Cross-validation of Everolimus LC-MS/MS and Thermo QMS Therapeutic Drug Monitoring Assays – The Zotracker 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.

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 retail 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.zotracker.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
- review and view collated data and associated statistical analyses

Site also provides:
- TDM related information and resources
- TDM discussion forum

Ordering process for kits:

- Guidelines for ordering process
- View and review collated data and associated statistical analyses
- Submit assay results for above kits

Results:

1) Ordering process for kits:

- Guidelines for ordering process
- View and review collated data and associated statistical analyses
- Submit assay results for above kits

2) Box and Whisker plots of LC-MS/MS (top) and QMS (bottom) measuring results show similar performance. Notation: interquartile (IQR) of the distribution (e.g. the IQR of IQR) is the middle part of the box. The horizontal line inside of each box is the median (50th percentile). The whiskers extend to the most extreme data points that are not outliers. Outliers are shown as separate data points. In this case, the IQR of IQR for the LC-MS/MS and QMS results closely showed similar performance, with a trend towards higher inter-laboratory variability among laboratories using LC-MS/MS than the QMS assay.

3) Box and Whisker plots of LC-MS/MS and QMS long-term park shows comparable. In case of the median results of the total means were used to create ‘time-dependent inaccuracy plots’, shown in these Whisker plots. The authors are presented as data in the graphs. For this long term test the monthly, inter-laboratory variability (IQR) ranged from 1.8 to 1.6 ng/mL in the QMS assay and 1.6 to 1.5 ng/mL in the LC-MS/MS assay.

4) Representative cross validation challenge result print-out:

- Assay results for each laboratory are collated and compared to the median results.
- Data is presented in a graphical format to demonstrate the performance of the laboratories.

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 Zotracker 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 Zotracker program and study will continue to enroll laboratories over the next years. Participation in this program is free of charge.