

Evaluation of a Dry Monoclonal Antibody Lymphocyte Subset Kit using the XF-1600 Flow Cytometer.

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Background

The development of pre-made monoclonal antibody cocktail dry tubes has allowed manufacturers to offer a variety of panel portfolios for clinical flow cytometry laboratories. The main benefits being workflow efficiency, stability, standardisation, easier logistics and room temperature storage⁽¹⁾.

Exbio, Prague, have recently launched the DryFlowEx TBNK 6-color dry tubes (DFE) for lymphocyte subset analysis. Sysmex Europe SE (SEU) evaluated the use and comparability of the DFE tubes with the KOMBITEST™ TBNK 6-color liquid cocktail (Exbio, Prague) using the XF-1600 flow cytometer TBNK automated volumetric counting software. The XF-1600 is calibrated and metrologically traceable to both the reference Sysmex XN-Series haematology analysers in the SEU quality control laboratory and the WBC international reference method⁽²⁾. To monitor the calibration, it was also decided to include WBC and lymphocyte absolute count analysis between the XN-Series and DFE / XF-1600 combination.

A limited study was performed comparing the following:

- Linear regression, Bland-Altman performance, and stain index (SI) between the predicate flow cytometry XF-1600 / TBNK SEU process and the XF-1600 / DFE tubes on normal patient samples, CD-Chex Plus® and CD-Chex Plus® - CD4 Low.
- Monitoring of WBC and lymphocyte count using the XN-Series and DFE / XF-1600.

Methods

- 40 samples were tested in total, these comprised of the following:
 - 10 x CD-Chex Plus®
 - 10 x CD-Chex Plus® - CD4 Low
 - 20 x normal patient samples
- 2 methodologies were then applied for flow cytometry:
 - The predicate and DFE flow cytometric methods are illustrated below in Table One.

Name	Reagent	Sample	Incubation	Lyse	Incubation	Total Volume
Predicate	20µL	50µL	15 mins	500µL	10 mins	570µL*
DFE	N.A	50µL	15 mins	520µL	10 mins	

Table 1: Illustrating the flow cytometric methods used for preparing the samples.

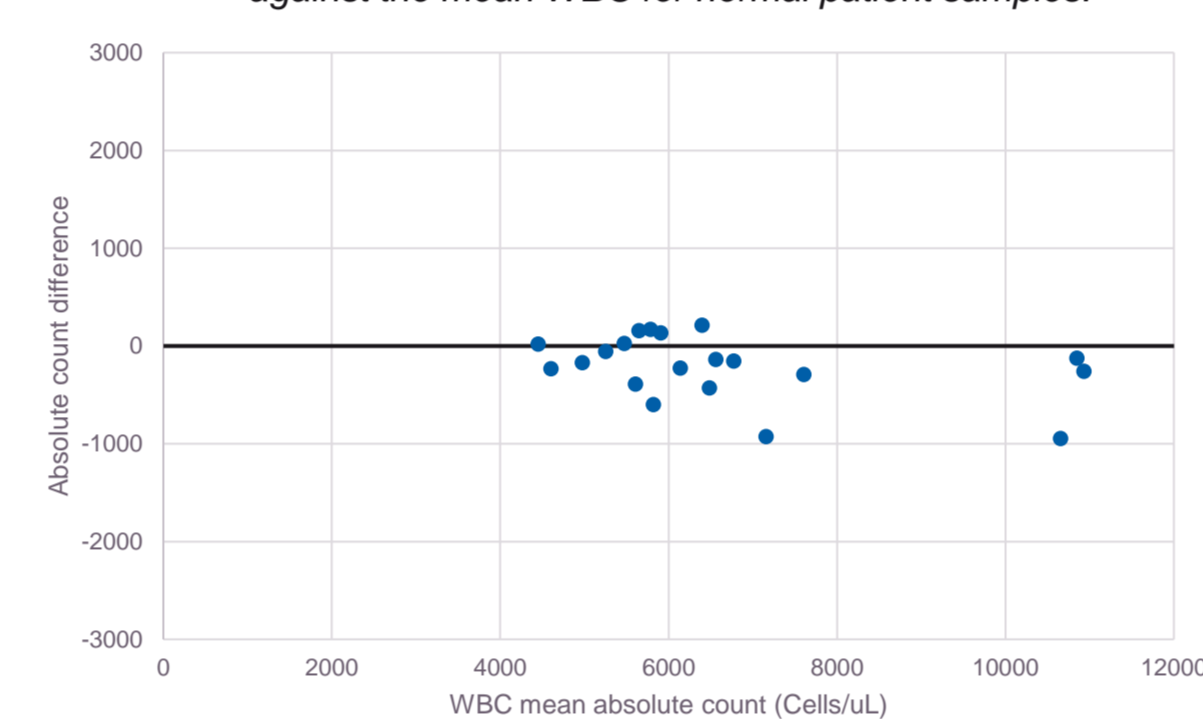
* 570µL total volume is required for accurate volumetric counting on the Sysmex XF-1600.

