ELSEVIER

Antiviral Research 51 (2001) 81–94

Antiviral Research

www.elsevier.com/locate/antiviral

Review

The importance of specific virus diagnosis and monitoring for antiviral treatment

Annika Linde [a,b,*]

[a] *Department of Virology, Swedish Institute for Infectious Disease Control, SE-171 82 Solna, Stockholm, Sweden*
[b] *Microbiology and Tumor Biology Center, Karolinska Institute, SE-171 77 Stockholm, Sweden*

Received 28 November 2000; accepted 24 January 2001

Abstract

Antiviral treatment, rapid viral diagnosis and point-of-care diagnostics are all relatively new, and their appropriate use not fully appreciated or evaluated. In this article, the need for laboratory diagnosis in relation to antiviral treatment, and practical approaches are discussed, with influenza and herpes simplex virus (HSV) as examples.

Keywords: Influenza; Herpes simplex; Antivirals; Diagnosis; Laboratory methods

1. Introduction

The demand for a laboratory verification of any clinical diagnosis before onset of treatment and for monitoring of effect varies. Factors that may affect the proneness of a clinician to use the laboratory are listed in Table 1. Most of these factors are relevant also for antiviral treatment, and differ for different kinds of viruses, the condition they cause depending on the host and their medications. However, the possibility to treat viruses and the availability of rapid virus diagnosis, especially to be used point-of-care, are relatively new. Neither their usefulness, nor the demands they impose on the doctors is yet fully appreciated. To illustrate how the problems may be approached, two groups of viruses will be dealt with; influenza viruses and herpes simplex viruses (HSV). The necessity for treatment and laboratory diagnosis in relation to the condition of the patient, and practical approaches will be discussed. Needs and practices concerning monitoring of effect and antiviral sensitivity will also be touched upon, as well as accuracy of assays and demands for standardization of evaluation, and of external quality control (EQC) of laboratories and point-of-care. Treatment of the human immunodeficiency virus (HIV) probably demands

* Corresponding author. Tel.: +46-8-4572652; fax: +46-8-337272.
E-mail address: annika.linde@smi.ki.se (A. Linde).

0166-3542/01/$ - see front matter
PII: S0166-3542(01)00129-2

more of laboratory-work up than any other virus today. However, the procedures and considerations concerning HIV are too complex to be included in this general review, and have also been dealt in other recent review articles (Carpenter et al., 2000; Hirsch et al., 2000).

2. Treatment of influenza

Amantadine and rimantadine have been available for influenza A treatment and prophylactics for many years (Aoki, 1998). Despite documented therapeutic, and above all preventive effects, they have not been widely used outside Russia and USA. This has been claimed to be due to fear of side effects, and also to the rapid development of antiviral resistance. The lack of efficacy against influenza B is a further complication. However, rimantadine causes far less adverse events than amantadine, and still it has not been licensed in many European countries. Lack among many doctors of appreciation of influenza as a severe disease that should be prevented and treated, at least in defined groups at risk of severe disease (Anonymous, 1999) might be an explanation. In many countries, patients with suspected influenza are discouraged to see the doctor unless the disease is very severe, or they suspect a bacterial complication. Since there has been the notion that there is no good, specific treatment to offer anyway, the health care systems are not dimensioned to urgently evaluate the large number of patients that would need treatment even during an ordinary, yearly influenza epidemic. In a developed, Western country like Sweden, influenza is estimated to, directly or indirectly, have killed $3/10^4$ inhabitants yearly between 1993 and 1999 (Linde, unpublished). It seems to be related to excess mortality in all kinds of diseases in all countries (Reichert, 2000). The realization that influenza is indeed one of the most severe infectious diseases that exists in the industrial world for those that are vulnerable, and that specific prevention and treatment exist, are slowly reaching the clinicians. What exists today concerning prophylactics, treatment and diagnostics, and how can we improve the management of influenza with the aid of medications and diagnostic techniques? As to prophylactics, amantadine and rimantadine are licensed as prophylactics against influenza in many countries, but vaccination is the most cost-effective preventive measure (Nichol et al., 1994; Gross et al., 1995). There are recommendations to vaccinate those at risk of severe disease in several countries (Fedson et al., 1995). Still, the majority belonging to the risk groups remains unvaccinated in most (Fedson et al., 1995, 1997) and post-exposure amantadine or rimanatadine prophylaxis to persons at risk seems extremely rare. This lends further support to the suspicion that health care providers, especially in many European countries, so far largely have failed to realize the severity of influenza infections in the risk groups.

In the past few years new drugs against influenza, the neuraminidase inhibitors, have been available (Gubareva et al., 2000). Whether lack of knowledge and logistics, or fear of side effects, antiviral resistance, and lack of promotion are

Table 1
Factors that affect the proneness of the clinician to obtain a laboratory verification of the diagnosis before onset of therapy

Factors related to diagnosis
Accuracy of the clinical diagnosis: the higher accuracy, the lesser need for laboratory confirmation
Availability of laboratory diagnosis: the higher the availability, the more likely it is to be used
Time for laboratory diagnosis: the shorter time, the more likely it is to be used
Reliability of available method/s for laboratory diagnosis: the more reliable, the more likely it is to be used
Ease by which the specimen can be obtained: the easier, the more specimen sent
Cost of laboratory diagnosis: the cheaper, the more likely it is to be used
Factors related to the disease and the treatment
Urgency of the disease: high degree of urgency; little time for pre-treatment verification
Severity of the disease: the more severe, the higher demand for verification of the diagnosis
Side effects of the treatment: the more side-effects, the higher demand for verification
Duration of treatment: long time, higher demand
Cost of treatment: high cost, higher demand
Risk of development of resistance against the treatment: the higher risk, the greater necessity to have a pre-treatment sample

major obstacles against influenza treatment will probably now be clarified, since these new drugs with little side effects and resistance development are being intensely promoted (Gubareva et al., 2000). They reduce the time for alleviation from influenza symptoms between 1–2 and 5 days in a febrile patient if given <48 h after onset of influenza (Hayden et al., 1997; Nicholson et al., 1998; Gubareva et al., 2000; Makela et al., 2000; Nicholson et al., 2000). Prevention of complications and reduction of time for disease in the patients at risk of severe disease have not been extensively documented, but there are indications that it might be cost-effective (Mauskopf et al., 1999). In vitro resistance has been show to develop in 1–2% of patients after oseltamivir treatment, but the frequency is not clinically documented for zanamivir (Gubareva et al., 2000).

