



Simple, Fast and Reproducible Phenotypic Resistance Determination for Herpes Simplex Virus by Flow Cytometry

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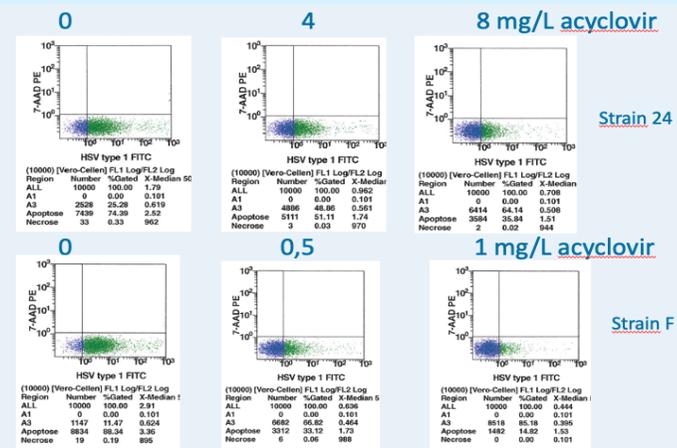
Introduction

Flow cytometry has been used for phenotypic resistance determination of HSV¹. The flow cytometry has evolved from a highly specialized technique to standard equipment in many laboratories. The goal of this study was to develop a simple, fast and reproducible method for resistance determination by flow cytometry.

Methods

- 25 previously characterized viral isolates (14 patients UL23, 30 sequence analysis, plaque reduction assay (PRA) and DNA reduction assay (DRA)².
- Different multiplicities of infection (MOI) were tested with a susceptible and resistant isolate.
- Reproducibility tests were performed by two different technicians.
- Different fresh isolates (4) were used to determine throughput time in clinical practice.
- Procedure:
- Ad virus with either acyclovir or foscarnet 2-fold dilution series, to Vero cells 6-well plate
- After 16-24 hrs remove cells incubate with HSV-1 glycoprotein (Diagnostic Hybrids; HSV-1 DFA reagent) (no permeabilization)
- Measure fluorescence (Beckman Coulter FC500-MCL) at 488nm wave length. Percentage of gated infected (fluorescent) cells were used.
- Determine susceptibility to antiviral drugs using the "trend" and "linest" functions in excel. The IC50 is the concentration of drug (micromolar) that reduced infected cells by 50%.

Example of results from the flow cytometer



Results

- Full agreement was obtained between the different methods.
- The results were similar in a wide range of MOI
- Reproducibility was high
- After a fresh isolate has been grown adequately antiviral resistance results are available within 16-24 hrs.

Conclusion

Resistance determination for Herpes simplex with flow cytometry is fast and reproducible if culture facilities are available.

Example of reproducibility

Strain F	AVG (IC50) mg/L	STDEV
1	0.29	0.1
2	0.35	0.07
3	0.35	0.14
Strain 24		
1	7.05	-1.11
2	7.70	-1.65
3	6.06	-1.66

References

1. Pavic I, et al. Flow Cytometric Analysis of Herpes Simplex Virus Type 1 Susceptibility to Acyclovir, Ganciclovir, and Foscarnet. AAC 1997; 41(12): 2686-92
2. Van der Beek et al. Rapid susceptibility testing for herpes simplex type 1 using real-time PCR. JCV 2013; 56: 19-24