- Following a lyse / no wash procedure the samples were acquired on the XF-1600 using the TBNK volumetric counting software.
- For the comparison of the haematology parameters the patient samples were then analysed on the XN-Series haematology analyser for WBC and lymphocyte absolute counts.

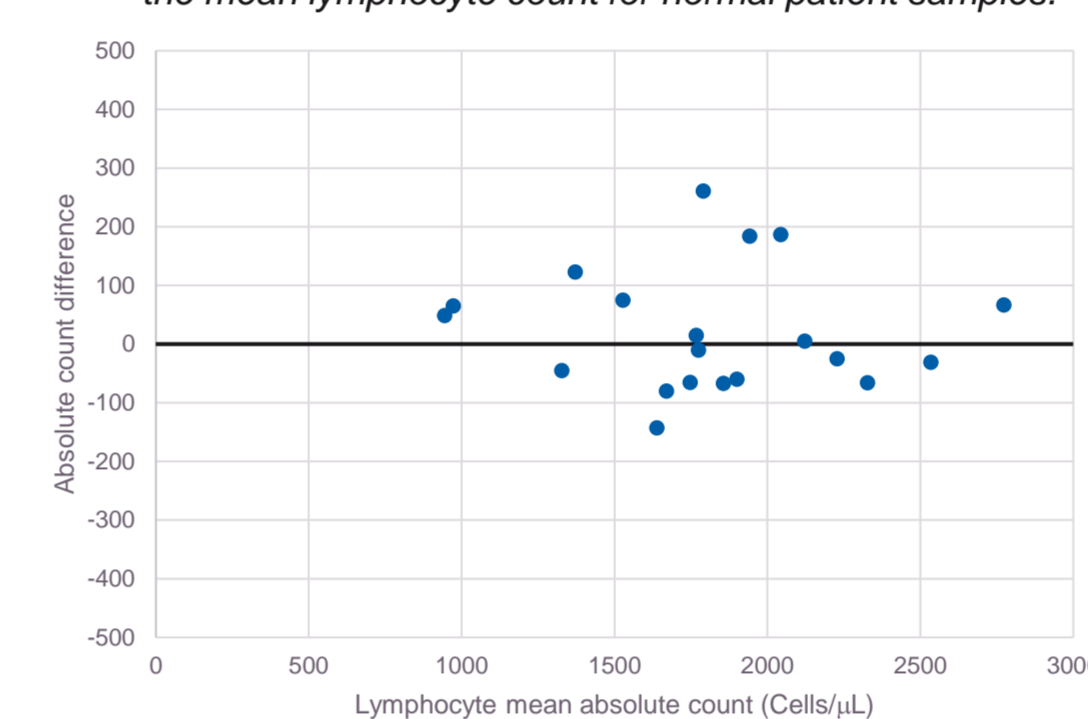
Note: CD-Chex Plus® products could not be analysed on the XN-Series due to the incompatibility of the liquid matrix.

