chain reaction (PCR) is a well characterised method for rapid and sensitive diagnosis of HSV, but, largely because of its cost and the requirement for appropriately trained technical staff, its role has hitherto been confined to investigation of suspected HSV encephalitis. However, with recent advances in automated PCR, the potential to apply molecular diagnosis to routine clinical diagnosis is enhanced and is likely to ultimately prove cost effective. Eight recent large studies have compared PCR with cell culture for detection of HSV, demonstrating consistently superior detection rates with PCR—specificity of PCR studies varied between 59% and 89% (table 1). As with application of new, highly sensitive molecular tests to diagnosis of other infections, there is no “gold standard” method against which PCR can be compared, so there is a theoretical risk of false positive results. However, in several of the above studies, discrepant samples—that is, those which were positive on PCR and negative on culture—were confirmed by a second PCR directed to a different gene, which endorses the specificity of PCR.

The clinical importance of typing HSV-1 has become an important cause of genital herpes in industrialised regions. This may be partly attributable to changing host susceptibility and also to changing sexual behaviour; the age specific population seroprevalence of HSV-1 is...
progressively declining in prepubertal children in the UK. However, the age specific incidence of HSV-1 infection rises rapidly (5–10% per year) in adolescents, increasing numbers of whom are now acquiring HSV-1 infection in association with sexual activity. As genital tract reactivation of latent HSV-1 infection is infrequent and of short duration, orogenital transmission is considered likely to account for most new cases of genital HSV-1 infection.

Knowledge of the causative type has important implications for patient management. Firstly, the natural history of first episode genital HSV-1 infection is more favourable than that of HSV-2. Three cohort studies have compared the natural history of genital HSV-1 with HSV-2 infection; the first of these demonstrated that 180 days following the resolution of a primary episode, 40% of 14 patients with HSV-1 primary infection and 90% of 123 patients with HSV-2 infection, experienced a symptomatic recurrence. Two subsequent studies observed that the mean monthly recurrence frequency following primary genital infection with HSV-1 was 0.02 to 0.08 months, compared with 0.33 to 0.34 months for HSV-2.

Secondly, the frequency of subclinical viral shedding following initial genital infection with HSV-1 is consistently lower than for HSV-2; this is likely to be associated with a reduced risk of future sexual transmission but no prospective studies have yet attempted to quantify this. Koelle et al conducted a large cohort study in 306 women with first episode genital herpes. Genital samples were cultured for HSV every four to six weeks, at times when genital symptoms and signs were absent, with a median follow up period of 63 weeks. The rate of asymptomatic genital tract shedding in 43 women with primary HSV-1 infection, was 11.9%, approximately half of the rate found with HSV-2 infection. Viral shedding was detected from the genital tract in 18.3% of 36 women with non-primary HSV-2 infection and 22.9% of 227 with primary HSV-2 infection. A more intensive study of asymptomatic shedding in 110 women with a history of genital herpes attending a research clinic, scheduled participants to collect daily anogenital specimens for a median follow up period of 105 days (range 5 to 799). Subclinical shedding occurred in 29% of 14 women with HSV-1 infection only, 55% of 65 women with HSV-2 infection, and 52% of 31 women with both types. The overall duration of subclinical shedding was also substantially lower for women with HSV-1 infection, occurring on 0.7% of days, compared with 2.0% for those with HSV-2.

### Type specific serology

Sero logical tests detect antibodies to HSV in blood and are indicative of past infection. Older, classical serological tests are vulnerable to cross reactivity between HSV-1 and HSV-2. Several type specific serological tests; developed over the last 20 years, can differentiate antibody responses to the two types and have been extensively applied both to epidemiological surveys and to studies of the transmission of genital herpes. Type specific tests are based on either western blot (which tests for a range of type specific antigens) or glycoprotein G (gG) assays. Western blot tests are expensive, take 2–5 days to complete the screening and confirmatory steps, and require expert interpretation. Therefore, they are unlikely to be commercially developed for use in routine clinical practice. Glycoprotein G assays detect antibodies to the type specific proteins gG-1 and gG-2. Very little sequence homology exists between gG-1 and gG-2, allowing differentiation between established infection with HSV-1 and HSV-2 respectively. A number of gG based tests have been commercially marketed, using a variety of test formats, most often using enzyme immunoassay (EIA) methods. Type specific tests have been used in epidemiological surveys for many years, but their diagnostic performance in individual patients is still a matter for debate and demands critical examination.

