Development and evaluation of i-Tracker Rituximab and i-Tracker Anti-Rituximab kits: fast and innovative chemiluminescent assays for the monitoring of patients treated with Rituximab

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INTRODUCTION

Rituximab is a chimeric monoclonal antibody directed against CD20 molecule expressed on the surface of B-cell. Rituximab is a drug widely used for the treatment of patients with non-Hodgkin's lymphoma, but also indicated for the treatment of active and severe rheumatoid arthritis. Therapeutic Drug Monitoring is currently proposed to provide useful information to clinicians to improve the efficacy of the treatment. Theradiag has just developed the innovative **i-Tracker** kits: fast quantification of Rituximab and Anti-Rituximab antibodies fully automated on the random access **i-Track[10]** chemiluminescent analyzer.

MATERIALS & METHODS

MATERIALS:

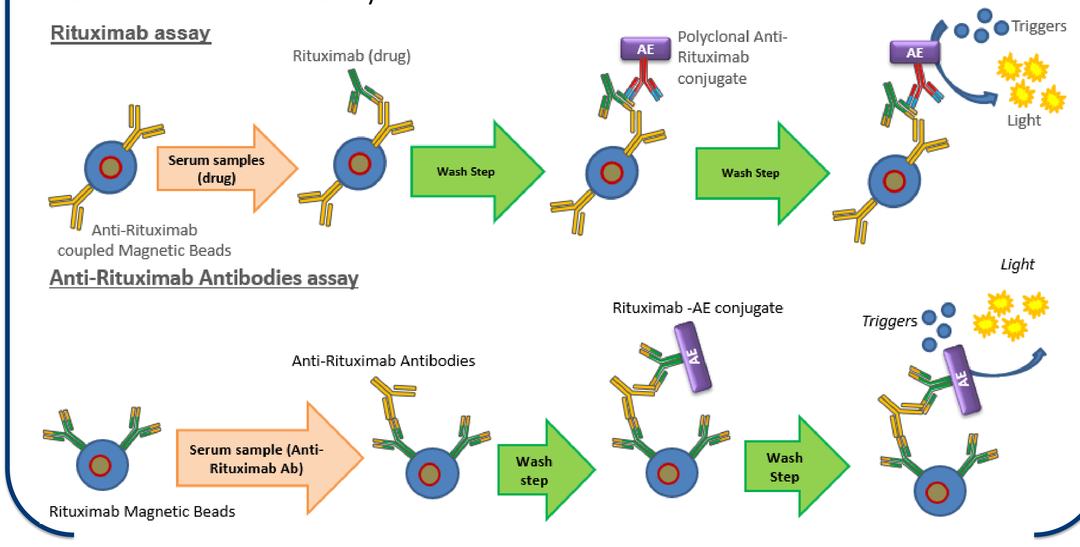
Rituximab SPIKED SAMPLES: 3 human serum matrices (from healthy donors) were used. The drug, Rituximab pharmaceutical solution (10 mg/mL), was spiked into these 3 matrices to reach 5 concentration levels spanning the dynamic range of the assay (0.5, 1.5, 6, 25 and 45 µg/mL). A total of 15 spiked samples were produced. % of recovery was calculated according to the following formula: *(quantified concentration/spiked concentration) x 100.*

CLINICAL SAMPLES: 51 serum samples from rheumatoid arthritis patients treated with Rituximab were collected. They arrived frozen and kept frozen until quantification at Theradiag. Additionally, 46 serum samples, previously quantified for Anti-Rituximab antibodies with LISA TRACKER Anti-Rituximab assay (#LTR 003, Theradiag) were used for correlation assessment.

i-Tracker Rituximab kit: composed of monoclonal Anti-Rituximab antibody (anti-idiotype) coated magnetic beads, polyclonal Anti-Rituximab antibodies conjugated to acridinium ester, and sample dilution buffers. **i-Tracker** Anti-Rituximab kit: composed of Rituximab coated magnetic beads, Rituximab conjugated to acridinium ester, and sample dilution buffer. Both types of kit contain 2 calibrators and 1 positive control dedicated for the calibration process (validation of the calibration vs. master curve) and for the validation of the run, respectively. Once performed, calibration is valid for 21 days.

