



**From: Immunology Quality Assessment (IQA) CD4 Program**

**Re: TransFix Implementation**

**Date: July 3, 2023**

## **1. Overview**

Stabilization of cellular surface markers is recommended for flow cytometry testing when the time from patient draw to testing is anticipated to extend past 48 hours. Stabilization may also be necessary when a sample must be shipped between laboratories due to inability to process. Any delay in testing the sample can have a negative impact on the results.

At the behest of NIAID leadership, the IQA explored TransFix tubes as a commercially available product for whole blood sample stabilization. TransFix tubes contain a solution of TransFix and EDTA at the correct volume to stabilize and anti-coagulate whole blood at the point of collection. After collection in TransFix tubes, the manufacturer states that the blood may be stored and transported for up to 3 days at room temperature (RT) and up to 14 days at 2-8°C.

The IQA compared TransFix tube performance to traditional EDTA tube performance on a clinical flow cytometry lymphocyte panel at both RT and 2-8°C temperature conditions according to the manufacturer's recommended storage times (3 days at RT and 14 days at 2-8°C), staining samples daily (excluding weekends).

At RT, the traditional EDTA tube out-performed the TransFix tube, exhibiting superior population separation on a CD45/SSC plot and better maintenance of lymphosum. The TransFix tube lymphosum value dropped below the recommended quality control lower limit by day 3 of processing.

In contrast, at 2-8°C, the traditional EDTA tube and the TransFix tube showed comparable performance for the first week before diverging, with the EDTA tube steadily declining in absolute counts and percentage values, while the TransFix tube maintained steady levels of the same parameters. Significance was determined by examining the slope of the absolute counts and percentage values over the course of the study period. A slope closer to zero indicates less change over time from initial values. A p-value of less than 0.05 indicates a slope is significantly different from zero, meaning there is significant change from initial values (see Table 1).

Therefore, the IQA recommends the use of TransFix tubes shipped at 2-8°C as an alternative to on-site testing. Laboratories performing flow cytometric enumeration of lymphocyte subsets for NIAID-sponsored studies may coordinate shipment of TransFix tubes at 2-8°C to other IQA CD4 program participants or to the IQA Central Laboratory with direction from NIAID leadership.

**Table 1. Comparison of Changes in Absolute Counts and Percent Values of TransFix Tubes and EDTA Tubes at 2-8°C**

**Slope and p-value estimates, 14-day observation**

Medium	Machine	Est.	CD3%	CD3#	CD3CD4%	CD3CD4#	CD3CD8%	CD3CD8#
TransFix 4C (raw)	FACSCalibur	Slope	-0.05	4.42	0.00	2.82	-0.04	0.77
		P-value	0.1837	0.2222	0.9720	0.2440	0.4464	0.5477
TransFix 4C (raw)	FACSCanto II	Slope	-0.02	0.71	-0.06	1.42	-0.09	-0.27
		P-value	0.7773	0.8538	0.3200	0.5140	0.2530	0.8611
TransFix 4C (converted)	FACSCalibur	Slope	-0.06	5.87	-0.04	3.81	-0.14	1.26
		P-value	0.2161	0.4301	0.5420	0.4609	<b>0.0482</b>	0.6933
TransFix 4C (converted)	FACSCanto II	Slope	0.00	-1.56	-0.04	0.17	-0.08	-1.00
		P-value	0.9756	0.7157	0.4535	0.9595	0.4161	0.4630
EDTA 4C	FACSCalibur	Slope	-0.04	-29.74	-0.26	-21.19	0.22	-5.60
		P-value	0.6539	<b>0.0015</b>	<b>0.0112</b>	<b>0.0009</b>	<b>0.0127</b>	<b>0.0242</b>
EDTA 4C	FACSCanto II	Slope	-0.17	-36.39	-0.19	-26.75	0.07	-11.11
		P-value	0.1880	<b>0.0004</b>	0.1389	<b>0.0003</b>	0.3220	<b>0.0004</b>

**2. Product Information**

TransFix Vacuum Blood Collection Tubes (TVTs) arrive pre-filled with both K3EDTA and stabilizing agent. TVTs fit most docking sheaths used with traditional EDTA tubes. Blood is directly collected into the TVT and mixed by inversion prior to transport. While TVTs come in both 3 mL and 9 mL sizes, only 3 mL tubes are approved for IVD used in the USA. Unused TVT tubes should be stored at 2-8°C until time of blood draw. See Table 2 for manufacturer purchasing information.

**Table 2. TransFix Product Information**

Manufacturer	Cytomark
Product Code: 3ml blood collection vacuum tube (Single Tube)	TVT-03-01
Product Code: 3ml blood collection vacuum tube (Pack of 50)	TVT-03-50

The manufacturer, Cytomark, recommends reaching out to local distributors if available in a laboratory's region. A list of available distributors may be found at <https://www.cytomark.co.uk/contact-us/distributors/>. Alternatively, the company may be contacted directly at [support@cytomark.uk](mailto:support@cytomark.uk) for quotes.



### **3. Shipping Instructions**

For best results, a TransFix tube filled with collection sample should be kept at 2-8°C until time of shipment. Some companies (World Courier, Biocair) that routinely ship biological specimens offer 2-8°C temperature controlled boxes for international transit. This is the preferred method of transport for international locations shipping to the IQA.

Please note that if shipping to the IQA, your materials will be listed as a Category B Biological Substance. Refer to shipping resources for Category B specimens online at:

<https://www.hanc.info/labs/labresources/procedures/Pages/actnShippingDemo.aspx>.

To organize shipping of TransFix tubes to the IQA for lymphocyte panel testing, please reach out to the IQA CD4 Program at [cd4iqa@duke.edu](mailto:cd4iqa@duke.edu).