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Long Term Stabilization of Tandem Dyes for Use in High Content, Multi Variant Flow Cytometry

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Long Term Stabilization of Tandem Dyes for Use in High Content, Multi Variant Flow Cytometry

ABSTRACT

Introduction: Multi parametric flow cytometry is a valuable tool for clinical research studies and requires use of multiple tandem dyes to utilize several markers simultaneously. However, the use of tandem dyes needs frequent re-compensation to account for changes in spectral spillover arising from the degradation of the tandem dye over time and manufacturing lot variability in tandem dye preparations. These drawbacks reduce the overall robustness of the process as it may result in incorrectly compensated data, and manual variations in staining. DuraClone technology, providing stable dry reagents, eliminates the need for manual reagent formulations or sustained cold chain storage, thereby substantially lowering variability coming from operator or workflow.

Study: A 10-color TBNK panel with multiple tandem dyes of R-Phycoerythrin (PE) including PE-Texas Red, PE-Cy5, PE-Cy7 and Allophycocyanin (APC) including APC-Alexa Fluor* 700, APC-AlexaFluor* 750 and single color antibody conjugates were used in the study. In order to expose the tandem dyes to extreme temperature stress, TBNK DuraClone reagents were placed in an environmental chamber controlled at 40±2°C with 75±5 % humidity or 60° at pre-scheduled time points and compared to reagents not exposed to stress (stored at 20-30°C). Single DuraClone reagents stored at 40±2° with 75±5 % humidity were compared to their liquid counterparts stored at 37°C.

Results: The DuraClone single color antibody conjugates at stress were found to have much better performance compared to their liquid counterparts stored at 37°C. The performance of the 10-color TBNK panel stored at 60°C compared to the reagent stored at room temperature (stored at 20-30°C) on the same instrument and compensation settings and fluorescent intensities were found to be comparable. Crosstalk Indices for tandem dyes were comparable across increasing days at stress at 40±2°C.

MATERIALS AND METHODS

To demonstrate the stability of the DuraClone reagents, CD3 conjugated to 10 different fluorophores and a 10 -color TBNK panel (Table 1) were dried down using DuraClone technology and their functional characteristics studied upon long term exposure to environmental stress. Single conjugates were exposed to 40±2°C with 75±5 % humidity while the TBNK panel cocktails were studied at 40°C / 75 % RH as well as at 60°C. The study was conducted using fresh human blood. Samples were processed according to a lyse-wash-stain protocol using ammonium chloride based lysing buffer. Processed samples were analyzed on a Navios flow cytometer equipped with 3 lasers capable to detect 12 parameters at 20-bit resolution. The flow cytometer was set up according to the manufacturers' recommendations. Obtained data was analyzed using Kaluza Software Version 1.2. Crosstalk indices were calculated as explained in Figure 1 and the indices were plotted for the tandems across various days at stress.

Table 1: Antibody conjugates used in the Study

Single Liquid and DuraClone Conjugates			TBNK DuraClone Conjugates		
Target	Fluorophore	Clone	Target	Fluorophore	Clone
CD3	FITC	UCHT1	CD45RA	FITC	ALB11
CD3	PE	UCHT1	CD7	PE	8H8.1
CD3	ECD	UCHT1	CD45RO	ECD	UCHL1
CD3	PC5.5	UCHT1	CD5	PC5.5	BL1a
CD3	PC7	UCHT1	CD27	PC7	1A4CD27
CD3	APC	UCHT1	CD56	APC	N901
CD3	APC-Alexa Fluor 700	UCHT1	CD8	APC-Alexa Fluor 700	B9.11
CD3	APC-Alexa Fluor 750	UCHT1	CD3	APC-Alexa Fluor 750	UCHT1
CD3	Pacific Blue*	UCHT1	CD4	Pacific Blue	13B8.2
CD3	Krome Orange	UCHT1	CD45	Krome Orange	J.33

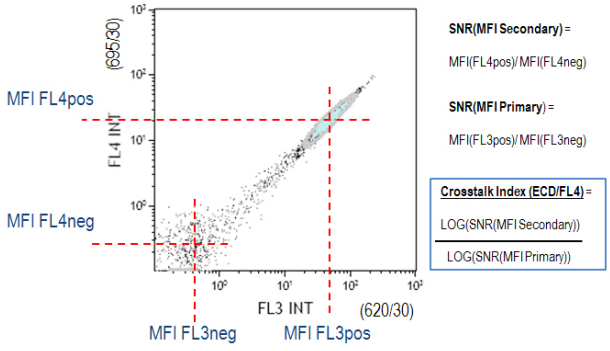

Figure 1: Calculation of crosstalk index of ECD on FL4.

