Cross-validation of Everolimus LC-MS/MS and Thermo Fisher QMS Therapeutic Drug Monitoring Assays – The Zortracker Cross-Validation and TDM Support Program

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Background:

Everolimus is an immunosuppressive agent used in kidney and liver transplantation to prevent rejection.

Therapeutic Drug Monitoring of everolimus is recommended for all transplant patients

Goal of this academic program and collaboration:

- To provide support to US transplant center laboratories , and US reference laboratories, to set up, validate, maintain and study the performance of clinical assays for the therapeutic drug monitoring (TDM) of everolimus in order to support its safe and effective use

Components of the program:

- Establishing an everolimus-treated patient blood sample bank
- Providing sample kits for assay set up and validation
- Run a study to allow participating laboratories to cross-validate their assays and monitor performance over time via an external control

Objective:

Assessment and comparison of the performance of

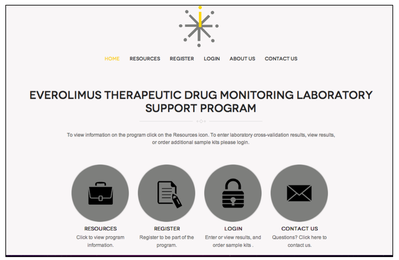
- 12 clinical TDM laboratories using LC-MS/MS and
- 7 laboratories using the Thermo Fisher QMS everolimus TDM immunoassay

in a cross validation study in the United States over 15 months.

Methods:

- Samples from transplant patients (different age, gender, ethnicity) receiving everolimus are collected in a prospective multicenter clinical trial
- 30-100 mL EDTA samples (less from pediatric patients) are collected and stored under controlled conditions in a sample bank
- Following the initial validation and implementation of an everolimus test method, each laboratory receives a set of 3 blinded samples on a monthly basis.
- All samples are shipped on dry ice to minimize freeze-thaw cycles (always ≤ 2)
- These samples include individual patient samples, patient sample pools to assess long-term performance and patient samples/pools enriched with isolated everolimus metabolites.

The www.zortracker.com Website:



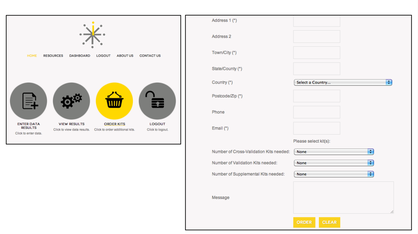
Allows laboratories to:

- register for program
- request validation kits
- request supplementary patient sample kits
- request cross validation kits
- submit assay results for above kits
- view and review collated data and associated statistical analyses

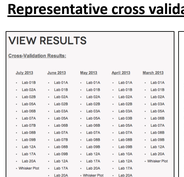
Site also provides:

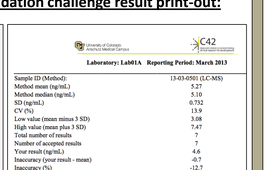
- TDM related information and resources
- TDM discussion forum

Ordering process for kits:



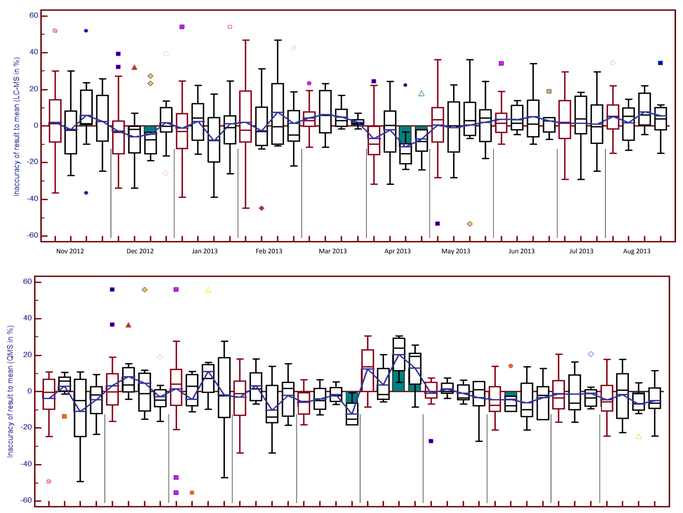
Representative cross validation challenge result print-out:





