



HAL
open science

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

Daniel Bertin, Mélanie Serrero, Jean Charles Grimaud, Ariadne Desjeux,
Sophie Desplat-Jégo

► To cite this version:

Daniel Bertin, Mélanie Serrero, Jean Charles Grimaud, Ariadne Desjeux, Sophie Desplat-Jégo. Monitoring of infliximab trough levels and anti-infliximab antibodies in inflammatory bowel diseases: A comparison of three commercially available ELISA kits. *Cytokine*, 2020, 126, pp.154859. 10.1016/j.cyto.2019.154859 . hal-02351814

HAL Id: hal-02351814

<https://hal.science/hal-02351814>

Submitted on 20 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

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

4 Daniel Bertin ^{1, 2}, Mélanie Serrero ³, Jean Charles Grimaud ³, Ariadne Desjeux ³, Sophie
5 Desplat-Jégo ^{1, 2}

6

7 ¹ Assistance Publique-Hôpitaux de Marseille, Hôpital de la Conception, Pôle de Biologie,
8 Service d'Immunologie, 13005 Marseille, France

9 ² Aix-Marseille Univ, CNRS, INP, Inst Neurophysiopathol, Marseille, France

10 ³ Service de Gastroentérologie, Hôpital Nord, Assistance Publique-Hôpitaux de Marseille,
11 France

12

13

14

15

16 Corresponding author:

17 Daniel Bertin - Service d'immunologie – Pôle de Biologie- Hôpital de la Conception –147,

18 Bd Baille – 13005 Marseille – France – Tel : +33 (0)491383907 – Fax : +33(0)491383633

19 E-mail : daniel.bertin@ap-hm.fr

20

21

22 Word Count: 3227 words

23

24 **Abstract**

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

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

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

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

47

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

50 **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

59 **1. Introduction**

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

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

90

91 **2. Materials and methods**

92 2.1 Sera selection

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

106 2.2 Laboratory assays

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

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

117

118 2.3 Data analysis

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

130 3 Results

131 3.1 Patients' characteristics

132 The patients' characteristics are detailed in Table 1. A total of 40 sera from 35 adult IBD
133 patients (17 men, 18 women; mean \pm SD age: 38.4 \pm 13 years, age range: 16-75 years), with
134 24 suffering from CD and 11 from UC, were evaluated.

135 3.2 Assays' properties

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

138

3.2.1 Properties of IFX through levels assays

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

150

3.2.2 Properties of ADA b assays

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

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

165 3.3 IFX trough levels

166 3.3.1 Quantitative analysis

167 Linear regression and Bland-Altman plots for each assay pair are shown in Figure 1. Linear
168 correlation was excellent for all assay pairs. Systematic bias from Bland-Altman analysis
169 indicated that i) IFX levels were, on average, 48% higher in Ridascreen® compared to Lisa-
170 Tracker® and 69% higher compared to Promonitor®, and ii) IFX levels were higher in
171 Promonitor® than in Lisa-Tracker® for IFX values above 7 µg/ml. The highest systematic
172 mean difference was found for Promonitor® vs Ridascreen® (-1.758 µg/ml, 95% CI: -3.995,
173 0.479). Bland-Altman plots of two assay pairs show that the difference between measured
174 IFX levels is not constant but varies proportionally with the average of measured IFX levels
175 (Figure 1). Indeed, Ridascreen® versus Lisa-Tracker® plots showed a positive proportional
176 bias (Fig 1e) whereas we observed a negative proportional bias for Promonitor® versus
177 Ridascreen® (Fig 1f). For Promonitor® versus Lisa-Tracker® pair, the difference of IFX
178 levels were around zero for an average IFX levels under 7 µg/ml but increased consistently
179 when IFX levels were above 7 µg/ml (Fig 1d).

180 3.3.2 Qualitative analysis

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

186 all these samples, which were therefore moved to the upper category. As each individual
187 measurement is associated with a certain measurement uncertainty and since all
188 manufacturers have provided coefficients of variation (CV%) from reproducibility tests (as
189 known as inter-assay variability) we considered these concentration changes as significant
190 when the difference in IFX levels between one method and Lisa-Tracker® exceeded the inter-
191 assay variability of this method (measured value * CV% / 100) . Therefore, among the 12
192 classification changes observed, 10 were really significant. Of interest, 2 patients with sub-
193 optimal IFX levels became classified as “optimal-low” with Ridascreen®. For Promonitor®
194 assay, the range classification was modified for 9 out of 40 samples (22.5%) with 4 lower and
195 5 upper. Among the 9 classification changes observed, 6 were really significant as the
196 difference in IFX levels exceeded the inter-assay variability of the evaluated assay. Of
197 interest, 2 patients with “optimal-low” IFX levels with Lisa-Tracker® were classified as “sub-
198 optimal” with Promonitor ®.

