EDTA Interference Study with the Tacrolimus Assay for the Dimension Integrated Chemistry System

SIEMENS Healthineers

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Abstract

Background

The Tacrolimus (TAC) assay on the Dimension® Integrated Chemistry Systems is an in vitro diagnostic test for the quantitative measurement of tacrolimus in human whole blood. Measurements of tacrolimus are used as an aid in the management of tacrolimus therapy in transplant patients. This new assay includes some important design changes to improve the analytical performance of the assay (Limit of quantification and precision) and to reduce matrix effects known to cause false positive results. To avoid errors, a built-in software check flags these false positive results. Nevertheless incorrect sample handling when collecting EDTA samples may depress the reported concentration of tacrolimus if the quantity of EDTA is too high. The objective of this study is to demonstrate the importance of properly filling the EDTA tubes with whole blood.

Methods

The automated Dimension® TAC assay uses an immunoassay technique in which free and tacrolimus-bound antibody-enzyme conjugate is separated using magnetic particles. The new assay retains the ACMIA* format but now employs a predecorated chrome particle. It also uses a new antibody. Furthermore, the automated pretreatment uses a new chemical component that helps reduce false signal. The assay is performed using a method specific Flex® reagent cartridge. The Flex® cartridge contains two pretreatment reagents, antibody-β-galactosidase conjugate, tacrolimus immobilized on chromium dioxide particles, chlorophenol red β-d-galactopyranoside (CPRG) substrate, and diluent to hydrate the tablets.

The EDTA interference of the TAC assay was evaluated on the three Dimension® Integrated Chemistry Systems (the Dimension Xpand®, RxL, and EXL™ Systems) according to CLSI/NCCLS EP7-A2. We observed the bias expressed in percent looking at the difference in the results between the fully filled tube (100% full, with appropriate EDTA concentration) and partially filled tube (EDTA concentration > 3 mg/mL).

Results

When the EDTA tube was only 60% filled, corresponding to an EDTA concentration of 3 mg/mL, the mean biases were -0.5% (from -7 to 9%) and 4.5% (from 3 to 7%) respectively at 5-6 and 15-20 ng/mL of tacrolimus on the Dimension® Xpand, RxL, and EXL systems. When the EDTA tube was filled to only 25% corresponding to a EDTA concentration of 7.2 mg/mL, a negative bias >10% was observed, with a mean value of -13.3% (from -10% to -19%) for a tacrolimus concentration of 5-6 ng/mL and a mean negative bias of –7.8% (-22% to +5.0%) for a tacrolimus concentration of 15-20 ng/mL.

Conclusion

We confirmed the need to be careful when collecting EDTA samples for TAC testing to ensure the collection tube is drawn to its intended capacity, in accordance with the tube manufacturer's recommendations. The higher concentration of EDTA present in the whole-blood sample when the tube is filled less than 33% may produce an absolute bias greater than 10%.

Background

While each immunosuppressant drug has its own mechanism of action, the utility of ISDs is always aimed at reaching the same goal: to provide adequate immunosuppression so that transplanted organs are not rejected by a recipient's immune system. Clinicians rely on immunosuppressant therapy to help prevent organ rejection. However, they must work carefully to reach an appropriate level of immunosuppression while minimizing the toxic side effects that these drugs have on the patient. Tacrolimus is used in liver, kidney, and heart transplants. In 2009, a report from the European Consensus Conference "Optimizing Tacrolimus Therapy in Organ Transplantation" was published that provided insight into laboratory practices for monitoring tacrolimus therapy[1]: 12-hour trough sampling (C0) target levels of 5–20 ng/mL. Today, low-dose tacrolimus treatment is becoming the standard of care to minimize CNI toxicity. Tacrolimus blood levels are generally maintained in the 4–10 ng/mL range with excellent clinical outcomes.

*Affinity Chrome-Mediated Immunoassay

Faster sample processing and result reporting are certainly an advantage, but the quality of results is the most important aspect of any assay. Several published studies have confirmed the improved performance of the TAC assay [2,3,4,5]. However, a deeper study of EDTA concentration interference was required, because sample handling when collecting EDTA samples may have an impact on the final result.

