

Comparison of EDTA and Acid-Citrate-Dextrose Collection Tubes for Detection of Cytomegalovirus Antigenemia and Infectivity in Leukocytes before and after Storage

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Received 8 July 1996/Returned for modification 18 September 1996/Accepted 18 October 1996

Duplicate blood samples collected in EDTA and acid-citrate-dextrose (ACD) were compared by cytomegalovirus (CMV) pp65 antigenemia and CMV infectivity on the day of sample collection and after 1 and 2 days of storage at 4°C. No significant difference was detected between EDTA and ACD. However, CMV antigenemia was more sensitive than culture at all time points tested.

The cytomegalovirus (CMV) pp65 antigenemia test measures viral load in peripheral blood leukocytes, and numerous studies have documented the association of high antigenemia levels with clinical disease (3, 4, 9). The test can be completed within 4 to 5 h of sample receipt and uses techniques available in routine clinical laboratories. For accurate quantitation, samples must be processed within 6 h of blood collection. Longer delays are associated with a variable decrease in antigenemia levels (1, 5, 7). If a delay is anticipated, storage at 4°C is preferable to room temperature (5).

Early studies showed that acid-citrate-dextrose (ACD) was superior to EDTA and heparin for preservation of neutrophil function in banked blood (6). However, all published studies of CMV antigenemia have used blood collected in either EDTA or heparin (1–5, 7–9). The purpose of the present study was to compare the anticoagulant ACD with EDTA for the preservation of both CMV pp65 antigenemia and CMV infectivity in blood samples before and after storage at 4°C.

Sixty-two blood samples from AIDS patients with suspected CMV disease were collected in paired EDTA and ACD tubes and submitted to the Clinical Virology Laboratory for testing. Aliquots of whole blood from both ACD and EDTA tubes were processed within 6 h of collection for both antigenemia and virus isolation. Tubes were then stored at 4°C (5). If the initial antigenemia assay was positive, additional aliquots were processed and tested 1 and 2 days after collection.

The CMV antigenemia assay was performed as previously described (4, 5). Leukocytes were separated in 6% dextran, and 1.5×10^5 cells were applied to each of two slides by using a cytospin. After fixation in 5% formaldehyde–2% sucrose and permeabilization in 0.5% Nonidet P-40, leukocytes were stained with anti-CMV pp65 (C10/C11) monoclonal antibodies (Clonab CMV, Biotest Diagnostics, Denville, N.J.) and fluorescein isothiocyanate-labeled sheep anti-mouse immunoglobulin G conjugate (Organon-Teknika-Cappel, Malvern, Pa.). Results were expressed as the sum of positive cells on two slides. For conventional culture, leukocytes were inoculated into two MRC-5 roller tubes and observed for 3 weeks (4).

For statistical analysis, McNemar's (r1) test was used for qualitative comparisons between pairs. Continuous paired variables were compared by using the Wilcoxon signed-rank

(r2) test. The ratios of sample to baseline were used to measure stability between pairs over the timepoints.

Of 62 blood samples tested on the day of collection (day 0), 32 were positive for CMV. Since the study was not done on weekends, only 27 of these were retested one day after collection (day 1) and 19 were retested by antigenemia and 17 by culture on day 2. As shown in Table 1, the number of samples positive by antigenemia or culture in EDTA and ACD were not significantly different. However, antigenemia was more sensitive than culture at each time point in both anticoagulants.

Only samples with low-level antigenemia (median, 1 positive cell) were culture negative on initial processing. However, two samples with intermediate-level antigenemia (median, 20 positive cells) were culture negative after 1 day of storage in both anticoagulants and three samples with high-level antigenemia (median, 341 positive cells) were culture negative after 2 days of storage. In contrast, samples that became antigenemia negative with delayed processing all had low-level antigenemia on initial testing (median, 1 positive cell).

A comparison of the number and stability of positive polymorphonuclear leukocytes (PMNL) detected in each anticoagulant indicated that, while CMV antigenemia levels tended to be higher in ACD than in EDTA, the differences were not significant (Tables 2 and 3). A progressive decline in the mean number of positive PMNL observed in 19 samples from 17 patients tested at all three time points was largely due to two samples with over 1,000 positive cells (Table 3).

