Objective

Varicella Zoster virus (VZV) may cause severe disseminated infections in immunocompromised children and adults and is a common cause of hospitalization among these patients population (1, 2). Individuals with deficiencies in cell-mediated immunity, such as those with AIDS or hematologic malignancies, are especially vulnerable to severe and fatal disease (3, 4, 5). Because the distribution and character of lesions in these patients are often atypical, rapid and sensitive detection of VZV from clinical specimens is important for a prompt identification of patients for treatment with antiviral therapy and to institute appropriate isolation procedure.

VZV infection is usually diagnosed using specific fluorescent antibody conjugates in a direct immunofluorescence assay (DFA) (6, 7), rapid shell vial culture (SV) system (8, 9) and real-time PCR assay. Proper collection of vesicle lesions specimens is important for rapid detection of VZV, especially for DFA testing. The objective of this study was to evaluate the performance of flocked swabs for collection of lesions specimens for the detection of VZV by DFA and SV culture.

Samples and method

197 clinical specimens were evaluated for this study. All vesicle specimens were collected with flocked swabs (FLOQSwabs™, Copan Italia, Brescia Italy) and placed into 3 ml UTM transport medium (Copan). All swabs for virus isolation were received and processed by the laboratory within 4 h from collection.

Vesicle specimens were first vortexed, the swabs removed, a sample aliquot of 500 ul was stored for PCR, 200 ul was inoculated into 2 HELF SV and the remaining volume was centrifuged to pellet the cells.

Cell pellets were resuspended with 50ul of PBS and 10ul were added to 3 wells on a glass slides. Smears were immediately fixed in cold (-20°C) methanol/acetone 2/1 for 5 min at room temperature, air dried, stained with 25 ul of Millipore (Temecula, CA USA) V2 Light Diagnostics™ DFA monodonal antibody containing an Evans blue counter stain for 30 min at 37°C and read with a UV microscope at a magnification of 40X. Specimens exhibiting at least 10 intact epithelial cells were considered adequate for cellular content, and a typical apple-green fluorescence in the nucleus of one or more infected epithelial cells in the entire smear was considered positive for VZV.

Two HELF SV cultures were inoculated and centrifuged 45 min at 2000 rpm to improve the efficacy of virus isolation, incubated for 48h at 37°C 5% CO2, stained with the same procedure and read at 10X magnification. Single or foci of infected fibroblast show a nuclear brilliant green fluorescence.

Discordant results were confirmed by PCR: 200ul sample were extracted with the Nuclisens easy MAG™ system (BioMérieux, Lior, France) and tested with an in-house real time RT-PCR assay using Ag-Path-ID one –step RT-PCR kit (Applied Biosystem, Foster City, CA, USA) and reported primer and probe sequences. The test run included the amplification of a DNA standards consisting of linearized plasmids obtained by cloning reference target sequences using the TA cloning kit (Invitrogen, Carlsbad, CA).

Results

With the present study, we summarize 5 years of our laboratory experience in utilizing both DFA and viral isolation techniques for the rapid detection of VZV from skin lesions. From January 2006 to December 2010, a total of 179 lesions samples were tested for VZV DFA and SV rapid isolation (tab.1). DFA detected 95 (53.07%) positive, 83 (46.5%) negative and one (0.5%) indeterminate, while SV culture detected 73 (40.78%) positive, 97 (54.18%) negative and 9 (5.2%) indeterminate samples (tab.2). The 5 DFA negative and cultures indeterminate samples were confirmed negative by PCR. DFA stained smears contained a high number of epithelial cells, which were well stained, easy to read and allowed the detection of 22 more positive samples compared to culture. Overall, DFA provide a rapid (turnaround time less than 1 h), simple, and practical method for early diagnosis of VZV.

Conclusions

Copan FLOCSwabs improved the DFA positivity rate for the detection of VZV compared to shell vial culture. Although, cells were not counted for each specimen, we found that the smear of lesions specimens, collected with flocked swabs and transported in UTM, were easier to read because a high number of cells were available for examination and the morphological structure was well preserved.

Direct DFA procedure is easy to perform and well suited for use in the routine diagnostic laboratory. UTM transport viral transport medium preserves cells and can be used for PCR confirmation.

References: