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

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INTRODUCTION

Golimumab, a monoclonal antibody directed against TNFα, is a drug widely used for the treatment of inflammatory diseases (ulcerative colitis, Crohn's disease, rheumatoid arthritis, ankylosing spondylitis, …). 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 Golimumab and Anti-Golimumab antibodies fully automated on the random access **i-Track[10]** chemiluminescent analyzer.

MATERIALS & METHODS

MATERIALS:

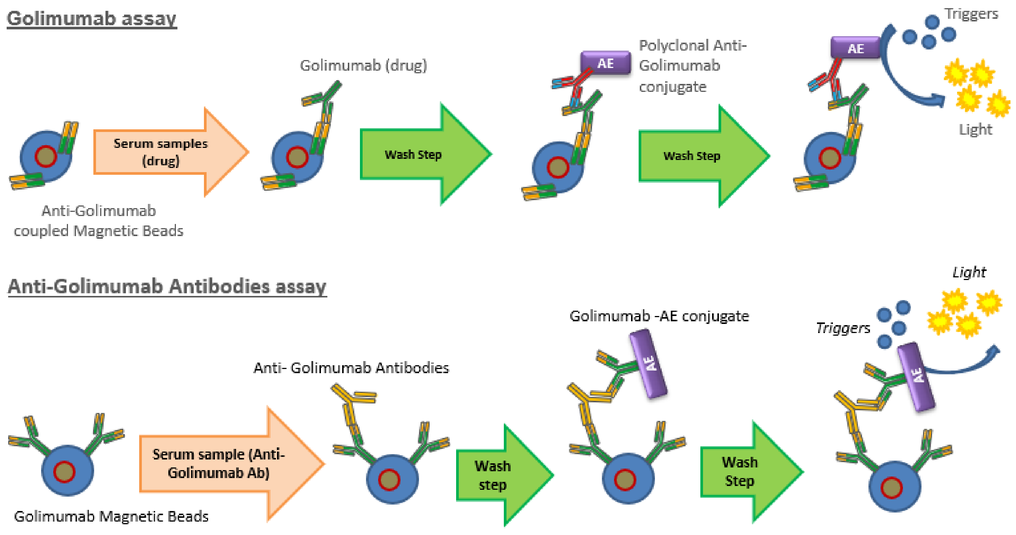
Golimumab SPIKED SAMPLES: 3 human serum matrices (from healthy donors) were used. The drug, Golimumab pharmaceutical solution (100 mg/mL), was spiked into these 3 matrices to reach 5 concentration levels spanning the dynamic range of the assay (0.5, 1, 3, 4.5 and 6 µg/mL). A total of 15 spiked samples were produced. % of recovery was calculated according to the following formula : *(quantified concentration/spiked concentration) x 100.*

CLINICAL SAMPLES: 60 serum samples from Crohn's disease patients treated with Golimumab were collected. They arrived frozen and kept frozen until quantification at Theradiag. Additionally, 24 serum samples, previously quantified for Anti-Golimumab antibodies with LISA TRACKER Anti-Golimumab assay (#LTG 005, Theradiag) were used for correlation assessment.

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

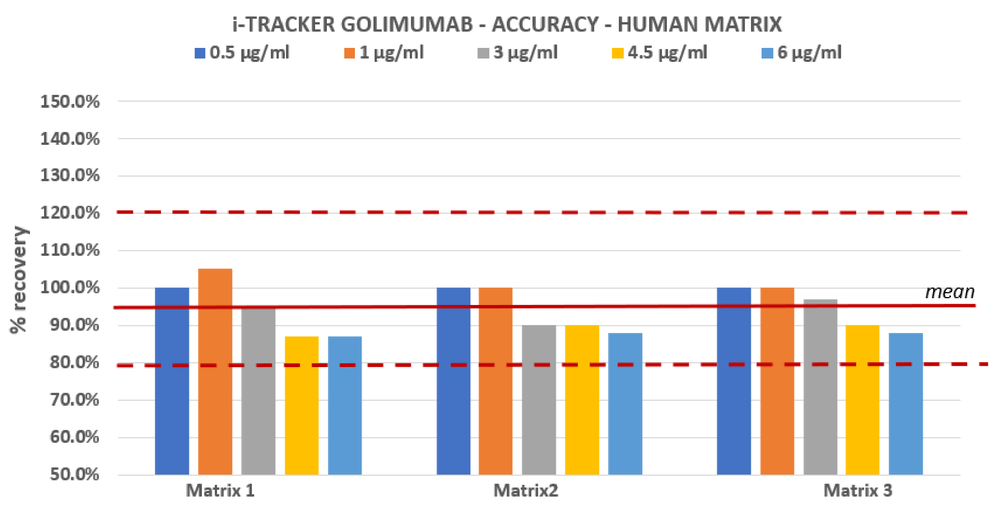
METHODS:

i-Tracker CHEMILUMINESCENT ASSAYS: quantification of Golimumab and Anti-Golimumab antibodies were performed with the **i-Track[10]** chemiluminescent analyzer according to the technical insert of **i-Tracker** kits (#CTG 002 and #CTG 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 Golimumab and Anti-Golimumab 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 8 µg/mL for **i-Tracker** Golimumab assay, 10 ng/mL and 2000 ng/mL for **i-Tracker** Anti-Golimumab assay.