A diagnosis of genital herpes may have significant psychosocial consequences for the patient and their partners; it is therefore essential that any diagnostic methods used are accurate, reproducible, and appropriate to the clinical situation to which they are applied. A recent review of commercial type specific antibody tests for HSV-1 and HSV-2 estimated their median sensitivity at 95% (range 81 to 100%) and specificity at 98% (range 97 to 100%). HSV-2 prevalence in two recent surveys among unselected UK GUM clinic attendees was estimated at 22.7% in a central London clinic and 14.3% in a district general hospital in Trafford. However, the UK general population prevalence is probably closer to 4–5%. Dependent on local population characteristics and variations in accessibility and case mix between GUM clinics, the HSV-2 prevalence in GUM settings across the UK may therefore vary between 5 and 20%. Numerous seroprevalence studies have been conducted in various populations worldwide, with widely varying rates of HSV-2 infection reflecting differences in population characteristics, notably in respect of sexual behaviour and socioeconomic attributes. There are few well conducted general population serosurveys. The NHANES III study was an important exception, this study rigorously conducted survey estimated that 22% of the US general population was infected with HSV-2, with higher rates in women and among black ethnic subgroups.

Table 1 quantifies the impact of population HSV-2 prevalence on performance of commercial type specific tests, assuming the sensitivity and specificity values above. Even in a clinic population with a very high prevalence of 20%, 10% of positive results would be false positives, rising to a 29% false positive rate in populations with a prevalence of 5%. Should the “real world” performance of currently available gG assays fall short of the sensitivity and specificity values quoted above, the likelihood of false positive results would increase considerably. In the case of investigations for other serious medical conditions, the risk of a false positive test is minimised by use of a second confirmatory test, however, this strategy is not always possible.

<p>| Table 1 Virus isolation compared with PCR for diagnosis of herpes simplex virus infection: summary of recent large* studies |</p>
<table>
<thead>
<tr>
<th>First author</th>
<th>Year of publication</th>
<th>Setting</th>
<th>Sample characteristics</th>
<th>Number of specimens analysed</th>
<th>Sensitivity (virus isolation of PCR) [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orel†</td>
<td>1996</td>
<td>Laboratory</td>
<td>Genital ulcers</td>
<td>298</td>
<td>72%</td>
</tr>
<tr>
<td>Safrin‡</td>
<td>1997</td>
<td>STD clinic</td>
<td>Oral &amp; genital lesions</td>
<td>246</td>
<td>79%</td>
</tr>
<tr>
<td>Slomka‡</td>
<td>1998</td>
<td>GUM clinic</td>
<td>Anogenital lesions</td>
<td>194</td>
<td>81%</td>
</tr>
<tr>
<td>Wolfthuber‡</td>
<td>1999</td>
<td>Sexual health centre</td>
<td>Genital ulcers</td>
<td>131</td>
<td>67%</td>
</tr>
<tr>
<td>Coyne‡</td>
<td>1999</td>
<td>Laboratory (various clinical settings)</td>
<td>Genital ulcers</td>
<td>134</td>
<td>59%</td>
</tr>
<tr>
<td>Espy‡‡</td>
<td>2000</td>
<td>Laboratory (various clinical settings)</td>
<td>Genital &amp; dermal</td>
<td>500</td>
<td>76%</td>
</tr>
<tr>
<td>Marshall‡‡</td>
<td>2001</td>
<td>Laboratory</td>
<td>Anogenital swabs</td>
<td>100</td>
<td>89%</td>
</tr>
<tr>
<td>Scouler‡‡</td>
<td>2002</td>
<td>GUM clinic</td>
<td>Anogenital ulcers</td>
<td>236</td>
<td>81%</td>
</tr>
</tbody>
</table>

* > 100 clinical specimens.
Currently not easily available in the context of HSV serology and absence of a contributory test remains a fundamental barrier to widespread application of type specific tests.