Extensive use of these agents poses the same logistic problems as amantadine and rimantadine, and the inclination to use them among doctors and patients may be as low for these new drugs as for the already very well documented, cost effective vaccination (Nichol et al., 1994; Gross et al., 1995). In addition, diagnosis and urgency is not a problem for vaccination. It should be given routinely each year, before the influenza season starts. The diagnostic demand before onset of anti-neuraminidase therapy has been widely discussed. There seems to be a general acceptance that treatment should at least not be considered unless surveillance systems have demonstrated influenza in the society. However, there is no agreement as to handling of the individual patient. In this article the diagnostic problem is scrutinized from different aspects presented in Table 1 and some solutions suggested.

2.1. Accuracy of the clinical diagnosis of influenza

In a characteristic influenza outbreak in an area where the presence of the virus is confirmed, the accuracy of the clinical diagnosis is high, but probably many atypical cases remain unrecognized. The clinical picture of typical influenza has been well described (Nicholson, 1998). Cough, fever and malaise in combination with a sudden onset seem to be the most frequent symptoms. Simple blood analyses such as C-reactive protein (CRP) and leukocyte count may help to indicate the diagnosis, but are in no way diagnostic. It has been show that only 60–80% of intentions to treat patients had laboratory confirmed influenza in clinical trials (Makela et al., 2000), despite strict diagnostic criteria, and verified influenza in the society. Studies in elderly outside clinical trials show diagnostic accuracy of only 30% (Govaert et al., 1998), and clinical hallmarks to differentiate influenza from other viral respiratory infection seem to be lacking (Carrat et al., 1997).

The elderly are at the greatest risk of severe influenza, but most of those who die during an influenza epidemic don't have the influenza disease that preceded the pneumonia or heart attack at all recognized. A few studies underline the importance of early specific diagnosis of index cases at outbreaks in nursing homes (Taylor et al., 1992; Staynor et al., 1994; Loeb et al., 2000; Read et al., 2000), but it certainly also would be valuable for handling of individual patients in the society. The reduction of antibiotic therapy in children as a result of a positive viral diagnosis is also an important gain (Serwint and Miller, 1993; Noyola and Demmler, 2000). The lack of accuracy of the clinical diagnosis certainly implicates the development and use of specific point-of-care assays for decision concerning treatment. Though probably better than the clinician, the ones available still lack substantially in performance related to reference methods (Table 2), and well equipped laboratories for confirmation of influenza diagnoses and for epidemiological surveillance remains a necessity. Only with the combination of point-of-care devices, the use of a well equipped laboratory for confirmation of all unclear cases, and a well structured surveillance system for monitoring of influenza in the society can optimal patient treatment be obtained.

2.2. Availability of laboratory diagnosis of influenza

When facilities for rapid diagnosis are close and allowing for a diagnosis on the day of sampling,

Table 2
Influenza diagnostic comparative analysis[a]

	ZstatFlu™ Zyme Tx	Directigen® Becton Dickinson	FLU OIA® BioStar	Quick Vue® Quidel
Detects influenza types	A and B	A only	A and B	A and B
CLIA categorization	Moderately complex	Moderately complex	Moderately complex	Moderately complex
Total assay time	20 min	15 min	15 min	10 min
Technician attended time	2 min	15 min	15 min	1 min
Specimens used	TS	TS, NPW, NPA, NPS	TS, NS, SP, NA	NS, NPW
Specificity	99%	88–97%	52–80%	95–99%
Sensitivity	62%	67–96%	62–88%	73–82%
Retail cost	$18.00	$18.00	$16.50	$18.99
Technology used*	Neuraminidase enzyme	Antigen–antibody	Antigen–antibody	Antigen–antibody
Detects all strains	Yes	No	No	No
Number of steps	4	9	7	2
Best market	POL/HOSP	HOSP	HOSP/POL	POL/HOSP
Product launch	Currently in market	Currently in market	Currently in market	Tentative 12/1/99 Limited availability
Storage conditions	Room temperature	Room temperature	4–8°C	Room temperature
Shelf life	3 years	Months	1 year	1 year
Portability	24 h	Yes with media	Yes	1 h

[a] *TS, throat swab; NA, nasal aspirate; NPW, nasal pharyngeal wash; NPA, nasal pharyngeal aspirate; NPS, nasal pharyngeal swab; S, nasal swab; SP, sputum.

frequent samples are likely to be sent, at least from hospital doctors with an interest in infectious diseases. During the last 3 years, about 20 influenza diagnoses per 10^6 population (2000 totally) have been reported in Sweden. This is probably a relatively high number; comparisons are lacking. The reason is the availability of rapid diagnosis at many laboratories, but it is still almost only from patients coming to the hospital that samples are taken. The rapid method used is almost invariably immunofluorescence (IF), and the recommended samples, nasopharyngeal aspirates, are regarded as major obstacles for the general practitioners (GPs). The analyses are also relatively expensive, the sending of samples is complicated for medical personnel not in the hospital setting, and the value of a confirmed influenza diagnosis is yet not appreciated by GPs. Though the assays for antigen detection in the laboratories can be performed within an hour, sending and handling of the specimen take time, and in reality it is an achievement if the result is reported on the same day as the specimen was taken. A conservative estimate of around 5% of the population falling ill with influenza yearly gives 500 000 cases each of these 3 years, and thus a reported laboratory diagnosis only in 0.4% of cases. This is probably too little to be the basis for decisions on treatment, both individually and if a local, epidemiological confirmation of influenza is regarded sufficient for initiation of treatment. Good point-of-care diagnostics are again a necessity for confirmation of diagnosis in the outpatient setting.

2.3. Diagnostic methods, their reliability and test evaluations for influenza

The main principles for diagnosis of influenza are virus isolation in embryonated hens eggs or

cell culture, including rapid culture with antigen detection; antigen detection in situ, most frequently by immunofluorescence; solid phase immune assays (EIAs) for antigen, and genome amplification (Harmon, 1999). One commercial rapid assay is based on detection of influenza neuraminidase activity (Noyola et al., 2000). Apart from the last mentioned it is mainly the EIA principle that has been used in the point-of-care KITs that are now coming on the market (Table 2). A prerequisite for their usefulness and success is that they are adequately evaluated, and that true information on their efficiency is spread to the users. A higher degree of standardization on what should be included such evaluations, and how they should be presented also in peer reviewed journals is desirable to facilitate comparison between evaluations, and identification of reasons for discrepant results when the same assay is used in different studies.

General standards to adhere to in test evaluation have been suggested, but very few published evaluations fulfil all, or even most, of the criteria (Reid et al., 1995). For example, when a new method is evaluated, it is extremely important to settle the reference method to which it is going to be related. Virus isolation has until recently been the reference method for diagnosis of influenza. Unfortunately, the isolation method varies in quality between laboratories, and might even do so at the same laboratory with time, if the susceptibility of the cells is not subject to continuos internal quality control. Different influenza strains might also vary in capacity to grow. The quality of submitted material may vary with e.g. long transports affecting the viability of the virus. Genome amplification which is more sensitive, easier to standardize and does not demand viable virus will probably soon replace virus isolation as the reference method. In the meantime it has become common to use combinations of methods as reference (Magnard et al., 1999), but if that is done all materials have to be examined with all methods, which is not always the case.

