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

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INTRODUCTION

Risankizumab, a monoclonal antibody directed against IL23, is a drug widely used for the treatment of inflammatory diseases (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 Risankizumab and Anti-Risankizumab antibodies fully automated on the random access i-Track^{10} chemiluminescent analyzer.

MATERIALS & METHODS

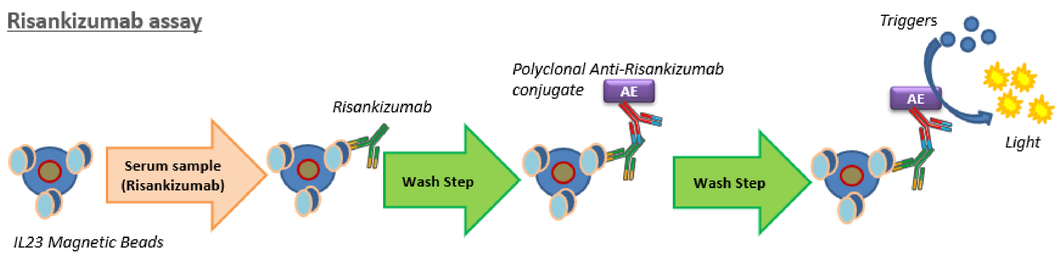
MATERIALS:

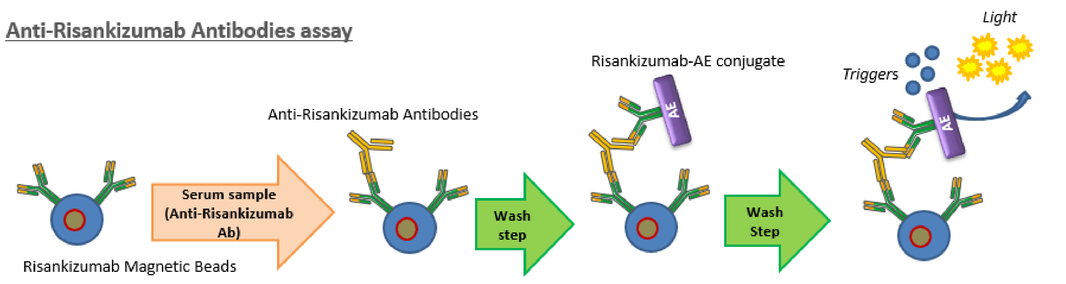
Risankizumab SPIKED SAMPLES: 3 human serum matrices (from healthy donors) were used. The drug, Risankizumab pharmaceutical solution (90 mg/mL), was spiked into these 3 matrices to reach 5 concentration levels spanning the dynamic range of the assay (2, 5, 10, 30 and 60 µg/mL). A total of 15 spiked samples were produced. % of recovery was calculated according to the following formula: *(quantified concentration/spiked concentration) x 100.*

i-Tracker Risankizumab kit: composed of recombinant IL23 coated magnetic beads, polyclonal anti-Risankizumab antibodies conjugated to acridinium ester, and sample dilution buffers. i-Tracker Anti-Risankizumab kit: composed of Risankizumab coated magnetic beads, Risankizumab 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:

