



HAL
open science

Reference values for IGF-I serum concentrations: Comparison of six immunoassays

P. Chanson, A. Arnoux, M. Mavromati, S. Brailly-Tabard, C. Massart, J. Young, M.-L. Piketty, J.-C. Souberbielle

► To cite this version:

P. Chanson, A. Arnoux, M. Mavromati, S. Brailly-Tabard, C. Massart, et al.. Reference values for IGF-I serum concentrations: Comparison of six immunoassays. *Journal of Clinical Endocrinology and Metabolism*, 2016, 101 (9), pp.3450–3458. 10.1210/jc.2016-1257 . hal-01380421

HAL Id: hal-01380421

<https://univ-rennes.hal.science/hal-01380421>

Submitted on 18 Oct 2016

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.

1 **Reference values for insulin-like growth factor I (IGF-I) serum concentrations:**
2 **comparison of six immunoassays.**

3 (Short title : Reference values for IGF-I with 6 immunoassays)

4

5 **Philippe Chanson, Armelle Arnoux, Maria Mavromati, Sylvie Brailly-Tabard,**
6 **Catherine Massart, Jacques Young, Marie-Liesse Piketty, and Jean-Claude Souberbielle**
7 **for the VARIETE Investigators*.**

8

9

10 Assistance Publique-Hôpitaux de Paris, Hôpitaux universitaires Paris-Sud, Hôpital de
11 Bicêtre, Service d'Endocrinologie et des Maladies de la Reproduction and Centre de
12 Référence des Maladies Endocriniennes Rares de la Croissance (P.C., M.M., J.Y.), and Unité
13 de Recherche Clinique (A.A.), and Service de Génétique Moléculaire, Pharmacogénétique et
14 Hormonologie (S.B.T), Le Kremlin-Bicêtre, F94275, France; Inserm 1185 (P.C., S.B.T.,
15 J.Y.), Fac Med Paris Sud, Université Paris-Saclay, Le Kremlin-Bicêtre, F-94276, France; and
16 Centre Hospitalier Universitaire de Rennes, Centre d'Investigation Clinique plurithématique,
17 Inserm 1414, Hôpital Pontchaillou, Laboratoire d'Hormonologie (C.M.), Rennes, F29000,
18 France; and Assistance Publique-Hôpitaux de Paris, Hôpital Necker-Enfants Malades, Service
19 des Explorations Fonctionnelles (M-L.P., J-C.S.), Paris, F75015, France

20 * a complete list of the VAleurs de Référence de l'IGF-I Et Transformation En Z-score
21 (VARIETE) study investigators is given in Supplemental Appendix

22

23

24 **Corresponding author and reprint requests:**

25 Philippe Chanson, MD

26 Service d'Endocrinologie et des Maladies de la Reproduction,
27 Hôpital de Bicêtre, 78 rue du Général Leclerc, 94275 Le Kremlin-Bicêtre, France
28 E-mail: philippe.chanson@bct.aphp.fr

29

30 **Abbreviations:** BMI, body mass index; IGFBP, IGF binding protein; SDS, standard
31 deviation score.

32

33 **Key terms:** IGF-I, Z-score, SD score, normative data, reference range, normal healthy
34 population, acromegaly, growth hormone deficiency

35

36 **Disclosure:** The authors have no conflicts of interest to disclose

37

38 **Word count** (for text only): 3581

39

40 **Abbreviated title:** Reference intervals for serum IGF-I

41

42

43 **Summary**

44 Context. Measurement of IGF-I is essential for diagnosis and management of patients with
45 disorders affecting the somatotrophic axis. However, even when IGF-I kit manufacturers
46 follow recent consensus guidelines, different kits can give very different results for a given
47 sample.

48 Objectives. We sought to establish normative data for six IGF-I assay kits, based on a large
49 random sample of the French general adult population.

50 Subjects and Methods: In a cross-sectional multicenter cohort study (ClinicalTrials.gov
51 Identifier: NCT01831648), we measured IGF-I in 911 healthy adults (18-90 years) with six
52 immunoassays (iSYS, LIAISON XL, IMMULITE, IGF1 RIACT, Mediagnost ELISA, and
53 Mediagnost RIA). Pairwise concordance between assays was assessed with Bland-Altman
54 plots for both IGF-1 raw data and standard deviation scores (SDS), as well as with the
55 percentage of observed agreement and the weighted Kappa coefficient for categorized IGF-I
56 SDS.

57 Results: Normative data included the range of values (2.5 to 97.5 percentiles) given by the six
58 IGF-I assays according to age group and sex. A formula for SDS calculation is provided.
59 While the lower limits of the reference intervals of the six assays were similar, the upper
60 limits varied markedly. Pairwise concordances were moderate to good (0.38 to 0.70).

61 Conclusion. Despite being obtained in the same healthy population, the reference intervals of
62 the six commercial IGF-1 assay kits showed noteworthy differences. Agreement between
63 methods was moderate to good.

64

65

66 Growth hormone (GH) exerts its effects on target tissues either directly or *via* the production
67 of insulin-like growth factor 1 (IGF-I). Accurate measurement of IGF-I in serum is crucial for
68 diagnosis and management of disorders affecting the somatotrophic axis, particularly GH
69 excess (acromegaly) and GH deficiency (GHD). However, even if manufacturers follow the
70 recommendations of the Consensus Group on the Standardization and Evaluation of GH and
71 IGF-I Assays (1), the different commercial IGF-I assay kits can give very different results for
72 the same sample, with up to a 2.5-fold difference between the lowest and highest values (2).
73 This inter-method variability is generally explained by calibration against different IGF-I
74 reference preparations (3), and differences in the efficiency of methods used to remove IGF-
75 binding proteins (IGFBPs) (4). In theory, this should not be a problem in clinical practice, as
76 kits that give higher values should have higher normal limits, and patients should thus be
77 consistently classified.