The result of an examination is still often depending on individual skill, such as preparation of slides and reading of IF assays. Like the clinical evaluation of a patient, results may thus vary depending on the examinator, and published evaluations may not be valid in the setting where they are later used. The most recent textbook on 'Laboratory Diagnosis of Viral Infections' gives a summary sensitivity of 70–100% of IF for influenza antigens in comparison to virus cultivation and of 53–91% of EIAs. In relation to cultivation the specificity of assays for antigen detection is seldom 100%, whether due to truly false positive reactions, or due to that virus has been inactivated during, e.g. transport has not till now not been firmly settled. However, gene-amplification techniques can detect also non-infectious viral genomes, and might solve the problem of specificity. Though information from literature is somewhat contradictory, gene amplification probably has 30–50% higher sensitivity that virus cultivation (Cherian et al., 1994; Ellis et al., 1997; Pregliasco et al., 1998; Magnard et al., 1999). The problems of contamination should be possible to solve, but the mere control of amplicon contamination frequently practiced is not sufficient. The whole laboratory process should be monitored by inclusion of negative samples from the onset of the processing of the sample at the laboratory in order to guarantee reliable positive results.

If an assay should be used for the determination of treatment, it is the diagnostic accuracy within 2 days after onset of disease in the patient that should be the goal, since treatment should start within that time limit to be of value. This prerequisite has to my knowledge not been included in any evaluation of diagnostic devices, and lowering of sensitivity of virus cultivation in comparison with PCR time after onset of disease has been shown (Cherian et al., 1994). Most likely, many rapid assays will prove more accurate during this early phase of disease than later, increasing their practical usefulness.

There are four registered point-of care KITs for rapid diagnosis of influenza A and B, and one that detects only influenza A (Table 2). Only evaluations of three of these have been published in peer-reviewed articles (Todd et al., 1995; Marcante et al., 1996; Kaiser et al., 1999; Noyola and Demmler, 2000; Noyola et al., 2000) to mention some. According to a recent summary of the producers own evaluations (Table 2) their sensi-

tivities and specificities are quite variable. The most important for treatment of influenza is specificity, since a positive influenza diagnosis implies also non-treatment with, e.g. antibiotics. Since influenza is normally self-limiting, but a septicemia or a bacterial pneumonia may be lethal, a false positive diagnosis may become truly deleterious. Definitive demands concerning accuracy of a diagnostic assay are difficult to settle, and depend on the specificity of the clinical diagnosis. If it is correct in 80% of cases, as in the best studies sampling from every patient, laboratory verification is hardly defendable. If it is 30%, as in studies of elderly, laboratory verification is far more important. A sensitivity of around 90% and a specificity of $>95\%$ with a sensitive PCR as the reference method, and during the first 2 days of disease, is probably a minimum requirement to defend the extra cost that testing implies. Future evaluations of point-of-care assays should, however, also include the evaluation of the accuracy of the clinical diagnosis, thereby allowing for an estimation of how testing should be used to be economically defendable.

Reliability of virological methods demands highly standardized performance from well-educated staff, KIT-independent controls and external quality control-quality control. There is an increasing awareness of these demands at laboratories, but maybe not that the demands have to be similar for point-of-care diagnostics as for tests performed at the laboratories. The expense of EQC may become another obstacle for the use of point-of-care diagnostics by the GPs, but experience points to that EQC is a necessity, and it should be a demand for accreditation of a method.

EQC can only encompass selected samples, and not the testing of large numbers of samples that are needed in the initial evaluation of a method. However, if one method fails in EQC at many places, both producers and users have to be informed so that the reason can be settled. The results of EQC for in situ detection of influenza antigens performed by the External Quality Control Assessment in Sweden (Equalis) are presented (Table 3). A low sensitivity for influenza B detection was identified repeatedly. Only assays used by a large enough number of participants can be evaluated. However, repeatedly the direct IFA with Imagen antibodies failed more frequently for influenza B than the indirect assay by Chemicon, and investigations are now being performed to find out whether this is due to the antibody quality or to a higher sensitivity of the indirect staining method.

2.4. Ease by which the specimen can be obtained for influenza diagnosis

Painful and complicated sampling is a major obstacle to obtain specimen, especially if children are involved. Throat- or nasal swabs are generally accepted. Influenza isolation is often made on nasal or throat swabs, or combined materials. However, for optimal results they should be sent in cultivation medium, and medium tubes could be difficult to store for infrequent users. For antigen detection with IFA, nasopharyngeal aspirates are ideal, and these can be used also for virus isolation, PCR and EIA, provided that they are not too diluted in medium or saline. Nasopharyngeal swabs eluted in a small amount of fluid, or spread on at a slide spot for in situ examination, probably give a higher sensitivity than nasal and throat swabs, but are more painful for the patient. In hospital settings, nasopharyngeal aspirates are not a major obstacle, but in my experience GPs are less prone to perform this kind of sampling, though mostly they can accept a

Table 3
External Quality Control in Sweden performed by External quality assurance in laboratory medicine in Sweden (Equalis)[a]

Source of anitbodies	Influenza A/H1	Influenza A/H3	Influenza B
Imagen*	97/102 (95%)	83/93 (89%)	89/114 (78%)
Chemicon**	52/52 (100%)	48/50 (96%)	56/58 (97%)
WHO**	14/14 (100%)	9/10 (90%)	14/15 (93%)
Biosoft/Argene*	5/6 (83%)	6/6 (100%)	5/7 (71%)
Biotrin*	4/4 (100%)	4/4(100%)	4/4(100%)

[a] Influenza antigendetection by in situ IFA 1994–1999. Frequency of correct analyses. *direct staining; **indirect staining.

nasopharyngeal swab. In one of the few studies of suitable samples for a point-of-care EIA method, nasal and bronchial aspirates also proved most sensitive (Covalciuc et al., 1999). More studies on suitable material should be performed. It is probably feasible to get acceptance for nasopharyngeal swabs instead of nasal or throat swabs, if the accuracy of an assay is dramatically changed. Aspirates will probably not be provided by GPs unless new, easy-to-use devices as used in the previously mentioned study (Covalciuc et al., 1999), are adapted and marketed for this kind of sampling.

