

Introduction of an Everolimus challenge sample in an immunosuppressant EQA program



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Introduction

The RCPAQAP offers an immunosuppressant program using a drug-free whole blood base spiked with parent drugs (Cyclosporin, Sirolimus, and Tacrolimus). 24 samples are provided across six linearly related levels (from low, Level 1 to high, Level 6). Within the survey year, each level is run 4 times. While Everolimus is a commonly prescribed immunosuppressant, potential interference with other immunosuppressants has limited its inclusion in the program to date. In 2022, Everolimus was spiked into a mid-level sample (Level 3) as part of a “challenge” to assess potential cross-reactivity and options to include in future programs.

Method

Everolimus was spiked (at a concentration of 11 µg/L) into two of the Level 3 immunosuppressant samples. The two equivalent samples (without Everolimus) were scheduled to run in parallel with the spiked samples.

61 laboratories were enrolled in the 2022 Special therapeutic drugs program.

- 5 LC-MS/MS users reported for both Everolimus and Sirolimus.
- 1 LC-MS/MS user reported for Sirolimus only.
- 1 LC-MS/MS user reported for Everolimus only.
- All of the 7 immunoassay users (1 Roche, 6 Abbott) reported for Sirolimus only.

The medians for each measurand in the immunosuppressant program submitted by participating laboratories were compared using RCPAQAP in-house software. The Analytical Performance Specifications for Everolimus were set at +/- 10% by the AACB-RCPAQAP Special Therapeutic drugs committee.

Results and Discussion

The Sirolimus immunoassay median for the spiked challenge sample (22-04) was 24.3 µg/L compared to 10.7 µg/L for the LC-MS/MS methods (Table 1). The LC-MS/MS Everolimus median was 12.6 µg/L which was consistent with the weighed-in spike (Table 1). There was no interference detected in the LC-MS/MS Sirolimus methods with medians of 10.4 µg/L and 10.7 µg/L in the unspiked and spiked samples, respectively (Table 1). These results confirmed that Sirolimus immunoassay methods have a significant (up to 100%) cross-reactivity with Everolimus in spiked samples (see also Figures 1–3). There was no evidence of Everolimus cross-reactivity with either Cyclosporin or Tacrolimus immunoassay methods (<0.5% difference in medians, Table 1). While there were no results submitted for Everolimus immunoassays, we note, for example, the Thermo Fisher reagent is only for use in patients that have not had any recent doses of Sirolimus due to similar cross-reactivity issues.¹

Conclusion

While combinations of Sirolimus and Everolimus (both mTOR inhibitors) are not commonly prescribed for patients, there is a potential risk of inappropriate dosing in those patients when measuring Sirolimus or Everolimus using immunoassays. LC-MS/MS is recommended for Sirolimus / Everolimus therapeutic drug monitoring.

References:

1. Thermo Fisher QMS Everolimus (EVER) IFU 2019. <https://assets.thermofisher.com/TFS-Assets/CDD/Package-Inserts/0160060-QMS-Everolimus-Assay-ROW-EN.pdf> (Accessed 30 Sep 2022).

Table 1. Comparison of Immunoassay and LC-MS/MS medians for all Immunosuppressant measurands.

	Sirolimus			Everolimus			Cyclosporin			Tacrolimus		
	n	Sample 22-03	Sample 22-04*	n	Sample 22-03	Sample 22-04*	n	Sample 22-03	Sample 22-04*	n	Sample 22-03	Sample 22-04*
Immunoassay Median ug/L	7	11.5	24.3	0	N/A	N/A	43	431	430	48	11.6	11.6
LC-MS/MS Median ug/L	6	10.4	10.7	6	<1.0	12.6	8	473	469	7	10.9	11.2

* Sample 22-04 is a duplicate of 22-03, with the addition of an Everolimus spike.