Clearly, the prevalence of HSV-2 infection is likely to be higher in specific subgroups of patients at higher risk of infection, such as those presenting with genital symptoms suggestive of herpetic infection and partners of patients with a known diagnosis of genital herpes. In these groups, test accuracy would be higher than in the clinic population as a whole. A final issue to bear in mind when interpreting gg-based tests is the influence of time. Seroconversion following initial infection may take up to six months and, secondly, the phenomenon of “seroreversion” (spontaneous reversal from seropositive to seronegative status), raises some concerns about the long term reliability of tests based on antibodies to a single protein, such as gg tests.

In summary, in assessing the merits of type specific serological testing for genital herpes, the crucial elements must include: firstly, knowledge of the prevalence of HSV infection in the population being offered testing, secondly, an appreciation of variation in test accuracy from one application to another and, most importantly, a strategy for pretest discussion, interpretation of the results, and communication of the meaning of the results to patients. Assuming that a valid, reproducible type specific test were available for use in individual patients, there are a number of potential clinical applications, which include assessment of pregnant women, evaluation of monogamous couples in a discordant relationship and investigation of symptomatic patients. The potential for offering HSV-2 screening as part of a sexual health “check up” also exists.

Type specific testing has been suggested as a means of identifying women at risk of acquiring HSV-1 or HSV-2 infections close to term, a setting in which there is a high risk (30–50%) of neonatal herpes. In theory, the couple could be counselled to adjust to the diagnosis. Between 1972 and 1999, diagnoses of genital herpes in GUM clinics in the UK increased fourfold in males and fourfold in females; similar patterns have been observed in many other regions of the world. In the United States, a well conducted seroepidemiological survey of the general population showed that HSV-2 seroprevalence rose
by 32% during the 1980s, from 16.4% in 1976–80 to 21.7% in 1988–94. HSV-1 is an increasingly important cause of genital infection. In Glasgow, the ratio of HSV-1:HSV-2 isolates in patients with genital herpes has risen progressively over the past 15 years; genital HSV-1 infection is independently associated both with being female and being young (aged ≤25 years). As risk factors for acquisition of HSV-1 and HSV-2 differ, it is crucially important that knowledge of the causative type informs counselling and information given to patients. Risk factors for HSV-1 infection are heterogeneous. In economically deprived populations, seroconversion occurs early in life, with around 70–80% of the population infected by late adolescence, many in the first few years of life. In more advantaged populations, acquisition of HSV-1 shows a more linear relationship with age; about 20% of individuals seroconvert in childhood, a further 20–40% in early adulthood and by the age of 50, a high proportion of the general population is usually infected. The rapid increase in age specific seroprevalence rates in early adulthood (occurring at an earlier age in females than males), together with the observed increase in the proportion of clinically manifest genital herpes attributable to HSV-1, suggest that sexual transmission of HSV-1 is progressively assuming a more important role. The majority of genital HSV-1 infection is presumed to be acquired as a result of oro-genital contact, but no evidence is available to quantify this more precisely. Only a third of HSV-1 antibody positive individuals have a known diagnosis of oral herpetic, but most regularly shed HSV-1 from the oral cavity. Overall, acquisition of HSV-1 in sexually active people occurs with ease. In a cohort study of 7046 pregnant women, 2033 of whom were initially HSV-1 and HSV-2 negative, the estimated mean risk of HSV-1 acquisition during pregnancy, adjusted for length of gestation, was 2.3% (SE 0.4). Another prospective study in a population at high risk of HSV-2 acquisition (two randomised controlled trials of a prophylactic HSV-2 vaccine which was subsequently shown to be ineffective), estimated an overall incidence rate of new HSV-1 infection at 1.6 per 100 patient years, which was similar for men and women. HSV-2 infections are usually sexually transmitted. The principal risk factors for acquisition are gender (women are at substantially greater risk than men),23 sexual behaviour,24 and socioeconomic deprivation. Asymptomatic shedding and unrecognized symptomatic infection in source partners play a major role in the acquisition of genital herpes. In Mertz’s well conducted, prospective study of discordant couples, nine of 14 (70%) of transmissions occurred when the source partner was asymptomatic, strengthening the evidence in this regard from older retrospective studies. The advent of type specific serology has been immensely helpful in prospective evaluation of new infections with HSV-1 and HSV-2 infections, of which 37% and 63% respectively are asymptomatic. Finally, 10–17% of patients who present with clinical features of initial episodes of genital herpes can be serologically confirmed as having recurrent disease. In summary, patients who present with an initial episode of genital herpes can be advised that they may not necessarily have acquired their infection within the recent past, that the infection is highly prevalent among the general population, and the partner from whom they acquired infection was unlikely to be aware of their infection, and the most likely acquisition route would depend on the viral type, HSV-1 or HSV-2. The availability of diagnostic tests to further define the HSV status of partners is limited by the factors discussed in the previous section.