Principles of the procedure

To perform the TAC assay, a sample cup (SSC) containing the whole-blood sample to be analyzed and a TAC Flex® reagent cartridge are placed on the Dimension system. The Dimension system mixes and lyses the whole-blood sample in the presence of a pretreatment reagent [1]. This reagent contains a displacer that acts to displace tacrolimus in the sample from binding proteins. The lysed sample is then mixed with the antibody enzyme conjugate. The tacrolimus present in the sample is bound by the tacrolimus antibody. Magnetic particles coated with tacrolimus are added to bind free (unbound) antibody-enzyme conjugate. TAC CrO_2 is first coated with anti-fluorescein antibody. Then the anti-fluorescein antibody-coated CrO_2 is predecorated with fluorescein-tacrolimus analog, resulting in a more stable antibody-KK506-fluoresecein combination. The reaction mixture is then separated magnetically. Following separation, the supernatant containing the tacrolimus-antibody-enzyme complex is transferred to a cuvette and mixed with the substrate; chlorophenol red β-d-galactopyranoside (CPRG). β-galactosidase catalyzes the hydrolysis of CPRG to produce chlorophenol red (CPR) that absorbs light maximally at 577 nm. The change in absorbance at 577 nm due to the formation of CPR is directly proportional to the amount of tacrolimus in the patient's sample and is measured using a bichromatic (577, 700 nm) rate technique.

Method

Four informed consent donors were enrolled, prospectively to from whom whole blood was drawn in BD VACUTAINER tubes containing K2 EDTA. Each donor was drawn at 4.0, 2.4, 2.0, 1.6, 1.3, and 1.0 mL, and each sample was aliquoted at 800µL.

A Dimension Tacrolimus stock solution (concentration 0.01 mg/mL) was prepared to spike each aliquoted sample. Aliquoted sample from two donors was spiked at the same volume with the Tacrolimus stock solution at 5–6 ng/mL, and aliquoted sample from two other donors was spiked at the same volume with the Tacrolimus stock solution at 15–20 ng/mL.

Flex reagent cartridges and corresponding TAC calibrator were used. Sampling, reagent delivery, mixing, and processing of the spiked samples were automatically performed on three Dimension systems (Dimension Xpand®, RxL, and EXL™ systems). Three-level Rap/Tac/CsA controls from More Diagnostics were used to validate the assays. (Table 1)

Table 1. More Diagnostics QC values for TAC assay on three Dimension systems.

MORE controls	QCs range ng/mL	Dimension EXL ng/mL	Dimension RxL ng/mL	Dimension Xpand ng/mL
Level-1	3.2 - 5.5	4.4	3.9	3.6
Level-2	8.6 - 13.8	11.0	10.7	9.6
Level-3	17.7 - 27.0	20.6	20.9	18.8

Results

Table 2. TAC values (ng/mL) for spiked samples on Dimension Xpand system.

Spiked TACR Solution (ng/mL)	Blood Draw Volume (mL)	% of Tube Fill	EDTA Conc. (mg/mL)	TAC Assay			
				Donor 1 (ng/mL)	% bias from full tube	Donor 2 (ng/mL)	% bias from full tube
=5 - 6 ng/mL	4	100%	1.8	6.3		5.6	
	2.4	60%	3	6.3	0%	6.1	9%
	2.0	50%	3.6	5.8	-8%	5.4	-4%
	1.6	40%	4.5	5.9	-6%	5.2	-7%
	1.3	33%	5.4	5.7	-10%	5.1	-9%
	1.0	25%	7.2	5.4	-14%	4.9	-13%
				Donor 3 (ng/mL)	% bias from full tube	Donor 4 (ng/mL)	% bias from full tube
=15 - 20 ng/mL	4	100%	1.8	16.0		15.1	
	2.4	60%	3	16.7	4%	15.6	3%
	2.0	50%	3.6	16.8	5%	15.8	5%
	1.6	40%	4.5	17.3	8%	14.3	-5%
	1.3	33%	5.4	16.9	6%	13.4	-11%
	1.0	25%	7.2	15.6	-3%	12.5	-17%

Table 3. TAC values (ng/mL) for spiked samples on Dimension RxL system.

