Use of a Single Monoclonal Antibody To Determine the Susceptibilities of Herpes Simplex Virus Type 1 and Type 2 Clinical Isolates to Acyclovir

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This report describes a flow cytometry drug susceptibility assay that uses a single fluorochrome-labeled monoclonal antibody to determine the acyclovir susceptibilities of herpes simplex virus (HSV) type 1 or type 2 clinical isolates. This assay yields 50% effective doses (drug concentrations that reduce the number of antigen-positive cells by 50%) for HSV clinical isolates that are equivalent to those obtained with the plaque reduction assay.

Herpes simplex virus (HSV) infections are ubiquitous, with approximately 80% of the adult population infected with HSV type 1 and approximately 20% of the adult population also infected with HSV type 2 (1, 22, 25). Current therapy for primary and recurrent HSV infections involves the use of acyclovir or one of its more bioavailable prodrugs, valacyclovir or famciclovir (2). Long-term use of these antiviral drugs in HSV-infected neonates and immunocompromised patients can lead to the selection of viral mutants that are resistant to these drugs (7, 8, 17). Less than 1% of the clinical isolates obtained from immunocompetent patients treated with acyclovir are resistant to acyclovir (3). However, 5 to 10% of the clinical isolates obtained from immunocompromised patients subjected to long-term treatment or multiple treatments with acyclovir are resistant to the drug due to mutations in the thymidine kinase (TK) gene and/or DNA polymerase genes (5, 7). Patients with acyclovir-resistant HSV clinical isolates caused by mutations in the TK gene, but not those infected with viruses with mutations in the DNA polymerase gene, can be successfully treated with the HSV DNA polymerase inhibitors foscarnet and cidofovir (9, 10).

There are no universally accepted methods for determining the drug susceptibilities of HSV clinical isolates. The most accurate assay for HSV is the plaque reduction assay (PRA) (19–21). The National Committee for Clinical Laboratory Standards (NCCLS) has established a standardized drug susceptibility assay for HSV based on the PRA, but it has not been validated and is seldom used because it is time-consuming, expensive to perform, and subjective. Other drug susceptibility assays are faster than the PRA, and some of the endpoints can be read automatically, but these assays are less sensitive than the PRA (6, 12, 23, 24). With the increased use of acyclovir and its derivatives among HSV-infected neonates and immunocompromised patients leading to the increased selection of drug-resistant HSV clinical isolates, there is a urgent need for a standardized drug susceptibility assay for HSV clinical isolates.

HSV-specific fluorochrome-labeled monoclonal antibodies and flow cytometry have been used to detect and quantify HSV-infected cells and to perform drug susceptibility testing of HSV clinical isolates (13, 18). These studies used a high multiplicity of infection and monitored the effect of antiviral drugs on HSV replication by measuring the effects of drugs on the synthesis of late antigens. In this report, we show that a single monoclonal antibody to an HSV antigen that is shared by both HSV type 1 and HSV type 2 and flow cytometry can be

| HSV clinical isolate | HSV type | Phenotype | EC50 (μM) | Reagent 5090 | MAb to late antigens |
|----------------------|----------|-----------|-----------|--------------|---------------------|
| SC16 (4)             | 1 TK     |           |           |              |                     |
| BW-S (21)            | 1 TK     |           | 1.17      | 1.53         |                     |
| BW-R (21)            | 1 TK     |           | 0.71      | 0.89         |                     |
| DM2.1 (4)            | 1 TK     |           | 59.01     | 58.24        |                     |
| SC16-S1 (4)          | 1 TK     |           | 92.66     | 82.65        |                     |
| KOST (11)            | 2 TK     |           | 43.10     | 47.79        |                     |
|                      |          |           | 20.61     | 19.15        |                     |

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a Each isolate was analyzed at least three times. Data from one representative experiment are presented.

b The original publications describing well-characterized HSV clinical isolates are shown.

c The phenotypes of these well-characterized HSV clinical isolates were determined using standard procedures at GlaxoSmithKline. TK<sup>def</sup>, defective TK; TK<sup>del</sup>, deletion of TK; TK<sup>alt</sup>, altered TK.

d EC<sub>50</sub> of HSV clinical isolates for acyclovir.

e Reagent 5090 is a monoclonal antibody that is directed against an unidentified HSV-specific antigen that is expressed in HSV type 1- and HSV type 2-infected cells.

f Monoclonal antibodies (MAb) from the HSV 1 or 2 Typing direct fluorescent antibody kit were used to detect HSV-infected cells expressing either the HSV type 1 or HSV type 2 late antigen.
used to determine the drug susceptibilities of HSV type 1 and type 2 clinical isolates to acyclovir.