78 However, it is very difficult to establish reference values for IGF-I. Indeed, serum IGF-I
79 concentrations increase with children's age and pubertal stage, while they fall with age in
80 adults (5). Furthermore, the distribution of IGF-I values in an apparently healthy population is
81 non Gaussian, and this necessitates complex mathematical transformation to obtain reference
82 intervals for each age group. For this reason, it is essential to generate reference values after
83 stratifying a large healthy population into age groups. Another problem is that IGF-I
84 concentrations are influenced by many factors other than GH concentrations, including
85 nutritional status and BMI, use of hormone replacement therapy by post-menopausal women,
86 depending on the administration route (6-8), kidney and liver function, and diabetic status (9).
87 Reference IGF-I values may therefore be influenced by the inclusion criteria used to select the
88 reference population sample. This could have important implications for diagnosis and
89 therapeutic decision-making, as a given patient could be classified as having a normal IGF-I
90 concentration with one method but an abnormal value with another method. Several studies

91 suggest that the main reason for inter-laboratory variability in patient classification is the use
92 of different populations to establish reference values for the different IGF-I assays (2,10,11).
93 It is currently difficult to monitor an individual patient with different IGF-I assays, even if the
94 results are all expressed in the same units (ng/ml). It is thus recommended to establish
95 specific reference ranges for each assay, and to apply common, well-defined inclusion criteria
96 to the reference population (1). It is also recommended, for the comparison of values obtained
97 with different assays in the same patient, to express each IGF-I result as an SD score (SDS)
98 with reference to the normative data for the assay in question, after appropriate transformation
99 for data non normality. We reasoned that the best way to overcome this variability would be
100 to apply all the commercial kits used in clinical laboratories to a battery of samples from the
101 same well-defined reference population, and to use the same mathematical transformation to
102 calculate reference ranges from the raw data.

103 The aim of this study was thus to establish normative data for six commercial IGF-1 assays in
104 a large random sample of healthy subjects from the French general population representing all
105 adult age groups (about 100 subjects per decade), as recommended by the Consensus Group
106 on the Standardization and Evaluation of GH and IGF-I assays (1). Serum samples from the
107 reference population were tested with six commercial assay kits available in France at the
108 time of this study, after careful exclusion of subjects with medical conditions or medications
109 that might affect their IGF-I concentration. The data were analyzed to obtain the range (2.5 to
110 97.5 percentiles) in mass units. The standard deviation scores were used to compare the six
111 assays.

112

113 **Subjects and Methods**

114 *IGF-I assay characteristics*

115 Six immunoassays (iSYS, LIAISON XL, IMMULITE, IGFI RIACT, Mediagnost ELISA, and
116 Mediagnost RIA) were used to measure the IGF-I concentration in each healthy subject. The
117 main characteristics of the assays, and the mathematical models used to determine normative
118 data, where relevant (12-14), are shown in Table 1.

119

120 *Healthy subjects*

121 The subjects were part of a large cohort of French healthy adults (VARIETE). The VARIETE
122 cohort was an open, prospective, national, multicenter, non randomized study of healthy
123 volunteers, designed to establish normative data for IGF-I and other hormones in the French
124 general adult population representing all age groups (about 100 subjects per decade from 18
125 to 90 years) (ClinicalTrials.gov Identifier: NCT01831648). A total of 972 healthy subjects
126 with BMI values between 19 and 28 kg/m² were recruited in 10 centers throughout France
127 between 2010 and 2011. Our objective of including 1000 subjects was not achieved due to
128 difficulties for obtaining an accurate number of subjects in the older age categories (>70
129 years) fulfilling all the inclusion criteria and without exclusion criteria before the end of our
130 inclusion period. Subjects with medical conditions or medications that might affect IGF-I
131 serum levels were excluded (see Supplemental Appendix). Each subject had a clinical
132 examination, personal medical history-taking and general examination, including careful
133 evaluation of nutritional and gonadal status. Standard laboratory tests (plasma sodium,
134 potassium, calcium, phosphate and creatinine, glycemia, total cholesterol, liver enzymes,
135 TSH, blood cell count, albuminemia, prothrombin time, as well as HIV and HCV serologies)
136 were then performed, and 80 mL of blood (50 mL without anticoagulant and 30 mL in
137 EDTA-containing tubes) was sampled and promptly centrifuged (2000 g, 4°C). Serum and
138 plasma were aliquoted, frozen, and stored at -80°C until hormone measurements.

139 All healthy subjects gave their written informed consent to participate in the study, which was
140 approved by the Paris-Sud Ethics committee before the beginning of the study.

141

142 **Statistical methods**

143 The distribution of IGF-1 values obtained with each assay was skewed, and was thus first
144 normalized by means of sex- and age-specific Box-Cox power transformation. Student's *t* test
145 and Levene's test were then used to assess equality of means and homogeneity of variances
146 between men and women in each age group. As men and women had significantly different
147 IGF-1 levels, centile curves were constructed separately for each sex.

148 Age- and sex-specific centile curves were constructed for each assay by using the LMS
149 method (12) implemented in the GAMLSS software package version 4.3-1 (15) of R software
150 version 3.1.2 (2014-10-31) (R Core Team (2014). R: A language and environment for
151 statistical computing. R Foundation for Statistical Computing, Vienna, Austria.URL
152 <http://www.R-project.org/>). The LMS method enables smooth curves to be estimated for
153 percentiles after normalization (by Box-Cox power transformation) and standardization of the
154 data. The parameters L (for skewness), M (for median) and S (for the coefficient of variation)
155 were also computed for each age and sex class. SD scores (SDS) were calculated as $z =$
156 $[(IGF-1 / M)^L - 1] / (L \times S)$, where IGF-I is the raw value given by the assay (in ng/mL). For
157 each technique, SDS were categorized as low, normal or high according to their positions
158 relative to both the 2.5th and 97.5th percentiles.