2.5. Urgency of influenza diagnosis

Influenza itself is normally not an urgently life-threatening disease, but to obtain effect of antiviral treatment an urgent onset of therapy is necessary. In most cases, there has already been a time delay for the patient to get in touch with the doctor, and the results of the laboratory diagnosis must be obtained within a few hours to be of guidance without jeopardizing the effect of treatment. If an influenza patient is visiting the doctor or the doctor the patient, there is a great likelihood that neither is willing to wait for an even few hours before a decision is taken. A clinical diagnosis or point-of-care devices seem to be the only options to allow for a rapid enough decision on whether to treat or not.

2.6. Severity of the influenza disease

The secondary bacterial complications are the main problems and big killers during influenza epidemics. It has, however, been indicated that early antiviral treatment of medical risk groups significantly diminishes the risk of bacterial complications. If proven, the antiviral treatment will be extremely important for prevention of severe disease the medical risk groups. New pandemic influenza strains may also cause more severe disease and be a threat per se, but when such a strain will appear no one knows. In the elderly, as mentioned above, the influenza diagnosis is more of a challenge than in young and middle-aged, and especially so in vaccinated persons. An urgent laboratory examination could be indicated in all patients at risk for severe disease to allow for a correct treatment and thereby diminish the risk for a lethal outcome.

2.7. Side effects of antivirals against influenza

The likelihood that doctors will prescribe medication without any laboratory confirmation is high with a treatment with the insidious side-effects so far documented for the neuraminidase inhibitors (Hayden et al., 1997; Freund et al., 1999; Gubareva et al., 2000). For many reasons this is still not desirable, one of the main being that the reputation of the medication will deteriorate if too many patients don't get alleviation since they don't have influenza. If the lack of severe side effects is indeed verified, the same diagnostic strategy as for herpes simplex encephalitis could be used; draw a diagnostic sample, give the first one or two doses, and await laboratory confirmation before continuation of therapy. Since the next dose will not be given until after 12 h this is a possible approach. The approach would be less costly than a full cure in a non-influenza patient, but impractical for out-patients. Since the neuraminidase inhibitors have no effects on any infectious agent but influenza, there is no risk of development of any resistance if influenza is not there. However, if the virus is indeed there, and the clinical diagnosis correct, but not verified due to inaccurate laboratory methods, the resulting, incomplete treatment may facilitate resistance. The approach demands very sensitive methods for laboratory detection of influenza.

2.8. The duration of treatment and the cost of treatment

Five days of treatment, twice daily, is probably no major problem for the individual patient. Whether or not cost will be a problem for the patients depends on rules for reimbursement, which vary between countries. If fully reimbursed, treatment including the necessary visit to the doctor may become a major concern for governments and insurance companies. The debate around the

cost-benefit of the new substances has been intensive in many countries. Probably the risk for overuse will increase the demand for laboratory diagnosis from the paying authorities. On the other hand someone has to pay for the examinations as well. These are often almost as expensive as the medication, leading to very difficult economic considerations.

2.9. Risk of resistance development

Since bacterial complications is the main problem for which patients go to the doctor in connection with influenza, it is maybe not the resistance against the antivirals, but aggravation of the resistance of bacteria to antibiotics that is the truly severe consequence**.** As mentioned, the risk for development of resistance against the neuraminidase inhibitors is not well evaluated, but there is no risk of resistance if a patient without influenza is treated since the substances are influenza-specific. In contrast to what is seen with bacteria, there is no known normal viral flora that can develop and spread resistance factors. If a certain virus strain becomes resistant, we are no worse off than before the introduction of the neuraminidase inhibitors. If we choose not to treat in order to save the drug for a pandemic, there will be no reason for companies to produce the drug, and no drug available when the crisis appears.

It is not only the risk of resistance to the antiviral agents that might be important, but also that treatment could increase the likelihood of critical mutations within the haemagglutinin, creating new antigenic variants more rapidly than normal. Though such mutations have only been demonstrated in the laboratory setting (Blick et al., 1998; Tai et al., 1998) they still might appear with widespread use of neuraminidase inhibitors. At present, a laboratory diagnosis in each patient is not defendable to prevent appearance of resistance, but influenza viruses must be isolated systematically, and the situation in the society closely monitored. An organization for monitoring resistance and mutations, involving among others the WHO influenza reference laboratories has been created.

2.10. Cost for diagnosis of influenza

The cost for the test KITs at the American market (Table 3) is probably more than what is acceptable by most GPs for a routine examination in European countries, using far less $ per capita for health care than the US (Fedson et al., 1997). In, e.g. Sweden a still higher price is accepted for influenza examinations at the microbiology laboratories, but these examinations are used for the epidemiological influenza surveillance as well. Further, the in-hospital patients from which the samples most frequently are drawn are probably more severely ill than out-patients, and a diagnosis therefore more urgent. In times of epidemics, there is also a shortness of hospital beds. It is important to identify influenza cases so that the patients can be put in the same rooms. For these purposes, a higher cost can be accepted than if the sample should be drawn to decide on whether or not to give a medication thought to be relatively free of side effects to a patient healthy enough to stay at home.

2.11. The importance of influenza isolation

For influenza, the diagnostic methods used for deciding whether to treat or not, can never replace the virus isolation that has to be performed for epidemiological purposes, and for monitoring of antiviral resistance. Samples must systematically and continuously be sent to the regional and central laboratories for these purposes, even if the diagnosis per se often can be rapidly established by a simple point-of-care assay.

3. Treatment of herpes simplex

There is an increasing number of antiviral substances against herpes simplex (Sasadeusz and Sacks, 1993). The nucleoside analogue aciclovir for treatment of herpes simplex (HSV) has been in use for 20 years. Side effects are few, and the risk for development of resistance limited at treatment of immune-competent persons, while it is frequently seen in severely immune-suppressed patients after long term use. The bioavailability of

aciclovir is low, and high doses needed at oral administration. Valaciclovir and famciclovir are better resorbed, and allow for efficient oral use. However, the cost of these preparations is higher than for aciclovir, and despite lower doses, the price for equal efficiency is not lower in all countries. If the nucleoside analogues fail, foscavir is the main alternative. This drug can only be given parenterally, and has severe side effects.

Treatment of HSV is depends upon the kind of disease caused in the individual patient. The agreement that herpes simplex infections of CNS, eye and generalized disease in newborn or atopic children should be treated is total. Severe, chronic herpes in, e.g. AIDS patients is probably also always treated in the Western world, despite the risk of resistance. In the long run, there will probably also be a consensus that severe, primary oral or genital herpes should be treated, provided that the doctor sees the patient early enough. Policies for treatment of recurrences, HSV in pregnancy and the use long-term prophylactics against recurrent genital HSV certainly may vary between and within countries, and probably also the likelihood that a laboratory diagnosis is demanded before onset. Below, various aspects on diagnosis of HSV are discussed in relation to Table 1.