RESULTS 1/2

ACCURACY *(see figure below)*: 15 Golimumab spiked samples were quantified with **i-Tracker** Golimumab. The % of recovery were comprised between 87% and 105% (mean % of recovery was 95%).



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

RESULTS 2/2

LLOQ (Lower Limit Of Quantification): on one hand, 115 serum samples from untreated patients were quantified with **i-Tracker** Golimumab: all samples were found below the selected LLOQ of 0.3 µg/mL. On the other hand, 108 samples from untreated patients were quantified with **i-Tracker** Anti-Golimumab : 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 0% to 11.5% for Golimumab assay and between 1.3% and 11.9% for Anti-Golimumab assay.

Golimumab			
ID	results (µg/ml)	mean (µg/mL)	CV
Sample 1	0.4	0.4	8.1%
	0.3		
	0.4		
	0.4		
	0.4		
	0.4		
	0.4		
	0.4		
	0.4		
	0.4		
Sample 2	1.0	1.0	0.0%
	1.0		
	1.0		
	1.0		
	1.0		
	1.0		
	1.0		
	1.0		
	1.0		
	1.0		
Sample 3	2.7	2.6	4.2%
	2.5		
	2.8		
	2.6		
	2.7		
	2.6		
	2.6		
	2.6		
	2.6		
	2.4		
Sample 4	4.3	4.1	11.5%
	3.5		
	3.5		
	4.5		
	3.7		
	4.2		
	4.5		
	4.2		
	4.7		
	3.5		
Sample 5	6.2	6.3	2.0%
	6.1		
	6.5		
	6.2		
	6.2		
	6.3		
	6.2		
	6.4		
	6.2		
	6.4		

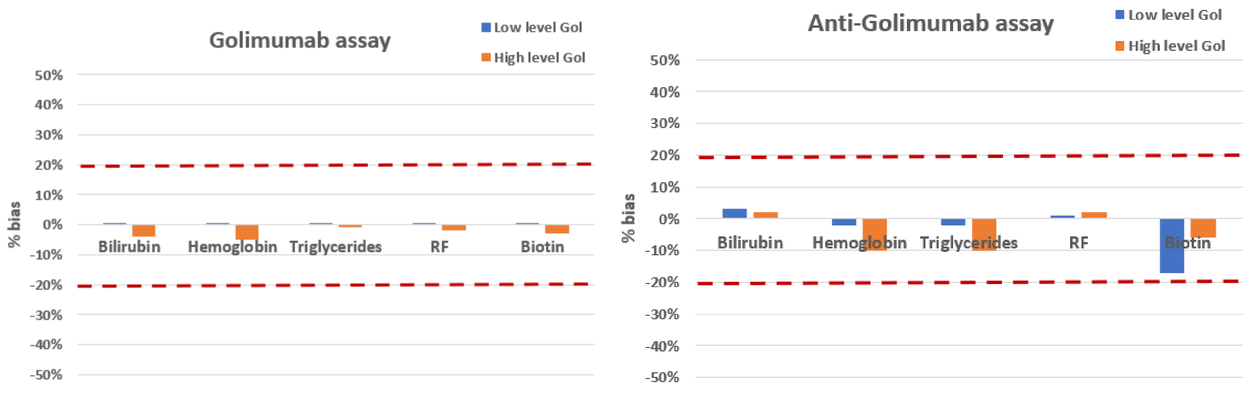
Anti-Golimumab			
ID	results (ng/ml)	mean (ng/mL)	CV
Sample 1	42	41	1.3%
	40		
	42		
	41		
	41		
	40		
	41		
	41		
	40		
	40		
Sample 2	78	77	1.4%
	77		
	78		
	75		
	78		
	77		
	76		
	77		
	77		
	77		
Sample 3	202	178	11.9%
	127		
	167		
	183		
	190		
	178		
	194		
	166		
	185		
	185		
Sample 4	760	765	1.3%
	763		
	757		
	780		
	753		
	751		
	769		
	777		
	766		
	771		
Sample 5	1 319	1352	1.9%
	1 319		
	1 344		
	1 378		
	1 344		
	1 405		
	1 357		
	1 344		
	1 347		
	1 363		

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 1.6% to 2.3% for Golimumab assay, and CV ranged from 0% to 4.4% for Anti-Golimumab assay. The acceptance criteria (CV<20%) was met. High precision is reached with **i-Tracker** Golimumab assay and **i-Tracker** Anti-Golimumab assay.