Spillover of a fluorophore over alternate channels can be best characterized by crosstalk indices. It takes into account the spillover intensity of the dye on the secondary channel and assigns a numerical value which is independent of the instrument settings.

This application is Research Use Only and not intended for diagnostic purposes even though the Navios was used for the analysis

RESULTS

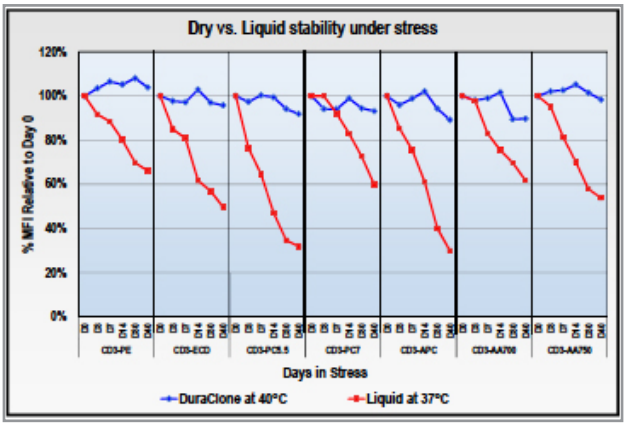

Figure 2: Liquid vs. DuraClone reagent MFI (Mean Fluorescent Intensity) comparison under stressed conditions.

Figure 2 shows the performance of the single CD3-PE and CD3-APC and their tandems in comparison to their liquid counterparts. The degradation is expressed in terms of % MFI relative to day 0. Over 40 days at stress, the liquid reagent degrades and MFI drop ranges from 35 % to 70 %; however the DuraClone reagents are observed to be stable with MFI drop of less than 10 % for all conjugates.

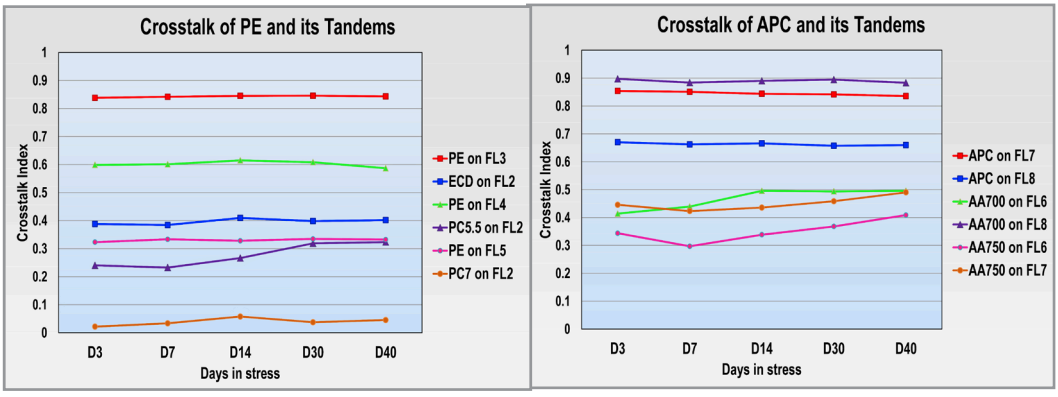

Figure 3: Monitoring crosstalk indices of PE and APC tandems over the study period

Crosstalk indices of tandem dyes on respective parent channels are plotted against the number of days in stress (40±2°C and 75±5 % RH). For all PE tandems (except PC5.5) and APC-A700, no increase in crosstalk index was observed over 40 days, indicating stability of tandem dyes over the time period studied. PC5.5 and APC-A750 shows increase in crosstalk with increasing days in stress. However, the shift is not significant, as indicated by consistent MFI over time (Figure 2) and consistent compensation matrix observed for 10 color cocktail over time (Figure 4).