159 Once the L, M and S parameters for each category of age and sex had been obtained, the
160 lower and upper reference interval limits were determined for each assay by fixing *z* at -1.96
161 and 1.96, respectively, and then mathematically back-transforming the SD score formula.

162 Pairwise concordance between assays was assessed with scatter plots and Bland-Altman plots
163 for both IGF-1 raw values and SDS values, as well as with the percentage of observed

164 agreement (total number of agreements divided by the total number of patients tested with
165 both assays) and the linearly weighted Kappa coefficient for categorized IGF-1 SDS (16,17).
166 An overall kappa coefficient (16) and Friedman's test were computed for global comparison
167 of all assays at the same time. Landis and Koch's table was followed for interpretation of
168 Kappa values (18).

169 Unless otherwise stated, SAS software was used for all statistical analyses (Statistical
170 Analysis System, version 9.4, SAS Institute, Cary, N.C., USA).

171

172 **Results**

173

174 *1- Description of the population*

175 Nine hundred seventy-two subjects were initially recruited, of whom 52 were excluded
176 because of abnormal values in the standard laboratory screening tests. A further 9 subjects
177 were excluded because of missing information on pregnancy status or viral serology. The
178 study population thus consisted of 911 subjects (470 males), comprising respectively 101,
179 118, 99, 98, 103, 102, 108, 97 and 85 subjects in the 18-20, 21-23, 24-26, 27-29, 30-39, 40-
180 49, 50-59, 60-69, 70-89 year age groups. Mean BMI was 23.0 ± 2.4 kg/m².

181

182 *2- IGF-I reference intervals obtained with the six assays*

183 The IGF-I reference intervals (2.5th-97.5th percentiles) obtained with the six immunoassays
184 are shown in Table 2 according to age and sex. Supplemental Figure 1 shows individual
185 points and fitted percentiles (2.5%, 50% and 97.5%) for males and females in each IGF-I
186 assay.

187 A calculator available online (http://ticemed.sa.upmc.fr/sd_score/) or by using Apps (IGF-I
188 SD_score) downloadable for Android from Google Play and for iOS from Apple Store (free

189 of charge) allows to obtain individual IGF-I SDS after entering the name of the assay, the
190 individual IGF-I value obtained with the assay, and the sex and age of the individual.

191 The six reference intervals for males and females are plotted on the same graph in Figure 1.
192 While the lower limits of the reference intervals (2.5th percentiles) were similar, the upper
193 limits (97.5th percentiles) varied markedly from one assay to another.

194 ***3- Comparison of IGF-I levels given by the six assays***

195 The results obtained with each IGF-I assay were compared with those obtained with each of
196 the other five assays. Scatter plots and Bland-Altman plots based on raw values and SDS for
197 each pair of assays are shown in Supplemental Figure 2

198 Whatever the assay, IGF-I concentrations were generally higher in women than in men until
199 the age of 59 years (this was significant for the age ranges 18-20 and 24-26 years). From the
200 age of 60 years, IGF-I levels were slightly higher in men than in women, although the gender
201 difference was smaller than in the younger age groups and was only significant for Immulite,
202 Mediagnost Elisa and Mediagnost RIA.

203 Two examples of inter-assay comparisons are shown in Figure 2. The results obtained with
204 iSYS and Mediagnost RIA were in good overall agreement, with no significant bias as
205 assessed by Bland-Altman plots (Figure 2 A, B, C and D). In contrast, the results obtained
206 with LIAISON XL and Mediagnost RIA were not in good agreement (Figure 2 E, F, G and
207 H).

208 Pairwise assay concordances assessed with the weighted Kappa coefficient for categorized
209 IGF-1 SDS are shown in Table 3. The concordances were moderate to good (0.38 to 0.70),
210 although the percentages of observed agreement were quite high (94% to 97%).

211 Overall agreement was moderate as overall Kappa coefficient was 0.55. Both in men and
212 women, global inter-assay comparison showed significant differences ($p < 0.0001$) on raw
213 values but not on SDS values ($p = 0.26$ and $p = 0.36$, respectively).

214 Table 4 shows pairwise concordances between the reference intervals provided by the
215 manufacturer and those obtained in the VARIETE cohort, as assessed by the Kappa
216 coefficient and the percentage agreement for each IGF-I assay. The concordances and
217 percentages of observed agreement were generally poor.

218

219 **Discussion**

220 We report reference intervals for IGF-I concentrations obtained with six
221 immunoassays in the same population of nearly 900 French healthy subjects aged from 18 to
222 90 years, in keeping with the 2011 recommendations of the Consensus Group on the
223 Standardization and Evaluation of GH and IGF-I assays (1). The population comprised about
224 100 subjects per age decade, and specific reference intervals were calculated for each sex and
225 age group. The reference intervals varied from one assay to another: the lower limits of the
226 normal range (2.5th percentile) were quite similar with the six methods, but the upper limits
227 (97.5th percentile) varied widely from one assay to another, in both men and women (Figure
228 1). Although the pre-analytic conditions were the same for the six kits, and although four of
229 the six kits were calibrated against the international reference standard 02/254, concordance
230 between the assays, as assessed with Bland-Altman plots and the Kappa coefficient, remained
231 quite variable, not only for raw IGF-I values but also for IGF-I SDS. This latter result was
232 somewhat surprising, as we expected that, by using the same healthy population, we would
233 obtain similar SDS.

234 In table 2, which shows the reference ranges for each assay, we have deliberately
235 omitted the mean and SD calculated for each age category from the raw values, in order to
236 avoid erroneous calculations of SDS. Indeed, the Box-Cox power transformation, which is
237 necessary because of the non Gaussian distribution in each age category, uses parameters (L
238 for skewness, M for median and S for the coefficient of variation) that are specific to each

239 assay and also to each age group and gender. We thus propose an online calculator available
240 either following this link (http://ticemed.sa.upmc.fr/sd_score/) or by using Apps (IGF-I
241 SD_score) downloadable for Android from Google Play and for iOS from Apple Store (free
242 of charge) which allows to determine SDS as a function of the assay method, the measured
243 IGF-I value, gender, and age. L, M and S parameters are also provided in Supplemental Table
244 1.

