Leukocyte Concentration in the Performance of the pp65 Antigenemia Assay

One of the more controversial and debatable issues presently confronting clinical virologists and infectious disease specialists is the application of the cytomegalovirus antigenemia (CMV-Ag) assay (pp65 antigenemia assay) as a marker and predictor of CMV disease. For example, recent reports have shown the CMV-Ag assay to be more accurate than conventional DNA PCR or the more recent nucleic acid sequence-based amplification (NASBA) as a predictor of CMV in patients with AIDS (2). In a second comparison study, the CMV-Ag assay was reported to be less accurate than the Digene Capture CM DNA System (Digene), a nongenome amplification assay, as a predictor of CMV disease in a similar (AIDS) patient population (13).

As recently pointed out by several researchers (13, 14), differences in the purported efficacy of the CMV-Ag assay might reflect such factors as antiviral management (prophylaxis against CMV versus no prophylaxis), duration of patient observation (e.g., 8 versus 12 months), the number of CD4+ T-lymphocyte counts at patient entry into a given study, the monitoring of CMV disease other than retinitis, and/or the laboratory protocol used in the performance of the CMV-Ag assay per se. Specifically, and perhaps most technically important, data ascertained from CMV-Ag assay evaluation testing might be skewed in relation to the input polymorphonuclear leukocyte (PMNL) concentration utilized in the preparation of the cytospot.

Wattanamano and colleagues (13) compared three assays (the CMV-Ag assay [Digene] and the AMPLICOR Qualitative PCR Test [Roche] as markers for the early detection of CMV infection in AIDS patients. Both the Digene and Roche assays were performed as described in the manufacturers’ specifications. However, the CMV-Ag assay was modified from that of earlier protocols (1, 4), wherein single cytospot preparations, each containing 150,000 polymorphonuclear leukocytes (PMNLs), were tested. In the Wattanamano et al. study, the pp65 antigenemia assay was found to be slightly less sensitive than the Digene assay (80% versus 85%), with both tests displaying equal specificities. Both assays displayed equal predictive values.

In the performance of evaluation-comparison testing, researchers must attempt to effect optimal assay parameters. Currently, most workers prepare and test cytospot preparations containing a minimum of 200,000 PMNLs, which is recommended in two FDA-approved CMV-Ag assay kits (12). The reporting of mean data obtained from duplicate cytospot slide preparations also addresses any subtle or unrecognized technical errors in cytospot preparation and/or immunostaining. Some workers, furthermore, routinely utilize cytospot preparations incorporating leukocyte input concentrations of 300,000 or 400,000 cells (3, 5, 6, 10). Lipson et al. (9) reported improved sensitivity and increased pp65 positive cell counts by utilizing an input population of 400,000 PMNLs/duplicate cytospot preparation. Most recently, Landry and Ferguson (7) addressed the relevance of different leukocyte inocula concentrations in an evaluation-comparison study incorporating a new 2-h quantitative CMV-Ag assay (7). The significance of increased PMNL inoculum concentrations in the detection of the CMV viremia in immunocompromised patients has been described earlier in detail by shell vial assay technology (8, 11).

In the evaluation-comparison study, failure to optimize one or another diagnostic test has the potential of misdirecting the novice or even confusing the experienced microbiologist. The readership should appreciate the importance of the leukocyte input concentration in the cytospot preparation as an important factor which may significantly impact interpretation of the CMV-Ag assay in evaluation-comparison testing, as well as in this assay’s accuracy in clinical diagnostics.

REFERENCES


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