

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





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### **INTRODUCTION**

Certolizumab, a monoclonal antibody directed against TNF $\alpha$ , is a drug widely used for the treatment of inflammatory diseases (rheumatoid arthritis, plaque psoriasis, 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 Certolizumab and Anti-Certolizumab antibodies fully automated on the random access i-Track<sup>10</sup> chemiluminescent analyzer.

## **MATERIALS & METHODS**

**MATERIALS:** 

## **RESULTS 2/2**

LLOQ (Lower Limit Of Quantification): on one hand, 116 serum samples from untreated patients were quantified with i-Tracker Certolizumab: all samples were found below the selected LLOQ of 3 µg/mL. On the other hand, 116 samples from untreated patients were quantified with i-Tracker Anti-Certolizumab: all samples were found below the selected LLOQ of 10 AU/mL.

	Certolizumab	ASSAY			ANTI-Certolizuma	b ASSAY	
ID	results (µg/ml)	mean (µg/mL) CV		ID	results (AU/ml)	mean (AU/mL)	CV
	4.2			Sample 1	12		1.0%
	4.2		2.7%		12		
	4.2				12	12	
	4.2				12		
Coursela 4	4.0				13		
Sample 1	4.1	4.2			12		
	4.4				12		
	4.1	-			13		
	4.1				12		
	4.0				13		
	9.7	9.8		Sample 2	58	59	1.3%
	10.0				59		
	9.9				58		
	9.6				59		
Samala 3	9.7		2.2%		59		
Sample 2	10.0				59		
	10.1				59		
	9.8				58		
	9.9				59		
	9.4				58		
Sample 3	20.1				236		
	20.0				222		2.0%
	19.5			Sample 3	234		
	19.8				233		
	18.8	19.6	2 1%		226	231	
	19.5		2.4%		230		

Certolizumab SPIKED SAMPLES: 3 human serum matrices (from healthy donors) were used. The drug, Certolizumab pharmaceutical solution (200 mg/mL), was spiked into these 3 matrices to reach 5 concentration levels spanning the dynamic range of the assay (4, 10, 20, 40 and 70  $\mu$ g/mL). A total of 15 spiked samples were produced. % of recovery was calculated according to the following formula: (quantified concentration) x 100.

CLINICAL SAMPLES: 56 serum samples from inflammatory disease patients treated with Certolizumab were collected. They arrived frozen and kept frozen until quantification at Theradiag. Additionally, 32 serum samples previously quantified for Anti-Certolizumab antibodies with LISA TRACKER Anti-Certolizumab assay (#LTC 005, Theradiag) were used for correlation assessment.

i-Tracker Certolizumab kit: composed of TNFα coated magnetic beads, polyclonal anti-Certolizumab antibodies conjugated to acridinium ester, and sample dilution buffers. i-Tracker Anti-Certolizumab kit: composed of Certolizumab coated magnetic beads, Certolizumab 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 Certolizumab and Anti-Certolizumab antibodies were performed with the i-Track<sup>10</sup> chemiluminescent analyzer according to the technical insert of i-Tracker kits (#CTC 002-50 and #CTC 003-50 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 Certolizumab and Anti-Certolizumab antibodies were calculated according to the calibration curve provided with the kit (master curve). The lower and the upper limits of quantification are 3  $\mu$ g/mL and 100  $\mu$ g/mL for i-Tracker Certolizumab assay, 10 AU/mL and 1500 AU/mL for i-Tracker Anti-Certolizumab assay. **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 2.1% to 4.5% for Certolizumab assay and between 1.0% and 3.2% for Anti-Certolizumab 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 1.5% to 4.8% for Certolizumab assay, and CV ranged from 3.3% to 10.6% for Anti-Certolizumab assay. The acceptance criteria (CV<20%) was met. High precision is reached with i-Tracker Certolizumab assay and i-Tracker Anti-Certolizumab assay.

Sample 3	10.0	19.6	2.4%			231	2.0%
	19.5			Sample S	230		
	20.1				237		
	18.8				234		
	19.9				231		
	19.3				230		
	40.7				398	393	1.5%
	38.1	40.4	4.5%	Sample 4	397		
Sample 4	39.5				399		
	42.0				390		
	39.2				387		
	39.8				394		
	39.9				397		
	39.9				380		
	39.9				397		
	44.6				391		
	79.3	81.0	2.1%		1 187	1189	3.2%
	78.8			Sampla 5	1 167		
	83.1				1 228		
Sample 5	83.0				1 219		
	82.2				1 239		
	79.5			Sumple S	1 215		5.2/0
	80.8				1 165		
	80.4				1 122		
	79.8				1 149		
	82.9				1 200		

RUNS	1	2	3	4	5	6	Total Mean (µg/ml)	CV
ample1 (low)	4.1	4.0	4.1	4.1	4.1	4.2	4.1	1.5%
ample2 (low)	10.5	10.1	10.5	10.6	10.5	9.8	10.3	2.9%
ample3 (mid)	21.2	20.2	21.4	21.1	20.9	20.1	20.8	2.6%
ample4 (high)	44.2	40.3	42.9	43.0	42.8	39.4	42.1	4.4%
ample5 (high)	75.9	68.3	73.2	73.4	73.1	79.1	73.8	4.8%

RUNS	1	2	3	4	5	6	Total Mean (ng/ml)	CV
Sample1 (low)	13	13	13	12	12	12	12	4.0%
Sample2 (mid)	69	68	71	57	58	62	64	9.5%
Sample3 (mid)	264	264	282	223	228	252	252	9.1%
Sample4 (high)	452	436	479	361	379	433	423	10.6%
Sample5 (high)	1152	1233	1218	1177	1135	1153	1178	3.3%



**ACCURACY** (see figure below): 15 Certolizumab spiked samples were quantified with i-Tracker Certolizumab. The % of recovery were comprised between 84% and 102% (mean % of recovery was 96%). **INTERFERENCES** *(see figures below)*: spiked samples (low and high level) were made with Certolizumab and Anti-Certolizumab antibodies with or without the presence of potential interfering agents, as bilirubin, hemoglobin, triglycerides, rheumatoid factors (RF) and biotin. Certolizumab spiked samples spiked with potential interfering agents were quantified with i-Tracker Certolizumab kit and compared to results obtained with Certolizumab spiked samples. Same method was performed with Anti-Certolizumab 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 (33 mg/mL), rheumatoid factors (1000 AU/mL) and biotin (2  $\mu$ g/mL).

**CORRELATIONS** *(see figure below)*: on one hand, 56 clinical samples (from Crohn's disease patients) were quantified for Certolizumab with i-Tracker Certolizumab and LISA TRACKER

On the other hand, 32 serum samples were quantified for Anti-Certolizumab antibodies with i-Tracker Anti-Certolizumab and LISA TRACKER Anti-Certolizumab (Theradiag). For



#### Conclusion:

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

Certolizumab (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 = 0.92.



both assays, concentrations were ranked, and high correlation was observed : Spearman's coefficient was found at 0.94 *(see figure below)*.



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