199

200 3.4 Antibodies to infliximab

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

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

211 (arbitrary units for Promonitor® and ng/ml for Lisa-Tracker® and Ridascreen®). Moreover,
212 for both assays quantitatively expressed in ng/ml, 3 out of 4 ADAb patients showed such high
213 ADAb levels that they were out of the standard curve. Quantitative analysis of ADAb levels
214 was only possible with Lisa-Tracker® and Ridascreen® kits and for patient #35. These results
215 were discrepant and we suspected correlation problems for ADAb values lower than
216 200ng/ml. All patient with detectable ADAb were under infliximab treatment only and they
217 didn't receive supplemental immunomodulatory treatment. The 36 remaining samples were
218 negative for ADAb and they all showed detectable IFX trough levels.

219 4 Discussion

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

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

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

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

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

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

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

270 We can observe that the manufacturers don't provide a cut-off or therapeutic window for the
271 IFX trough or ADA_b level interpretation. Yet, these target levels are extremely important
272 since they potentially influence clinical decision leading to significant therapeutic changes
273 [11]. IFX trough levels superior to 3 µg/ml have been proposed by several authors to achieve
274 clinical remission in IBD [27–29]. However, in a recent study, Cookson *et al.* showed that
275 the threshold for IFX trough levels leading to dose escalation differs between CD and UC
276 [30]. Therefore, specific pathology thresholds still need to be validated.

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

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

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

296 **Conflict of interest**

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

299 **Acknowledgments**

300 The authors thank Laurence Louis for kindly proofreading this paper

301

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

305

306

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

308

309 LLOQ: Lower limit of quantification; LLOD: Lower limit of detection; IFX: infliximab, HRP: horseradish
 310 peroxidase. ADAb: anti-drug antibodies.