The flow cytometry drug susceptibility assay is essentially that described previously for human cytomegalovirus (14–16). Briefly, confluent BSC-1 cell monolayers were infected with HSV clinical isolates at a multiplicity of infection of 0.001 in the presence of various concentrations of acyclovir. After overnight incubation, the cells were harvested, permeabilized, and treated with the appropriate fluorochrome-labeled monoclonal antibody to HSV antigens, and the number of antigen-positive cells was determined by flow cytometry. The EC50s (the drug concentration that reduces the number of antigen-positive cells by 50%) were determined by plotting the percent reduction in the number of antigen-positive cells versus the drug concentration using SlideWrite Plus software. Reagent 5090 is a fluorochrome-labeled monoclonal antibody that detects an unknown HSV-specific antigen expressed in cells infected with either HSV type 1 or HSV type 2. The HSV 1 Typing Reagent contains two fluorescein-labeled monoclonal antibodies to HSV type 1 late antigens, glycoprotein C and ICP35. The HSV 2 Typing Reagent contains two fluorescein-labeled monoclonal antibodies that react with HSV type 2-specific glycoproteins of 78 to 82 and 110 to 120 kDa. All monoclonal antibodies were obtained from Chemicon International, Temecula, Calif. The PRA followed standard procedures (20, 21).

Previous studies have demonstrated that fluorochrome-labeled monoclonal antibodies to a type-specific HSV late antigen and flow cytometry can be used for drug susceptibility assays of HSV type 1 (18). We tested the ability of reagent 5095 and flow cytometry to determine the drug susceptibilities of HSV type 1 and HSV type 2 clinical isolates. Six phenotypically and genotypically characterized HSV clinical isolates (4, 11, 21) were tested by the drug susceptibility assay using either reagent 5095 or a type-specific monoclonal antibody for HSV type 1 or HSV type 2 late antigens. The data are presented in Table 1. Using any of these monoclonal antibodies, the assay correctly identified the drug-susceptible and -resistant HSV isolates. The EC50s for the drug-susceptible clinical isolates for acyclovir were very similar. The EC50s for the acyclovir-resistant isolates were 10 to 100 times greater than the EC50s for the acyclovir-susceptible isolates.

The most accurate method for determining drug susceptibilities of HSV clinical isolates is the PRA. To determine how well the flow cytometry drug susceptibility assay compares with the PRA, the EC50s of a large number of drug-susceptible and -resistant HSV clinical isolates were determined by these assays. The results are presented in Table 2. The average EC50s for acyclovir for 42 drug-susceptible HSV clinical isolates by all three assays were similar. The average EC50s for acyclovir for 25 drug-resistant HSV clinical isolates by all three assays were similar and approximately 50 times higher than the EC50s of the drug-susceptible isolates.

The data presented show that reagent 5095 in conjunction with flow cytometry can be used to determine the drug susceptibilities of acyclovir-susceptible and -resistant HSV type 1 and type 2 clinical isolates. The assay can be completed in less than 24 h, is easy to perform, is not subjective, and yields EC50s that are equivalent to values obtained with the more-labor-intensive PRA. The rapid quantitative nature of the flow cytometry drug susceptibility assay makes it more useful than the PRA for the appropriate treatment of patients. If a cell line that metabolizes the prodrugs valacyclovir and famciclovir to acyclovir is used, the assay should also be useful for determining the drug susceptibilities of these drugs for HSV clinical isolates. This assay could replace the currently used assays for determining the drug susceptibilities of HSV clinical isolates.

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| Samples              | No. of samples | Avg EC50 (µM) for acyclovir (range) by:                     |
|----------------------|----------------|-------------------------------------------------------------|
| Acyclovir susceptible | 42             | 0.73 ± 0.37 (0.34–1.61)                                      |
| Acyclovir resistant   | 25             | 38.71 ± 21.27 (8.02–92.66)                                   |

* a FACS, fluorescence-activated cell sorting.
* b Reagent 5095 detects cells infected with HSV type 1 and HSV type 2.
* c The HSV 1 or 2 Typing direct fluorescent antibody kit reagents were used to detect HSV-infected cells expressing either HSV type 1 or HSV type 2 late antigens.

### Table 2. Comparison of flow cytometric drug susceptibility assay (fluorescence-activated cell sorting) and PRA

| Samples              | No. of samples | Avg EC50 (µM) for acyclovir (range) by:                     |
|----------------------|----------------|-------------------------------------------------------------|
| FACS* assay with reagent 5090b | FACS assay using HSV 1 or 2 Typing Kit for late antigens | PRA |
| Acyclovir susceptible | 42             | 0.82 ± 0.45 (0.34–1.85)                                      | 1.11 ± 0.62 (0.21–1.48) |
| Acyclovir resistant   | 25             | 44.71 ± 20.97 (14.82–99.0)                                   | 60.78 ± 47.49 (32.17–102.34) |
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