## INTRODUCTION

The use of flow cytometric analysis of peripheral whole blood to enumerate lymphocyte subsets is commonly used to assess the immunological status of patients in a wide variety of clinical conditions. The testing to enumerate up to 24 hours at 2-8°C in the dark.

## RESULTS

The use of flow cytometric analysis of peripheral whole blood to enumerate lymphocyte subsets is commonly used to assess the immunological status of patients in a wide variety of clinical conditions. The testing to enumerate up to 24 hours at 2-8°C in the dark.

### MATERIALS AND METHODS

Approximately 30 normal and clinical specimens were included in the data analysis for each study. Among them ≥ 50%, had CD4+ absolute counts lower than 500/μL. Specimens were stored at room temperature for the following times after collection: Fresh (0 h), 24, 48, and 72 hours post-venipuncture. Additionally, samples at each time point were prepared and stored refrigerated up to 48 hours prior to analysis.

The following time points were tested for specimen age and prepared sample:

<table>
<thead>
<tr>
<th>Time</th>
<th>Specimen age</th>
<th>Prepared Sample age</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>24</td>
<td>48</td>
</tr>
</tbody>
</table>

Samples were analyzed using automated Navios tetra Algorithm on Navios™ instrument or Tetra CXP algorithm on Cytometrics FC500™ instrument.

Data were modeled as a linear function of samples, specimen age, and prepared time for each test case. A mixed model was used to analyze the data. Samples constituted the random component of the model while "age" and "prepared" were used as regressor fixed effects. The PROC MIXED routine of SAS was used for data analysis. Drift at different combinations of "age" and "prepared" was calculated based on the slopes of age and prepared (2-variable regression).

Average drift (δ) at a certain time point was calculated as the difference between the response at that time point and the response at time zero (t₀).

\[ \delta = \mu + \beta_1 t + \beta_2 \delta t - \mu - \beta_0 \delta_t + \beta_2 \delta_2 \]

Where \( \mu \) was the intercept, \( \beta_1 \) was the coefficient for specimen age, and \( \beta_2 \) was the coefficient for prepared sample age.

Since \( t_0 = 0 \), and intercepts cancelling out, the resulting equation for calculating drift was:

\[ \delta = \beta_1 t + \beta_2 \delta t \]

Standard error (se) of drift \( \sigma \) was calculated based on the standard errors of the two components (\( \sigma_1 \) and \( \sigma_2 \)) and their covariance Cov(\( \beta_1, \beta_2 \)).

\[ \sigma = \sqrt{\sigma_1^2 + \sigma_2^2 + 2 \text{Cov}(\beta_1, \beta_2)} \]

The upper confidence limit of the drift was calculated based on the standard error of the drift and 95% confidence. Upper limit of the drift represented the worst case scenario of the drift ("95% Upper Limit" in the tables below).

### Table below shows Navios tetra and Tetra CXP results for up to 48 hours of specimen age and prepared sample. It is recommended that samples are prepared with CYTOSTAT tetraCHROME reagents and analyzed with Navios tetra or Tetra CXP from specimens within 24 hours post venipuncture. Prepared samples can be stored to up 24 hours at 2-8°C in the dark.

#### Table 1: Drift analysis approach to investigate the effect of specimen age and prepared sample on the stability of lymphocyte subsets.

<table>
<thead>
<tr>
<th>Specimen Age</th>
<th>Prepared Sample age</th>
<th>Drift</th>
<th>95% Confidence Limits of Drift</th>
<th>95% Confidence Limits of Drift in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>24</td>
<td>1.42</td>
<td>0.36 - 2.48</td>
<td>-10% - 18%</td>
</tr>
<tr>
<td>48</td>
<td>24</td>
<td>2.85</td>
<td>1.28 - 4.42</td>
<td>-31% - 23%</td>
</tr>
</tbody>
</table>

### CONCLUSION

Derived regression models allow to predict the drift at any time points within tested interval of Specimen age and/or Prepared sample stability time. Tables below show prediction of the drift at 36 h for both Navios tetra and Tetra CXP systems.

#### NAVIOS TETRA RESULTS

<table>
<thead>
<tr>
<th>Specimen Age</th>
<th>Prepared Sample age</th>
<th>Drift</th>
<th>95% Confidence Limits of Drift</th>
<th>95% Confidence Limits of Drift in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>24</td>
<td>1.60</td>
<td>-0.74 - 3.94</td>
<td>-32% - 24%</td>
</tr>
</tbody>
</table>

#### CONCLUSION

The derived models provide a robust method for prediction of the drift at different time points within the tested range.

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