245 Reliable reference intervals are crucial for interpreting IGF-I values in adults with
246 acromegaly (for diagnosis and assessment of disease control during treatment), and also for
247 diagnosing GH deficiency and monitoring GH therapy (4,5,19,20). Reference intervals
248 obtained with the IGF-I Nichols Advantage assay in a very large population of healthy
249 subjects (21) were once widely used for research and clinical practice. However, market
250 withdrawal of this assay, together with the availability of numerous automated methods with
251 considerable heterogeneity, led to calls for improved comparability and reliable normative
252 data. One important first step was the creation of the recombinant international IGF-I standard
253 preparation 02/254 (22). A consensus conference held in 2011 proposed that all assays be
254 calibrated against this standard, and advocated precise pre-analytical and analytical conditions
255 (1). Another recommendation was to establish normative data based on a random selection of
256 individuals from the background population, with representation of all age groups (1). The
257 first normative data for the iSYS IGF-I assay, based on these recommendations and on a very
258 large healthy population, were published by Bidlingmaier et al (23). We now propose
259 reference intervals for six IGF-I assays also based on a large population of healthy subjects. It
260 should be noted that we used very stringent inclusion criteria. Indeed, despite the large sample
261 size (almost one thousand healthy subjects, with about 100 subjects per age group), all the
262 subjects had a clinical examination, including assessment of gonadal status, and also a careful
263 medical history taking that included ongoing medications. Furthermore, all the subjects had

264 an extensive standard biological work-up in order to exclude those with disorders capable of
265 influencing IGF-I levels or their measurement. These very strict inclusion and exclusion
266 criteria allow to define a population as “healthy” as possible; however this implies that these
267 normative data will not be strictly applicable to patients with BMI > 28 kg/m² or to patients
268 with oral treatment with estrogens.

269 As expected, IGF-I concentrations fell gradually with age in both sexes, irrespective of the
270 assay. Contrary to previous reports (21,23), we found a gender difference, with higher IGF-I
271 levels in women than in men, whatever the assay, until the 5th decade. After 50 years of age,
272 however, IGF-I levels were higher in men than in women, as reported elsewhere (21,23). We
273 therefore propose separate normative data for men and women. One possible explanation for
274 the discrepancy between this work and previous reports is that we excluded all subjects
275 receiving steroid hormones such as estrogens. Indeed, oral estrogen is known to lower IGF-1
276 levels (6-8). In premenopausal women, for example, contraceptive pills containing ethinyl
277 estradiol reduce IGF-I levels by up to an average of 30% (24-27). Another explanation might
278 be the size of our population. Indeed, in their study involving a larger number of subjects
279 (15,000), Bidlingmaier et al. did not find differences in terms of gender differences (23).

280 Inter-assay differences in IGF-I reference intervals are a well-known issue that has
281 previously been underlined by one of us (28,29) and by many other researchers
282 (2,11,23,30,31). In this study, as expected, the largest inter-centile intervals (and highest
283 values) were obtained with the two assays calibrated with the old standard IRP 87/518
284 (IMMULITE and IGFI RIACT). Moreover, the three automated methods (iSYS, Liaison XL
285 and IMMULITE), which should theoretically be the most reproducible, did not yield narrower
286 reference intervals. For example, the iSYS automated method and the Mediagnost RIA
287 manual method gave very similar intervals for both men and women in all age groups. Thus,
288 the main source of variation does not appear to be analytical reproducibility. Using the same

289 iSYS method and a similar transformation for normalizing data and constructing specific
290 centile curves in the LMS method, our 2.5th and 97.5th percentiles were generally slightly
291 higher and our intervals generally narrower than those reported by Bidlingmaier et al. (23).
292 Although inter-laboratory variability may play a role in these discrepancies, they are likely
293 due mainly to differences in the population samples (our population was smaller, and the
294 inclusion criteria were different). Another issue raised by our study is the poor concordance
295 between our reference intervals and those proposed by the assay manufacturers. Once again
296 this might be related to the use of different background populations: indeed, those used by
297 manufacturers may not fulfill all the criteria recommended by the consensus group in 2011,
298 particularly with respect to their size, the definition of healthy subjects, and the use of
299 hormonal contraceptives (Supplemental Material).

300 Likewise, one obvious explanation for the discordance between assays is the use of different
301 populations to establish reference intervals. This is why we used the same reference
302 population for all the kits. However, although the six assays showed comparable analytical
303 performance in terms of their reproducibility and detection limits (Table 1), and despite the
304 fact that they use the same non-competitive “sandwich” format and similar methods to avoid
305 IGFBP interference (IGF-II addition), the reference values obtained in our well-controlled
306 adult population differed strikingly from one assay to another. Two of the six assays
307 (IMMULITE and IGF-I RIACT) are still calibrated against the old IRR 87/518 standard,
308 whereas the other four are calibrated against the new IRR 02/254 standard, as currently
309 recommended (1). As expected, the former two assays gave the highest upper reference range
310 for both sexes until the age of 50 (Table 2, Figure 1). However, the reference ranges of two
311 differently calibrated kits may be either similar (e.g. LIAISON XL and IGFI RIACT in men),
312 or significantly different (e.g. iSYS lower than IMMULITE) (Table 2). Likewise, reference
313 ranges determined with kits calibrated against the same reference preparation may also be

314 significantly different, even for kits from the same manufacturer (e.g. the RIA and ELISA kits
315 from Mediagnost). It therefore seems likely that the observed differences are related to other
316 analytical factors, such as the efficiency of IGFBP interference removal and the specificity
317 and/or affinity of the antibody used. For example, since the 2.5th percentile is at least similar
318 between the assays, the broadening of the interval for the IMMULITE assay is probably not
319 related to the calibrator, but to relatively higher measurement results at the upper end:an
320 explanation could be that IMMULITE assay preferentially recognizes the high free IGF-I at
321 high concentrations, while the other 2 assays more efficiently remove the impact of BPs.
322 This could have important implications in patients with disorders affecting their IGFBP
323 profile, such as acromegaly and chronic kidney disease. If confirmed in further studies, this
324 implies that a given individual must be monitored with the same IGF-I assay.
325 Another limitation of our study is that it lies on a single measurement of IGF-I while it is well
326 known that there is some within-subject variability when an individual is sampled on different
327 days (32,33).