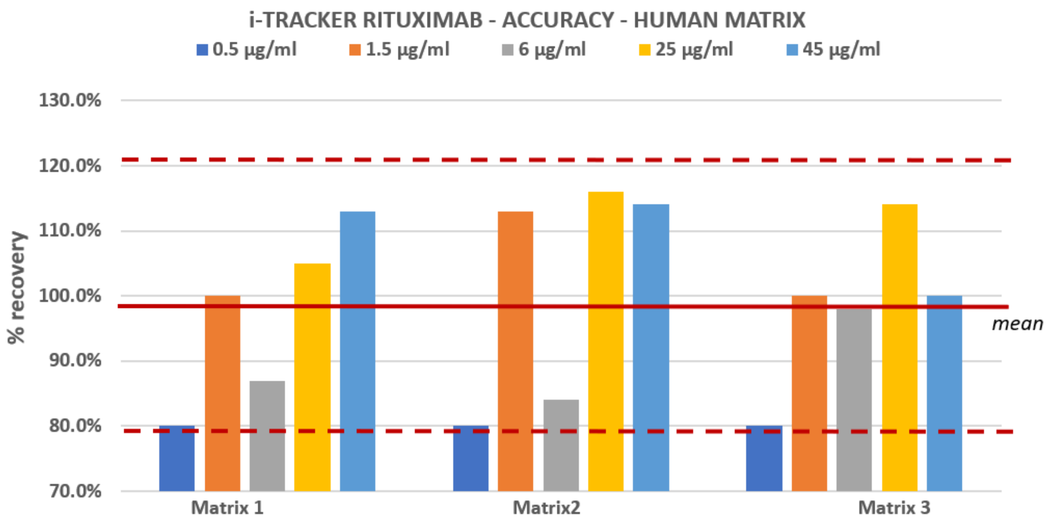
METHODS:

i-Tracker CHEMILUMINESCENT ASSAYS: quantification of Rituximab and Anti-Rituximab antibodies were performed with the **i-Track[10]** chemiluminescent analyzer according to the technical insert of **i-Tracker** kits (#CTR 002 and #CTR 003 respectively). Briefly, serum samples were diluted and added to the coated magnetic beads suspension. After incubation of 15 minutes at +37°C, beads were washed and acridinium ester (AE) conjugate was added. After 15 minutes of incubation at +37°C, beads were washed, and triggers were added. Instantly, relative light emissions (RLU) were detected and quantified by **i-Track[10]** chemiluminescent analyzer. Concentrations of Rituximab and Anti-Rituximab antibodies were calculated according to the calibration curve provided with the kit (master curve). The lower and the upper limits of quantification are 0.3 µg/mL and 60 µg/mL for **i-Tracker** Rituximab assay, 10 ng/mL and 2000 ng/mL for **i-Tracker** Anti-Rituximab assay.



RESULTS 1/2

ACCURACY *(see figure below)*: 15 Rituximab spiked samples were quantified with **i-Tracker** Rituximab. The % of recovery were comprised between 80% and 116% (mean % of recovery was 99%).



Conclusion:
*The acceptance criteria were met (% recovery comprised +/- 20% of spiked concentrations for > 80% samples). Quantification of Rituximab with **i-Tracker** Rituximab is not affected by serum matrix.*

RESULTS 2/2

LLOQ (Lower Limit Of Quantification): on one hand, 118 serum samples from untreated patients were quantified with **i-Tracker** Rituximab: all samples were found below the selected LLOQ of 0.3 µg/mL. On the other hand, 119 samples from untreated patients were quantified with **i-Tracker** Anti-Rituximab : all samples were found below the selected LLOQ of 10 ng/mL.

INTRA-RUN PRECISION *(see figures on the right):* for both assays, 5 clinical samples spanning the dynamic range of the respective assays were quantified 10 times within a run. The coefficients of variation (CV) were calculated for each sample: the CV ranged from 2% to 6.8% for Rituximab assay and between 0.7% and 4.5% for Anti-Rituximab assay.

