A Novel Genotyping Test for Tacrolimus Dose Optimization

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Abstract

Tacrolimus is an immunosuppressant commonly prescribed for transplant recipients. It has a narrow therapeutic window, with acute rejection resulting from insufficient dosing, and increased risk of kidney injury and drug toxicity associated with increased blood concentrations. Tacrolimus is metabolized by the CYP3A5 enzyme, where reductions in CYP3A5 activity cause decreased metabolism leading to more rapid increases in blood concentration level for a given dose of the drug. Identifying individual CYP3A5 expression level is therefore essential for proper tacrolimus dosing. Three CYP3A5 alleles (*3, *6 and *7) are associated with full or partial loss of protein activity depending on zygosity. Both the Dutch Pharmacogenetic Working Group (DPWG) as well as the Clinical Pharmacogenetics Implementation Consortium (CPIC) agree on recommendations for tacrolimus dosing based on CYP3A5 genotyping for the *3, *6 and *7 alleles.

We have developed a test for CYP3A5 *3, *6 and *7 genotyping. The test uses a 6-plex qPCR assay format with a high level of reliability. We completed analytical performance testing by assessing accuracy, sensitivity, precision and robustness of TacroType using multiple sources of human DNA. Accuracy was confirmed for each possible genotype at all three alleles using well-characterized reference samples. TacroType exhibited accurate and robust performance within a broad range of DNA input. Precision studies indicated consistent assay results across operators, instruments and lots of reagent. Accurate and consistent assay performance was demonstrated using EDTA and ACD blood and buccal swabs prepared by a variety of DNA extraction methods.

Introduction

- FDA indicates that CYP3A5 expressors take longer to achieve their target tacrolimus concentration, supporting therapeutic management recommendations based on CYP3A5 genotype (1)
- A joint recommendation of the Association for Medical Pathology, Clinical Pharmacogenetics Implementation Consortium, College of Medical Pathologists, Dutch Pharmacogenetics Working Group, European Society for Pharmacogenomics and Personalized Therapy and Pharmacogenomics Knolwedgebase recommend CYP3A5 Genotyping for Tacrolimus dosing (2)
- Prospective clinical trials have indicated improvement in percentage of patients who achieve target Tacrolimus post transplant when CYP3A5 genotype guides dosage (**Figure 1**) (3):

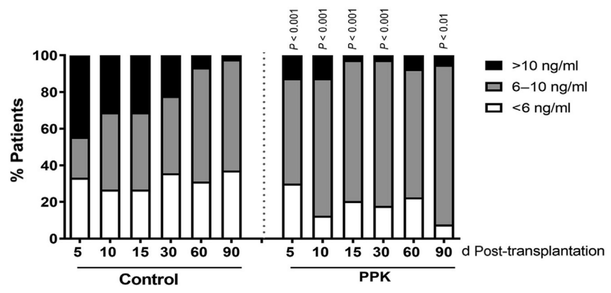


Figure 1: Tacrolimus dosage adjustment incorporating *CYP3A5* genotype ("PPK") resulted in more patients in the optimal range (6-10 ng/mL) following kidney transplantation (45 control vs. 40 PPK) (3).

Tacrolimus Dosing Recommendations

CPIC and DPWG recommend the following adjustments to Tacrolimus dosing based on CYP3A5 genotype (**Table 1**).

Genotype	Description	CPIC (4)	DPWG (5)
*1/*1	Extensive metabolizer	1.5x-2x standard dosing	2.5x standard dosing
*1/*3, *1/*6, *1/*7	Intermediate metabolizer	1.5x-2x standard dosing	1.5x standard dosing
*3/*3, *6/*6, *7/*7, *3/*6, *3/*7, *6/*7	Poor metabolizer	Standard dosing	Standard dosing

Table 1. CPIC and DPWG recommendations for Tacrolimus dosing based on CYP3A5 genotype

Genotyping Method

- Six qPCR assays were developed for three biallelic SNPs (rs776746, rs10264272 and rs41303343) in *CYP3A5.* Assays were optimized until they achieved robust discrimination of each allele (**Figure 2A**)
- Fluorescence signals generated during qPCR reactions were used to assign genotype (**Figure 2B**) and an analysis script was developed to genotype 6 alleles in a single reaction.
- 95% confidence intervals were generated by the Clopper-Pearson test.