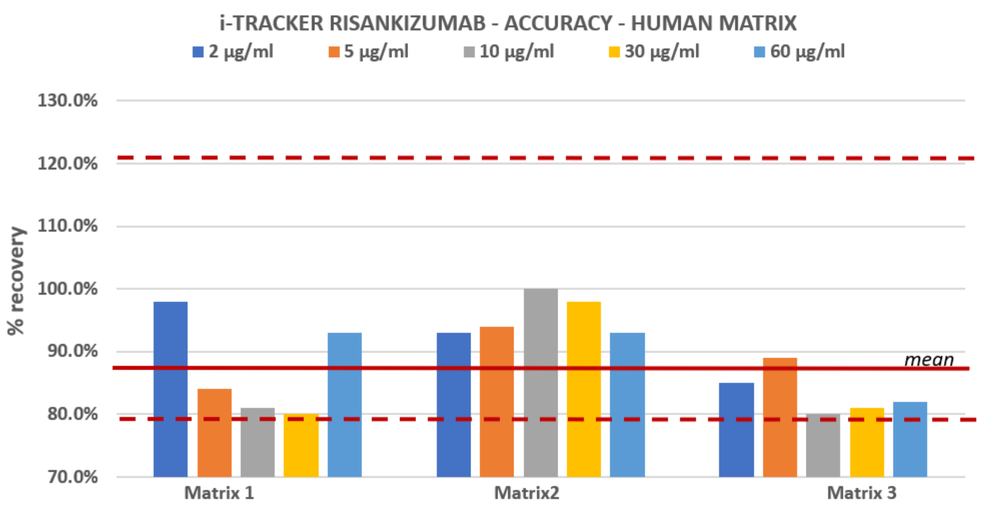
i-TRACKER CHEMILUMINESCENT ASSAYS: quantification of Risankizumab and Anti-Risankizumab antibodies were performed with the i-Track^{10} chemiluminescent analyzer according to the technical insert of i-Tracker kits (#CTR 002-50 and #CTR 003-50 respectively). Briefly, serum samples were diluted and added 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^{10} chemiluminescent analyzer. Concentrations of Risankizumab and Anti-Risankizumab antibodies were calculated according to the calibration curve provided with the kit (master curve). The lower and the upper limits of quantification are 0.5 µg/mL and 80 µg/mL for i-Tracker Risankizumab assay, 10 ng/mL and 2000 ng/mL for i-Tracker Anti-Risankizumab assay.





RESULTS 1/2

ACCURACY *(see figure below)*: 15 Risankizumab spiked samples were quantified with i-Tracker Risankizumab. The % of recovery were comprised between 80% and 100% (mean % of recovery was 88%).



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

RESULTS 2/2

LLOQ (Lower Limit Of Quantification): on one hand, 120 serum samples from untreated patients were quantified with i-Tracker Risankizumab: all samples were found below the selected LLOQ of 1 µg/mL. On the other hand, 120 samples from untreated patients were quantified with i-Tracker Anti-Risankizumab: all samples were found below the selected LLOQ of 10 ng/mL.

INTRA-RUN PRECISION *(see figure below):* for both assays, 5 spiked 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 4.0% to 7.3% for Risankizumab assay and between 1.3% and 5.7% for Anti-Risankizumab assay.

Risankizumab ASSAY			
ID	results (µg/ml)	mean (µg/mL)	CV
Sample 1	1.2	1.2	**4.7%**
	1.1		
	1.2		
	1.1		
	1.1		
	1.2		
	1.2		
	1.2		
	1.1		
	1.2		
Sample 2	4.8	5.3	**5.1%**
	5.4		
	5.4		
	5.6		
	4.9		
	5.5		
	5.4		
	5.3		
	5.3		
	5.3		
Sample 3	10.0	10.5	**4.0%**
	10.4		
	10.8		
	10.0		
	11.1		
	10.4		
	10.9		
	10.0		
	10.8		
	10.6		
Sample 4	37.3	33.4	**7.3%**
	30.6		
	34.2		
	36.9		
	30.7		
	34.1		
	32.6		
	31.7		
	31.3		
	34.6		
Sample 5	74.3	72.7	**4.5%**
	70.8		
	68.8		
	69.4		
	72.0		
	78.0		
	72.0		
	77.5		
	69.7		
	74.6		