Results

Graph 1. Bland-Altman illustrating the dry tube bias against the mean WBC for normal patient samples.



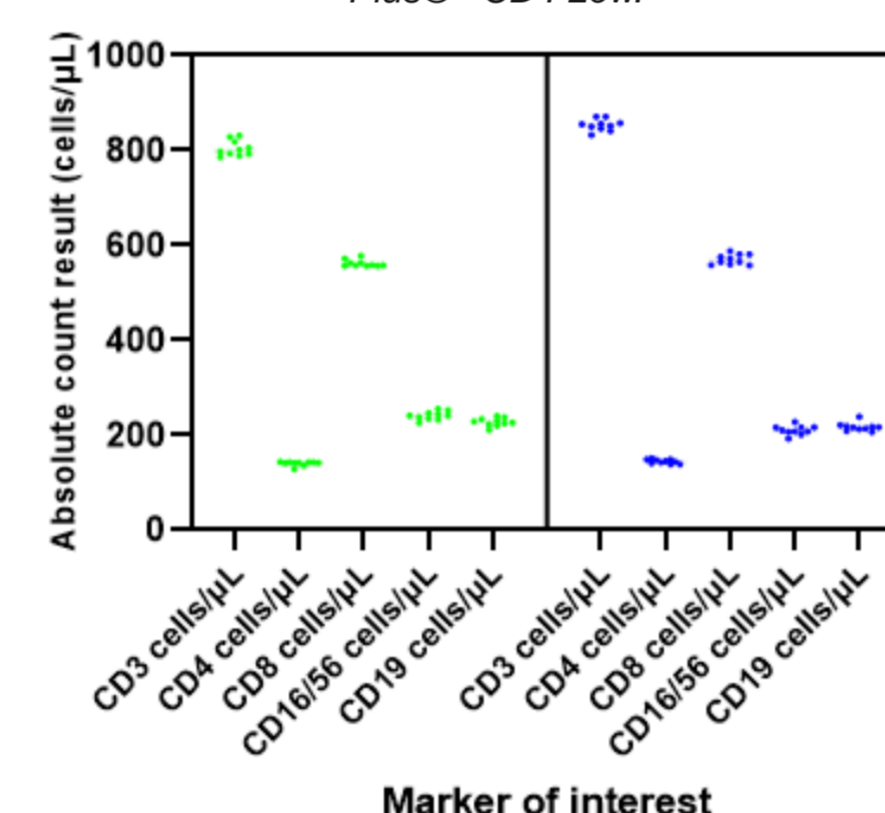
Graph 2. Bland-Altman illustrating the dry tube bias against the mean lymphocyte count for normal patient samples.



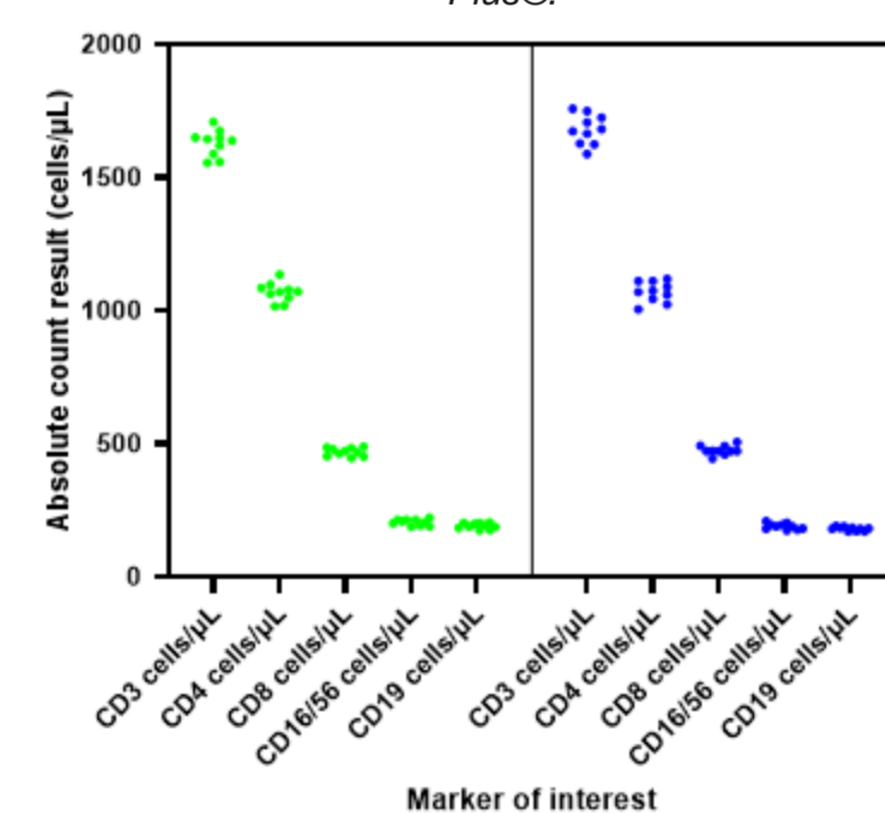
Parameter	R ² Value
WBC	0.9981
Lymph	0.997
CD3	0.9984
CD3/4	0.9976
CD3/8	0.9974
CD16/56	0.9873
CD19	0.9953