3.1. Accuracy of the clinical diagnosis of HSV

The general concept is probably that HSV lesions are easy to diagnose clinically, but most systematic studies prove the opposite. Typical cases of HSV skin lesions, often seen at the primary infection, can mostly be diagnosed accurately, but VZV might frequently be misdiagnosed as HSV (Rubben et al., 1997), and there are atypical presentations. Herpetic gingivostomatitis in children can often be misdiagnosed, and regarded, e.g. as teething difficulty (King et al., 1992). Genital herpes is often atypical, or not recognized at all (Cowan et al., 1996; Morse et al., 1997; Behets et al., 1999). Encephalitis, meningitis, visceral or generalized herpes and neonatal herpes are often atypical in presentation (Schlesinger et al., 1995; Linde et al., 1997; Malm and Forsgren, 1999), and laboratory confirmation necessary. So are manifestations among immune-compromised individuals. Probably a plethora of neurology symptoms might be ascribed to HSV2 recurrences. These are difficult to recognize clinically and to diagnose accurately even by PCR on cerebrospinal fluid (CSF).

3.2. Diagnostic methods, their reliability and test evaluations for HSV

In principle, the methods that can be used to identify HSV are the same as for influenza (Cohen, 1994). The choice of method varies depending on disease. Virus isolation is regarded the gold standard for diagnosis of herpes in blisters, and especially during the first days of infection it is probably a very accurate method. Antigen detection in lesion material, either by ELISA or IFA usually has a sensitivity of about 80–90% in comparison to cultivation, but results from different studies are highly variable. The specificity of antigen detection in relation to virus isolation is often similar, and some of this lack of specificity is instead lack of sensitivity of the standard cultivation. Like for influenza, genome amplification techniques have turned out to be most sensitive (Rubben et al., 1997; Slomka et al., 1998; Coyle et al., 1999; Fang et al., 1999; Madhavan et al., 1999; Espy et al., 2000), and are on their way replace virus isolation as reference method. For CSF, it has offered completely new diagnostic possibilities (Lakeman and Whitley, 1995; Klapper and Cleator, 1997; Linde et al., 1997; Mitchell et al., 1997), and the technology is developing rapidly (Hofgartner et al., 1999). Quantitative DNA assays for monitoring of therapy will probably be used in patients with suspected therapy failure in the future. DNA detection has also become increasingly important for ocular disease (Kowalski et al., 1993; Knox et al., 1998). With improved methodology allowing assays for specific DNA to be performed within less than 1 h, they may replace both antigen detection and virus isolation at many laboratories for all kinds of herpetic lesions. There is a fear that the high amount of HSV in, e.g. blister material will increase the risk of contamination, but with care contamination problems can probably be fully

abolished. Assays for DNA will certainly improve the accuracy of the laboratory diagnosis and thereby the proneness of the clinician to send specimens.

Antibody detection is seldom useful as guidance for treatment since antibodies appear too late after the primary infection, and titre changes are difficult to detect after recurrences. However, some authors strongly encourage the use of serological assays for detection of HSV2 antibodies in patients, in order to identify those with genital herpes (Ashley, 1998; Ashley and Wald, 1999). However, the accuracy of the assays (Field et al., 1993) and consequences and measures if the patient is seropositive have to be well defined before general screening is introduced, and the increasing commonness of genital HSV1 may lessen the value of screening for HSV2 antibodies for identification of genital HSV.

The demand for quality monitoring and control is as high for HSV as for influenza, and the details will not be repeated here. Within the framework of European Union Concerted Actions, quality assurance panels for HSV nucleic acid detection has been sent to 68 laboratories in 1999. Most laboratories perform acceptable, but for 31% the results were poor or mediocre, and in 5% contamination of negative specimen were detected (see www.qcca.org.uk).

3.3. Availability of laboratory diagnosis for HSV

Diagnostic methods for HSV are normally confined to the well-equipped microbiology laboratory. Since the cultivation of virus is relatively easily performed, laboratories that have any type of cell culture usually perform HSV isolation. HSV genome detection is also routine at most large microbiology laboratories. Antigen detection, mainly by in situ methods in preparations from blister cells but also with ELISA is the most rapid method for diagnosis, but still the handling of the samples delays the result. A response on the same day as the sample arrives is normally the best that can be obtained. Availability of antigen detection varies, to some extent due to the tradition of the laboratory. Those laboratories that have tradition of antigen detection by in situ methods will use these extensively for many agents, but others find them to laborious and subjective.

There are diagnostic devices for point-of-care use. There is also one for HSV2 antibody detection. Easy-to-use KITs for demonstration of antigens have started to appear. However, the non-epidemic character of HSV with only sporadic cases by most GPs may make them less prone to store HSV KITs for infrequent use than KITs for influenza. Specialists, such as dermatovenereologists and ophthalmologists, who might also prescribe treatment to out-patients. If they are not in a hospital setting with easy access to laboratory facilities these would probably gain from having diagnostic facilities of their own.

3.4. Urgency of an HSV diagnosis

Herpes simplex encephalitis and neonatal herpes infections are life threatening, and immediate onset of therapy is demanded at a likely diagnosis. Herpes of the eye and visceral manifestation in immune-compromised patients should also be treated as soon as possible. Since aciclovir has few side effects, the normal procedure is to start therapy, and stop it after 1–2 days, if the diagnosis can not be verified. Diagnostic approaches for verification of herpes encephalitis have been reviewed (Linde et al., 1997). Genome amplification on CSF is the method of choice for a rapid diagnosis today, and few virological methods have been so thoroughly verified to be optimal for their purpose. Samples to be sent to the laboratory should be drawn as soon a possible, but genomes detectable by amplification techniques do not disappear immediately with treatment. If pre-treatment samples have not been obtained, later sampling for verification of diagnosis is worth-while since genomes can be detected in CSF for up to weeks after onset of treatment (Aurelius et al., 1991). For many other manifestations of herpes simplex, the reason to obtain a rapid diagnosis is the same as for influenza- if therapy is not started rapidly it has no effect.

3.5. Severity, side effects, duration, cost and risk of resistance for antiviral drugs against HSV

Only the very urgent manifestations of herpes have consequences severe enough for the doctor to feel obliged to verify the cause of disease. Herpetic ulcers are painful and contagious, but not life threatening. This probably can induce certain neglect in the diagnostic work-up, and the very limited side effects and risk of resistance of the treatment in otherwise healthy individuals (Laufer and Starr, 1995) may further encourage a less demanding attitude towards a confirmed diagnosis. However, the cost of treatment, especially if long-term treatment is planned, demands a firm diagnosis before onset of therapy. An isolate before therapy also will help in monitoring of resistance, and should definitely be encouraged in immune-compromised patients. Ideally, in any suspected recurrence occurring during therapy and prophylactics, virus should be examined for resistance. However, monitoring of resistance on an individual basis is probably not a feasible strategy for long-term prophylactics for genital herpes, but projects to keep control of the situation in different settings should be encouraged.