Can I pass it on?

A major concern for patients with genital herpes is fear of transmission to sexual partners. In general, transmission of HSV infections occurs by close contact with an individual who is shedding virus at a mucocutaneous surface and/or in oral or genital secretions. Infection occurs by inoculation of susceptible mucosal surfaces or through breaches in skin that are invisible to the naked eye. Since HSV is readily inactivated at room temperature and by drying, transmission by means other than direct contact is rare.

Research into transmission of genital herpes has focussed virtually exclusively on HSV-2 infection in heterosexual people. Limited indirect evidence on shedding patterns suggests that the advice we should be giving to patients about risk of transmission following a diagnosis of genital HSV-1 may differ substantially. Similarly, some information is available on HSV shedding frequency and patterns in a small study of 30 HIV negative gay men, but not on absolute transmission rates within gay partnerships.

As detailed above, both the proportion of patients who asymptotically shed HSV from the anogenital region and the duration of shedding following initial genital infection with HSV-1 are consistently lower than that for HSV-2. Although these observations are likely to translate into a reduced risk of genital transmission, no prospective studies have yet been conducted to investigate this and the advice we give to patients must therefore be based on biological plausibility combined with a summary of the above data on shedding rates. Much better data are urgently required to inform the advice and counselling process we provide in this respect, particularly in view of the increasing predominance of genital HSV-1 infection.

In contrast, several large prospective studies of HSV-2 transmission have been published in recent years (table 3), which followed on from an early couple study of 29 HSV-2 seronegative partners; this had reported a male to female transmission rate of 14% (23% in HSV-1 seronegative susceptible partners and 6% in those who were HSV-1 seropositive). Of the large prospective studies shown in table 3, two studied HSV discordant couples, one a population at high risk of HSV acquisition, and the fourth a lower risk population, comprising 7046 pregnant women receiving routine antenatal care in Seattle. The first three of these studies were designed and powered to investigate the efficacy of prophylactic HSV-2 vaccines, not the determinants of transmission. Brown et al’s study of HSV acquisition in pregnant women has the advantage of reflecting more closely the situation in the general population. In this study, the estimated mean risk of HSV-1 acquisition during pregnancy, adjusted for length of gestation, was 2.3% (0.4) with a risk of 1.4% (0.3) for HSV-2 acquisition in women with no serological evidence of pre-existent HSV infection and 1.7% (0.3) in those with pre-existent HSV-1 antibodies.

