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Monitoring of infliximab trough levels and anti-infliximab antibodies in inflammatory bowel diseases: a comparison of three commercially available ELISA kits

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Abstract

Background: There are many studies presenting trough-data of biologics and several ELISA kits commercially available for monitoring infliximab trough levels (s-IFXt) and anti-drug. We propose to compare technical characteristics and results of three different assays on a cohort of 35 patients under infliximab (IFX) and suffering from inflammatory bowel disease (IBD).

Patients and methods: s-IFXt and ADA_b were systematically measured with three ELISA kits: Lisa-Tracker® Duo infliximab (Théradiag®), Ridascreen® IFX Monitoring (R-Biopharm AG®) and Promonitor® IFX (Progenika Biopharma SA®).

Results: The main technical features that differed between kits for measuring s-IFXt were: i) TNF coating, ii) immune complexes revelation strategy and/or iii) interference with other anti-TNF α agents. As for kits measuring ADA_b, it was revelation steps and unit of results. There was an excellent mathematical correlation of s-IFXt between assays however Bland-Altman analysis denoted i) s-IFXt were on average 48 to 69% higher in Ridascreen® than in the other two assays, and ii) high s-IFXt were overestimated with Promonitor® compared to Lisa-Tracker®. As a consequence, there were some substantial discrepancies between assays for classification of s-IFXt into concentration ranges. Despite unstandardized units, pairwise qualitative comparison showed a perfect agreement between the three pairs of ADA_b assays

Conclusion: Our data show that the evaluated assays are not interchangeable due to substantial variations in some results that could lead, for some patients, to divergent therapeutic decisions. We remind to be cautious when comparing study results issued from different kits and recommend using the same assay for the longitudinal follow-up of IBD patients.

Keywords: anti-infliximab antibodies, inflammatory bowel disease, infliximab trough levels, comparison of assays, immunomonitoring, anti-drug antibodies

Bullet points:

- 51 - Several ELISA kits for monitoring infliximab trough levels (s-IFXT) and anti-drug
52 antibodies (ADAb) are commercially available
- 53 - s-IFXt and ADAb detection performed on 35 IBD patients sera showed good
54 **mathematical** correlation of results between the three evaluated assays
- 55 - However, because of **some substantial** discrepancies in quantitative interpretation and
56 **potential** changes in subsequent clinical decision, we suggest to keep the same kit
57 from the same manufacturer to perform the longitudinal follow-up of IBD patients
- 58

1. Introduction

Infliximab (IFX) is a chimeric monoclonal therapeutic antibody that targets soluble and transmembrane Tumor Necrosis Factor alpha (TNF α) with proven efficiency in treatment of inflammatory bowel diseases (IBD) [1]. However, about 10-30% of IBD patients have no primary response after treatment induction and up to one-third lose response becoming secondary non-responders [2]. Low levels of trough infliximabemia and immunogenicity, with production of anti-drug antibodies (ADAb) increasing IFX serum clearance, have been identified as possible mechanisms of primary inefficiency and loss of response [3,4]. On-demand treatment and/or absence of associated immunosuppressive drugs are two identified factors which increase the probability of ADAb occurrence in patients with IBD [5]. Moreover, acute infusion reactions are more frequent in patients with ADAb than in those without [6,7]. High concentrations of IFX are associated with a higher risk of infection episode(s) in patients with rheumatic disease [8]. Considering that anti-TNF trough-levels are predictive of mucosal healing [9], all these data suggest that monitoring IFX and ADAb concentrations could be very useful for optimizing IFX therapy. Several decision algorithms using these parameters have already been published [10–12]. Depending on IFX trough levels and ADAb results, they propose dose intensification or de-escalation [13] as well as treatment interruption or therapeutic switch. Medico-economic studies performed in many countries have demonstrated that IFX treatment immuno-monitoring leads to major cost savings with no negative impact on the treatment efficacy [14–17]. Several ELISA kits for the quantitative determination of IFX or ADAb are commercially available. They were used in many studies presenting through data of biologics, including company-driven clinical trials, trials on biosimilars, and academic post-marketing. It is well known that ADAb positive subjects frequencies substantially vary, depending on the detection method [18,19], but the s-IFXt quantitative results are often regarded as comparable especially because they are expressed in

the same measuring unit. In addition, comparison of results issued from different studies using different assays requires great caution particularly in the absence of standardization for these assays. In this context, we propose in this study to compare and evaluate technical characteristics and performance of three different assays on a cohort of 35 IFX IBD patients under maintenance treatment. We further analyzed the impact of assay choice on IBD patient management related to IFX monitoring.