Table 2. R² value for DFE tubes compared to predicate methods for normal patient samples.

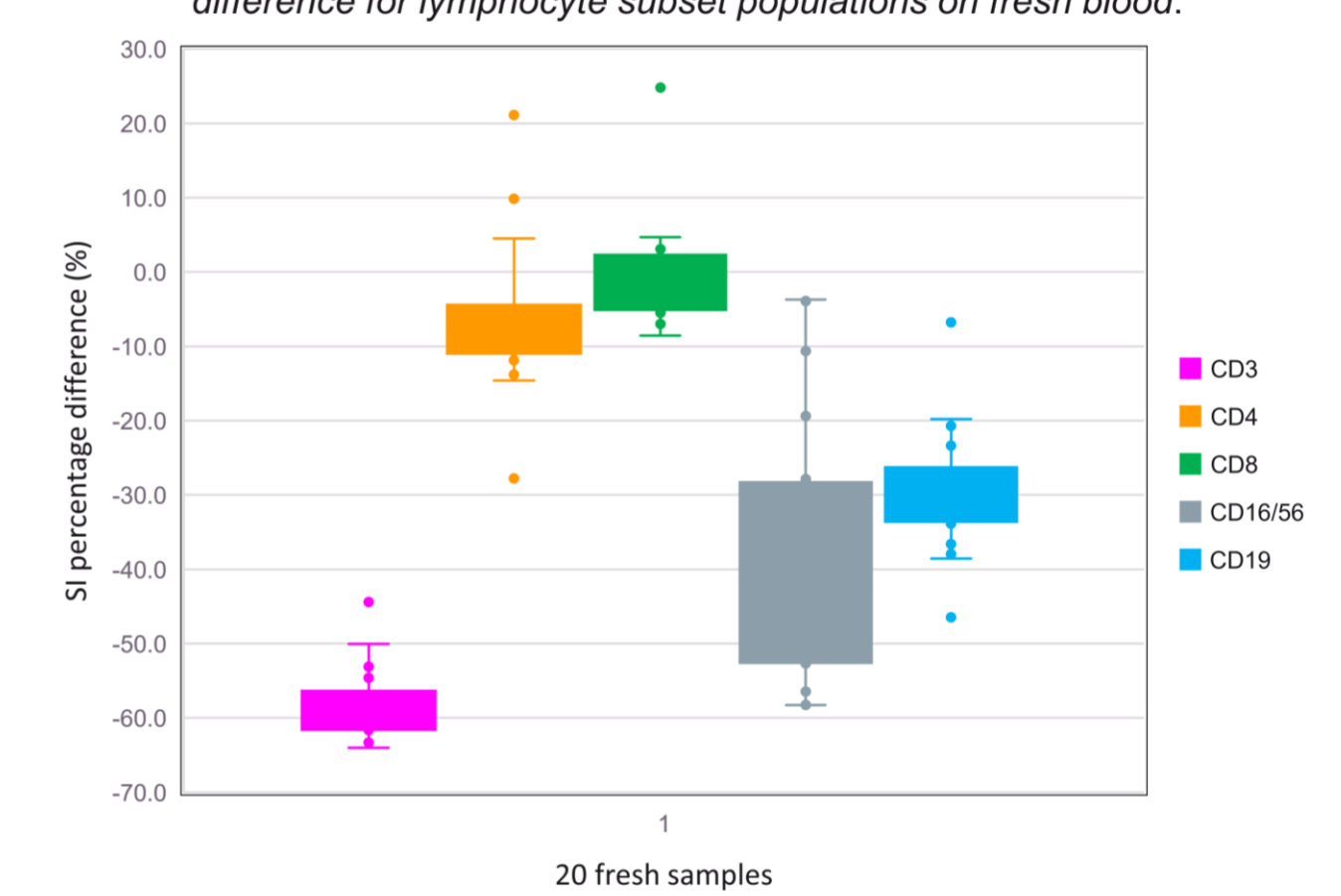
Graph 3. Illustrating the absolute count (cell/µL) results for the predicate and dry tube methods using CD-Chex Plus® - CD4 Low.



