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

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INTRODUCTION

Infliximab, a monoclonal antibody directed against TNFα, is a drug widely used for the treatment of inflammatory diseases (Rheumatoid Arthritis, Crohn's disease…). 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 Infliximab (princeps and biosimilar molecules) and Anti-Infliximab antibodies are fully automated on the random access i-TRACK[10] chemiluminescent analyzer.

MATERIALS & METHODS

- **MATERIALS:**

INFLIXIMAB SPIKED SAMPLES: 3 human serum matrix (from healthy donors) were used. The drug, Infliximab pharmaceutical solution (10mg/ml), was spiked into these 3 matrix to reach 5 levels of concentrations spanning the dynamic range of the assay (0.75, 2, 6, 12 and 18µg/ml). A total of 15 spiked samples were produced. % of recovery was calculated according to the following formula: *(quantified concentration/spiked concentration) x 100.*

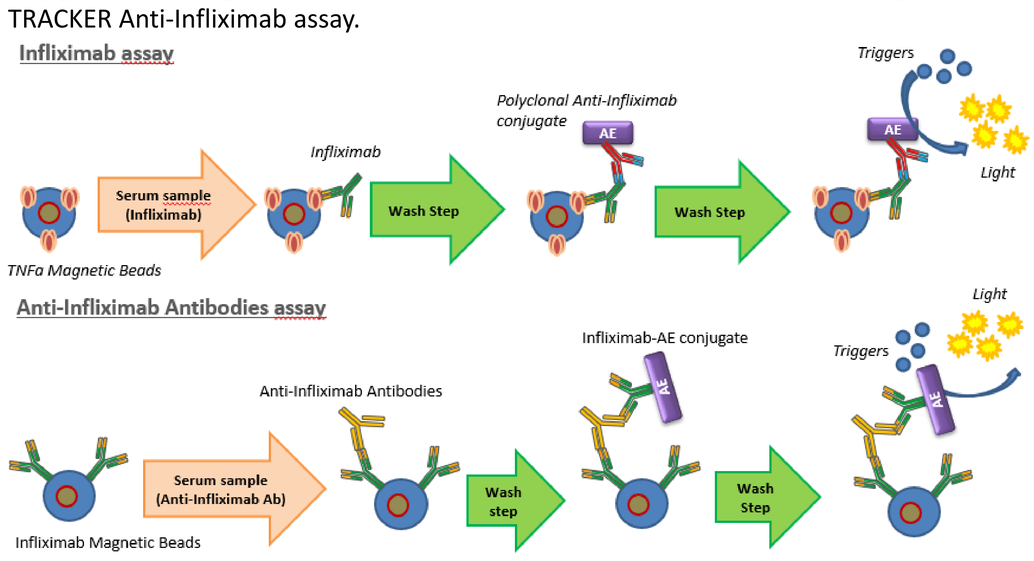
CLINICAL SAMPLES: 41 serum samples from IBD patients (Inflammatory Bowel Disease patients) treated with Infliximab were collected. They arrived frozen and kept frozen until quantification at Theradiag. Additionally, 57 serum samples from IBD patients, previously quantified for Anti-Infliximab antibodies with LISA-TRACKER Anti-Infliximab assay (#LTI 005, Theradiag) were used for correlation assessment.

i-TRACKER Infliximab kit: composed of recombinant human TNFα coated magnetic beads, polyclonal anti-Infliximab antibodies conjugated to acridinium ester, and sample dilution buffers. i-TRACKER Anti-Infliximab kit: composed of Infliximab coated magnetic beads, Infliximab conjugated to acridinium ester, and sample dilution buffer. Both types of kit contain 2 calibrators and 1 positive control dedicated for the calibration processing (master curve) and for the validation of the run, respectively. Once performed, calibration is validated for 21 days.