2. Materials and methods

2.1 Sera selection

Whole blood samples were collected from consecutive adult IBD patients treated with IFX just before IFX infusion at the gastroenterology unit of the University Hospital, Hôpital Nord, Marseille, France. All patients were included in the maintenance phase of treatment (>6 weeks of treatment) for Crohn's disease (CD) or ulcerative colitis (UC). The CD and UC diagnosis were based on clinical, radiological, endoscopic examination and histological findings using European Crohn's and Colitis Organisation (ECCO) criteria [1,20]. Blood samples were sent to immunology laboratory for immunomonitoring of IFX treatment. After centrifugation, sera were collected and stored at -80°C until further analysis. A total of 40 sera from 35 IBD patients treated with infliximab were routinely tested for IFX trough concentration measurement and systematically assessed for anti-IFX antibodies using enzyme linked immunosorbent assays (ELISA) commercialized by Théradiag (Lisa-Tracker, Théradiag, Marne la Vallée, France), R-Biopharm AG (Ridascreen, Darmstadt, Germany) and Progenika Biopharma SA (Promonitor, Derio-Bizkaia, Spain).

2.2 Laboratory assays

Trough infliximab concentrations were measured with three different ELISA kits: Lisa-Tracker® Duo infliximab (Théradiag, Marne la Vallée, France), Ridascreen® IFX Monitoring (R-Biopharm AG, Darmstadt, Germany), and Promonitor® IFX (Progenika Biopharma SA, Derio-Bizkaia, Spain). Anti-infliximab antibodies detection was performed using cognate ELISA methods commercialized by the same manufacturers: Lisa-Tracker® Duo infliximab (Théradiag, Marne la Vallée, France), Ridascreen® anti-IFX Antibodies (R-Biopharm AG, Darmstadt, Germany) and Promonitor® Anti-IFX (Progenika Biopharma SA, Derio-Bizkaia, Spain). All tests were performed following manufacturers' instructions. The absorbance was

read within 30 min using a spectrophotometer InfiniteTM TECAN, (Mannedorf, Switzerland)
at a wavelength of 450 nm.

2.3 Data analysis

The agreement between results obtained with the different manufacturers was assessed using Cohen's Kappa coefficient which takes the value of i) zero if there is no more agreement between two tests than can be expected by chance, ii) 1 if there is perfect agreement. Kappa result are commonly interpreted as follows: values lower than 0.2 as indicating slight agreement, values between 0.2 and 0.4 as fair, values between 0.4 and 0.6 as moderate, values between 0.6 and 0.8 as substantial and values greater than 0.8 as almost perfect agreement while negative Kappa indicates poor agreement [21]. Linear regression with R² calculation and Bland-Altman plots [22] were performed with GraphPad Prism version 6.00 (GraphPad Software, La Jolla California, USA). A R² value of 1 indicates a perfect linear correlation while a R² value of 0 translates no correlation. Cohen's Kappa coefficients were calculated with R statistical software (R Foundation for Statistical Computing, Vienna, Austria)

3 Results

3.1 Patients' characteristics

The patients' characteristics are detailed in Table 1. A total of 40 sera from 35 adult IBD patients (17 men, 18 women; mean \pm SD age: 38.4 \pm 13 years, age range: 16-75 years), with 24 suffering from CD and 11 from UC, were evaluated.

3.2 Assays' properties

Properties of commercially available assays used for determination of trough IFX levels and ADAb have been collected from manufacturer's datasheets and gathered in Table 2.

3.2.1 Properties of IFX through levels assays

Main differences between kits rely on i) TNF coating, ii) immune complexes revelation strategy and/or iii) interference with other anti-TNF α agents such as Adalimumab (Table 2). All assays for infliximab quantification are directly using a soluble recombinant TNF α for coating except Promonitor® kit which uses anti-TNF α -human monoclonal antibody for coating and presenting TNF α . Both Ridascreen® and Promonitor® use HRP-labelled anti-IFX monoclonal antibody to reveal bounded IFX whereas Lisa-Tracker® uses a two-step revelation method with anti-human IgG biotinylated antibodies and HRP streptavidin. As a consequence, anti-TNF α biotherapies other than IFX may interfere with IFX levels measured with Lisa-Tracker® but not with Promonitor® or Ridascreen®. Lisa-tracker is also the test taking the longest time to be performed as many revelation steps are required. Of interest, neither of manufacturers provided a cut-off for results' interpretation in their datasheet.