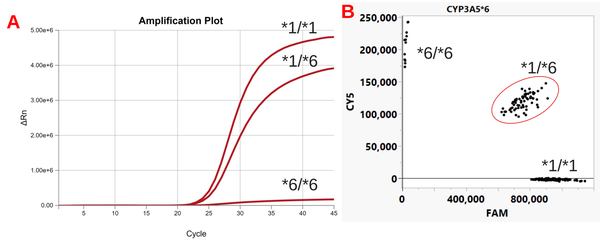


Figure 2: (**A**) Representative FAM amplification curves for *1/*1 (top), *1/*6 (middle), *6/*6 (bottom). (**B**) Clustering graph for *1/*1, *1/*6 and *6/*6 using fluorescence values from FAM (**A**) and CY5 (not shown) amplification curves. To illustrate clusters of fluorescence values associated with a particular genotype, the *1/*6 heterozygous genotype cluster is circled in red.

Verification Plate Overview and Accuracy

- Accuracy, Lot-to-Lot and Repeatability and Reproducibility studies used the same plate layout: 32 unique DNA's were sourced from either One Lambda or the Coriell Repository, including heterozygotes and homozygotes for all alleles (**Table 2**). The 32 DNA samples were dotted in triplicate on each plate for a total of 96 reactions on each plate.
- Coriell repository samples are well characterized, and we independently confirmed the genotype of One Lambda samples.
- TacroType generated genotyping calls with an accuracy of 100% (192/192, lower bound of 95% CI = 98.1%)

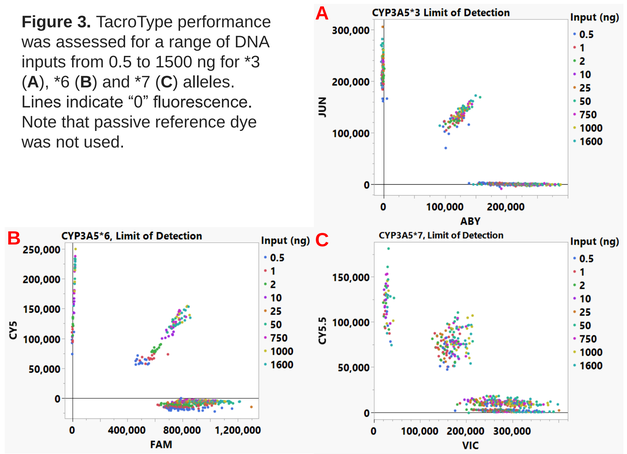
Source	*1/*1	*1/*3	*1/*6	*1/*7	*3/*6	*3/*7	*6/*7	*3/*3	*6/*6	*7/*7
Coriell	2	0	0	0	2	2	3	2	2	2
One Lambda	3	2	3	2	2	2	1	2	0	0

Table 2. Verification studies included CYP3A5 *1, *3, *6 and *7 alleles as homozygotes and heterozygotes.

Limit of Detection

A total of 12 samples were tested for limit of detection studies. The 12 samples included 9 One Lambda samples and 3 well-characterized samples from the Coriell repository, and represented all genotypes listed in **Table 2**. Each sample was tested over a range of DNA inputs from 0.5 ng to 1600 ng using two separate lots of primer/probe-mix. Reactions were performed in triplicate for each lot (**Figure 3**).

