



Evaluation of the Serodyn Everolimus Immunoassay for the Abbott TDx/Flx.



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Introduction

- ❖ Everolimus, also known as RAD (Rapamycin Analogue Derivative) is a macrolide immunosuppressant derived by chemical modification of the natural product rapamycin (sirolimus).
- ❖ The Serodyn Everolimus kit trades as Innofluor® Certican® Assay System.
- ❖ The assay protocol recommends single extractions for patient samples, calibrators and quality control materials. The supernatants are run in duplicate on the analyser.
- ❖ The therapeutic interval is 3 – 8 ng/mL.



Aim

- ❖ The aim of this study was to validate the Serodyn Everolimus assay on the TDx/FLx for routine analysis of clinical samples.

Methods

Precision Studies:

Study 1 - Within-run analytical precision

- Precision of replicate measurements of the same extraction (QC material). N=63

Study 2 - Between-extraction precision

- Precision of repeat extractions, 3 levels of QCs were extracted in triplicate over 3 days.

Study 3 - Between-run precision

- Precision of QC measurements on separate days. The data was analysed for average of 2 measurements (same extraction) and using only the first measurement. N=32

Study 4 - Patient duplicate analyses

- Within-run analytical precision for patient samples. N=249.

Study 5 - Functional Sensitivity

- Between-day precision of patient pool at 2 ng/mL.

The QC materials are whole blood hemolysates spiked with 3 levels of everolimus (Innofluor® Certican® Control set Cat # 0373399). The Innofluor® Certican® kit is Cat #0373290.

Linearity

Linearity was assessed with serial dilutions of the highest kit calibrator and everolimus free blood.

Cross-reactivity

Cross-reactivity was measured using samples with known concentrations of tacrolimus, cyclosporin and sirolimus.

Accuracy relative to LCMS has been addressed elsewhere¹

Results (1)

Precision

- ❖ At QC material values of approximately 4 ng/mL, 12 ng/mL and 25 ng/mL the within-run precision showed CVs of <5% at all levels (n=63). Table 1.
- ❖ The between-extraction CVs were < 5.5% at all levels (n=3 in triplicate). Table 2.
- ❖ The total precision was <8% at all levels using single measurements (n=32). Table 3.
- ❖ Within-run analytical precision for patient samples was <7% for all samples >3 ng/mL. Table 4.
- ❖ The results of the functional sensitivity study showed at an everolimus level of 1.97ng/mL the CV is 15.5%.

COMMENT: Precision using singlicate measurement of QC and patient material was acceptable for use. This data is summarised in Figure 1.

	L1	L2	L3
Count	64	63	65
Average	4.4	12.5	24.3
SD	0.22	0.54	1.02
CV	5.0%	4.3%	4.2%

Table 1. Within-run precision based on duplicate analysis of QC samples (Study 1)

	L1	L2	L3
Concentration	4.8	11.9	24.7
Day 1 CV	3.2%	3.9%	4.2%
Day 2 CV	2.1%	1.4%	4.3%
Day 3 CV	3.1%	1.2%	5.3%
Average CV	2.8%	2.2%	4.6%

Table 2. Between-extraction precision based on QC samples (Study 2).

	L1	L2	L3
Count	32	33	33
Average	4.4	13.2	25.7
SD (1)	0.37	1.08	2.05
CV (1)	8.5%	8.1%	8.0%
SD (2)	0.37	1.02	2.04
CV (2)	8.4%	7.6%	7.9%

Table 3. Between-run precision based on QC samples (Study 3). (1) singlicate measurements; (2) average of duplicate measurements.

	Bin 1	Bin 2	Bin 3	Bin 4	Bin 5
Bin range	0 - 3	3 - 5.5	5.5 - 8.0	8 - 12.0	12 - 50
n	12	70	58	65	44
Average	2.0	4.4	6.7	9.8	14.8
CV	10.3%	6.2%	7.0%	5.1%	4.7%

Table 4. Within-run analytical precision based on patient samples (Study 4)

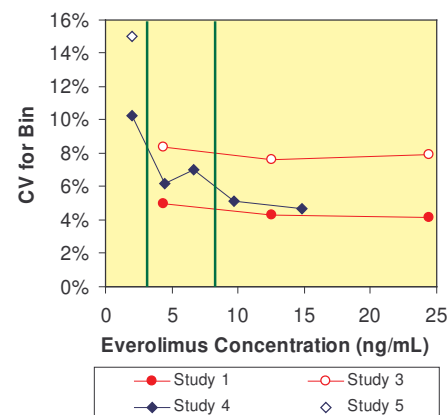


Figure 1. Precision profiles. Red data – QC material; Blue data – patient samples; Closed symbols – within run; Open symbols – between run. Green lines - therapeutic interval.

Results (2)

Linearity

- ❖ The linearity study gave recoveries from 72 – 108% with linearity to the highest standard tested. Table 5.

Cross-reactivity

- ❖ No cross-reactivity was seen with tacrolimus up to 30ug/L mg/L or cyclosporin up to 730ug/L. However cross-reactivity between 35 and 128% was found with sirolimus concentrations between 2 and 12.8ug/L.

COMMENT: At therapeutic concentrations of sirolimus, cross reactivity was significant in the everolimus assay.

Dilution	Expected results	Observed results	Recovery %
Neat	42.3	40.9	97
1/2	21.1	22.8	108
1/4	10.6	10.2	97
1/8	5.3	4.5	85
1/16	2.6	1.9	72

Table 5. Linearity Study.

Conclusions

- ❖ The results indicate that the Serodyn Everolimus assay is acceptable for routine clinical use on the parameters tested here.
- ❖ The data supports analysis in singlicate rather than the recommended duplicate.
- ❖ False positive results may occur in patients receiving sirolimus treatment.
- ❖ The variability in extraction of patient samples at low concentrations initially raised doubts about the claimed limit of quantitation however further analyses with the functional sensitivity study supports the manufacturer's claim (2ng/mL).
- ❖ Any low everolimus results should be repeated to minimise false negatives.

(1) Salm P et al, Ther Drug Monit, 2005. Vol 27, p251-252