The CMV antigenemia assay has many advantages for clinical laboratories, such as utilizing routinely available methods and equipment, minimal space requirements, low reagent costs, high sensitivity, ease of quantitation, and a 4- to 5-h total assay time. However, the number of CMV antigenemia-positive neutrophils in both heparinized and EDTA blood has been found to decline after storage of whole blood for 24 (1, 7) to 48 (5) h at either room temperature or 4°C.

In the present study, ACD was compared with EDTA for preservation of CMV antigenemia and infectivity since citrate has been found to be a better preservative of neutrophil function (6). While the number of positive leukocytes detected per sample was higher for ACD, the total number of CMV antigenemia-positive samples detected was slightly higher for EDTA, and the differences were not significant for the number of samples tested.

Most importantly, in both ACD and EDTA, CMV antigenemia was significantly more sensitive than culture at all time

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TABLE 1. Comparison of two anticoagulants for qualitative detection of CMV in blood samples over a 2-day period

Day after collection and test method	No. of patients ^a	No. (%) of samples positive in:	
		EDTA ^b	ACD ^c
0			
Antigenemia	32	29 (91)	27 (84)
Culture	32	17 (53)	15 (47)
1			
Antigenemia	27	22 (82)	21 (78)
Culture	27	9 (33)	11 (41)
2			
Antigenemia	19	17 (90)	15 (79)
Culture	17	4 (24)	4 (24)

^a Includes patients positive by either method or anticoagulant on initial testing.

^b Antigenemia was more sensitive than culture at days 0 ($P = 0.003$), 1 ($P < 0.001$), and 2 ($P = 0.004$) (McNemar's test).

^c Antigenemia was more sensitive than culture at days 0 ($P < 0.001$), 1 ($P = 0.004$), and 2 ($P = 0.008$) (McNemar's test).

points tested. These results have been recently confirmed in a study conducted by a reference laboratory that found antigenemia significantly more sensitive than both shell vial and conventional cultures in specimens 3 to 75 h old (2). In our study, negative cultures were seen on the day of collection only in samples with low-level antigenemia, but with delayed processing, several samples with intermediate- and even high-level antigenemia after 1 or 2 days of storage were culture negative. In contrast, samples that became antigenemia negative after storage had very low level antigenemia on initial testing and were less likely to be of clinical significance.

Nevertheless, when comparing serial CMV antigenemia levels to assess changes in viral load or response to treatment, specimens are best processed without delay to avoid an artifactual decrease. In addition, consistent trends (increasing or decreasing levels) over several samples are more reliable than a single test result. Fortunately, the rapid turnaround time of

TABLE 2. Comparison of EDTA and ACD for quantitative CMV antigenemia

Day after collection	No. of samples ^a	No. (%) of CMV antigenemia-positive PMNL detected ^b :		
		EDTA>ACD	ACD>EDTA	EDTA=ACD
0	32	12 (37.5)	16 (50)	4 (12.5)
1	27	7 (26)	12 (44)	8 (30)
2	19	8 (42)	8 (42)	3 (16)

^a Includes samples positive by either method or anticoagulant on initial testing.

^b There were no significant differences between EDTA and ACD at any time point (Wilcoxon signed-rank test).

TABLE 3. Number and stability of CMV antigenemia-positive PMNL detected in EDTA and ACD collection tubes^a

Anticoagulant	No. of CMV antigenemia-positive PMNL:					
	Day 0		Day 1		Day 2	
	Me-dian	Mean (range)	Me-dian	Mean (range)	Me-dian	Mean (range)
EDTA	8	204 (1–1,228)	5	176 (0–818)	10	128 (0–593)
ACD	5	264 (0–1,619)	9	223 (0–1,145)	10	173 (0–908)

^a Data are from 19 patient samples tested at all three time points. There was no significant difference between anticoagulants (Wilcoxon signed-rank test).

CMV antigenemia facilitates repeat testing with another sample or staining of a third slide if needed.

A previous report found heparin and EDTA collection tubes to be equivalent for detection of CMV in leukocytes by shell vial, antigenemia, and PCR (8). The present study has shown that ACD collection tubes are also acceptable for CMV antigenemia and culture. Serial samples should be collected in the same anticoagulant to minimize variability. Most importantly, while concerns about stability of antigenemia with delayed processing are valid, antigenemia remains more sensitive than infectivity even after storage.

We thank the staff of the Nathan Smith Clinic for collecting the samples and Karol Katz for performing the statistical analyses.

This work was supported in part by the Adult AIDS Clinical Trial Group (UOI-AI-32766).

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