328 What refinements may be expected in the measurement of this very demanding
329 analyte? The LC-MSMS method may prove to be a valid alternative and is now being used to
330 assess inter-laboratory agreement on IGF-I concentrations (34) or for validation of IGF-I
331 measures (35). Reference intervals for IGF-I provided with this LC-MS (36) seem very
332 comparable with those obtained with immunoassays. When compared with our data, lower
333 limit of normal range is similar and upper limit corresponds more or less with those observed
334 with Liaison XL or IGF1 RIACT immunoassays. However, LC-MSMS is a time-consuming
335 and complex method that requires expensive machines and high technical expertise, because
336 many variables need to be controlled for providing accurate quantitative results
337 (e.g. extraction strategies, approaches to detect and quantify IGF-I, calibration
338 protocols...)(37). Furthermore, a recent preliminary study of an LC-MS method suggested

339 that it might miss some IGF-I protein variants (pathogenic or physiological), which are
340 present in 0.6% of the population (38). Thus, despite their limitations, immunoassays will
341 continue to be widely used, at least in the near future (39).

342 In conclusion, we have established reference intervals for six commercial IGF-I
343 assays, in a study conforming to recent international recommendations. Despite being
344 obtained in the same large population of French healthy subjects, the reference intervals
345 differed somewhat from one assay to another, and agreement between assays was moderate to
346 good. Finally, concordances between the manufacturers' reference intervals and those
347 obtained in our cohort were generally poor. These findings confirm the need to establish
348 reference intervals for each commercial IGF-I assay in a large background population. Inter-
349 assay concordance with respect to the classification of patients with acromegaly or GH
350 deficiency remains to be determined, and the IGF-I standard deviation scores obtained with
351 the six assays in these subjects need to be compared.

352

353 **Acknowledgments**

354 The study was supported by a grant from Programme Hospitalier de Recherche Clinique,
355 French Ministry of Health, N° P081216 / IDRCB 2009-A00892-55 and by a grant from Fond
356 National Suisse , P1GEP3_155694, to Dr Maria Mavromati.

357

358 We thank for Dr H  l  ne Agostini and Prof. Bruno Falissard for their helpful comments.

359 We also thank Cisbio International, DiaSorin, IDS, Mediagnost, and Siemens for the kind
360 donation of IGF-I kits.

361

362 **Legends of Figures**

363 **Figure 1.**

364 Reference intervals (Upper panel, males; lower panel, females) according to the age intervals
365 of the 6 immunoassays tested. Lower limits (2.5th percentile) and upper limits (97.5th
366 percentile) of the normal range are drawn as full lines and means as dotted lines.

367

368 **Figure 2.**

369 Comparisons between iSYS and Mediagnost RIA expressed as scatter plots (A) or Bland-
370 Altman plots (B) for raw data, or scatter plots (C) and Bland-Altman plots (D) for SDS
371 showing a good overall agreement between both immunoassays, with no significant bias.

372 Comparisons between Liaison XL and Mediagnost RIA expressed as scatter plots (E) or
373 Bland-Altman plots (F) for raw data, or scatter plots (G) and Bland-Altman plots (H) for SDS
374 showing a bad overall agreement between these two immunoassays.

375

376

377

378

379 **References**

380

381

382 **1.** Clemmons DR. Consensus Statement on the Standardization and Evaluation of
383 Growth Hormone and Insulin-like Growth Factor Assays. *Clin Chem* 2011; 57:555-
384 559

385 **2.** Pokrajac A, Wark G, Ellis AR, Wear J, Wieringa GE, Trainer PJ. Variation in GH and
386 IGF-I assays limits the applicability of international consensus criteria to local
387 practice. *Clin Endocrinol (Oxf)* 2007; 67:65-70

388 **3.** Quarmby V, Quan C, Ling V, Compton P, Canova-Davis E. How much insulin-like
389 growth factor I (IGF-I) circulates? Impact of standardization on IGF-I assay accuracy.
390 *J Clin Endocrinol Metab* 1998; 83:1211-1216

391 **4.** Frystyk J, Freda P, Clemmons DR. The current status of IGF-I assays--a 2009 update.
392 *Growth Horm IGF Res* 2010; 20:8-18

393 **5.** Brabant G, Wallaschowski H. Normal levels of serum IGF-I: determinants and validity
394 of current reference ranges. *Pituitary* 2007; 10:129-133

395 **6.** Juul A. Serum levels of insulin-like growth factor I and its binding proteins in health
396 and disease. *Growth Horm IGF Res* 2003; 13:113-170

397 **7.** Leung KC, Johannsson G, Leong GM, Ho KK. Estrogen regulation of growth
398 hormone action. *Endocr Rev* 2004; 25:693-721

399 **8.** Meinhardt UJ, Ho KK. Modulation of growth hormone action by sex steroids. *Clin*
400 *Endocrinol (Oxf)* 2006; 65:413-422

401 **9.** Clemmons DR. Value of insulin-like growth factor system markers in the assessment
402 of growth hormone status. *Endocrinol Metab Clin North Am* 2007; 36:109-129

403 **10.** Massart C, Poirier JY, Jard C, Pouchard M, Vigier MP. Determination of serum
404 insulin-like growth factor-I reference values for the immunometric Cisbio method on a

