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The question of the effect of anticoagulants on virological assays has been of interest lately, due to the large amounts of blood being collected for clinical trials. The purpose of this review is to document the studies which have evaluated the effects of anticoagulants, specifically ACD and EDTA, on virological assays, and to determine if specimens could be consolidated from a single anticoagulant.

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Several investigators have compared viral RNA recovery in the various anticoagulants (1,3,4). The consensus was that viral loads were higher in EDTA than in ACD-anticoagulated plasma. This was attributed to a dilution effect caused by the liquid formulation of ACD, which resulted in as much as a 14% reduction in viral load. This is especially a concern in pediatric samples where obtaining full blood volumes are not always possible. The consequence is increased variability. The conclusion from these studies is that regardless of the anticoagulant used, all measurements within a study should be done using one type of anticoagulant. Furthermore, if ACD is used, care must be taken to ensure that the blood containers are properly filled.

Specimens often have to be shipped to remote laboratories, which motivated several investigators to look at the effect of anticoagulants and holding time on viral RNA stability (1,3,4,6,7). Dickover et al. (1) evaluated RNA stability in plasma processed 1, 3, 6, 24 and 48 hours post collection. Their data suggested that there was a loss in viral RNA recovery, which peaked during the first six hours post collection. During the first

six hours after collection, they documented a 1.8% loss of RNA per hour for EDTA and 3.3% loss per hour for ACD. The rate of loss declined after six hours, but the process continued. The total loss in viral RNA was 20% and 29% for ACD vs. 10% and 20% for EDTA, 6 and 24 hours after collection, respectively. Holodniy et al. (4) showed similar results with a 13% reduction in viral RNA recovery for EDTA and a 36% reduction for ACD samples processed 30 hours after collection. Moye et al. (7) demonstrated a mean change of 20% between samples processed rapidly (within 6 hours) and 30 hours post collection with ACD anticoagulant. Even though 59% of matched pairs in this data set showed a decrease in RNA recovery, the loss was not statistically significant ($p=0.29$). Finally, Sebire et al. (8) investigated changes in viral loads associated with samples held at room temperature and under refrigerated conditions for up to 72 hours. The samples were either held as whole ACD- treated blood or plasma. A loss in viral load of 5% and 7% respectively was associated with plasma and blood samples held at room temperature for 72 hours ($p= 0.59, 0.70$); while a 34% and 45% reduction was noted after 72 hours in the refrigerator ($p= 0.06, 0.60$).

Studies have also been done to evaluate the effect of freeze/thaw cycles on RNA stability and recovery (3,8). These data suggested that as many as three freeze/thaw cycles had little to no effect on RNA recovery (8). When multiple anticoagulants were evaluated (3), an average loss in RNA of 0.219 $\log_{10}$ was noted for ACD plasma, while a loss of only 0.050 $\log_{10}$ was observed for EDTA plasma after one freeze/thaw cycle. These data were not significant.

Finally, RNA recovery after long-term storage at -70°C was evaluated (3,8). Using Nasba™ (Organon Teknika, Durham, NC) for the evaluation, Ginocchio et al. (3) lysed plasma in the guanidine buffer after 0, 1, 2, 3, 4, and 6 months time points, and then stored the lysates at –70°C. All timepoints were evaluated within the same assay to minimize inter-assay variability. No significant loss of RNA was observed in either ACD or EDTA plasma after 6 months. Sebire et al. (8) looked at RNA stability in samples stored at –70°C using the Roche Monitor™ assay. They noted a significant loss in RNA recovery after 5 months of storage, but then did not note a significant loss after 12 months of storage, when compared to baseline results. This discrepancy was probably due to the inability to control for operator and assay lot variability in this assay.

Though none of the RNA stability papers discussed thus far compared the effect of anticoagulant on the ability to culture HIV, Sebire et al. (8) did compare viral load with the ability to culture HIV from peripheral blood mononuclear cells (PBMCs) from ACD-anticoagulated blood. They demonstrated a direct correlation between viral load and the ability to culture HIV. They achieved a 94% positive culture rate for samples with a viral load of greater than 15,000 copies/mL and only a 75%, 64% and 20% positivity rate for samples with viral loads at 1,000-15,000, 500-1,000, and <500 copies/mL, respectively.

Fiscus et al. (2) evaluated the effect of anticoagulant and processing time on the ability to culture HIV in qualitative macrocultures. They set up cultures from ACD and EDTA-anticoagulated whole blood, which was processed fresh or after an overnight incubation at room temperature. Their data demonstrated that the choice of anticoagulant did not

have an effect on the ability to culture HIV, nor did it affect the length of time for a culture to turn positive. They also noted that the only factor that did affect the ability to culture the virus was viral load. They achieved an 89% positive culture rate for samples with a viral load of greater than 5,000 copies/mL, and a 79% and 41% positivity rate for samples with viral loads at 400-5,000, and <500 copies/mL, respectively. This supported the observations noted by Sebire et al. (8).

Finally, the VQA laboratory evaluated the effects of anticoagulants and processing time on the ability to quantify infectious HIV in a microculture (5). Twenty quantitative microcultures were set up from ACD and EDTA-anticoagulated blood, within six hours of collection and again thirty hours after collection. These data noted that there was neither a significant difference between the baseline titers in EDTA vs. ACD ($p=0.78$), nor a difference between the average loss in titers ($p=0.16$). However, the data did demonstrate a significant loss of IUPM with both anticoagulants when compared to baseline titers, suggesting time is critical for quantifying infectious virus.

Based on these data, it is reasonable to conclude that all virology studies could be done from a single anticoagulant, either EDTA or ACD, with little affect on the result.

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