311

312

313

314

315

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

317

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

319

320

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

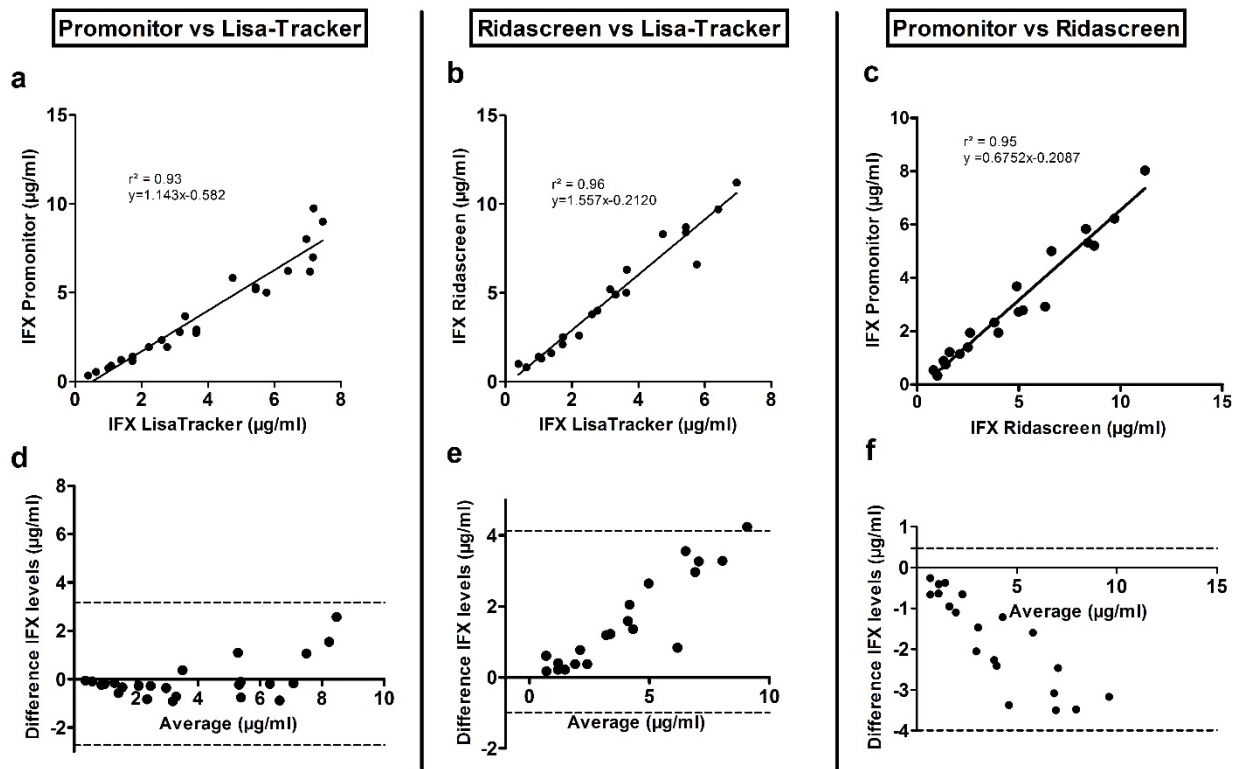
322

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.

328

329

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



331

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 ($\mu\text{g/ml}$) is plotted on the y-axis and the average of
 336 the two measurements ($\mu\text{g/ml}$) on the x-axis. Dashed lines represent the 95% limits of
 337 agreement of the bias. (d) Promonitor® vs. Lisa-Tracker® (Bias= $-0.029 \mu\text{g/ml}$, 95% CI:-
 338 $1.655, 1.597$), (e) Ridascreen® vs. Lisa-Tracker® (Bias= $1.570 \mu\text{g/ml}$, 95% CI: $-0.994, 4.133$),
 339 and (f) Promonitor® vs. Ridascreen® (Bias= $-1.758 \mu\text{g/ml}$, 95% CI: $-3.995, 0.4791$).

340

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

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

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

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

343
 344
 345
 346
 347
 348
 349
 350
 351

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

353 **Legends**

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

355 qXw: every X weeks, IFX: infliximab

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

357 LLOQ: Lower limit of quantification; LLOD: Lower limit of detection; IFX: infliximab,
358 HRP: horseradish peroxydase

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

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

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

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

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

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

371 a,b,c: Linear regression of IFX levels to compare tests (a) Promonitor® vs. Lisa-Tracker®
372 ($R^2=0.93$), (b) Ridascreen® vs. Lisa-Tracker® ($R^2=0.96$), and (c) Promonitor® vs.
373 Ridascreen® ($R^2=0.95$). d,e,f : Bland-Altman plots of IFX levels to compare different assays,
374 the difference between two measurements($\mu\text{g/ml}$) is plotted on the y-axis and the average of
375 the two measurements ($\mu\text{g/ml}$) on the x-axis. Dashed lines represent the 95% limits of
376 agreement of the bias. (d) Promonitor® vs. Lisa-Tracker® (Bias=-0.029 $\mu\text{g/ml}$, 95% CI:-
377 1.655, 1.597), (e) Ridascreen® vs. Lisa-Tracker® (Bias=1.570 $\mu\text{g/ml}$, 95% CI:-0.994, 4.133),
378 and (f) Promonitor® vs. Ridascreen® (Bias=-1.758 $\mu\text{g/ml}$, 95% CI:-3.995, 0.4791).