Mertz et al studied 144 heterosexual couples in whom each source partner had symptomatic, recurrent genital herpes (97% due to HSV-2) and each susceptible partner was HSV-2 antibody negative. Transmission occurred in 14 couples, 11 with male source partners and three with female source partners. In nine couples, transmission occurred when the source partner was asymptomatic. Only 15% of couples in the study who were discordant, that is, one partner was HSV-2 seropositive, had HSV-2 seroconversion, compared with 13.6% in those who did not (p = 0.19). Overall, pre-existent HSV-1 infection in the susceptible partner did not protect against HSV-2 infection, but subgroup analysis by gender suggested a protective effect in women.

Useful data on HSV acquisition have recently been generated from two parallel phase three trials of an ineffective HSV-2 vaccine. One trial enrolled 351 HSV-2 seronegative individuals, each of whom was in a monogamous relationship with a partner with HSV-2 infection. The second enrolled 1862 STD clinic attendees at high risk of HSV-2 acquisition. Langenberg et al used combined data from both trials to describe the acquisition rate of HSV-1 and HSV-2 and describe the characteristics of new infection. In contrast to the much
smaller study conducted by Mertz et al. pre-existent HSV-1 infection did not reduce the rate of HSV-2 acquisition, but it did increase the likelihood of asymptomatic seroconversion. Sixty three per cent and 37% of newly acquired HSV-1 and HSV-2 infections respectively were symptomatic. The sensitivity and specificity of a clinical diagnosis of genital herpes was poorly studied. The Seattle research group has conducted several short term studies and one longer term observational study over the past two decades. The findings of the shorter studies, which had median follow up periods of six to 12 months, are described in the section above (the clinical importance of typing).

Benedetti et al described the longer term natural history of genital herpes in an observational study of 664 patients recruited to their research clinic between 1974 and 1991. Three hundred and six patients had first episode genital herpes (60 had primary HSV-1 infection, 205 primary HSV-2 infection, and 41 non-primary initial HSV-2 infection). The remaining 358 patients had recurrent HSV-2 infection at enrolment. In the 169 patients who were followed up for more than 6 years, clinically significant reductions in recurrence frequency occurred in the majority of patients. However, 25% had an increase in recurrence frequency.

In summary, the expected course of genital HSV infection is strongly dependent on the infecting viral type. Although the long term natural history of genital HSV-2 infection is very variable, reductions in recurrence frequency can be expected in the majority of patients.

### Should my partner come in for a test?

This is essentially a question about the value of partner notification in patients recently diagnosed with genital herpes, up to 20% of whom have established infection and are presenting with a first symptomatic recurrence at a variable time interval following asymptomatic seroconversion. The aims of partner notification (PN) are to break the chain of transmission of STIs and reduce the population rates of infections, firstly through identifying, counselling, and offering treatment if appropriate and, secondly, by educating and promoting sexual health on an individual basis.

### Will it keep coming back for ever?

The long term natural history of genital herpes has been poorly studied. The Seattle research group has conducted several short term studies and one longer term observational study over the past two decades. The findings of the shorter studies, which had median follow up periods of six to 12 months, are described in the section above (the clinical importance of typing).
transmission. Availability of an accurate diagnostic test to offer asymptomatic individuals is an essential element of PN for viral STIs, as antimicrobial therapy does not have the potential to eliminate the disease. Finally, in evaluating any clinical intervention, the potential for harm must be weighed against any perceived benefits. Few, if any, of the above criteria would support a systematic strategy of PN for asymptomatic partners of individuals recently diagnosed with genital herpes. However, as a high proportion of partners of newly diagnosed individuals have symptomatic but undiagnosed disease, there is some evidence to support an offer to see partners for a general sexual health consultation, to identify any symptoms suggestive of previously unrecognized HSV infection, leading towards a formal diagnosis (currently, this should ideally be made by direct confirmation of HSV—type specific serology) and appropriate counselling and information provision about transmission.

CONCLUSION
There have been several major advances in the range of available diagnostic tests for genital HSV infection in recent years, as well as substantially improved understanding of its epidemiology and transmission. In reviewing the recent literature, this article demonstrates the application of some of this new evidence to clinical care, focussing on the issues which are most important to patients and their partners.

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