GOLIMUMAB ASSAY								
RUNS	1	2	3	4	5	6	Total Mean (µg/ml)	CV
Sample1 (low)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	3.2%
Sample2 (low)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0%
Sample3 (mid)	2.6	2.6	2.6	2.6	2.6	2.7	2.6	1.9%
Sample4 (high)	4.5	4.2	4.0	4.3	4.5	4.4	4.3	4.4%
Sample5 (high)	6.2	6.4	6.1	6.3	6.3	6.3	6.3	1.6%
Sample6 (high)	7.9	7.9	8.0	8.1	8.1	8.1	8.0	1.2%

ANTI-GOLIMUMAB ASSAY								
RUNS	1	2	3	4	5	6	Total Mean (ng/ml)	CV
Sample1 (low)	36	38	38	38	37	38	37	2.2%
Sample2 (mid)	65	68	70	70	75	74	70	5.2%
Sample3 (mid)	168	178	181	183	178	178	177	2.9%
Sample4 (high)	709	731	734	745	736	743	733	1.8%
Sample5 (high)	1311	1339	1387	1367	1397	1379	1364	2.4%

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

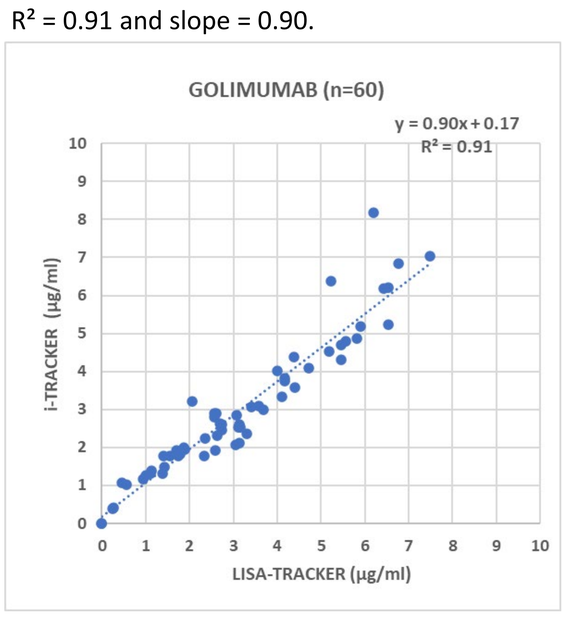


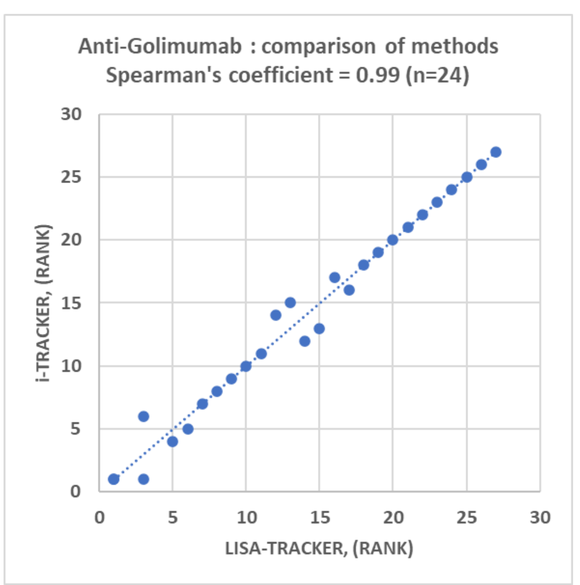
***Conclusion: i-Tracker** assays are not disrupted by the presence of biologic agents as bilirubin (0.2 mg/mL), hemoglobin (2 mg/mL), triglycerides (33mg/ml), rheumatoid factors (1000 AU/mL) and biotin (2µg/mL).*

CORRELATIONS *(see figure below)*:

On one hand, 60 clinical samples (from Crohn's disease patients) were quantified for Golimumab with **i-Tracker** Golimumab and LISA TRACKER Golimumab (Theradiag). Concentrations were plotted on a "x/y" axis and a linear regression was performed. High correlation was observed: $R^2 = 0.91$ and slope = 0.90.

On the other hand, 24 samples were quantified for Anti-Golimumab antibodies with **i-Tracker** Anti-Golimumab and LISA TRACKER Anti-Golimumab (Theradiag). For both assays, concentrations were ranked, and high correlation was observed : Spearman's coefficient was found at 0.99 *(see figure below).*





CONCLUSION : i-Tracker Golimumab and i-Tracker Anti-Golimumab 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 Golimumab and allowing rapid treatment adjustment.