3.2.2 Properties of ADA b assays

All evaluated tests are using bridging assay strategy to capture free ADA b. This strategy takes advantage of ADA b bridging properties, which are bivalent antibodies. ADA b serve as a bridging element between a coated IFX playing the role of the capture antibody and a labelled IFX being the detection antibody. The main assay differences regard the units and immune complex revelation steps. Indeed, only Lisa-Tracker® and Ridascreen® express ADA b results in ng/ml whereas Promonitor® gives results as arbitrary units. Another difference is the revelation strategy which is a two-step biotin/streptavidin revelation for Lisa-Tracker® and Ridascreen® as opposed to Promonitor® which is a one-step. As aforementioned for the IFX levels measurements, there is no cut-off proposed for ADA b result interpretation. As a consequence, ADA b are considered as positive only just when they are detectable. There is no serum concentration threshold for ADA b associated with clinical significance. In the absence

of prior dissociation of immune-complexes all the evaluated kits in this study are drug-sensitive. The circulating free IFX are likely to bind to free ADAb, resulting in either a false ADAb decrease or undetectable measurement.

3.3 IFX trough levels

3.3.1 Quantitative analysis

Linear regression and Bland-Altman plots for each assay pair are shown in Figure 1. Linear correlation was excellent for all assay pairs. Systematic bias from Bland-Altman analysis indicated that i) IFX levels were, on average, 48% higher in Ridascreen® compared to Lisa-Tracker® and 69% higher compared to Promonitor®, and ii) IFX levels were higher in Promonitor® than in Lisa-Tracker® for IFX values above 7 µg/ml. The highest systematic mean difference was found for Promonitor® vs Ridascreen® (-1.758 µg/ml, 95% CI:-3.995, 0.479). Bland-Altman plots of two assay pairs show that the difference between measured IFX levels is not constant but varies proportionally with the average of measured IFX levels (Figure 1). Indeed, Ridascreen® versus Lisa-Tracker® plots showed a positive proportional bias (Fig 1e) whereas we observed a negative proportional bias for Promonitor® versus Ridascreen® (Fig 1f). For Promonitor® versus Lisa-Tracker® pair, the difference of IFX levels were around zero for an average IFX levels under 7 µg/ml but increased consistently when IFX levels were above 7 µg/ml (Fig 1d).

3.3.2 Qualitative analysis

The results for each assay were sorted into categories according to s-IFXt. Ranges were defined as follows: < 3 µg/ml : sub-therapeutic, between 3 µg/ml and 5µg/ml : optimal-low, between 5 µg/ml and 8µg/ml: optimal-target range, > 8µg/ml: supra-optimal. As summarized in Table 3, we can observe that Ridascreen® gave higher s-IFXt than Lisa-Tracker® in 15 out of 40 samples (37.5%). This s-IFXt overestimation led to a change of classification range for

all these samples, which were therefore moved to the upper category. As each individual measurement is associated with a certain measurement uncertainty and since all manufacturers have provided coefficients of variation (CV%) from reproducibility tests (as known as inter-assay variability) we considered these concentration changes as significant when the difference in IFX levels between one method and Lisa-Tracker® exceeded the inter-assay variability of this method (measured value * CV% / 100) . Therefore, among the 12 classification changes observed, 10 were really significant. Of interest, 2 patients with sub-optimal IFX levels became classified as “optimal-low” with Ridascreen®. For Promonitor® assay, the range classification was modified for 9 out of 40 samples (22.5%) with 4 lower and 5 upper. Among the 9 classification changes observed, 6 were really significant as the difference in IFX levels exceeded the inter-assay variability of the evaluated assay. Of interest, 2 patients with “optimal-low” IFX levels with Lisa-Tracker® were classified as “sub-optimal” with Promonitor ®.