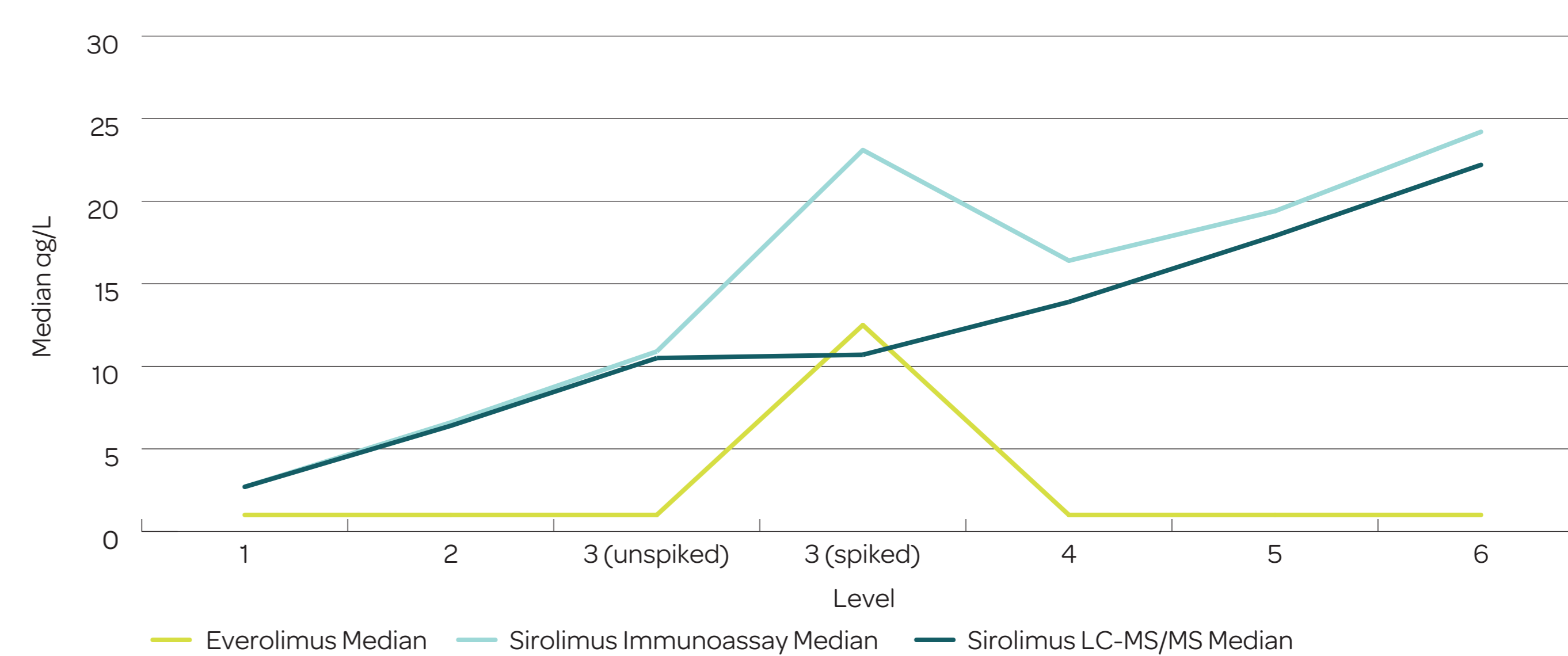


Figure 1. Sirolimus and Everolimus medians for LC-MS/MS and Immunoassays for levels 1 to 6. Level 3 unspiked (Sample 22-03) is a duplicate of level 3 spiked (Sample 22-04).