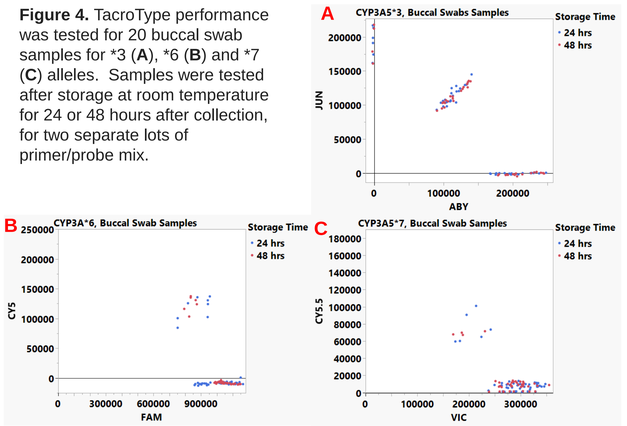
Figure 3. TacroType performance was assessed for a range of DNA inputs from 0.5 to 1500 ng for *3 (**A**), *6 (**B**) and *7 (**C**) alleles. Lines indicate "0" fluorescence. Note that passive reference dye was not used.



Sample Preparation (Buccal Swabs)

DNA was extracted from buccal swabs from 20 individuals and was tested with TacroType (**Figure 4**). Positives for each allele were represented in our sample, and all genotyping calls were independently verified.

Figure 4. TacroType performance was tested for 20 buccal swab samples for *3 (**A**), *6 (**B**) and *7 (**C**) alleles. Samples were tested after storage at room temperature for 24 or 48 hours after collection, for two separate lots of primer/probe mix.



Sample Preparation Summary

We validated TacroType performance for samples types listed in **Table 3**. Genotyping calls were independently verified for all samples tested.

Source	Anticoagulant	DNA Extraction Method	Samples	Concordance
Blood	ACD	Thermo Fisher Scientific	30	100%
Blood	ACD	Qiagen	10	100%
Blood	ACD	Maxwell	10	100%
Blood	EDTA	Thermo Fisher Scientific	30	100%
Blood	EDTA	Qiagen	10	100%
Blood	EDTA	Maxwell	10	100%
Buccal	N/A	Thermo Fisher Scientific	20	100%
Buccal	N/A	Qiagen	10	100%

Table 3. Consistent TacroType performance for multiple sample preparation methods.

Precision

Lot-to-lot variability was assessed using 32 samples across 3 lots of primer/probe mix in two duplicate plates for a total of 192 reactions. Concordance was 100% with a lower-bound 95% CI of 99%. Repeatability and reproducibility was tested in 24 plates dotted with 32 unique samples in triplicate, for a total of 2304 reactions. Concordance of all reportable genotypes was 100% (**Table 4**). Four of 2304 total reactions (0.17%) failed to generate genotyping results due to technical errors.

Factor Tested	Groups Tested	Plates/ Group	Samples per Group	Concordance
Time of Day	AM, PM	12 / AM or PM	1152	100% (99.8%)
Day	Six non-consecutive days	4 / day	384	100% (99.5%)
Operator	1, 2	12 / operator	1152	100% (99.8%)
Instrument	1, 2, 3, 4	6 / instrument	576	100% (99.6%)

Table 4. TacroType performance was not impacted across multiple days, times of day, operators or instruments used. Lower bound of 95% CI are in parentheses.

Conclusion

We have developed a robust and rapid test to detect CYP3A5 *3, *6 and *7 genotyping. TacroType is accurate, sensitive, precise and is suitable for use with blood and buccal swab samples (**Table 5**).

Study	Unique DNAs	Total Samples Tested	Conditions Tested	Concordance
Accuracy	32	96	All genotypes	100% (98.1%)
Limit of Detection	12	72/input	0.5, 1600 ng input	100% (97.4%)
Lot-to-Lot Variability	32	192	3 lots of primer/probe mix	100% (99%)
R/R	32	2304	Time, operator, instrument, day	100% (99.9%)

Table 5.Summary of studies performed for verification of TacroType. Lower bound of 95% CI are in parentheses.

References

1) https://www.fda.gov/medical-devices/precision-medicine/table-pharmacogenetic-associations
2) Pratt et al., 2023. PMID: 37419245
3) Lloberas et al., 2023. PMID 37391040. EudraCT 2016-00340-34;
4) Birdwell et al., 2015. PMID 25801146
5) https://www.pharmgkb.org/page/dpwgMapping#cyp3a5

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