3.4 Antibodies to infliximab

Pairwise comparison by Cohen’s kappa showed a perfect agreement between the three pairs of assays (Table 4). We found four samples issued from four different patients with detectable ADAAb. Among them, three patients suffered from CD and one patient suffered from UC (Table 5). There were no trough IFX detectable for all four patients with ADAAb. Three out of four patients displayed ADAAb levels above 200 ng/ml. Only the patient with UC presented ADAAb levels into the measure range of all the assays. It is worth highlighting that absence of IFX detection in four patients of our study was observed with all the kits and was associated with ADAAb in all cases.

Quantitative comparison of ADAAb levels were not performed here because of i) the low number of ADAAb positive samples and ii) the difference in result expression between assays

(arbitrary units for Promonitor® and ng/ml for Lisa-Tracker® and Ridascreen®). Moreover, for both assays quantitatively expressed in ng/ml, 3 out of 4 ADAb patients showed such high ADAb levels that they were out of the standard curve. Quantitative analysis of ADAb levels was only possible with Lisa-Tracker® and Ridascreen® kits and for patient #35. These results were discrepant and we suspected correlation problems for ADAb values lower than 200ng/ml. All patient with detectable ADAb were under infliximab treatment only and they didn't receive supplemental immunomodulatory treatment. The 36 remaining samples were negative for ADAb and they all showed detectable IFX trough levels.

4 Discussion

The aim of this study was to assess and compare three different assays detecting IFX and ADAb in a cohort of 35 IFX treated IBD patients. We found a good mathematical correlation of IFX trough levels measured with all the tested kits. This is in line with results that have been already reported for ELISA kits using close methods of detection based on the same strategy of capturing the unbound circulating IFX by immobilized TNF α [23–25]. However a good mathematical correlation is not sufficient to prove that these tests are interchangeable. Indeed, they have significant design differences and we have shown that the results produced by different kits can vary substantially, upwards or downwards, depending on the pair of kits compared. For instance, Promonitor® assay, unlike the other two, uses a pre-coated microplate with an anti-TNF α human monoclonal antibody bound to human recombinant TNF α . According to the manufacturer, this format ensures a better capture of infliximab by avoiding TNF α disruption. Another difference between the IFX kits tested regards the immune complex revelation method. Only Lisa-Tracker® uses an anti-human IgG antibody for the revelation step, whose main drawback is IFX interference with other circulating anti-TNF agent. This could happen when switching drugs but, in those cases, it is very uncommon that drug-levels are measured within the time-window where there is still relevant level of the

old drug in the serum. In our cohort of patients, we did not have any patient undergoing a switch with another anti-TNF α but this interference, however, reveals the importance of having detailed information about the patient's treatment in order to interpret the test in an optimal way. Ridascreen® and Promonitor® use specific anti-IFX antibodies which theoretically would suppress this cross-reactivity interference.

Systematic biases of IFX trough levels, as well as differences in their classification into concentration ranges based on therapeutic window, showed that subsequent therapeutic decisions could differ according to the assay used. Our results point out that these category changes are relatively frequent and differ between compared manufacturers. However the subsequent therapeutic changes are less frequent. Indeed there were two patients with “optimal” IFX levels with Lisa-Tracker® and Ridascreen ® that became classified as “optimal” with Ridascreen®. Conversely, there were two patients with “optimal” IFX levels with Lisa-Tracker® and Ridascreen ® that became classified as “sub-optimal” with Promonitor ®. Therefore, subtle variations exist for few patients that can't be explained by inter-assay variability. This demonstrate that the three assays are not fully interchangeable, especially during the longitudinal follow-up of a given patient. This statement is in accordance with Pérez et al [24] that suggest that the same assay should be used during the follow-up of patients.

When a humoral immune response is generated against an immunogenic therapeutic antibody, the ADA_b and the biotherapeutic drug are both present in free form and bound form as immune complexes. With the methodology of the ELISA commercial kits evaluated in this study, the biologist must bear in mind that there is no prior step of immune complexes' dissociation before testing. Therefore, these assays are subject to drug interference where the free form of drug found in circulation limits the detection of ADA_b. In other words, the lack

of detection of ADA_b does not mean that there is no ADA_b bound with infliximab into circulating immune complexes.