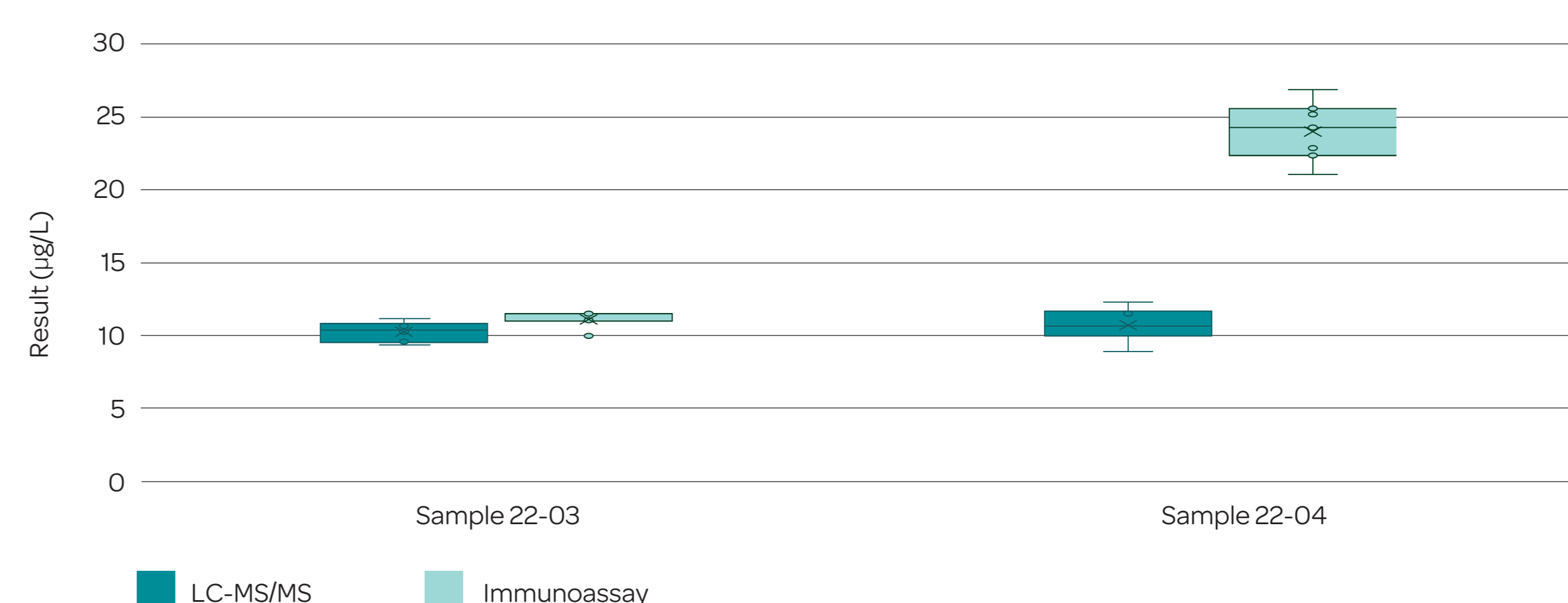


Figure 2. Box and whisker plot summarising Sirolimus results for duplicate samples 22-03 and 22-04, where 22-03 is unspiked and 22-04 is spiked with Everolimus.

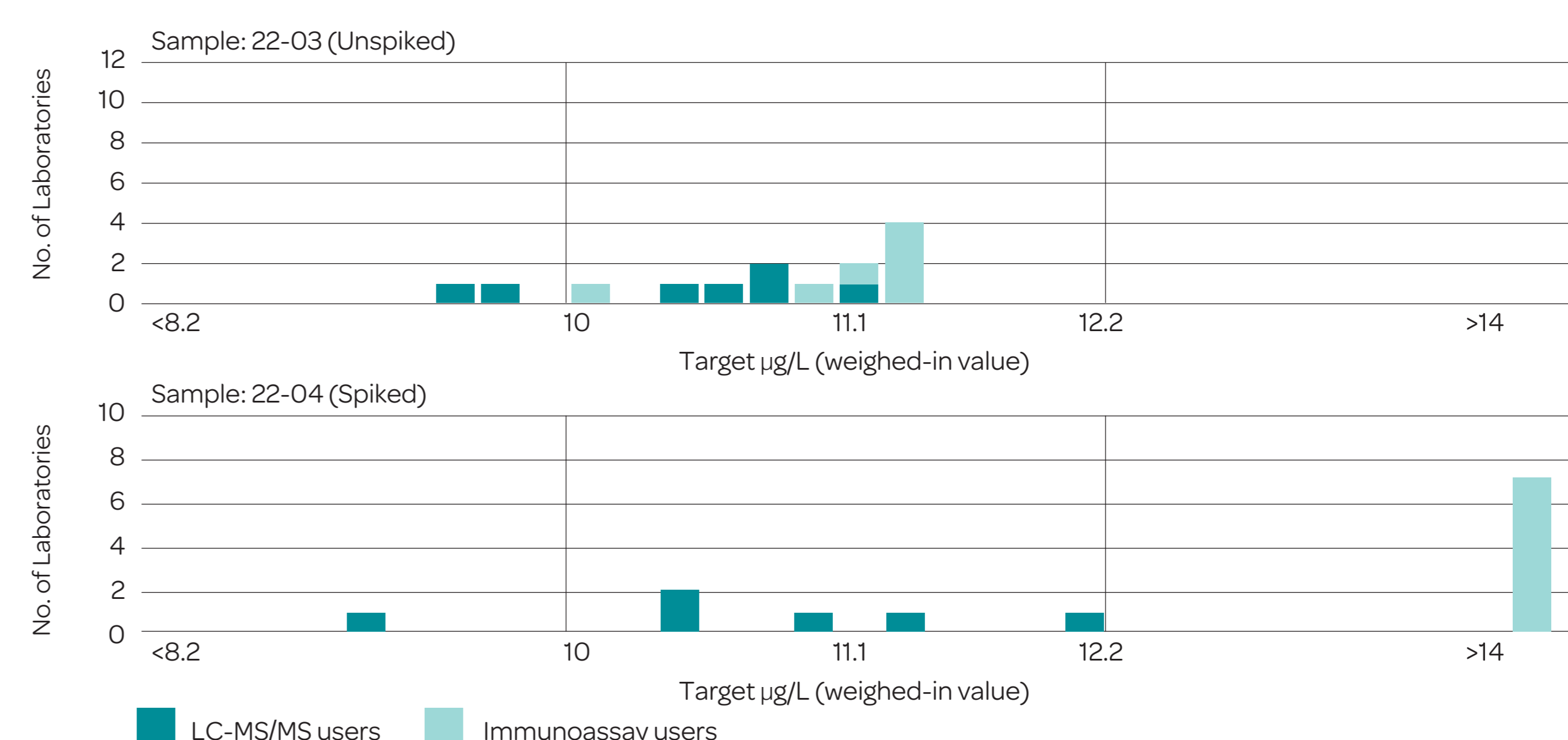


Figure 3. Distribution of all Sirolimus results for unspiked (sample 22-03) and spiked challenge sample spiked with Everolimus (sample 22-04). Light blue represents the Immunoassay users and dark blue represents the LC-MS/MS users. The light blue bar for sample 22-04 highlights the positive interference Everolimus has on the Sirolimus measurement for the Immunoassay group.