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## Influence of sample storage time and temperature on lymphocyte subset counts using a FACScount system

**We report the influence of storage and temperature on the count of lymphocyte subsets from HIV positive patients using a FACSCount flow cytometer. The results show that the quality of the counts remains high in the samples first stained and then stored for 24 hours, preferably at 4ºC.**

Sir,

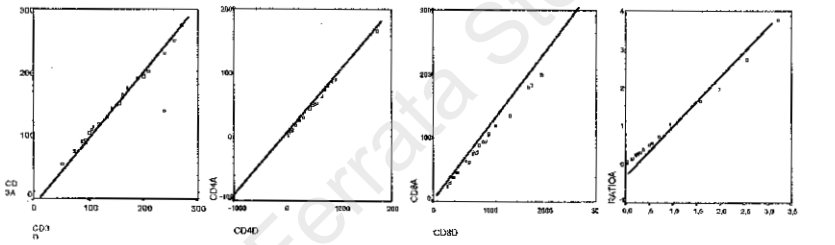
The study of lymphocyte subsets in peripheral blood has become the most commonly used method to follow-up and monitor HIV positive patients.[1,2] We count lymphocyte subsets in approximately 2,500 samples/year from HIV positive patients in our department. Counting is carried out daily by flow cytometry (FC).[3,4] However, these counts may not always be carried out daily, e.g. when samples are delayed or FC devices are unavailable. The present study was aimed to evaluate the quality of service provided, even when analytical procedures are delayed.

The phenotypic characterization of lymphocyte subsets was assessed using a flow cytometer: FACSCount (Becton & Dickinson, San José, CA, USA), an easy technique, as clearly explained by Strauss *et al*.[5]

A total of 43 samples were randomly taken from patients attending the HIV clinic in our hospital. These samples were divided into two groups: in the first one (GROUP 1, #23), samples were stained and analyzed on the same day they were taken, then stored at room temperature for 24 hours. In the second group (GROUP 2, #20), samples were also stained and analyzed on the same day as they were taken and then stored at 4ºC for 24 hours. The analysis was repeated in both groups after the planned delay.

A precision and accuracy control system was designed which analyzed standard deviations (SD) and coefficient of variations (CV). A random blood sample drawn into $K_3$ EDTA from a patient who had been admitted to our hospital was used for the reproducibility and accuracy tests. Statistical analyses were performed applying a Student's t-test for paired samples, with Pearson's coefficient of correlation also being assessed.

Results obtained for both groups were considered as satisfactory, since no statistically significant differences were found in either of them ($p$>0.1). Nevertheless, Group 2 (storage at 4°C) showed better coefficients of correlation, as shown in Figure 1. A few irregularities were detected in the results obtained for Group 1 (storage at room temperature), since slightly higher mean values for CD3 positive cell counts were found after incubation at room temper-



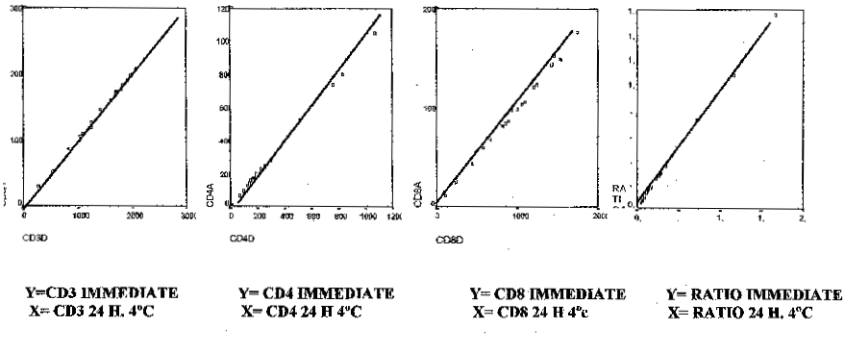




**Figure 1. Regression scatterplots of both groups.**

**Table 1. Comparison of counts of samples analyzed on the same day as they were taken and after 24 hours' storage.**

| | Means | | |
|---|---|---|---|
| | *Inmediate* | *24 hours* | *p* |
| Group 1 | | | |
| CD3 | 1434.78 | 1500.6 | |
| CD4 | 476.34 | 489.0 | |
| CD8 | 888.65 | 893.8 | >0.1 |
| RATIO | 0.803 | 0.775 | |
| Group 2 | | | |
| CD3 | 1338.45 | 1342.9 | |
| CD4 | 284.35 | 291.1 | |
| CD8 | 978.75 | 979.5 | >0.1 |
| RATIO | 0.37 | 0.39 | |

ature, although these differences were not statistically significant ($p$>0.1)(Table 1). According to previously established limits (10% of the mean value in all cases for SD and 8% for CV)[6] good results were obtained for both reproducibility and accuracy control tests.

Previous studies have demonstrated the reliability of the FACSCount cytometer for unstained samples stored for different periods.[5,6] This study, using the same technical procedure, investigates the reliability of tests on samples stored after having been incubated with monoclonal antibodies. We show that samples, stored for 24 hours after preparation, remain stable and, therefore, that FACSCount flow cytometry is reliable, especially when the samples were stored at 4ºC. These results refer only to this specific method and may not be necessarily be extended to other techniques.[7]

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*Flow cytometry, fluorescence gating, HIV monitoring, lymphocyte subsets, monoclonal antibodies.*

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## Acute hepatomegaly with severe liver toxicity due to all-*trans*-retinoic acid

**All *trans*-retinoic acid (ATRA) has improved the outcome of patients with acute promyelocytic leukemia (APL). Despite the fact that ATRA is usually well tolerated, major adverse effects can be observed in a minority of cases. We report here a case of acute life-threatening hepatic toxicity caused by ATRA in a patient with APL.**

Sir,

A 40-year old male was admitted to hospital because of gingival and nasal bleeding. His leukocyte count was $15.3\times10^9$/L (89% blastic forms), hemoglobin level 70 g/L and platelet count $14\times10^9$/L. His serum lactate dehydrogenase was 2,244 U/L. All other biochemical parameters were in normal ranges (NR) and markers for hepatitis virus were negative. A prolonged prothrombin time and hypofibrinogenemia were noted. Microscopic examination of a bone marrow aspirate revealed acute promyelocytic leukemia (APL). Cytogenetic analysis showed the t(15;17)(q22;q21) and PML/RAR$\alpha$ was detected using reverse transcription polymerase chain reaction. The patient began induction treatment consisting of all-trans retinoic acid (ATRA) (45 mg/m$^2$/d) and intravenous idarubicin (12 mg/m$^2$/d on days 2, 4, 6 and 8). He also started prophylaxis against a potential ATRA syndrome with dexamethasone for ten days.

Twenty-one days later, the patient's general condition worsened. Alkaline phosphatase and $\gamma$-glutamyltranspeptidase rose to 370 U/L (normal range, NR: 82-198 U/L) and 198 U/L (NR: 7-43 U/L) respectively, followed by an elevation of direct bilirubin to 39 umol/L (NR:<10 µmol/L). Aminotransferases levels were normal. At that time, a fast-growing painful hepatomegaly was noted without splenomegaly. Abdominal Doppler ultrasound examination did not find intra- or extrahepatic biliary tract injury