- **METHODS:**

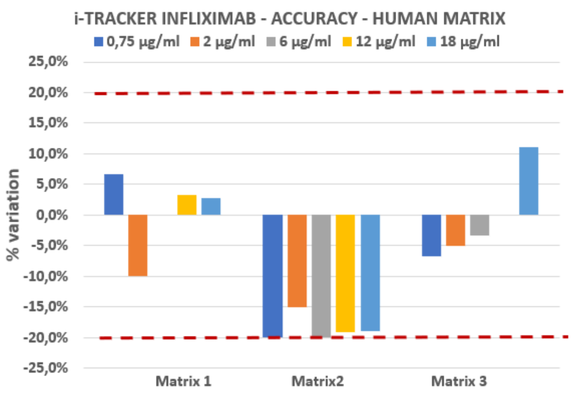
i-TRACK[10] chemiluminescent analyzer

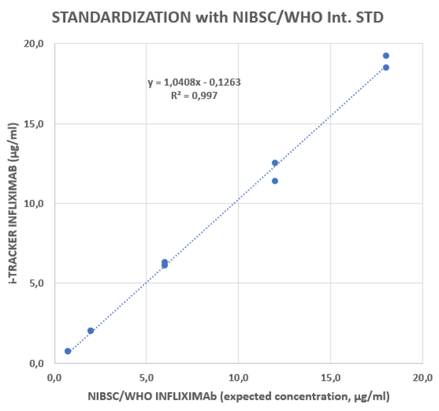
i-TRACKER CHEMILUMINESCENT ASSAYS: quantification of Infliximab and Anti-Infliximab antibodies were performed with the i-TRACK[10] chemiluminescent analyzer according to the technical insert of i-TRACKER kits (#CTI 002 and #CTI 003 respectively). Briefly, serum samples were diluted and added to the coated 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 Infliximab and Anti-Infliximab antibodies were calculated according to the calibration curve provided with the kit (master curve). The lower and the upper limits of quantification are 0.3µg/ml and 24µg/ml for i-TRACKER Infliximab assay, 10ng/ml and 2000ng/ml for i-TRACKER Anti-Infliximab assay.



RESULTS (1/2)

- **ACCURACY & STANDARDIZATION** *(see figures below)*: on one hand, 15 Infliximab spiked samples were quantified with i-TRACKER Infliximab. The % of recovery were comprised between 80% and 111% (mean % of recovery was 93%). On the other hand, 10 spiked samples were prepared with the *NIBSC/WHO Infliximab International standard (#16/170)* and quantified. The % of recovery were comprised between 93% and 107% (mean % of recovery was 100%).





Conclusion:
The acceptance criteria were met (% recovery comprised +/- 20% of spiked concentrations) and similar results were obtained with spiked samples made with biosimilars of Infliximab (CT-P13 and SB2). Quantification of Infliximab with i-TRACKER Infliximab is not affected by serum matrix. i-TRACKER Infliximab kit is standardized according to the Infliximab international standard.

RESULTS 2/2

- **LLOQ (Lower Limit Of Quantification):** on one hand, 149 serum samples from untreated patients were quantified with i-TRACKER Infliximab: all samples were found below the selected LLOQ of 0.3µg/ml. On the other hand, 110 samples from untreated patients were quantified with i-TRACKER Anti-Infliximab : all samples were found below the selected LLOQ of 10ng/ml.

INFLIXIMAB ASSAY			
ID	results (µg/ml)	mean (µg/mL)	CV
Sample1	0,8	0,8	8,1%
	0,7		
	0,9		
	0,8		
	0,8		
	0,8		
	0,7		
	0,7		
	0,8		
	0,8		
Sample2	1,0	1,0	5,0%
	1,0		
	1,1		
	1,0		
	1,1		
	1,1		
	1,0		
	1,0		
	1,0		
	1,1		
Sample3	7,9	7,9	5,1%
	8,1		
	8,1		
	7,0		
	8,1		
	8,2		
	7,3		
	8,1		
	8,0		
	7,7		
Sample4	11,0	10,8	5,2%
	11,4		
	10,6		
	11,0		
	11,1		
	10,0		
	10,9		
	9,7		
	11,0		
	11,4		
Sample5	17,0	16,9	1,6%
	17,0		
	16,5		
	16,6		
	16,7		
	17,0		
	17,2		
	17,3		
	16,8		
	17,2		