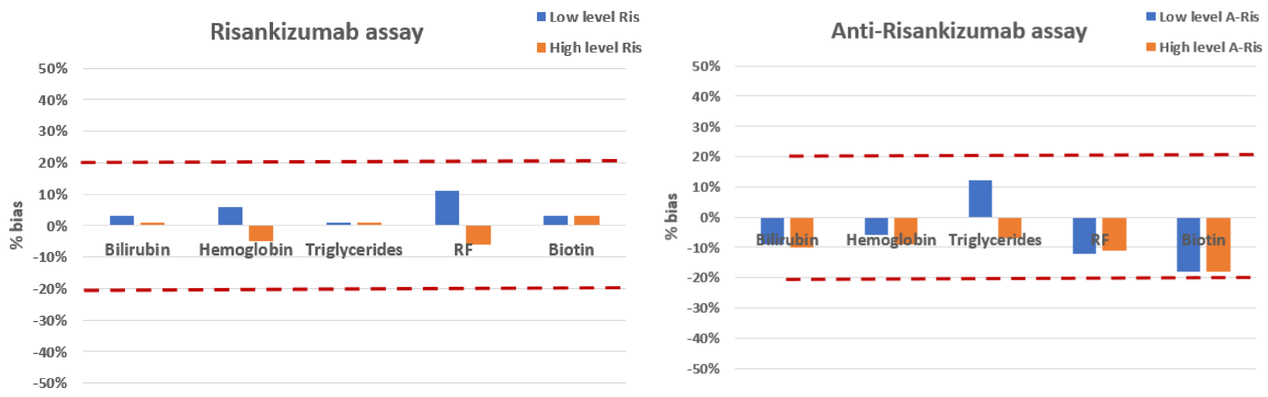
ANTI-Risankizumab ASSAY			
ID	results (ng/ml)	mean (ng/mL)	CV
Sample 1	22	24	**5.7%**
	22		
	25		
	24		
	26		
	26		
	25		
	25		
	25		
	25		
Sample 2	103	103	**2.1%**
	103		
	109		
	103		
	105		
	102		
	101		
	103		
	103		
	101		
Sample 3	466	471	**1.3%**
	469		
	471		
	480		
	470		
	473		
	478		
	472		
	474		
	459		
Sample 4	1 048	1038	**3.8%**
	1 006		
	978		
	1 027		
	993		
	1 051		
	1 027		
	1 063		
	1 108		
	1 079		
Sample 5	1 406	1386	**3.0%**
	1 365		
	1 390		
	1 426		
	1 403		
	1 318		
	1 439		
	1 318		
	1 390		
	1 408		

INTER-RUN PRECISION *(see figures on the right):* for both assays 5 spiked 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 6.0% to 15.2% for Risankizumab assay, and CV ranged from 2.7% to 8.0% for Anti-Risankizumab assay. The acceptance criteria (CV<20%) was met. High precision is reached with i-Tracker Risankizumab assay and i-Tracker Anti-Risankizumab assay.

Risankizumab ASSAY								
RUNS	1	2	3	4	5	6	Total Mean (µg/ml)	CV
Sample1 (low)	1.1	1.1	0.9	1.0	1.1	1.1	1.0	**6.0%**
Sample2 (low)	5.3	5.4	4.4	4.7	4.8	5.4	5.0	**8.6%**
Sample3 (mid)	10.3	11.2	9.3	9.6	9.8	11.5	10.3	**8.9%**
Sample4 (high)	32.9	37.3	27.8	26.1	35.8	37.9	32.9	**15.2%**
Sample5 (high)	66.2	73.3	59.6	67.8	69.6	75.5	68.6	**8.2%**

ANTI-Risankizumab ASSAY								
RUNS	1	2	3	4	5	6	Total Mean (ng/ml)	CV
Sample1 (low)	23	22	22	23	24	22	22	**3.1%**
Sample2 (mid)	127	115	108	106	118	103	113	**8.0%**
Sample3 (mid)	505	497	485	456	443	484	478	**5.0%**
Sample4 (high)	1127	1093	1062	1012	994	959	1041	**6.1%**
Sample5 (high)	1386	1467	1469	1464	1450	1390	1437	**2.7%**

INTERFERENCES *(see figures below)*: spiked samples (low and high level) were made with Risankizumab and Anti-Risankizumab antibodies with or without the presence of potential interfering agents, as bilirubin, hemoglobin, triglycerides, rheumatoid factors (RF) and biotin. Risankizumab spiked samples spiked with potential interfering agents were quantified with i-Tracker Risankizumab kit and compared to results obtained with Risankizumab spiked samples. Same method was performed with Anti-Risankizumab 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 µg/mL).*

CONCLUSION : i-Tracker Risankizumab and i-Tracker Anti-Risankizumab kits are innovative assays which exhibits fast, accurate and reproducible results. i-Tracker kits are valuable tools for the monitoring of patients treated with Risankizumab and allowing rapid treatment adjustment.