

## INTRODUCTION

Adalimumab, a monoclonal antibody directed against TNF $\alpha$ , 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 Adalimumab (princeps and biosimilar molecules) and Anti-Adalimumab antibodies are fully automated on the random access i-TRACK<sup>10</sup> chemiluminescent analyzer.

### **RIALS & METHODS**

### **MATERIALS:**

**METHODS:** 

Adalimumab SPIKED SAMPLES: 3 human serum matrices (from healthy donors) were used. The drug, Adalimumab pharmaceutical solution (50mg/ml), was spiked into these 3 matrices to reach 5 levels of concentration 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: 32 serum samples from IBD patients (Inflammatory Bowel Disease patients) treated with Adalimumab were collected. They arrived frozen and were kept frozen until quantification at Theradiag. Additionally, 46 serum samples from IBD patients, previously quantified for Anti-Adalimumab antibodies with LISA-TRACKER Anti-Adalimumab assay (#LTA 005, Theradiag) were used for correlation assessment.

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



i-TRACK<sup>10</sup> chemiluminescent analyzer

i-TRACKER CHEMILUMINESCENT ASSAYS: quantification of Adalimumab and Anti-Adalimumab antibodies were performed with the i-TRACK<sup>10</sup> chemiluminescent analyzer according to the technical insert of i-TRACKER kits (#CTA 002 and #CTA 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<sup>10</sup> chemiluminescent analyzer. Concentrations of Adalimumab and Anti-Adalimumab antibodies were calculated according to the calibration curve provided with the kit (master curve). The lower and the upper limits of quantification are 0.5µg/ml and 24µg/ml for i-TRACKER Adalimumab assay, 10ng/ml and 2000ng/ml for i-TRACKER Anti-Adalimumab assay.



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

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### **RESULTS (1/2)**

ACCURACY & STANDARDIZATION (see figures below): on one hand, 15 Adalimumab spiked samples were quantified with i-TRACKER Adalimumab. The % of recovery were comprised between 77% and 118% (mean % of recovery was 96%). On the other hand, 10 spiked samples were prepared with the NIBSC/WHO Adalimumab International standard (#17/236) and quantified. The % of recovery were comprised between 97% and 107% (mean % of recovery was 96%).



### Conclusion:

The acceptance criteria were met (% recovery comprised +/- 20% of spiked concentrations, for >80% of the samples) and similar results were obtained with spiked samples made with biosimilars of Adalimumab (ABP501 and SB5). Quantification of Adalimumab with i-TRACKER Adalimumab is not affected by serum matrix. i-TRACKER Adalimumab kit is standardized according to the Adalimumab International standard.

LLOQ (Lower Limit Of Quantification): on one hand, 161 serum samples from untreated patients were quantified with i-TRACKER Adalimumab: all samples were found below the selected LLOQ of 0.5µg/ml. On the other hand, 110 serum samples from untreated patients were quantified with i-TRACKER Anti-Adalimumab : all samples were found below the selected LLOQ of 10ng/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 2.4% to 5.3% for Adalimumab assay and between 1.2% and 3.1% for Anti-Adalimumab 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 3.0% to 10.6% were obtained for the Similar results quantification of Adalimumab biosimilars (ABP501 and SB5) : CV ranged from 3.4% to 9.2%. For Anti-Adalimumab assay, CV ranged from 0.8% to 4.8%. The acceptance criteria (CV<20%) was met. High precision is reached with i-TRACKER Adalimumab assay and i-TRACKER Anti-Adalimumab assay.

ADALIMUMAB - ASSAY					ANTI-ADALIMUMAB ASSAY					
ID	results (ug/ml)	mean (ug/mL)	CV	10	)	results (ng/ml)	mean (ng/mL)	CV		
	0.8	······(µ8,····-)				32				
	0.9	_				32				
	0.9					32				
	0.9		4,8%	Sample1	31	31	3,1%			
Sample1	0.9				30					
	0,9	0,9			31					
	0,9				30					
	0,5				31					
	0,5	-			33					
	0,8					32				
	2.0					258				
	2,0	-		Sample2	251	251	3,0%			
	2,0	-			233					
	1,5				254					
	1,9				244					
Sample2	1,0	1,9	3,5%		257					
	1,5					253	-			
	1,9				257	-				
	1,9	-				252				
	1,8	-				255				
	1,9	-				585				
	7,3	7,2				592	-			
	7,1				576	1				
	7,1					567	-			
	7,4			Sample3	574	578	1,2%			
Sample3	6,2		5,3%		573					
	7,4				582					
	6,9				581					
	7,4				577					
	7,2				575					
	7,5				1111					
	16,6	16,6	3,0%	Sample4	1150	1146	3,0%			
	16,6				1133					
	16,0				1171					
	17,1				1124					
Sample4	16,0				1105					
	1/,1				1110					
	16,2					1107	-			
	16,7	-				1107				
	16,4	-				1133				
	17,5	-			1205	<b>├</b>				
Sample5	19,4	19,0			1205	-				
	19,3		2,4%			1221	1328			
	18,5					1252				
	19,5					1353				
	19,5			Sam	Sample5	13/9		2,6%		
	19,1		-			1312				
	18,3	-				1292				
	18,9	-				1283				
	19,3					131/				
	18 5	1				1303				

ADALIMUMAB ASSAY (µg/ml)								
RUNS	1	2	3	4	5	6	Mean (µg/ml)	CV
Sample1	1,0	1,1	1,2	1,1	1,1	1,1	1,1	7,3%
Sample2	2,0	2,0	2,1	2,1	2,0	2,0	2,0	3,0%
Sample3	6,0	7,2	7,9	7,6	8,0	7,9	7,4	10,4%
Sample4	9,2	9,5	10,3	9,9	10,1	9,5	9,7	4,2%
Sample5	12,0	16,6	15,2	15,4	16,0	15,5	15,1	10,6%

ANTI-ADALIMUMAB ASSAY (ng/ml)								
RUNS	1	2	3	4	5	6	Mean (ng/ml)	CV
Sample1	36	36	36	36	36	37	36	1,7%
Sample2	301	299	298	300	301	305	300	0,8%
Sample3	667	625	636	651	653	701	655	4,0%
Sample4	1297	1208	1259	1306	1281	1394	1291	4,8%
Sample5	1459	1388	1338	1385	1391	1474	1406	3,6%

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# **RESULTS (2/2)**

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



biotin (2µg/ml).

**CORRELATIONS** (see figure below): on one hand, 32 clinical samples (from IBD patients) were quantified for Adalimumab with i-TRACKER Adalimumab and LISA-TRACKER Adalimumab (Theradiag). Concentrations were plotted on a "x/y" axis and a linear regression was performed High correlation was observed:  $R^2 = 0,97$  and slope = 0.94.



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



**Conclusion:** i-TRACKER assays are not disrupted by the presence of biologic agents such as bilirubin (0.2mg/ml), hemoglobin (2mg/ml), triglycerides (10mg/ml), rheumatoid factors (1000 UI/ml) and

On the other hand, 46 clinical samples were quantified for Anti-Adalimumab antibodies with i-TRACKER Anti-Adalimumab and LISA-TRACKER Anti-Adalimumab (Theradiag). For both assays, concentrations were ranked and high correlation was observed : Spearman's coefficient was found at 0.97 *(see figure*) below).