A completely neglected reason to be careful with the use of nucleoside analogues is that there is a replicating normal flora of herpesviruses such as cytomegalovirus, Epstein–Barr virus, human herpesvirus 6 and 7 and possibly varicella-zoster virus in seropositive individuals. These herpesviruses are all more or less sensitive to ACV. Like bacteria of the normal flora, they may develop resistance with the sub-optimal treatment given when the more sensitive HSV is treated. To see severe consequences from this may seem farfetched, but it is an addition to other factors indicating strict indications and a firm diagnosis before onset of long term therapy of HSV ulcers.

3.6. Ease by which the HSV specimen can be obtained

Blisters are easily accessible, and the main problem in specimen collection is to get representative material from the blisters, especially in preparations made for antigen detection in situ. Instructions for specimen collection should be available from the laboratory, as well as transport medium for virus isolation. Since HSV is relatively stable, transport is not a major problem. In encephalitis and meningitis, CSF is the material of choice, and since it is normally drawn in meningitis or encephalitis of any severity it is not a major problem to obtain. With suspicion of neonatal disease, a variety of samples, including serum for PCR are of value (Malm and Forsgren, 1999), and none of them is complicated to draw. For keratoconjunctivitis and retinal necrosis, PCR on swab material from the conjunctival sac and on vitreous fluid, respectively, will probably be the method of choice. Problems concerning specimen collection might occur in suspected visceral manifestations in immune-compromised patients, where lavages and biopsies may be necessary. How to routinely demonstrate HSV in various neurological conditions such as facial palsy and various neuralgic conditions probably due to HSV is still not firmly settled.

3.7. Cost for HSV diagnosis

The cost for HSV diagnosis is usually somewhat lower than for influenza, since most methodologies are simpler to perform. The systems for virus isolation are more stable, and many cell-lines can be used. Preparations made by the clinician and not at the laboratory are used for in situ antigen detection, and genome amplification does not demand reverse transcription. Still, the price is much higher than for most bacterial cultivation, and the price for point of care devices will probably be similar as for influenza. At least for short term treatment there might be a resistance to use laboratory verification due to the cost.

3.8. The importance of HSV isolation

A laboratory verification of a herpes diagnosis is always desirable before onset of treatment, but especially in severe disease and before long-term prophylactics. Like for influenza, virus isolation must be retained despite the availability of simpler methods, to allow for monitoring of resistance and strain changes.

References

Anonymous., 1999. Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR, Report 48, 1–28.

Aoki, F.Y., 1998. Amantadine and Rimantadine. In: Nicholson, K.G., Hay, R.G. (Eds.), Textbook of Influenza. Balckwell Science, Oxford, pp. 457–475.

Ashley, R.L., 1998. Genital herpes. Type-specific antibodies for diagnosis and management. Dermatol. Clin. 16, 789–793.

Ashley, R.L., Wald, A., 1999. Genital herpes: review of the epidemic and potential use of type-specific serology. Clin. Microbiol. Rev. 12, 1–8.

Aurelius, E., Johansson, B., Sköldenberg, B., Staland, Å., Forsgren, M., 1991. Rapid diagnosis of herpes simplex encephalitis by nested polymerase chain reaction assay of cerebrospinal fluid. Lancet 337, 189–192.

Behets, F.M., Brathwaite, A.R., Hylton-Kong, T., Chen, C.Y., Hoffman, I., Weiss, J.B., Morse, S.A., Dallabetta, G., Cohen, M.S., Figueroa, J.P., 1999. Genital ulcers: etiology, clinical diagnosis, and associated human immunodeficiency virus infection in Kingston, Jamaica. Clin. Infect. Dis. 28, 1086–1090.

Blick, T.J., Sahasrabudhe, A., McDonald, M., Owens, J.J., Morley, P.J., Fenton, R.J., McKimm-Breschkin, J.L., 1998. The interaction of neuraminidase and hemagglutinin mutations in influenza virus in resistance to 4-guanidino-Neu5Ac2en. Virology 246, 95–103.

Carpenter, C.C., Cooper, D.A., Fischl, M.A., Gatell, J.M., Gazzard, B.G., Hammer, S.M., Hirsch, M.S., Jacobsen, D.M., Katzenstein, D.A., Montaner, J.S., Richman, D.D., Saag, M.S., Schechter, M., Schooley, R.T., Thompson, M.A., Vella, S., Yeni, P.G., Volberding, A., 2000. Antiretroviral therapy in adults: updated recommendations of the International AIDS Society-USA Panel. J. Am. Med. Assoc. 283, 381–390.

Carrat, F., Tachet, A., Housset, B., Valleron, A.J., Rouzioux, C., 1997. Influenza and influenza-like illness in general practice: drawing lessons for surveillance from a pilot study in Paris, France. Br. J. Gen. Pract. 47, 217–220.

Cherian, T., Bobo, L., Steinhoff, M.C., Karron, R.A., Yolken, R.H., 1994. Use of PCR-enzyme immunoassay for identification of influenza A virus matrix RNA in clinical samples negative for cultivable virus. J. Clin. Microbiol. 32, 623–628.

Cohen, P.R., 1994. Tests for detecting herpes simplex virus and varicella-zoster virus infections. Dermatol. Clin. 12, 51–68.

Covalciuc, K.A., Webb, K.H., Carlson, C.A., 1999. Comparison of four clinical specimen types for detection of influenza A and B viruses by optical immunoassay (FLU OIA test) and cell culture methods. J. Clin. Microbiol. 37, 3971–3974.

Cowan, F.M., Johnson, A.M., Ashley, R., Corey, L., Mindel, A., 1996. Relationship between antibodies to herpes simplex virus (HSV) and symptoms of HSV infection. J. Infect. Dis. 174, 470–475.

Coyle, P.V., Desai, A., Wyatt, D., McCaughey, C., O'Neill, H.J., 1999. A comparison of virus isolation, indirect immunofluorescence and nested multiplex polymerase chain reaction for the diagnosis of primary and recurrent herpes simplex type 1 and type 2 infections. J. Virol. Methods 83, 75–82.

Ellis, J.S., Fleming, D.M., Zambon, M.C., 1997. Multiplex reverse transcription-PCR for surveillance of influenza A and B viruses in England and Wales in 1995 and 1996. J. Clin. Microbiol. 35, 2076–2082.

