Poster 212

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Can EDTA samples be used to measure CD4 counts at the weekend?

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Background

There is a push nationally towards Saturday opening for HIV clinics. However, viability of lymphocyte subset EDTA samples is thought to decrease with time[1]. The more expensive Cyto-Chex tube[2] (£5 vs £0.07) extends the 'shelf life' of the samples up to 7 days[3], facilitating venepuncture when laboratories are closed. We aimed to evaluate CD4 stability over time in EDTA tubes compared to Cyto-Chex.

Methods

Patient samples were taken into both (K2)EDTA and Cyto-Chex tubes and CD4 counts were determined on Days 1, 3 and 7. Four groups were compared:

Group 1: EDTA (Day 1) vs. Cyto-Chex (Day 1) [n=13]

Group 2: Cyto-Chex (Day 1) vs. Cyto-Chex (Day 3) [n=11]

Group 3: Cyto-Chex (Day 1) vs. Cyto-Chex (Day 7) [n=6]

Group 4: EDTA (Day 1) vs. EDTA (Day 3) [n=39]

Results

Groups 1, 2 and 3

On Day 1, there was a small but statistically significant reduction in CD4 counts (7%) for samples taken in Cyto-Chex tubes compared to EDTA. Additionally, CD4 count differences between Cyto-Chex and comparative EDTA samples varied considerably between individual samples from -22.5% to +3.2%. Nevertheless, the two sets of data displayed a good correlation (R2= 0.954) (figure 1).

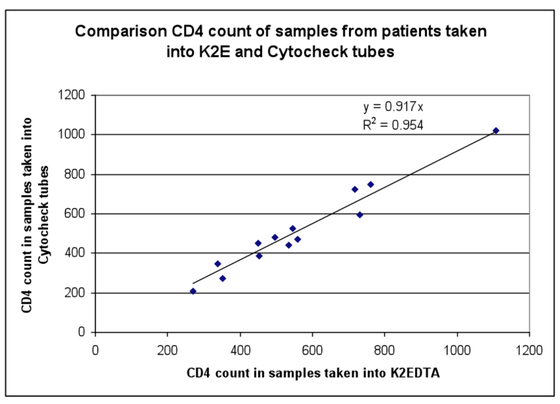


Figure 1

Samples stored in Cyto-Chex tubes remained stable. There was no statistically significant difference between Cyto-Chex CD4 results analysed on Days 1, 3 and 7

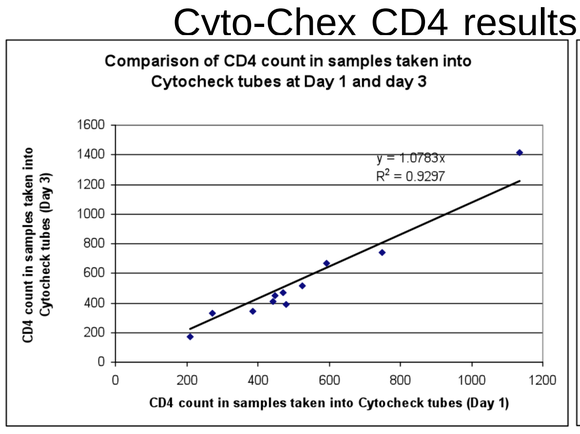


Figure 2

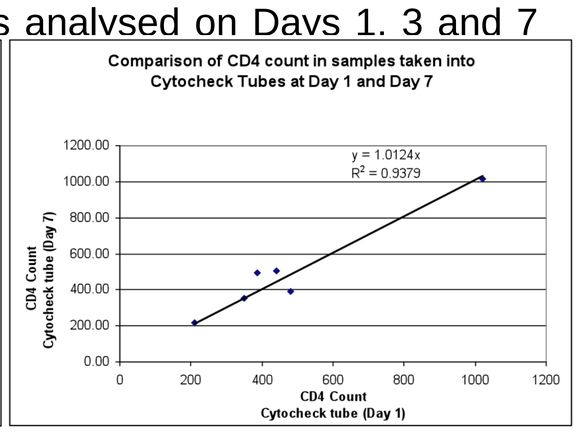


Figure 3

Group 4

For samples stored in EDTA tubes, there was a small but statistically significant reduction in CD4 counts over time. The average count for the test group dropped from 563.31 on Day 1 to 531.74 on Day 3 (5%). Concurrently, CD4 percentage increased slightly from an average of 29.8% to 30.9% (1%). Importantly, there was a good correlation between the Day 1 and Day 3 CD4 counts and CD4%. In addition, Bland and Altman Plots for CD4 counts and CD4% indicated that there is no bias between measurements on Day 1 and Day 3 for samples collected in EDTA tubes (Figures 4-7).