ANTI-INFLIXIMAB ASSAY			
ID	results (ng/ml)	mean (ng/mL)	CV
Sample1	37	36	11,8%
	37		
	29		
	36		
	35		
	35		
	30		
	44		
	38		
	38		
Sample2	162	164	1,8%
	164		
	160		
	165		
	168		
	160		
	163		
	166		
	169		
	164		
Sample3	378	369	3,1%
	373		
	375		
	375		
	339		
	365		
	368		
	372		
	368		
	378		
Sample4	819	819	1,7%
	846		
	828		
	818		
	804		
	834		
	819		
	799		
	814		
	810		
Sample5	1490	1462	3,2%
	1513		
	1407		
	1443		
	1479		
	1363		
	1460		
	1509		
	1471		
	1483		

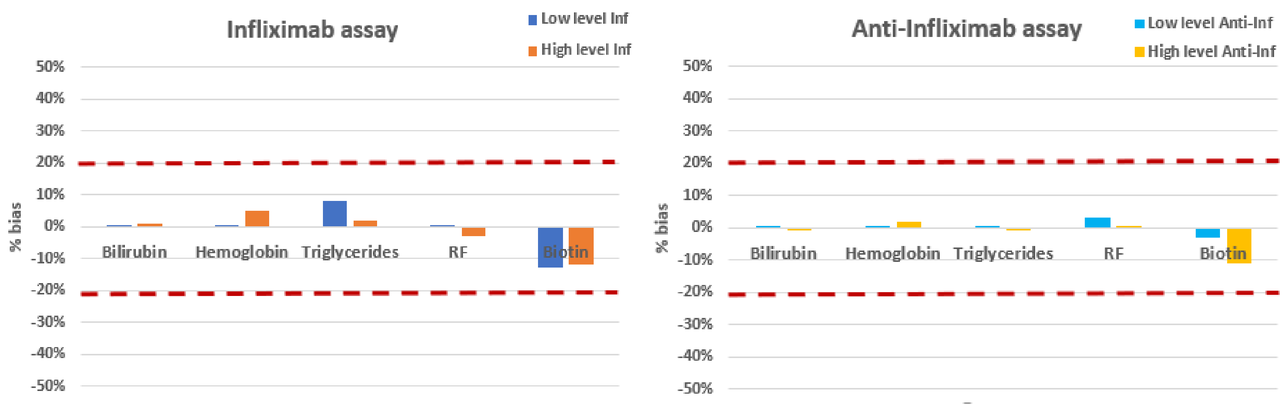
- **INTRA-RUN PRECISION** *(see figures on the right):* for both assays, 5 clinical samples spanning the dynamic range of the respective assays were quantified 10 times within a run. The coefficients of variation (CV) were calculated for each sample: the CV ranged from 1.6% to 8.1% for Infliximab assay and between 1.7% and 11.8% for Anti-Infliximab assay.

- **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%. Similar results were obtained for the quantification of Infliximab biosimilars (CT-P13 and SB2): CV ranged from 2.4% to 9.2%. For Anti-Infliximab assay, CV ranged from 2.3% to 4.8%. The acceptance criteria (CV<20%) was met. High precision is reached with i-TRACKER Infliximab assay and i-TRACKER Anti-Infliximab assay.