Bridging assays for ADA_b detection rely on ternary complexes formation where a “bridge” is formed between the capture reagents (e.g., coated IFX), ADA_b, and the detection reagent (e.g. biotin or HRP IFX). This method has the ability to detect multivalent monospecific antibodies such as IgG or IgM antibodies but not bispecific antibodies such as IgG4.

We detected ADA_b in 4 out of 35 patients. These 4 patients with ADA_b were only treated with infliximab and didn't receive concomitant immunosuppressants. This observation supports previous findings showing that immunomodulatory treatments, primarily methotrexate, are able to reduce infliximab immunogenicity [5,26].

We can observe that the manufacturers don't provide a cut-off or therapeutic window for the IFX trough or ADA_b level interpretation. Yet, these target levels are extremely important since they potentially influence clinical decision leading to significant therapeutic changes [11]. IFX trough levels superior to 3 µg/ml have been proposed by several authors to achieve clinical remission in IBD [27–29]. However, in a recent study, Cookson *et al.* showed that the threshold for IFX trough levels leading to dose escalation differs between CD and UC [30]. Therefore, specific pathology thresholds still need to be validated.

We observed a full concordance between kits in terms of ADA_b detection since each kit detected successfully the same 4 patients with ADA_b. Promonitor® expresses ADA_b results in arbitrary units which impedes comparison with other kits. Among ADA_b positive patients with Ridascreen® and Lisa-Tracker®, 2 out of 3 had high ADA_b levels and only one patient had ADA_b levels falling out into the detection range, however these levels were very different suggesting a poor quantitative correlation. A dedicated study with more ADA_b positive patients should be of interest to conclude.

It is important to highlight that absence of IFX detection in four patients was observed with all the kits and was associated with ADAb in all cases. Interestingly, an improvement of clinical symptoms was observed for two of them despite the detection of circulating ADAb. In this uncommon situation, we could hypothesize that i) drug is still active in tissues and on target cells despite absence of detection in serum and/or ii) transient IFX binding with ADAb in excess may impair serum IFX detection but not ADAb detection iii) a clinical response could occur independently of the treatment [31].

In conclusion, the detailed analysis of this study results showed that, despite a good mathematical correlation, these assays are not substitutable due to substantial variations for some patients. That's why we recommend being extremely cautious when comparing study results that have been produced using different kits. We also suggest sticking to one assay, from the same manufacturer, for the entire longitudinal follow-up of IBD patients.

Conflict of interest

R-Biopharm AG, (Darmstadt, Germany), Progenika Biopharma SA (Derio-Bizkaia, Spain) and Théradiag (Marne la Vallée, France) provided complimentary ELISA kits for testing

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302 **Results**

303 **Table 1: Clinical characteristics of the 35 patients included in the study.**

Variable	Result
Nb of Patients	35
Male/female ratio	16/19
Age, mean (min-max)	38 years (16-75)
Diagnosis	
- Crohn's disease	24 (68.6%)
- Ulcerative colitis	11 (31.4%)
Disease duration (n=29), mean (min-max)	12 years (1-39)
Improvement of clinical symptoms: Yes/No	17/18
Other active immunomodulating treatment	5 (14.3%)
Nb of Patients in induction/maintenance phase	0/35
IFX dose and schedule:	
- 5 mg/kg q8w	1 (2.9%)
- >5 mg/kg q8w	13 (36.1%)
- Other dose and/or schedule	21 (60%)

304 qXw: every X weeks, IFX: infliximab

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307 **Table 2: Comparison of assays' features**