Espy, M.J., Uhl, J.R., Mitchell, P.S., Thorvilson, J.N., Svien, K.A., Wold, A.D., Smith, T.F., 2000. Diagnosis of herpes simplex virus infections in the clinical laboratory by LightCycler PCR. J. Clin. Microbiol. 38, 795–799.

Fang, X.F., Song, B., Tu, Y.Y., Tong, J.Z., Faul, J.L., Bai, H., 1999. Rapid detection of glycoprotein G gene for the diagnosis and typing of herpes simplex virus infection in genital herpes. Sex. Transm. Infect. 75, 396–397.

Fedson, D.S., Hannoun, C., Leese, J., Sprenger, M.J., Hampson, A.W., Bro-Jorgensen, K., Ahlbom, A.M., Nokelby, H., Valle, M., Olafsson, O., et al., 1995. Influenza vaccination in 18 developed countries, 1980–1992. Vaccine 13, 623–627.

Fedson, D.S., Hirota, Y., Shin, H.K., Cambillard, P.E., Kiely, J., Ambrosch, F., Hannoun, C., Leese, J., Sprenger, M.J., Hampson, A.W., Bro-Jorgensen, K., Ahlbom, A.M., Nokleby, H., Valle, M., Olafsson, O., Salmeron, F., Cloetta, J., Rebelo de Andrade, H., Snacken, R., Donatelli, I., Jennings, L.C., Strikas, R.A., 1997. Influenza vaccination in 22 developed countries: an update to 1995. Vaccine 15, 1506–1511.

Field, P.R., Ho, D.W., Irving, W.L., Isaacs, D., Cunningham, A.L., 1993. The reliability of serological tests for the diagnosis of genital herpes: a critique. Pathology 25, 175–179.

Freund, B., Gravenstein, S., Elliott, M., Miller, I., 1999. Zanamivir: a review of clinical safety. Drug Saf. 21, 267–281.

Govaert, T.M., Dinant, G.J., Aretz, K., Knottnerus, J.A., 1998. The predictive value of influenza symptomatology in elderly people. Family Practice 15, 16–22.

Gross, P.A., Hermogenes, A.W., Sacks, H.S., Lau, J., Levandowski, R.A., 1995. The efficacy of influenza vaccine in elderly persons. A meta-analysis and review of the literature. Ann. Intern. Med. 123, 518–527.

Gubareva, L.V., Kaiser, L., Hayden, F.G., 2000. Influenza virus neuraminidase inhibitors. Lancet 355, 827–835.

Harmon, M.W., 1999. Influenza virus. In: Lennette, E.H., Smith, T.F. (Eds.), Laboratory Diagnosis of Viral Infections, 3rd. Marcel Dekker, New York, pp. 587–602.

Hayden, F.G., Osterhaus, A.D., Treanor, J.J., Fleming, D.M., Aoki, F.Y., Nicholson, K.G., Bohnen, A.M., Hirst, H.M., Keene, O., Wightman, K., 1997. Efficacy and safety of the neuraminidase inhibitor zanamivir in the treatment of influenzavirus infections. GG167 Influenza Study Group. New Engl. J. Med. 337, 874–880.

Hirsch, M.S., Bruin-Vezinet, F., D'Aquila, R.T., Hammer, S.M., Johnson, V.A., Kuritzkes, D.R., Loveday, C., Mellors, J.W., Clotet, B., Conway, B., Demeter, L.M., Vella, S., Jacobsen, D.M., Richman, D.D., 2000. Antiretroviral drug resistance testing in adult HIV-1 infection: recommendations of an International AIDS Society-USA Panel. J. Am. Med. Assoc. 283, 2417–2426.

Hofgartner, W.T., Huhmer, A.F., Landers, J.P., Kant, J.A., 1999. Rapid diagnosis of herpes simplex encephalitis using microchip electrophoresis of PCR products. Clin. Chem. 45, 2120–2128.

Kaiser, L., Briones, M.S., Hayden, F.G., 1999. Performance of virus isolation and Directigen® Flu A to detect influenza A virus in experimental human infection. J. Clin. Virol. 14, 191–197.

King, D.L., Steinhauer, W., Garcia-Godoy, F., Elkins, C.J., 1992. Herpetic gingivostomatitis and teething difficulty in infants. Pediatr. Dentist. 14, 82–85.

Klapper, P.E., Cleator, G.M., 1997. Herpes simplex virus. Intervirol. 40, 62–71.

Knox, C.M., Chandler, D., Short, G.A., Margolis, T.P., 1998. Polymerase chain reaction-based assays of vitreous samples for the diagnosis of viral retinitis. Use in diagnostic dilemmas. Ophthalmologie 105, 37–44.

Kowalski, R.P., Gordon, Y.J., Romanowski, E.G., Araullo-Cruz, T., Kinchington, P.R., 1993. A comparison of enzyme immunoassay and polymerase chain reaction with the clinical examination for diagnosing ocular herpetic disease. Ophthalmologie 100, 530–533.

Lakeman, F.D., Whitley, R.J., 1995. Diagnosis of herpes simplex encephalitis: application of polymerase chain reaction to cerebrospinal fluid from brain-biopsied patients and correlation with disease. National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group. J. Infect. Dis. 171, 857–863 see comments.

Laufer, D.S., Starr, S.E., 1995. Resistance to antivirals. Pediatr. Clin. North Am. 42, 583–599.

Linde, A., Klapper, P.E., Monteyne, P., Echevarria, J.M., Cinque, P., Rozenberg, F., Vestergaard, B.F., Ciardi, M., Lebon, P., Cleator, G.M., 1997. Specific diagnostic methods for herpesvirus infections of the central nervous system: a consensus review by the European Union Concerted Action on Virus Meningitis and Encephalitis. Clin. Diagn. Virol. 8, 83–104.

Loeb, M., McGeer, A., McArthur, M., Peeling, R.W., Petric, M., Simor, A.E., 2000. Surveillance for outbreaks of respiratory tract infections in nursing homes. Can. Med. Assoc. J. 162, 1133–1137.

Madhavan, H.N., Priya, K., Anand, A.R., Therese, K.L., 1999. Detection of herpes simplex virus (HSV) genome using polymerase chain reaction (PCR) in clinical samples comparison of PCR with standard laboratory methods for the detection of HSV. J. Clin. Virol. 14, 145–151.

Magnard, C., Valette, M., Aymard, M., Lina, B., 1999. Comparison of two nested PCR, cell culture, and antigen detection for the diagnosis of upper respiratory tract infections due to influenza viruses. J. Med. Virol. 59, 215–220.