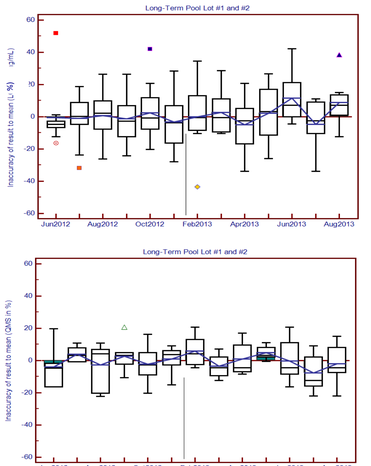
Results:

1)



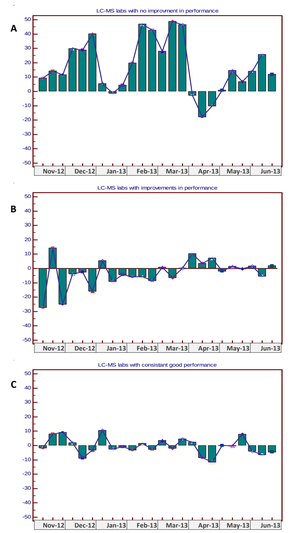
Box and Whisker blots of LC-MS/MS (top) and QMS (bottom) inaccuracy results show similar performance. Relative inaccuracies [%] of the individual results to the total mean were used to create a "time-dependant inaccuracy plot", shown in these Whisker blots. The outliers are presented as dots in the graphs. Comparison of these Box and Whisker blots of LC-MS/MS and QMS inaccuracy results showed similar performance, with a trend towards higher inter-laboratory variability among laboratories using LC-MS/MS than the QMS assay.

2)



Box and Whisker blots of LC-MS/MS and QMS long-term pools were comparable. Inaccuracies of the individual results to the total mean were used to create "time-dependant inaccuracy plots", shown in these Whisker blots. The outliers are presented as dots in the graphs. For this long term pool the monthly inter-laboratory variability (CV%) ranged from 14.9-19.1% (LC-MS/MS) and 4.8-15.4 (QMS).

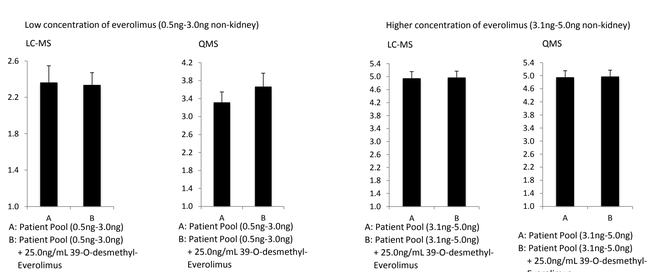
3)



LC-MS/MS laboratory performance patterns over time. Laboratories could be classified into the following three types of laboratories:
- Consistently irregular performers (A)
- Initial irregular performers improving over time (B) and
- Consistently good performers (C)

It was interesting to note that the consistently irregular performing laboratories were responsible for most of the inter-laboratory variability of the LC-MS/MS laboratories. On the other hand, consistently good performing LC-MS/MS laboratories matched the inter-laboratory variability of the QMS laboratories.

4)



LC-MS/MS and QMS results of everolimus concentrations showed similar results when purified everolimus metabolites were added to patient sample pools. Enrichment of patient sample pools with purified the major everolimus metabolites 39-O-desmethyl everolimus resulting in clinically relevant concentrations (1-5 ng/mL) did not affect the results of either LC-MS/MS or QMS assays. In addition, patient sample pools enriched with 46-hydroxy or 12,24-dihydroxy everolimus were tested (not shown) and did not affect the results of QMS or LC-MS/MS assays.

Summary:

- Both LC-MS/MS and QMS everolimus assays gave similar results and showed similar performance.
- Presence of major everolimus metabolites did not significantly impact the performance of either LC-MS/MS or QMS everolimus assays
- Laboratories using LC-MS/MS showed a trend towards higher inter-laboratory variability in comparison to the QMS everolimus assay that interestingly seemed to be caused by a few consistently irregular performing laboratories.

Conclusions and Next Steps:

- LC-MS/MS and QMS are both acceptable assays for the TDM of everolimus in transplant patients
- The Zortracker program and its resources have been successful to facilitate the proper validation of laboratories implementing everolimus testing, and to improve the performance of several LC-MS/MS laboratories over time.
- The Zortracker program and study will continue to enroll laboratories over the next years. Participation in this program is free of charge.