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Comparison of Blood Collected in Acid-Citrate-Dextrose and EDTA for Use in Human Immunodeficiency Virus Peripheral Blood Mononuclear Cell Cultures

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Paired blood samples collected in acid-citrate-dextrose and EDTA were compared for human immunodeficiency virus (HIV) infectivity on the day of collection or after 1 day of storage at room temperature. No significant differences between the anticoagulants were observed. Culture positivity was significantly associated with HIV RNA viral loads for both anticoagulants.

EDTA is widely recognized as the best anticoagulant for plasma specimens used for human immunodeficiency virus (HIV) RNA determinations (3, 6, 8, 10), while heparin and acid-citrate-dextrose (ACD) are usually used for obtaining cells to be used in HIV culture (2, 4, 7, 11). Drawing two separate tubes of blood for these two assays is an inconvenience and, especially in the case of children, may be limited by a small total blood volume. In an attempt to find one anticoagulant that could be used for both assays, we performed a series of HIV cultures with samples from consecutive patients enrolled in a variety of clinical trials who were having both ACD and EDTA tubes of blood drawn for protocol-specified HIV RNA and HIV peripheral blood mononuclear cell (PBMC) cultures.

All patients gave informed consent for the studies, which were approved by the Institutional Review Board for the University of North Carolina at Chapel Hill. A total of 105 paired specimens were obtained. The first 50 paired specimens were obtained from 17 individuals and cultured within 4 h of blood collection (cohort 1). Concerns over the possible fragility of cells stored overnight in EDTA prompted a second round of testing. As a result, 55 paired specimens from 43 individuals were cultured after an overnight, room-temperature incubation simulating shipping conditions (cohort 2). Plasma HIV RNA from blood collected in EDTA was performed by the Amplicor monitor assay (Roche Diagnostic Systems, Branchburg, N.J.), following the manufacturer's instructions and including Division of AIDS Virus Quality Assurance Laboratory standards. Qualitative HIV PBMC cultures were set up from cells isolated either from the ACD or the EDTA blood by established techniques (11). A culture was deemed positive by guidelines established by the AIDS Clinical Trials Group. Basically, a culture is considered positive if one of the following three conditions is met: (i) two consecutive HIV p24 antigen values greater than 30 pg/ml and the second value is at least four times greater than the first value or has an optical density of >2.0, (ii) two consecutive HIV p24 antigen values with optical densities of >2.0, or (iii) three consecutive increasing HIV p24 antigen values greater than 30 pg/ml, where neither consecutive value is four times greater than the previous sample but the third value is four times greater than the first (11). The laboratory was certified as proficient for these assays by the Virus Quality Assurance Laboratory.

The first day that a culture had at least 30 pg of p24 antigen per ml in the culture supernatant was recorded as the day it became positive. For statistical purposes, negative cultures were arbitrarily set at 25 days. Specimens with concentrations of <400 HIV RNA copies/ml were assigned a value of 400 copies/ml in the analysis. The McNemar's test was used to test the differences between the two anticoagulants' abilities to produce a positive culture. The *t* test was used to test the difference in mean number of days before a culture became positive. Pearson's correlation coefficient was used to evaluate the relationship between HIV RNA concentration and the number of days required to obtain a positive culture.

First, we determined whether there was any difference between the two anticoagulants and their ability to culture HIV from PBMCs. Overall concordance in culture sensitivity was 88% (92 of 105 pairs were concordant). Seventy-two pairs were positive in both cultures, and 20 were negative in both cultures. Of the 13 discordant pairs, seven ACD-collected PBMC cultures were positive and six EDTA-collected PBMC cultures were positive. Overall, 75% of the cultures were positive, and it took about 6.7 days for the p24 antigen concentration to reach 30 pg/ml for those cultures which actually became positive (Table 1). Negative cultures were not used in the analysis of days to positive culture.