RITUXIMAB ASSAY			
ID	results (µg/ml)	mean (µg/mL)	CV
Sample 1	0.6	0.6	6.8%
	0.6		
	0.6		
	0.6		
	0.7		
	0.6		
	0.6		
	0.6		
	0.6		
	0.7		
Sample 2	1.7	1.8	4.0%
	1.7		
	1.7		
	1.7		
	1.8		
	1.7		
	1.7		
	1.8		
	1.8		
	1.9		
Sample 3	7.1	7.5	3.2%
	7.1		
	7.4		
	7.5		
	7.7		
	7.5		
	7.6		
	7.5		
	7.5		
	7.9		
Sample 4	17.3	18.1	4.2%
	17.9		
	17.8		
	18.7		
	18.8		
	16.7		
	17.9		
	19.3		
	17.9		
	18.2		
Sample 5	37.1	38.0	2.0%
	38.0		
	38.6		
	39.7		
	37.3		
	38.2		
	37.6		
	38.0		
	37.4		
	38.1		

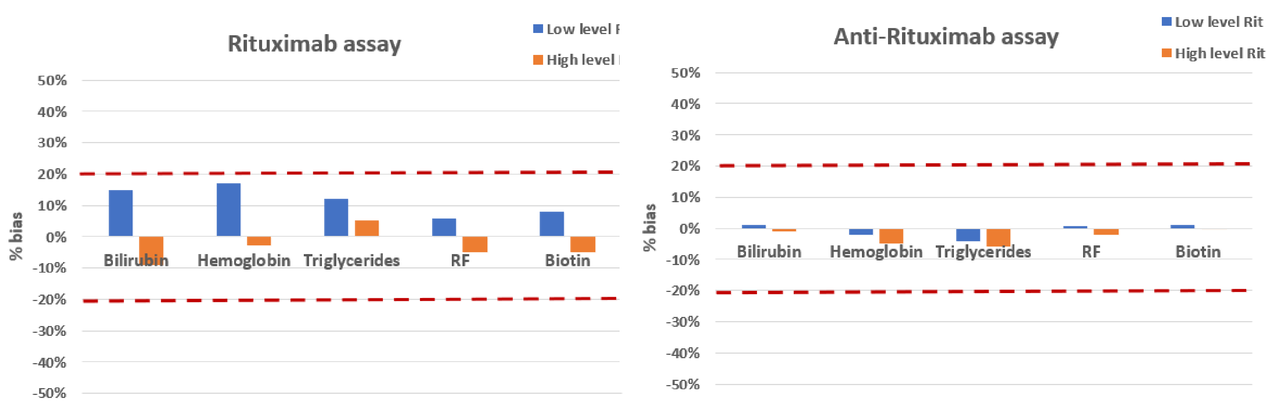
ANTI-RITUXIMAB ASSAY			
ID	results (ng/ml)	mean (ng/mL)	CV
Sample 1	11	11	2.8%
	11		
	12		
	11		
	11		
	11		
	11		
	11		
	11		
	11		
Sample 2	116	117	0.7%
	117		
	118		
	118		
	116		
	118		
	116		
	117		
	117		
	117		
Sample 3	384	372	1.9%
	380		
	376		
	365		
	372		
	370		
	368		
	360		
	369		
	375		
Sample 4	710	730	4.5%
	734		
	719		
	707		
	723		
	706		
	716		
	703		
	797		
	782		
Sample 5	1 208	1246	3.8%
	1 236		
	1 295		
	1 234		
	1 222		
	1 176		
	1 338		
	1 261		
	1 274		
	1 216		

INTER-RUN PRECISION *(see figures on the right):* for both assays 5 clinical samples spanning the dynamic range of the respective assays were quantified on 6 independent runs. The coefficients of variation (CV) were calculated for each sample: the CV ranged from 2.0% to 6.7% for Rituximab assay, and CV ranged from 2.2% to 4.4% for Anti-Rituximab assay. The acceptance criteria (CV<20%) was met. High precision is reached with **i-Tracker** Rituximab assay and **i-Tracker** Anti-Rituximab assay.

RITUXIMAB ASSAY								
RUNS	1	2	3	4	5	6	Total Mean (µg/ml)	CV
Sample1 (low)	1.6	1.6	1.6	1.6	1.6	1.5	1.6	2.6%
Sample2 (low)	6.3	6.1	6.2	6.2	6.2	5.9	6.1	2.0%
Sample3 (mid)	14.2	14.2	14.2	14.4	13.7	13.5	14.0	2.4%
Sample4 (high)	34.5	29.7	30.5	30.6	30.6	28.4	30.7	6.7%
Sample5 (high)	46.9	47.6	44.0	44.9	42.5	42.7	44.7	4.7%