Graph 4. Illustrating the absolute count (cell/µL) results for the predicate and dry tube methods using CD-Chex Plus®.



Graph 5. Box and Whisker plot illustrating the SI percentage difference for lymphocyte subset populations on fresh blood.



Statistical results on normal patient samples displayed the following:

- Bland-Altman analysis (Graph 1 and 2) exhibited no bias for the lymphocyte count, CD16/56% and CD16/56#. Then a minor negative bias for WBC, CD3% and #, CD4% and #, CD8% and #, CD19% and #.
- A two tailed unpaired t-test applied to the absolute and percentage count displayed no significant difference with each parameter having a P value >0.05.
- Linear regression analysis (Table 2) illustrated that all parameters including the WBC and lymphocyte comparison with the XN-Series were comparable with R² values >0.95.
- SI information (Graph 5) showed a negative difference from the predicate for CD3 and CD16/56 (Mean percentage difference of -55.2% and -37.3% respectively) all other parameters were comparable.

Statistical results on CD-Chex Plus® and CD-Chex Plus® - CD4 Low displayed the following:

- All percentage and absolute parameter results (Graph 3 and 4) were within the data sheet expected range.
- Bland-Altman analysis exhibited no bias for CD4 and CD8, a minor positive bias for CD3 and minor negative bias for CD16/56 and CD19.
- SI information showed a negative difference from the predicate for CD16/56 (Mean percentage difference of -39.5%) all other parameters were comparable.
- Linear regression analysis illustrated that all parameters were comparable with R² values >0.99.

Conclusion

Dry tube technology is of benefit to users through extended expiry dates, storage, standardisation and logistics⁽¹⁾. Exbio (Prague) have recently launched a dry tube for lymphocyte subset analysis which was evaluated by SEU in terms of comparability with two predicate methods (flow cytometric and a haematology counting analyser).

Although minor biases were observed for some parameters, these would not alter any clinical decisions on the management of patients. All other statistical analysis showed excellent correlation.

The SI for CD3 and CD16/56 exhibited a negative difference from the predicate likely due to the XF-1600 being set up specifically for the KOMBITEST™ TBNK 6-color liquid cocktail and not optimised for the DFE.

The DFE demonstrates great potential for users, further testing should include specific set up criteria and the use of patient samples with a low CD4 count in a clinical setting.

Literature cited

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2. No authors listed. 1994. Reference method for the enumeration of erythrocytes and leucocytes. International Council for Standardization in Haematology; prepared by the Expert Panel on Cytometry. *Clin Lab Haem*, 16(2), pp. 131-138.

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