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Clinical Virology Laboratory Newsletter

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Herpes simplex and varicella zoster: Direct PCR to replace DFA for lesions

For over 20 years, direct immunofluorescence antibody (DFA) staining has been used at YNH for rapid diagnosis of herpes simplex virus (HSV) and varicella zoster virus (VZV) for lesion swab samples. Compared to the time delay (1-14 days) for culture results, DFA can provide a result within 2 hours of sample receipt in the Virology Laboratory. Although PCR is more sensitive and has been available in Virology for many years, DFA has retained utility due to the faster time to result and lower costs. It has been done continuously during operating hours, whereas PCR is performed once a day.

Limitations of DFA: DFA is manual, labor intensive, and requires substantial expertise. Cells from the patient sample are centrifuged onto microscope slides, fixed, then stained with monoclonal antibodies, and finally examined under a fluorescence microscope. The protein target is not amplified and sufficient cells must be examined for a valid result. If the sample submitted for DFA has insufficient cells, it is reported as "Inadequate".

DFA and inadequate cells: Recently, the proportion of samples for DFA testing with inadequate cells has increased to over 50%. These samples are designated inadequate and no DFA result is reported. However, these samples can be PCR-positive.

"Direct" PCR: PCR can be simplified and made more economical by direct testing of samples, without first extracting the nucleic acids. Impact on clinical sensitivity for this sample type is minimal due to the relatively high virus titers in swab samples. In a recent comparison, 120 samples were tested by DFA and direct PCR in our lab. While HSV direct PCR detected 38 positives, DFA detected only 21 positives (55%). Thirteen of the 17 discrepant samples had inadequate cells for a valid DFA result. Of 5 samples positive by VZV direct PCR, DFA detected 4 (80%).

HSV/VZV DFA discontinued: Thus, HSV and VZV DFA will be discontinued on July 1st, 2015. Instead, "HSV 1 & 2 Direct PCR, swab" and "VZV Direct PCR, swab" will be offered for rapid diagnosis. HSV culture will also still be available if needed.

Table: Tests for HSV and VZV detection at YNH Virology Laboratory

Virus	Test	Sample types	Test schedule
HSV	HSV-1 & HSV-2 Direct PCR, swab	Swab samples only	Once a day, afternoon
	HSV PCR*	CSF, BAL, amniotic fluid, plasma, tissue	Once a day, morning
	HSV culture	All samples except CSF and blood	Daily
VZV	VZV Direct PCR, swab	Swab samples only	Once a day, afternoon
	VZV PCR*	CSF, BAL, amniotic fluid, plasma, tissue	Once a day, morning

*Routine PCR uses nucleic acids that have been extracted, purified and concentrated from the clinical samples

Note: VZV culture is not sensitive and is generally not recommended.

Test information:

Test name: Order HSV 1 & 2 Direct PCR, swab or VZV Direct PCR, swab as needed.

Collection/ transport medium: Unroof vesicles or crusts, absorb vesicular fluid onto the swab if present, and swab the base of the lesions. Place swab in viral transport medium.

Performed: HSV and VZV Direct PCRs for swab samples will be performed once a day in the afternoon.

Results: Results will be reported by 5 PM. Positives by HSV direct PCR will be reported as HSV-1 or HSV-2.