ANTI-RITUXIMAB ASSAY								
RUNS	1	2	3	4	5	6	Total Mean (ng/ml)	CV
Sample1 (low)	64	66	69	70	68	65	36	4.4%
Sample2 (mid)	120	122	126	121	122	119	198	4.3%
Sample3 (mid)	393	381	405	388	387	385	686	3.4%
Sample4 (high)	832	815	835	808	813	806	979	2.2%
Sample5 (high)	1496	1415	1439	1405	1370	1379	1202	2.5%

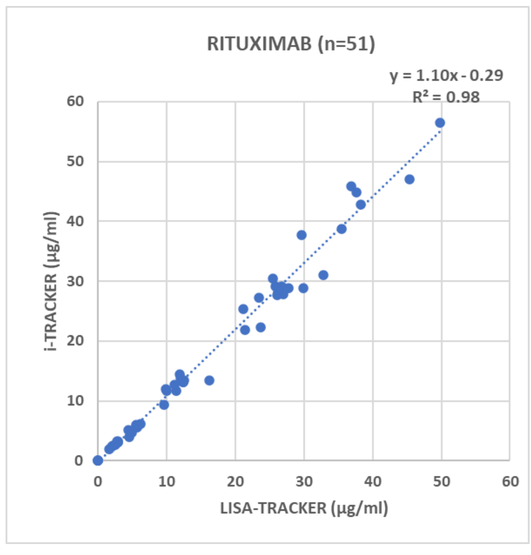
INTERFERENCES *(see figures below)*: spiked samples (low and high level) were made with Rituximab and Anti-Rituximab antibodies with or without the presence of potential interfering agents, as bilirubin, hemoglobin, triglycerides, rheumatoid factors (RF) and biotin. Rituximab spiked samples spiked with potential interfering agents were quantified with **i-Tracker** Rituximab kit and compared to results obtained with Rituximab spiked samples. Same method was performed with Anti-Rituximab antibodies spiked samples. The percentages of bias (% of variation between samples with/without interfering agents) were low (within +/- 20%).

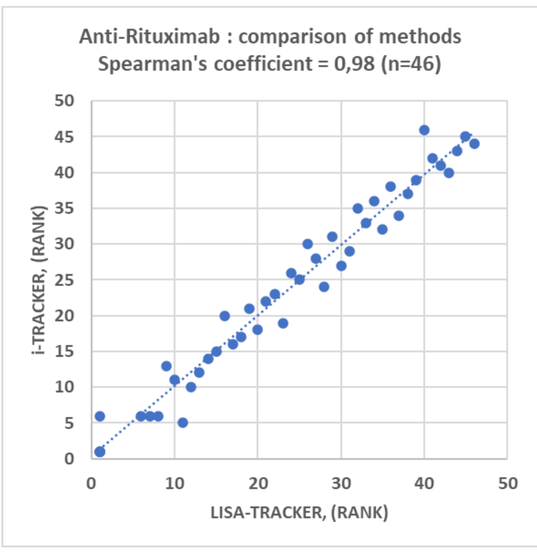


***Conclusion: i-Tracker** assays are not disrupted by the presence of biologic agents as bilirubin (0.2mg/ml), hemoglobin (2mg/ml), triglycerides (33mg/ml), rheumatoid factors (1000 AU/ml) and biotin (2µg/ml).*

CORRELATIONS *(see figure below)*: on one hand, 51 clinical samples (from rheumatoid arthritis patients) were quantified for Rituximab with **i-Tracker** Rituximab and LISA TRACKER Rituximab (Theradiag). Concentrations were plotted on a "x/y" axis and a linear regression was performed. High correlation was observed: $R^2 = 0.98$ and slope = 1.10.

On the other hand, 46 samples were quantified for Anti-Rituximab antibodies with **i-Tracker** Anti-Rituximab and LISA TRACKER Anti-Rituximab (Theradiag). For both assays, concentrations were ranked, and high correlation was observed: Spearman's coefficient was found at 0.98 *(see figure below).*





CONCLUSION : i-Tracker Rituximab and i-Tracker Anti-Rituximab kits are innovative assays which exhibit fast, accurate and reproducible results. Excellent agreements were observed with LISA TRACKER assays. i-Tracker kits are valuable tools for the monitoring of patients treated with Rituximab and allowing rapid treatment adjustment.