Makela, M.J., Pauksens, K., Rostila, T., Fleming, D.M., Man, C.Y., Keene, O.N., Webester, A., 2000. Clinical efficacy and safety if the orally inhaled neuraminidase inhibitor zanamivir in the treatment of Influenza: a randomized double-blind, Placebo-contolled European study. J. Infect. 40, 42–48.

Malm, G., Forsgren, M., 1999. Neonatal herpes simplex virus infections: HSV DNA in cerebrospinal fluid and serum. Arch. Dis. Childh. Fetal and Neonat. Edit. 81, F24–29.

Marcante, R., Chiumento, F., Palu, G., Cavedon, G., 1996. Rapid diagnosis of influenza type A infection: comparison of shell-vial culture, directigen flu-A and enzyme-linked immunosorbent assay. New Microbiol. 19, 141–147.

Mauskopf, J.A., Cates, S.C., Griffin, A.D., Neighbors, D.M., Lamb, S.C., Rutherford, C., 1999. Cost effectiveness of zanamivir for the treatment of influenza in a high risk population in Australia. Pharmacoeconomics 17, 611–620.

Mitchell, P.S., Espy, M.J., Smith, T.F., Toal, D.R., Rys, P.N., Berbari, E.F., Osmon, D.R., Persing, D.H., 1997. Laboratory diagnosis of central nervous system infections with herpes simplex virus by PCR performed with cerebrospinal fluid specimens. J. Clin. Microbiol. 35, 2873–2877.

Morse, S.A., Trees, D.L., Htun, Y., Radebe, F., Orle, K.A., Dangor, Y., Beck-Sague, C.M., Schmid, S., Fehler, G., Weiss, J.B., Ballard, R.C., 1997. Comparison of clinical diagnosis and standard laboratory and molecular methods for the diagnosis of genital ulcer disease in Lesotho: association with human immunodeficiency virus infection. J. Infect. Dis. 175, 583–589.

Nichol, K.L., Margolis, K.L., Wuorenma, J., Von Sternberg, T., 1994. The efficacy and cost effectiveness of vaccination against influenza among elderly persons living in the community. New Engl. J. Med. 331, 778–784.

Nicholson, K.G., 1998. Human Influenza. In: Nicholson, K.G., Webster, R.G., Hay, A.J. (Eds.), Textbook of Influenza. Blackwell Science, Oxford, pp. 219–265.

Nicholson, K.G., Nguyen-Van-Tam, J.S., Ahmed, A.H., Wiselka, M.J., Leese, J., Ayres, J., Campbell, J.H., Ebden, P., Eiser, N.M., Hutchcroft, B.J., Pearson, J.C., Willey, R.F., Wolstenholme, R.J., Woodhead, M.A., 1998. Randomised placebo-controlled crossover trial on effect of inactivated influenza vaccine on pulmonary function in asthma. Lancet 351, 326–331.

Nicholson, K.G., Aoki, F.Y., Osterhaus, A., Trottier, S., Carewicz, O., Mercier, C.H., Rode, A., Kinnersley, N., Ward, P., 2000. Efficacy and safety of oseltamivir in treatment of acute influenza: a randomised controlled trial. Lancet 355, 1845–1850.

Noyola, D.E., Demmler, G.J., 2000. Effect of rapid diagnosis on management of influenza A infections. Pediatr. Infect. Dis. J. 19, 303–307.

Noyola, D.E., Paredes, A.J., Clark, B., Demmler, G.J., 2000. Evaluation of a neuraminidase detection assay for the rapid detection of influenza A and B virus in children. Pediatr. Dev. Pathol. 3, 162–167.

Pregliasco, F., Mensi, C., Camorali, L., Anselmi, G., 1998. Comparison of RT-PCR with other diagnostic assays for

rapid detection of influenza viruses. J. Med. Virol. 56, 168–173.

Read, C.A., Mohsen, A., Nguyen-Van-Tam, J.S., McKendrick, M., Kudesia, G., 2000. Outbreaks of influenza A in nursing homes in Sheffield during the 1997-1998 season: implications for diagnosis and control. J. Public Health Med. 22, 116–120.

Reichert, T.A. (2000) The seasonality of human mortality: the role of influenza. Abstract, Options for the Control of Influenza IV, W21, Crete, Sept. 2000.

Reid, M.C., Lachs, M.S., Feinstein, A.R., 1995. Use of methodological standards in diagnostic test research. Getting better but still not good. J Am. Med. Assoc. 274, 645–651.

Rubben, A., Baron, J.M., Grussendorf-Conen, E.I., 1997. Routine detection of herpes simplex virus and varicella-zoster virus by polymerase chain reaction reveals that initial herpes zoster is frequently misdiagnosed as herpes simplex. Br. J. Dermatol. 137, 259–261.

Sasadeusz, J.J., Sacks, S.L., 1993. Systemic antivirals in herpesvirus infections. Dermatol. Clin. 11, 171–185.

Schlesinger, Y., Buller, R.S., Brunstrom, J.E., Moran, C.J., Storch, G.A., 1995. Expanded spectrum of herpes simplex encephalitis in childhood. J. Pediatr. 126, 234–241.

Serwint, J.R., Miller, R.M., 1993. Why diagnose influenza infections in hospitalized pediatric patients? Pediatr. Infect. Dis. J. 12, 200–204 see comments.

Slomka, M.J., Emery, L., Munday, P.E., Moulsdale, M., Brown, D.W., 1998. A comparison of PCR with virus isolation and direct antigen detection for diagnosis and typing of genital herpes. J. Med. Virol. 55, 177–183.

Staynor, K., Foster, G., McArthur, M., McGeer, A., Petric, M., Simor, A.E., 1994. Influenza A outbreak in a nursing home: the value of early diagnosis and the use of amantadine hydrochloride. Can. J. Infect. Contr. 9, 109–111 see comments.

Tai, C.Y., Escarpe, P.A., Sidwell, R.W., Williams, M.A., Lew, W., Wu, H., Mendel, D.B., 1998. Characterization of human influenza virus variants selected in vitro in the presence of the neuraminidase inhibitor GS 4071. Antimicrob. Agents Chemother. 42, 3234–3241.

Taylor, J.L., Dwyer, D.M., Coffman, T., Groves, C., Patel, J., Israel, E., 1992. Nursing home outbreak of influenza A (H3N2): evaluation of vaccine efficacy and influenza case definitions. Infect. Contr. Hosp. Epidemiol. 13, 93–97.

Todd, S.J., Minnich, L., Waner, J.L., 1995. Comparison of rapid immunofluorescence procedure with TestPack RSV and Directigen FLU-A for diagnosis of respiratory syncytial virus and influenza A virus. J. Clin. Microbiol. 33, 1650–1651.