	IFX assays			Anti-IFX assays		
	Lisa-Tracker®	Ridascreen®	Promonitor®	Lisa-Tracker®	Ridascreen®	Promonitor®
Test Duration (min)	165	100	135	165	130	165
Measuring Range	0.3-8 µg/ml	0.5-12 µg/ml	0.2-14.4 µg/ml	10-200 ng/ml	2.5-125 ng/ml	5-255 AU/ml
Analyzed form	Free drug	Free drug?	Free drug	Not stated	Not stated	Free ADAb
Coated antigen	TNFα	TNFα	anti-TNFα-human monoclonal antibody + TNFα	IFX	IFX	IFX
Immune complexes revelation step(s)	Two Steps: 1 st Step: Anti-human IgG biotinylated antibodies 2 nd Step: HRP Streptavidin	One Step: HRP-labelled anti-IFX monoclonal antibody	One Step: HRP-labelled anti-IFX monoclonal antibody	Two Steps: 1 st Step: Biotinylated IFX 2 nd Step: HRP Streptavidin	Two Steps: 1 st Step: Biotinylated IFX 2 nd Step: HRP Streptavidin	One Step: HRP-labelled IFX
Lower Limit of Detection (LLOD)	LLOD=0.3 µg/ml	LLOD <1 ng/ml	Not stated	LLOD = 10 ng/ml	Not stated	Not stated
Lower Limit of Quantification (LLOQ)	Not stated	Not stated	LLOQ=1.7 ng/ml	Not stated	Not stated	LLOQ=2 AU/ml
Automation	Yes	Yes	Yes	Yes	Yes	Yes
Serum dilution	1/101	1/100	1/200	1/2	1/25	1/2
Repeatability	< 8.8%	<6.8%	4.5%	<7.6%	<7.5%	10%
Reproducibility	<10.6%	3.7-9.6%	4.3%	<8.6%	<12.4%	8%
ELISA method	Sandwich	Sandwich	Sandwich	Bridging	Bridging	Bridging
Interference	Present with other anti-TNF antibodies	Absent with Adalimumab and golimumab	Absent with Adalimumab and etanercept	Not stated	Not stated	Not stated

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309 LLOQ: Lower limit of quantification; LLOD: Lower limit of detection; IFX: infliximab, HRP: horseradish

310 peroxidase. ADAb: anti-drug antibodies.

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316 **Table 3: Range and posology classification of trough IFX levels for each assay**

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IFX POSOLOGY	LISA-TRACKER® IFX levels (µg/ml)			
	<3	3-5	5-8	>8
5 mg/kg q6w	35	No patient	No patient	No patient
5 mg/kg q7w	No patient	23	15, 22	No patient
5 mg/kg q8w	18	No patient	16	No patient
7.5 mg/kg q4w	No patient	No patient	No patient	32
7.5 mg/kg q8w	11	2	1	No patient
10 mg/kg q6w	7, 12, 30	34	5, 8, 9	4, 6, 6.1, 20, 27, 29
10 mg/kg q7w	12.1, 13	No patient	No patient	No patient
10 mg/kg q8w	10, 10.1, 14, 19, 21, 28	17, 21.1	25, 26, 31	3, 24, 24.1
10 mg/kg q10w	33	No patient	No patient	No patient

318

IFX POSOLOGY	RIDASCREEN® IFX levels (µg/ml)			
	<3	3-5	5-8	>8
5 mg/kg q6w	35	No patient	No patient	No patient
5 mg/kg q7w	No patient	23	No patient	15*, 22*
5 mg/kg q8w	18	No patient	No patient	16
7.5 mg/kg q4w	No patient	No patient	No patient	32
7.5 mg/kg q8w	11	No patient	2	1*
10 mg/kg q6w	12, 30	7*	8, 34*	4, 5, 6, 6.1, 9*, 20, 27, 29
10 mg/kg q7w	12.1, 13	No patient	No patient	No patient
10 mg/kg q8w	10, 10.1, 14, 19, 21	28*	17	3, 21.1 24, 24.1, 25*, 26*, 31*
10 mg/kg q10w	33	No patient	No patient	No patient

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IFX POSOLOGY	PROMONITOR® IFX levels (µg/ml)			
	<3	3-5	5-8	>8
5 mg/kg q6w	35	No patient	No patient	No patient
5 mg/kg q7w	No patient	23	22	15*
5 mg/kg q8w	18	No patient	16	No patient
7.5 mg/kg q4w	No patient	No patient	32*	No patient
7.5 mg/kg q8w	2*, 11	No patient	No patient	1*
10 mg/kg q6w	7, 12, 30, 34	No patient	5, 8, 9	4, 6, 6.1, 20, 27, 29
10 mg/kg q7w	12.1, 13	No patient	No patient	No patient
10 mg/kg q8w	10, 10.1, 14, 17*, 19, 21, 28	No patient	21.1*, 31	3, 24, 24.1, 25*, 26
10 mg/kg q10w	33	No patient	No patient	No patient