We next investigated differences between the anticoagulants for when cells are cultured soon after collection compared with waiting 20 to 24 h, which frequently occurs when blood is shipped to a central laboratory for culture. We saw no difference either in sensitivity or in the number of days it took for a culture to turn positive (Table 1). Surprisingly, however, only 62 to 66% of the fresh cultures (cohort 1) were positive, compared with 82 to 87% of the specimens stored overnight (cohort 2), and it took an average of 8.6 to 8.9 days for the fresh specimens to first turn positive, compared with 5.0 to 5.6 days for the overnight specimens.

The answer to this apparent paradox was found in the plasma HIV RNA values of the specimens and the way in which the study was conducted. The specimens for cohort 1 were largely obtained from subjects already enrolled in clinical trials while the other 55 subjects were primarily selected from patients being screened or at the baseline for other clinical trials. The average viral load of the first 50 patients was 17,784

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TABLE 1. Comparison of ACD and EDTA as anticoagulants for blood used in PBMC cultures

Group[a]	Characteristic	Result obtained with:	
		ACD	EDTA
Cohort 1 (n = 50)	Avg time between blood draw and culture	4 h	4 h
	Sensitivity [no. positive (%)]	31 (62)	33 (66)
	Days to positive culture (range)	8.6 (2–23)	8.9 (4–22)
	HIV-1 RNAcopies/ml		
	Mean	ND[b]	17,784
	Median	ND	1,739
Cohort 2 (n = 55)	Avg time between blood draw and culture	22 h	22 h
	Sensitivity [no. positive (%)]	48 (87)	45 (82)
	Days to positive culture (range)	5.6 (2–24)	5.0 (2–19)
	HIV-1 RNA copies/ml		
	Mean	ND	336,164
	Median	ND	169,946
Total (n = 105)	Sensitivity [no. positive (%)]	79 (75)	78 (74)
	Days to positive culture (range)	6.8 (2–24)	6.7 (2–22)
	HIV-1 RNA copies/ml		
	Mean	ND	184,555
	Median	ND	7,394

[a] The two patient cohorts are not directly comparable since viral loads were, in general, much higher in cohort 2.
[b] ND, not done.

copies/ml (median, 1,739 copies/ml) and ranged from <400 to 291,269 copies/ml compared with an average of 336,164 copies/ml (median, 169,946 copies/ml) for cohort 2 patients (range, <400 to 2,527,778 copies/ml).

We paired specimens from cohort 1 and cohort 2 based on HIV type 1 (HIV-1) RNA levels (within 0.2 log) and reanalyzed the length of time it took for a culture to become positive (Table 2). A total of 27 pairs were compared, with viral loads ranging from <400 to 291,269 copies/ml. Mean viral loads were 23,448 HIV-1 RNA copies/ml for the fresh specimens (cohort 1A) and 24,737 copies/ml for the overnight specimens (cohort 2A). The mean numbers of days for a culture to become positive were 9.1 and 9.6 for ACD and EDTA, respectively, in the fresh cultures. In the case of both anticoagulants, 66.7% of the cultures in this group were positive. For the 27 overnight cultures, 74% of the ACD specimens produced a positive culture in an average of 7.8 days while 66.7% of the EDTA specimens became positive on average by 6.3 days.

We found that the sensitivity of the HIV PBMC cultures was significantly associated with plasma viral loads ($P < 0.0001$). Only 26% (13 of 50) of specimens with plasma HIV RNA concentrations of <400 copies/ml yielded a positive culture, compared with 79% (38 of 48) of specimens with viral loads between 400 and 5,000 HIV RNA copies/ml, 89% (39 of 44) of specimens with viral loads between 5,000 and 100,000 copies/ml, and 99% (67 of 68) of specimens with viral loads greater than 100,000 copies/ml (Fig. 1). There was a weak inverse relationship between RNA concentration and number of days to a positive culture ($r = -0.33$, $P < 0.0001$). Nine of the 13 specimens for which discrepant culture results were obtained had low viral loads that were less than 2,000 copies/ml.