	INFLIXIMAB ASSAY (µg/ml)							
RUNS	1	2	3	4	5	6	Mean (µg/ml)	CV
Sample1	1,0	1,0	0,9	1,1	1,1	1,1	1,0	**6,7%**
Sample2	2,0	2,1	2,1	2,0	2,0	2,0	2,0	**2,6%**
Sample3	8,0	8,0	7,1	8,2	8,0	7,9	7,8	**5,1%**
Sample4	11,2	11,1	10,6	11,2	11,7	10,5	11,0	**4,1%**
Sample5	18,9	19,3	18,7	19,2	19,4	19,7	19,2	**2,0%**

	ANTI-INFLIXIMAB ASSAY (ng/ml)							
RUNS	1	2	3	4	5	6	Total Mean (ng/ml)	CV
Sample1	56	54	53	51	51	49	52	**4,8%**
Sample2	175	184	187	185	185	186	184	**2,3%**
Sample3	390	404	413	410	415	424	409	**2,8%**
Sample4	903	965	977	963	984	958	958	**3,0%**
Sample5	1700	1786	1790	1815	1822	1824	1789	**2,6%**

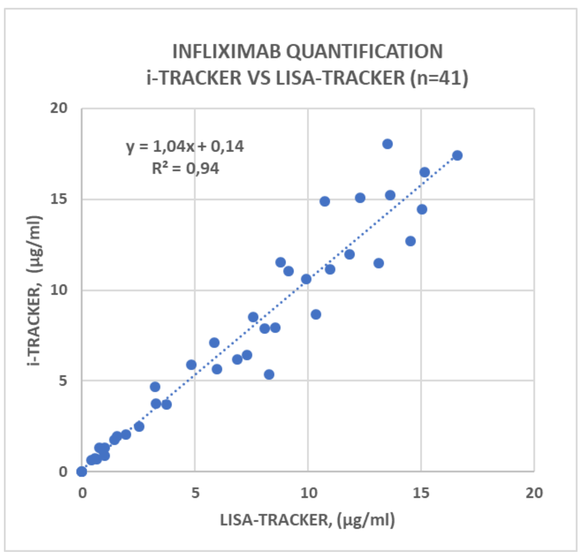
- **INTERFERENCES** *(see figures below)*: spiked samples (low and high level) were made with Infliximab and Anti-Infliximab antibodies with or without the presence of potential interfering agents, as bilirubin, hemoglobin, triglycerides, rheumatoid factors (RF) and biotin. Infliximab spiked samples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

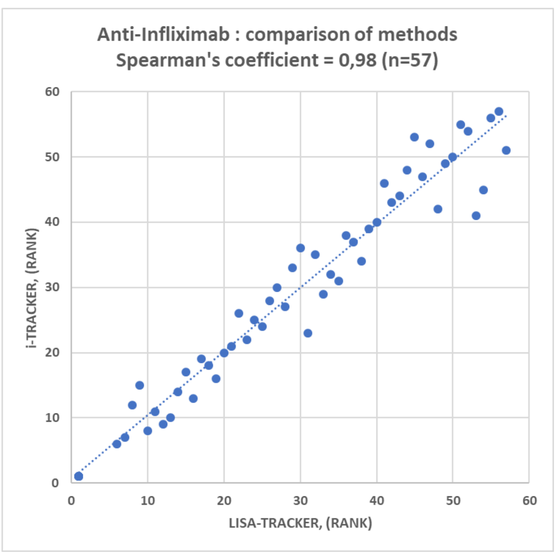


Conclusion: *i-TRACKER assays are not disrupted by the presence of biologic agents as bilirubin (2mg/ml), hemoglobin (0,2mg/ml), triglycerides (33mg/ml), rheumatoid factors (1000 AU/ml) and biotin (2µg/ml).*

- **CORRELATIONS** *(see figure below)*: on one hand, 41 clinical samples (from IBD patients) were quantified for Infliximab with i-TRACKER Infliximab and LISA-TRACKER Infliximab (Theradiag). Concentrations were plotted on a "x/y" axis and a linear regression was performed. High correlation was observed: R^2 = 0,94 and slope = 1.04.

On the other hand, 57 clinical samples were quantified for Anti-Infliximab antibodies with i-TRACKER Anti-Infliximab and LISA-TRACKER Anti-Infliximab (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 Infliximab and i-TRACKER Anti-Infliximab kits are innovative assays which exhibits 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 Infliximab (princeps and biosimilars) and allowing rapid treatment adjustment.