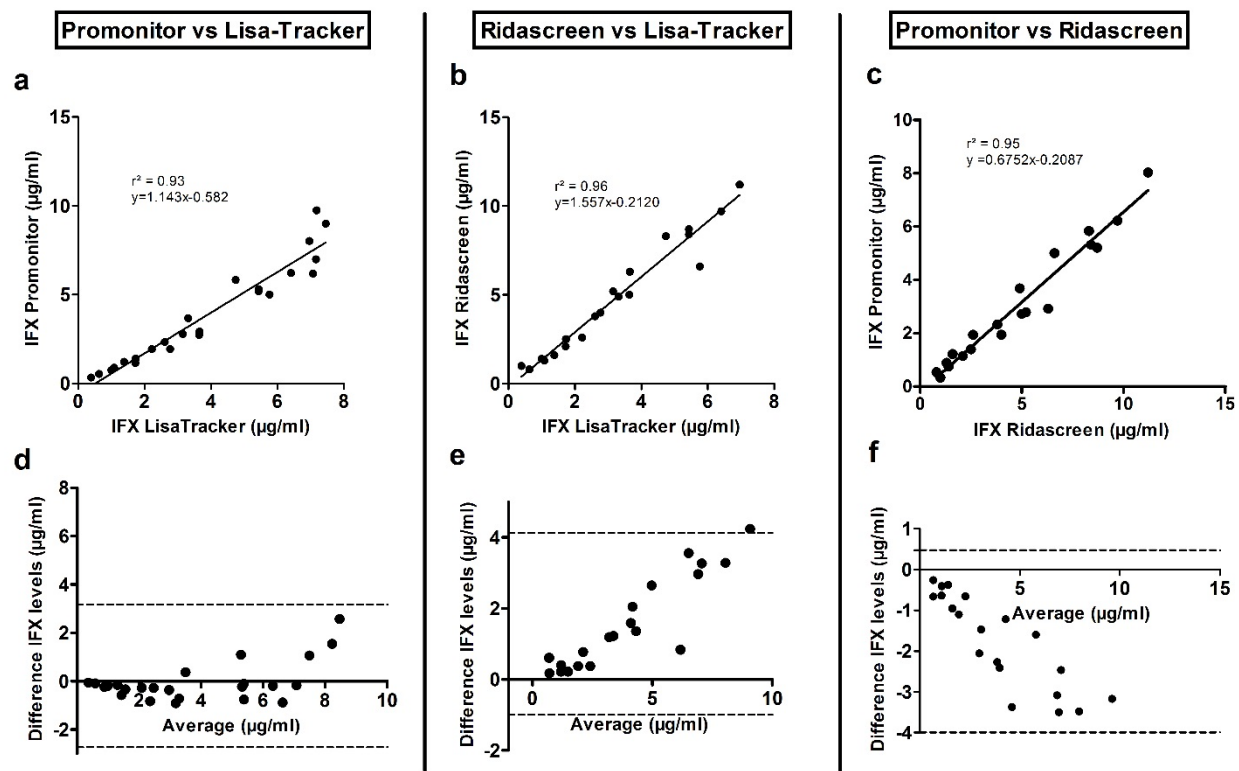
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323 qXw: every X weeks, IFX: infliximab, numbers indicate patients by their inclusion number,
 324 “bis” means that a later supplemental sample was available for a same patient, underlined
 325 numbers depict patients whose range classification differ from the one issuing from Lisa-
 326 Tracker®’s results. Asterisk depicts patients with variation of IFX trough levels exceeding the
 327 inter-assay variability of the evaluated test.

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330 **Figure 1: Serum trough IFX levels correlation between different assays**



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332 a,b,c: Linear regression of IFX levels to compare tests (a) Promonitor® vs. Lisa-Tracker®
333 ($R^2=0.93$), (b) Ridascreen® vs. Lisa-Tracker® ($R^2=0.96$), and (c) Promonitor® vs.
334 Ridascreen ® ($R^2=0.95$). d,e,f : Bland-Altman plots of IFX levels to compare different assays,
335 the difference between two measurements (µg/ml) is plotted on the y-axis and the average of
336 the two measurements (µg/ml) on the x-axis. Dashed lines represent the 95% limits of
337 agreement of the bias. (d) Promonitor® vs. Lisa-Tracker® (Bias=-0.029 µg/ml, 95% CI:-
338 1.655, 1.597), (e) Ridascreen® vs. Lisa-Tracker® (Bias=1.570 µg/ml, 95% CI:-0.994, 4.133),
339 and (f) Promonitor® vs. Ridascreen ® (Bias=-1.758 µg/ml, 95% CI:-3.995, 0.4791).