- 405 large number of healthy subjects: clinical utility in the follow-up of patients with
406 treated acromegaly. *Clin Chim Acta* 2007; 381:176-178
- 407 **11.** Varewijck AJ, Lamberts SW, van der Lely AJ, Neggers SJ, Hofland LJ, Janssen JA.
408 The introduction of the IDS-iSYS total IGF-1 assay may have far-reaching
409 consequences for diagnosis and treatment of GH deficiency. *J Clin Endocrinol Metab*
410 2015; 100:309-316
- 411 **12.** Cole TJ, Green PJ. Smoothing reference centile curves: the LMS method and
412 penalized likelihood. *Stat Med* 1992; 11:1305-1319
- 413 **13.** Blum WF, Breier BH. Radioimmunoassays for IGFs and IGFbps. *Growth Regul*
414 1994; 4 Suppl 1:11-19
- 415 **14.** Royston P, Wright EM. A method for estimating age-specific reference intervals
416 based on fractional polynomials and exponential transformation. *J Royal Stat Soc:*
417 *Series A* 1998; 161:79-101
- 418 **15.** Rigby RA, Stasinopoulos DM. Generalized additive models for location, scale and
419 shape. *Journal of the Royal Statistical Society: Series C (Applied Statistics)* 2005;
420 54:507-554
- 421 **16.** Fleiss J-L, Levin B, Paik MC. *Statistical methods for rates and proportions*. Third
422 Edition ed. New York: Wiley.
- 423 **17.** Cicchetti DV, Allison T. A new procedure for assessing reliability of scoring EEG
424 sleep recordings. *American Journal of EEG Technology* 1971; 11:101-109
- 425 **18.** Landis JR, Koch GG. The measurement of observer agreement for categorical data.
426 *Biometrics* 1977; 33:159-174
- 427 **19.** Clemmons DR. IGF-I assays: current assay methodologies and their limitations.
428 *Pituitary* 2007; 10:121-128

- 429 **20.** Junnila RK, Strasburger CJ, Bidlingmaier M. Pitfalls of insulin-like growth factor-I
430 and growth hormone assays. *Endocrinol Metab Clin North Am* 2015; 44:27-34
- 431 **21.** Brabant G, von zur Muhlen A, Wuster C, Ranke MB, Kratzsch J, Kiess W,
432 Ketelslegers JM, Wilhelmssen L, Hulthen L, Saller B, Mattsson A, Wilde J, Schemer
433 R, Kann P. Serum insulin-like growth factor I reference values for an automated
434 chemiluminescence immunoassay system: results from a multicenter study. *Horm Res*
435 2003; 60:53-60
- 436 **22.** Burns C, Rigsby P, Moore M, Rafferty B. The First International Standard For
437 Insulin-like Growth Factor-1 (IGF-1) for immunoassay: preparation and calibration in
438 an international collaborative study. *Growth Horm IGF Res* 2009; 19:457-462
- 439 **23.** Bidlingmaier M, Friedrich N, Emeny RT, Spranger J, Wolthers OD, Roswall J,
440 Korner A, Obermayer-Pietsch B, Hubener C, Dahlgren J, Frystyk J, Pfeiffer AF,
441 Doering A, Bielohuby M, Wallaschofski H, Arafat AM. Reference intervals for
442 insulin-like growth factor-1 (igf-i) from birth to senescence: results from a multicenter
443 study using a new automated chemiluminescence IGF-I immunoassay conforming to
444 recent international recommendations. *J Clin Endocrinol Metab* 2014; 99:1712-1721
- 445 **24.** Jernstrom H, Deal C, Wilkin F, Chu W, Tao Y, Majeed N, Hudson T, Narod SA,
446 Pollak M. Genetic and nongenetic factors associated with variation of plasma levels of
447 insulin-like growth factor-I and insulin-like growth factor-binding protein-3 in healthy
448 premenopausal women. *Cancer Epidemiol Biomarkers Prev* 2001; 10:377-384
- 449 **25.** Elkazaz AY, Salama K. The effect of oral contraceptive different patterns of use on
450 circulating IGF-1 and bone mineral density in healthy premenopausal women.
451 *Endocrine* 2015; 48:272-278

- 452 **26.** Blackmore KM, Wong J, Knight JA. A cross-sectional study of different patterns of
453 oral contraceptive use among premenopausal women and circulating IGF-1:
454 implications for disease risk. *BMC Womens Health* 2011; 11:15
- 455 **27.** Balogh A, Kauf E, Vollanath R, Graser G, Klinger G, Oettel M. Effects of two oral
456 contraceptives on plasma levels of insulin-like growth factor I (IGF-I) and growth
457 hormone (hGH). *Contraception* 2000; 62:259-269
- 458 **28.** Massart C, Poirier JY. Serum insulin-like growth factor-I measurement in the follow-
459 up of treated acromegaly: comparison of four immunoassays. *Clin Chim Acta* 2006;
460 373:176-179
- 461 **29.** Massart C, Poirier JY. Determination of serum insulin-like growth factor-I reference
462 values for the automated chemiluminescent Liaison(R) assay. Clinical utility in the
463 follow-up of patients with treated acromegaly. *Clin Chim Acta* 2011; 412:398-399
- 464 **30.** Krebs A, Wallaschofski H, Spilcke-Liss E, Kohlmann T, Brabant G, Volzke H, Nauck
465 M. Five commercially available insulin-like growth factor I (IGF-I) assays in
466 comparison to the former Nichols Advantage IGF-I in a growth hormone treated
467 population. *Clin Chem Lab Med* 2008; 46:1776-1783
- 468 **31.** Cowan DA, Bartlett C. Laboratory issues in the implementation of the marker method.
469 *Growth Horm IGF Res* 2009; 19:357-360
- 470 **32.** Milani D, Carmichael JD, Welkowitz J, Ferris S, Reitz RE, Danoff A, Kleinberg DL.
471 Variability and reliability of single serum IGF-I measurements: impact on determining
472 predictability of risk ratios in disease development. *J Clin Endocrinol Metab* 2004;
473 89:2271-2274
- 474 **33.** Nguyen TV, Nelson AE, Howe CJ, Seibel MJ, Baxter RC, Handelsman DJ,
475 Kazlauskas R, Ho KK. Within-subject variability and analytic imprecision of