379

380

381

382

383

384

385 **References**

- 386 1. Gomollón F, Dignass A, Annese V, Tilg H, Van Assche G, Lindsay JO, et al. 3rd European
387 Evidence-based Consensus on the Diagnosis and Management of Crohn's Disease 2016: Part
388 1: Diagnosis and Medical Management. *J Crohns Colitis*. 2017;11:3–25.
- 389 2. Qiu Y, Chen B-L, Mao R, Zhang S-H, He Y, Zeng Z-R, et al. Systematic review with meta-
390 analysis: loss of response and requirement of anti-TNF α dose intensification in Crohn's
391 disease. *J Gastroenterol*. 2017;52:535–54.
- 392 3. Danese S, Fiorino G, Reinisch W. Review article: Causative factors and the clinical
393 management of patients with Crohn's disease who lose response to anti-TNF- α therapy.
394 *Aliment Pharmacol Ther*. 2011;34:1–10.
- 395 4. Nanda KS, Cheifetz AS, Moss AC. Impact of antibodies to infliximab on clinical outcomes
396 and serum infliximab levels in patients with inflammatory bowel disease (IBD): a meta-
397 analysis. *Am J Gastroenterol*. 2013;108:40–7; quiz 48.
- 398 5. Ben-Horin S, Waterman M, Kopylov U, Yavzori M, Picard O, Fudim E, et al. Addition of
399 an immunomodulator to infliximab therapy eliminates antidrug antibodies in serum and
400 restores clinical response of patients with inflammatory bowel disease. *Clin Gastroenterol*
401 *Hepatol Off Clin Pract J Am Gastroenterol Assoc*. 2013;11:444–7.
- 402 6. O'Meara S, Nanda KS, Moss AC. Antibodies to infliximab and risk of infusion reactions in
403 patients with inflammatory bowel disease: a systematic review and meta-analysis. *Inflamm*
404 *Bowel Dis*. 2014;20:1–6.
- 405 7. Steenholdt C, Svenson M, Bendtzen K, Thomsen OØ, Brynskov J, Ainsworth MA. Severe
406 infusion reactions to infliximab: aetiology, immunogenicity and risk factors in patients with
407 inflammatory bowel disease. *Aliment Pharmacol Ther*. 2011;34:51–8.
- 408 8. Bejan-Angoulvant T, Ternant D, Daoued F, Medina F, Bernard L, Mammou S, et al. Brief
409 Report: Relationship Between Serum Infliximab Concentrations and Risk of Infections in
410 Patients Treated for Spondyloarthritis. *Arthritis Rheumatol Hoboken NJ*. 2017;69:108–13.
- 411 9. Barnes EL, Allegretti JR. Are Anti-Tumor Necrosis Factor Trough Levels Predictive of
412 Mucosal Healing in Patients With Inflammatory Bowel Disease?: A Systematic Review and
413 Meta-Analysis. *J Clin Gastroenterol*. 2016;50:733–41.
- 414 10. Bendtzen K, Ainsworth M, Steenholdt C, Thomsen OØ, Brynskov J. Individual medicine
415 in inflammatory bowel disease: monitoring bioavailability, pharmacokinetics and
416 immunogenicity of anti-tumour necrosis factor-alpha antibodies. *Scand J Gastroenterol*.
417 2009;44:774–81.
- 418 11. Afif W, Loftus EV, Faubion WA, Kane SV, Bruining DH, Hanson KA, et al. Clinical
419 utility of measuring infliximab and human anti-chimeric antibody concentrations in patients
420 with inflammatory bowel disease. *Am J Gastroenterol*. 2010;105:1133–9.
- 421 12. Vermeire S, Gils A. Value of drug level testing and antibody assays in optimising
422 biological therapy. *Frontline Gastroenterol*. 2013;4:41–3.