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Table 4: Qualitative agreement (Cohen’s Kappa) between assay pairs for anti-IFX antibodies detection in serum samples

Ridascreen® anti –IFX Antibodies					Kappa
		Positive	Negative	TOTAL	
Lisa-Tracker®	Positive	4	0	4	1.000
	Negative	0	31	31	
	TOTAL	4	31	35	
Promonitor® Anti-IFX Antibodies					Kappa
		Positive	Negative	TOTAL	
Lisa-Tracker®	Positive	4	0	4	1.000
	Negative	0	31	31	
	TOTAL	4	31	35	
Promonitor® Anti-IFX Antibodies					Kappa
		Positive	Negative	TOTAL	
Ridascreen®	Positive	4	0	4	1.000
	Negative	0	31	31	
	TOTAL	4	31	35	

Table 5: Characteristics of patients with circulating anti-infliximab antibodies

	Patient #12	Patient #14	Patient #19	Patient #35
IBD Disease: CD / UC	CD	CD	CD	UC
Infliximab posology	10mg/kg q6w	10mg/kg q8w	10mg/kg q8w	5mg/kg q6w
Serum IFX trough levels				
- Lisa-Tracker® (µg/ml)	<0.3	<0.3	<0.3	<0.3
- Ridascreen® (µg/ml)	<0.5	<0.5	<0.5	<0.5
- Promonitor® (µg/ml)	<0.2	<0.2	<0.2	<0.2
Anti-IFX antibodies serum levels				
- Lisa-Tracker® (ng/ml)	>200	>200	>200	105
- Ridascreen® (ng/ml)	>200	>200	>200	9.2
- Promonitor® (AU/ml)	117.1	114.1	85.8	15.5
IFX response	Yes	No	Yes	No
Treatment duration	7 years	13 months	>1 year	1 year
Other associated Immunomodulator?	No	No	No	No

Legends

Table 1: Clinical characteristics of the 35 patients included in the study.

qXw: every X weeks, IFX: infliximab

Table 2: Comparison of assays' features

LLOQ: Lower limit of quantification; LLOD: Lower limit of detection; IFX: infliximab,
HRP: horseradish peroxidase

Table 3: Range and posology classification of trough IFX levels for each assay

qXw: every X weeks, IFX: infliximab, numbers indicate patients by their inclusion number, “bis” means that a later supplemental sample was available for a same patient, underlined numbers depict patients whose range classification differ from the one issuing from Lisa-Tracker®'s results. Asterisk depicts patients with variation of IFX trough levels exceeding the inter-assay variability of each test

Table 4: Qualitative agreement (Cohen's Kappa) between assay pairs for anti-IFX antibodies detection in serum samples

Table 5: Characteristics of patients with circulating anti-infliximab antibodies

IBD: Inflammatory Bowel Disease, CD: Crohn Disease, UC: Ulcerative colitis, qXw: every X weeks, IFX: infliximab

Figure 1: Serum trough IFX levels correlation between different assays

a,b,c: Linear regression of IFX levels to compare tests (a) Promonitor® vs. Lisa-Tracker® ($R^2=0.93$), (b) Ridascreen® vs. Lisa-Tracker® ($R^2=0.96$), and (c) Promonitor® vs. Ridascreen® ($R^2=0.95$). d,e,f : Bland-Altman plots of IFX levels to compare different assays, the difference between two measurements($\mu\text{g/ml}$) is plotted on the y-axis and the average of the two measurements ($\mu\text{g/ml}$) on the x-axis. Dashed lines represent the 95% limits of agreement of the bias. (d) Promonitor® vs. Lisa-Tracker® (Bias= $-0.029 \mu\text{g/ml}$, 95% CI:-1.655, 1.597), (e) Ridascreen® vs. Lisa-Tracker® (Bias= $1.570 \mu\text{g/ml}$, 95% CI:-0.994, 4.133), and (f) Promonitor® vs. Ridascreen® (Bias= $-1.758 \mu\text{g/ml}$, 95% CI:-3.995, 0.4791).

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