476 insulinlike growth factor axis and collagen markers: implications for clinical diagnosis
477 and doping tests. *Clin Chem* 2008; 54:1268-1276

478 **34.** Cox HD, Lopes F, Woldemariam GA, Becker JO, Parkin MC, Thomas A, Butch AW,
479 Cowan DA, Thevis M, Bowers LD, Hoofnagle AN. Interlaboratory agreement of
480 insulin-like growth factor 1 concentrations measured by mass spectrometry. *Clin*
481 *Chem* 2014; 60:541-548

482 **35.** Kay R, Halsall DJ, Annamalai AK, Kandasamy N, Taylor K, Fenwick S, Webb A,
483 Wark G, Pleasance S, Gurnell M. A novel mass spectrometry-based method for
484 determining insulin-like growth factor 1: assessment in a cohort of subjects with
485 newly diagnosed acromegaly. *Clin Endocrinol (Oxf)* 2013; 78:424-430

486 **36.** Bystrom C, Sheng S, Zhang K, Caulfield M, Clarke NJ, Reitz R. Clinical utility of
487 insulin-like growth factor 1 and 2; determination by high resolution mass
488 spectrometry. *PLoS One* 2012; 7:e43457

489 **37.** Hoofnagle AN, Whiteaker JR, Carr SA, Kuhn E, Liu T, Massoni SA, Thomas SN,
490 Townsend RR, Zimmerman LJ, Boja E, Chen J, Crimmins DL, Davies SR, Gao Y,
491 Hiltke TR, Ketchum KA, Kinsinger CR, Mesri M, Meyer MR, Qian WJ, Schoenherr
492 RM, Scott MG, Shi T, Whiteley GR, Wrobel JA, Wu C, Ackermann BL, Aebersold R,
493 Barnidge DR, Bunk DM, Clarke N, Fishman JB, Grant RP, Kusebauch U, Kushnir
494 MM, Lowenthal MS, Moritz RL, Neubert H, Patterson SD, Rockwood AL, Rogers J,
495 Singh RJ, Van Eyk JE, Wong SH, Zhang S, Chan DW, Chen X, Ellis MJ, Liebler DC,
496 Rodland KD, Rodriguez H, Smith RD, Zhang Z, Zhang H, Paulovich AG.
497 Recommendations for the Generation, Quantification, Storage, and Handling of
498 Peptides Used for Mass Spectrometry-Based Assays. *Clin Chem* 2016; 62:48-69

- 499 **38.** Hines J, Milosevic D, Ketha H, Taylor R, Algeciras-Schimnich A, Grebe SK, Singh
500 RJ. Detection of IGF-1 protein variants by use of LC-MS with high-resolution
501 accurate mass in routine clinical analysis. Clin Chem 2015; 61:990-991
- 502 **39.** Ketha H, Singh RJ. Clinical assays for quantitation of insulin-like-growth-factor-1
503 (IGF1). Methods 2015; 81:93-98
- 504

Table 1 : Characteristics of the tested IGF-I assays as provided by the manufacturers. These 6 assays are sandwich assays that use a couple of monoclonal antibodies directed against epitopes whose exact nature is not disclosed by the manufacturers. In all cases, IGFBPs are said to be removed by displacement of endogenous IGF-I by an excess of IGF-II (or analog) as initially proposed by Blum and Breier (13). The limit of quantification (LOQ) is the lowest amount of IGF-I that can be accurately quantified with an allowable error $\leq 20\%$. The limit of detection (LOD) is the IGF-I concentration corresponding to the 95th percentile value from a number of determinations of IGF-I concentration in free serum samples.

Name of the assay	Manufacturer	Automated	Tracer	International standard against which the assay is calibrated	Intra-assay CV	Inter-assay CV	LOQ or LOD in ng/mL	Highest measurable value without dilution (ng/mL)	Reference adult population recruited by the manufacturer
iSYS	IDS	Yes	Acridinium ester	WHO/NIBSC 02/254	2.9% at 22 ng/mL 1.9% at 163 ng/mL 4.2% at 304 ng/mL	5.4% at 22 ng/mL 3.9% at 163 ng/mL 7.2% at 304 ng/mL	8.8 (LOQ)	1200	6500 adults. Reference values provided according to the method of Cole and Green) (12)
LIAISON XL	DiaSorin	Yes	isoluminol	WHO/NIBSC 02/254	5.1% at 70 ng/mL 3.5% at 183 ng/mL 3% at 589 ng/mL	9.6% at 80 ng/mL 7.1% at 187 ng/mL 5.6% at 317 ng/mL	3 (LOD) 10 (LOQ)	1500	1606 adults. Reference values provided by age according to the method of Royston and Wright (14)
IMMULITE 2000	Siemens	Yes	Alkaline phosphatase	WHO/NIBSC 1 st IRR 87/518	3.9% at 77 ng/mL 6.5% at 169 ng/mL 2.9% at 380 ng/mL 3.0% at 689 ng/mL 2.3% at 1053 ng/mL 2.4% at 1358 ng/mL	7.7% at 77 ng/mL 5.4% at 169 ng/mL 7.4% at 380 ng/mL 8.1% at 689 ng/mL 3.7% at 1053 ng/mL 4.7% at 1358 ng/mL	20 (LOQ)	1600	1499 pediatric and adult samples from an apparently healthy population (no indication is given concerning the respective numbers of adult and children)
IGFI-RIACT	Cisbio	No	¹²⁵ I	WHO/NIBSC 1 st IRR 87/518	3.8% at 49 ng/mL 3.4% at 162 ng/mL 3.2% at 496 ng/mL	3.8% at 39 ng/mL 8.2% at 352 ng/mL 5.9% at 509 ng/mL	1 (LOD)	900	693 adults 29-70 years
Mediagnost ELISA	MEDIA-GNOST	No	Peroxydase enzyme conjugate	WHO/NIBSC 02/254	5.7% at 138 ng/mL 5.1% at 141 ng/mL 6.6% at 145 ng/mL	6.1% at 142 ng/mL 6.8% at 174 ng/mL 2.2% at 494 ng/mL	1.9 (LOD)	1050	Based on the data reported by Blum and Breier (13)
Mediagnost RIA	MEDIA-GNOST	No	¹²⁵ I	WHO/NIBSC 02/254	4.6% at 56 ng/mL 3.4% at 140 ng/mL 2.5% at 180 ng/mL	4.9% at 55 ng/mL 6.2% at 140 ng/mL 4.5% at 186 ng/mL	2.6 (LOD)	780	Based on the data reported by Blum and Breier (13) The reference values for the different age ranges are the same as those used for the Mediagnost ELISA kit