Spiked TACR Solution (ng/mL)	Blood Draw Volume (mL)	% of Tube Fill	EDTA Conc. (mg/mL)	TAC Assay			
				Donor 1 (ng/mL)	% bias from full tube	Donor 2 (ng/mL)	% bias from full tube
=5 - 6 ng/mL	4	100%	1.8	6.4		5.4	
	2.4	60%	3	6	-6%	5.3	-2%
	2.0	50%	3.6	6	-6%	5.7	6%
	1.6	40%	4.5	5.9	-8%	5.5	2%
	1.3	33%	5.4	5.5	-14%	4.9	-9%
	1.0	25%	7.2	5.2	-19%	4.7	-13%
				Donor 3 (ng/mL)	% bias from full tube	Donor 4 (ng/mL)	% bias from full tube
=15 - 20 ng/mL	4	100%	1.8	17.8		16.6	
	2.4	60%	3	18.3	3%	17.3	4%
	2.0	50%	3.6	18.8	6%	16.8	1%
	1.6	40%	4.5	17.9	1%	15.3	-8%
	1.3	33%	5.4	17.8	0%	15.5	-7%
	1.0	25%	7.2	17.4	-2%	12.9	-22%

Table 4. Spiked each samples TAC value (ng/mL) on Dimension EXL

Spiked TACR solution (ng/mL)	Blood draw Volume (mL)	% of tube fill	EDTA conc. (mg/mL)	TAC Assay			
				Donor 1 (ng/mL)	% bias from full tube	Donor 2 (ng/mL)	% bias from full tube
=5 - 6 ng/mL	4	100%	1.8	6.2		5.9	
	2.4	60%	3	6.4	3%	5.5	-7%
	2.0	50%	3.6	5.8	-6%	5.8	-2%
	1.6	40%	4.5	5.7	-8%	5.4	-8%
	1.3	33%	5.4	5.6	-10%	5.3	-10%
	1.0	25%	7.2	5.5	-11%	5.3	-10%
				Donor 3 (ng/mL)	% bias from full tube	Donor 4 (ng/mL)	% bias from full tube
=15 - 20 ng/mL	4	100%	1.8	17.6		15.5	
	2.4	60%	3	18.8	7%	16.4	6%
	2.0	50%	3.6	18.2	3%	16	3%
	1.6	40%	4.5	17.8	1%	16.1	4%
	1.3	33%	5.4	19.9	13%	16.1	4%
	1.0	25%	7.2	18.4	4.5%	14.3	-8%

Percentage bias results were calculated for each Dimension system from a reference value obtained when the tube was 100% filled and the values obtained when the tubes were partially filled (to levels of 60%, 50%, 40%, 33%, and 25%) (Tables 2–4).

Decreases in concentration of less than 10% or equal to 10% were acceptable according to CLSI guideline NCCLS EP7-A2.

We found that a risk of under-reporting occurs when the tube was filled less than 33% and 25%, corresponding to EDTA concentrations of 5.4 and 7.2 mg/mL. These concentrations are much higher than the expected concentration of EDTA in a fully filled collection tube (1.8 mg/mL).

Conclusion

EDTA concentrations exceeding 3 mg/mL may depress Dimension Tacrolimus (TAC) Assay results in whole blood. Under-reporting greater than -10% may occur if the K2-EDTA tube is filled with whole blood to a level below 33% of capacity, because at 33% capacity the EDTA concentrations exceeds 5.4 mg/L, compared to a concentration of 1.8 mg/mL when the tube is correctly filled.

References:

1. Therapeutic Drug Monitoring. 2009 Apr; 31(2):139-52.
2. Suzuki Y. Improved open-well stability of ACMIA reagent for determining blood tacrolimus. Ther Drug Monit. 2017.
3. Brugnolo, Plebani. Performance evaluation of a new automated tacrolimus assay on Dimension system. Poster, IATDMCT 2015.
4. Ko D-H. Performance evaluation of a restored Dimension tacro assay: an automated platform for measuring the whole blood tacrolimus concentration. Clin Lab. 2016:7-12.
5. Bargnoux AS. Evaluation of the new Siemens tacrolimus assay on the Dimension EXL Integrated Chemistry System analyzer: comparison with an ultra-performance liquid chromatography-tandem mass spectrometry. Ther Drug Monit. 2016 Dec;38(6):808-12.

Product availability varies from country to country and is subject to varying regulatory requirements. Please contact your local representative for availability.

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