Figure 4

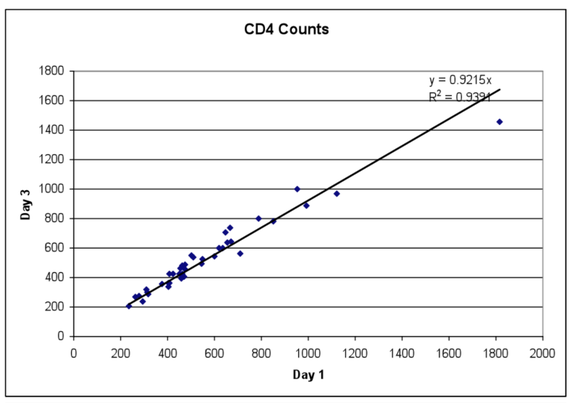
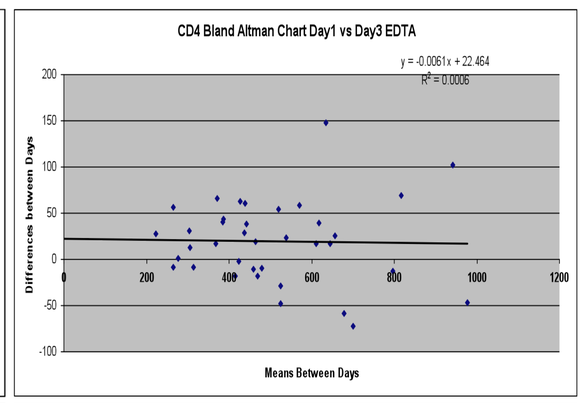


Figure 5



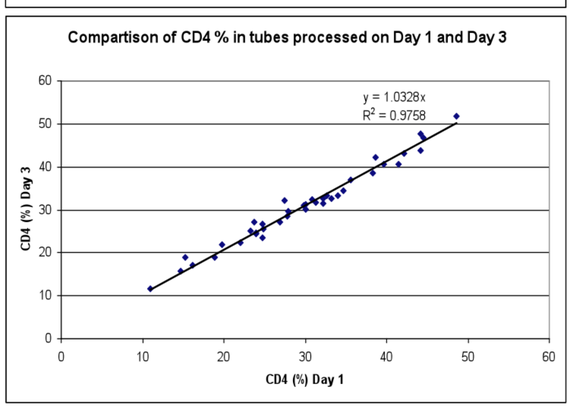


Figure 6

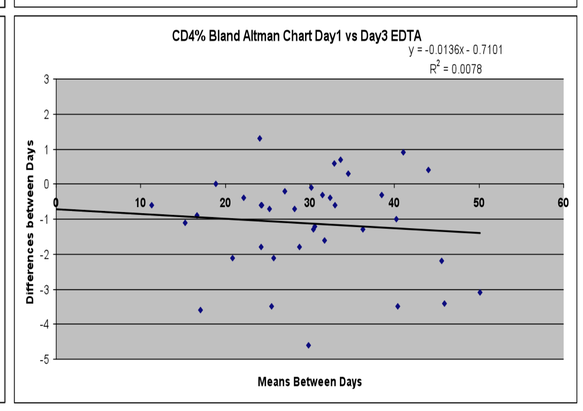


Figure 7

Conclusion

Collection in Cyto-Chex tubes results in an initial significant drop in CD4 counts, which then, however, remain stable for up to 7 days, as found by other studies[3]. Analysis of EDTA samples after 2 days results in a small reduction in CD4 count. This deviation is unlikely to be clinically significant as it is within the natural variability of this assay.

Thus, for a period of 2 days (which covers samples collected on Saturday and tested on Monday), storage of blood samples in EDTA tubes should provide clinically acceptable CD4 counts. Based on these data, there is no advantage in using of Cyto-Chex tubes over this short

References

1. **CDC. 1997 revised Revised Guidelines for Performing CD4+ T-Cell Determinations in Persons Infected with Human Immunodeficiency Virus (HIV**). MMWR 1997 / Vol. 46 / No. RR-2

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1. **Stabilization of white blood cells and immunologic markers for extended analysis using flow cytometry**. Warrino DE, DeGennaro LJ, Hanson M, Swindells S, Pirruccello SJ, Ryan WL. J Immunol Methods. 2005 Oct 30; 305(2):107-19.

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