- 423 13. Vande Casteele N, Ferrante M, Van Assche G, Ballet V, Compennolle G, Van Steen K, et
424 al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel
425 disease. *Gastroenterology*. 2015;148:1320-1329.e3.
- 426 14. Velayos FS, Kahn JG, Sandborn WJ, Feagan BG. A test-based strategy is more cost
427 effective than empiric dose escalation for patients with Crohn's disease who lose
428 responsiveness to infliximab. *Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol*
429 *Assoc*. 2013;11:654–66.
- 430 15. Steenholdt C, Brynskov J, Thomsen OØ, Munck LK, Fallingborg J, Christensen LA, et al.
431 Individualised therapy is more cost-effective than dose intensification in patients with Crohn's
432 disease who lose response to anti-TNF treatment: a randomised, controlled trial. *Gut*.
433 2014;63:919–27.
- 434 16. Roblin X, Attar A, Lamure M, Savariego B, Brunel P, Duru G, et al. Cost savings of anti-
435 TNF therapy using a test-based strategy versus an empirical dose escalation in Crohn's
436 disease patients who lose response to infliximab. *J Mark Access Health Policy*. 2015;3.
- 437 17. Martelli L, Olivera P, Roblin X, Attar A, Peyrin-Biroulet L. Cost-effectiveness of drug
438 monitoring of anti-TNF therapy in inflammatory bowel disease and rheumatoid arthritis: a
439 systematic review. *J Gastroenterol*. 2017;52:19–25.
- 440 18. Imaeda H, Andoh A, Fujiyama Y. Development of a new immunoassay for the accurate
441 determination of anti-infliximab antibodies in inflammatory bowel disease. *J Gastroenterol*.
442 2012;47:136–43.
- 443 19. Wang S-L, Ohrmund L, Hauenstein S, Salbato J, Reddy R, Monk P, et al. Development
444 and validation of a homogeneous mobility shift assay for the measurement of infliximab and
445 antibodies-to-infliximab levels in patient serum. *J Immunol Methods*. 2012;382:177–88.
- 446 20. Magro F, Gionchetti P, Eliakim R, Ardizzone S, Armuzzi A, Barreiro-de Acosta M, et al.
447 Third European Evidence-based Consensus on Diagnosis and Management of Ulcerative
448 Colitis. Part 1: Definitions, Diagnosis, Extra-intestinal Manifestations, Pregnancy, Cancer
449 Surveillance, Surgery, and Ileo-anal Pouch Disorders. *J Crohns Colitis*. 2017;11:649–70.
- 450 21. Landis JR, Koch GG. The measurement of observer agreement for categorical data.
451 *Biometrics*. 1977;33:159–74.
- 452 22. Bland JM, Altman DG. Measuring agreement in method comparison studies. *Stat*
453 *Methods Med Res*. 1999;8:135–60.
- 454 23. Vande Casteele N, Burman DJ, Sturkenboom MGG, Kleibeuker JH, Vermeire S,
455 Rispens T, et al. Detection of infliximab levels and anti-infliximab antibodies: a comparison
456 of three different assays. *Aliment Pharmacol Ther*. 2012;36:765–71.
- 457 24. Pérez I, Fernández L, Sánchez-Ramón S, Alba C, Zatarain A, Cañas M, et al. Reliability
458 evaluation of four different assays for therapeutic drug monitoring of infliximab levels. *Ther*
459 *Adv Gastroenterol*. 2018;11:1756284818783613.
- 460 25. Nasser Y, Labetoulle R, Harzallah I, Berger A-E, Roblin X, Paul S. Comparison of Point-
461 of-Care and Classical Immunoassays for the Monitoring of Infliximab and Antibodies Against
462 Infliximab in IBD. *Dig Dis Sci*. 2018;63:2714–21.

- 463 26. Garcês S, Demengeot J, Benito-Garcia E. The immunogenicity of anti-TNF therapy in
464 immune-mediated inflammatory diseases: a systematic review of the literature with a meta-
465 analysis. *Ann Rheum Dis*. 2013;72:1947–55.
- 466 27. Feuerstein JD, Nguyen GC, Kupfer SS, Falck-Ytter Y, Singh S, American
467 Gastroenterological Association Institute Clinical Guidelines Committee. American
468 Gastroenterological Association Institute Guideline on Therapeutic Drug Monitoring in
469 Inflammatory Bowel Disease. *Gastroenterology*. 2017;153:827–34.
- 470 28. Mitrev N, Vande Casteele N, Seow CH, Andrews JM, Connor SJ, Moore GT, et al.
471 Review article: consensus statements on therapeutic drug monitoring of anti-tumour necrosis
472 factor therapy in inflammatory bowel diseases. *Aliment Pharmacol Ther*. 2017;46:1037–53.
- 473 29. Roblin X, Boschetti G, Duru G, Williet N, Deltedesco E, Phelip JM, et al. Distinct
474 Thresholds of Infliximab Trough Level Are Associated with Different Therapeutic Outcomes
475 in Patients with Inflammatory Bowel Disease: A Prospective Observational Study. *Inflamm*
476 *Bowel Dis*. 2017;23:2048–53.
- 477 30. Cookson TA, Fedorak R, Halloran BP, Dieleman LA, Wong K, Huang V, et al. The
478 threshold for infliximab trough levels leading to dose escalation differs between Crohn's
479 disease and ulcerative colitis. *J Can Assoc Gastroenterol*. 2018;1:233–4.
- 480 31. Krieckaert C, Rispens T, Wolbink G. Immunogenicity of biological therapeutics: from
481 assay to patient. *Curr Opin Rheumatol*. 2012;24:306–11.

482