It thus appears that EDTA can be used as an anticoagulant when setting up HIV PBMC cultures. Other investigators have compared EDTA with heparin in HIV whole-blood cultures, but results have been mixed. Some reported higher isolation rates with EDTA (1), some obtained better results with heparin (12), while others found no difference between the two anticoagulants (5). The cultures performed in this study em-

TABLE 2. Specimens from cohort 1 were paired with specimens from cohort 2 based on viral load (within 0.2 log), and effects of the anticoagulants were reanalyzed

Group	Characteristic	Result obtained with:	
		ACD	EDTA
Cohort 1A (n = 27)	Avg time between blood draw and culture	4 h	4 h
	Sensitivity [no. positive (%)]	18 (66.7)	18 (66.7)
	Days to positive culture	9.1	9.6
	HIV-1 RNA copies/ml		
	Mean	ND[a]	23,448
	Median	ND	10,937
Cohort 2A (n = 27)	Avg time between blood draw and culture	22 h	22 h
	Sensitivity [no. positive (%)]	20 (74.1)	18 (66.7)
	Days to positive culture	7.8	6.3
	HIV-1 RNA copies/ml		
	Mean	ND	24,737
	Median	ND	11,807

[a] ND, not done.

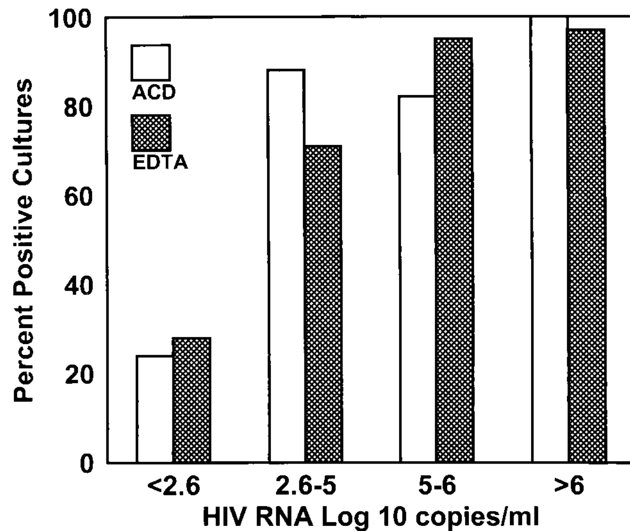

FIG. 1. Sensitivity of HIV PBMC cultures according to $\log_{10}$ HIV RNA plasma viral loads with ACD or EDTA as the anticoagulant.

ployed PBMCs isolated on Ficoll-Hypaque gradients and washed extensively prior to culture, which would remove most, if not all, of the anticoagulant. In our study, we found no significant differences between the two anticoagulants as measured by the sensitivity of the culture assays (ability to culture HIV from the specimen) or in the number of days it took a culture to become positive. Similarly, in a comparison of ACD- and EDTA-collected blood samples and cytomegalovirus infectivity and pp65 antigenemia assays, no significant differences between the two anticoagulants were observed (9).

Not surprisingly, we observed an association in the ability to culture HIV from PBMCs and the plasma viral load. This had been reported by others (M. B. Bouzas, I. Zapiola, J. Waisman, and G. R. Muchinik, Intern. Conf. AIDS, abstr. 42176, 1998). However, it was somewhat surprising to find that 26% of specimens with viral loads of <400 copies/ml had recoverable infectious virus. In a substudy of ACTG 320, approximately 44% of PBMC cultures from patients with viral loads of <500 copies/ml were positive (L. Demeter, M. Hughes, M. Fischl, J. Grimes, R. Bosch, K. Squires, and S. Hammer for the ACTG 320 Study Team, 5th Conf. Retrovir. Opportunistic Infect., abstr. 509, 1998). Using a cutoff of 500 copies/ml in our data set, we find that 41% of patients have recoverable virus.

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