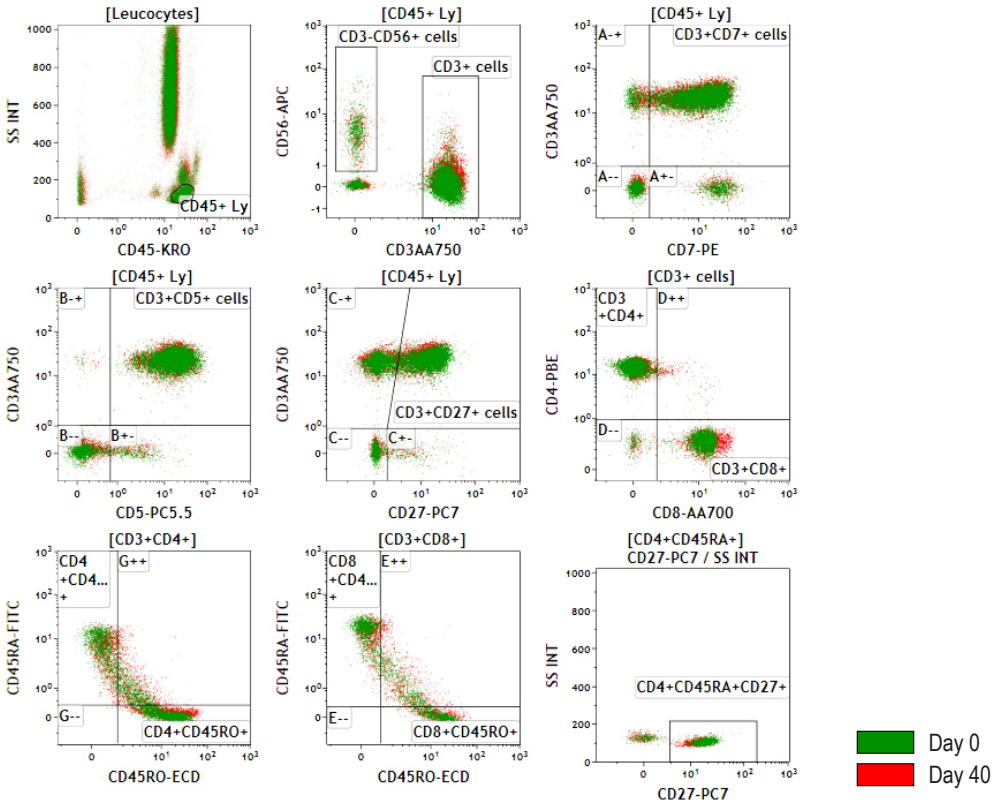

Figure 4: Overlay of Day 0 and Day 40 data of the 10 color TBNK panel stressed at 60°C

Figure 4 depicts that the multicolor cocktail under stressed conditions has comparable performance with Day 0, using same compensation settings and gating strategy.

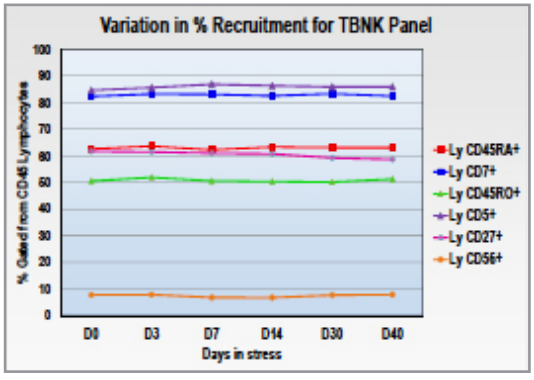

Figure 5: Percentage observed for the TBNK Panel over the study period.

Percentage or gated populations were monitored across reagents placed at 60°C for up to 40 days and found to be consistent over time. This shows that the changes in compensation matrix are not significant enough to affect the recruitment of the marker. Comparable results were obtained with studies at 40°C and 75 % RH (data not shown).

SUMMARY

- The study demonstrates that the DuraClone reagent can withstand high temperature and humidity stress over 40 days.

- There is minimal change in crosstalk indices of tandem dyes over time under stressed conditions, indicating no deterioration of tandem dyes over time.

- DuraClone process stabilizes the tandem dyes without loss of activity. This characteristic can help researchers plan for robust studies, being conducted by multiple laboratries and across multiple time points, without tandem deterioration or requirement of changing compensation.

Research Use Only, Not Intended for Diagnostic Purposes

§ Navios is CE marked for 10-color in-vitro diagnostic use. In the U.S., Navios is intended for use as an in-vitro diagnostic device for immunophenotyping with Navios tetra software and CYTOSTAT tetraCHROME reagents. All other uses are for research use only.

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