Table 2. Normative reference intervals (95% confidence interval : CI) of IGF-I measured by 6 assay methods according to age range and sex in a cohort of 899 healthy subjects

Age range	N	iSYS IGF-I (ng/mL) 95%CI	LIAISON XL IGF-I (ng/mL) 95%CI	IMMULITE 2000 IGF-I (ng/mL) 95%CI	IGFI-RIACT IGF-I (ng/mL) 95%CI	Mediagnost ELISA IGF-I (ng/mL) 95%CI	Mediagnost RIA IGF-I (ng/mL) 95%CI
Males							
18-20 years	56	168-391	186-453	195-537	197-486	177-430	168-374
21-23 years	61	147-346	168-411	171-477	173-430	159-388	150-337
24-26 years	53	132-313	153-377	152-430	155-389	144-355	135-308
27-29 years	49	122-292	142-351	138-396	143-363	133-331	126-289
30-39 years	56	108-265	124-310	118-348	127-329	115-295	112-265
40-49 years	51	91-233	106-271	98-301	107-286	98-261	97-237
50-59 years	54	81-214	97-252	85-273	94-262	88-245	86-218
60-69 years	49	75-208	92-245	77-260	87-250	80-237	82-214
70-89 years	34	64-192	80-220	66-242	75-231	71-233	72-200
Females							
18-20 years	41	155-421	191-483	180-586	169-517	169-487	161-412
21-23 years	54	144-383	176-448	166-541	159-476	156-446	149-379
24-26 years	45	134-353	163-418	153-501	150-440	144-412	139-353
27-29 years	48	126-330	152-391	142-467	142-410	134-385	131-332
30-39 years	47	113-294	131-345	121-403	126-356	118-341	118-298
40-49 years	50	97-253	109-296	98-331	107-297	100-296	103-258
50-59 years	54	80-209	93-253	80-271	90-247	82-248	97-220
60-69 years	47	64-170	84-222	68-227	76-209	68-208	75-190
70-89 years	50	56-154	81-204	60-188	67-189	60-187	68-175

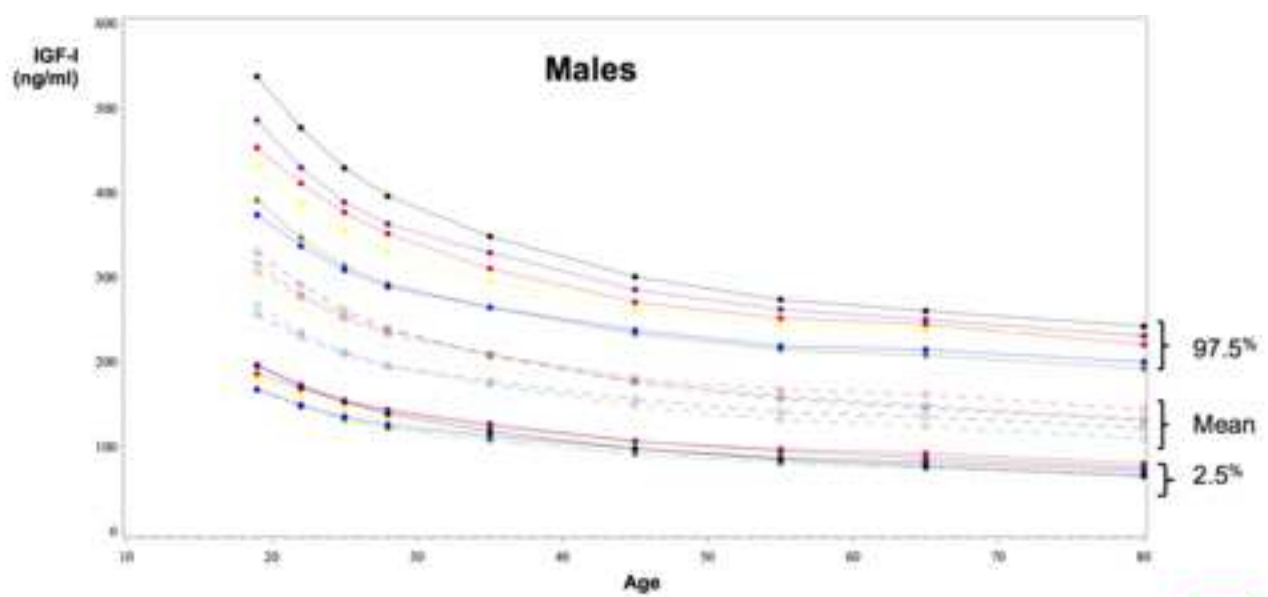
Table 3. Agreement of each IGF-1 assay method against each of the other, expressed as weighted kappa and percentages of observed agreement.

	LIAISON XL	iSYS	IMMULITE 2000	Mediagnost ELISA	Mediagnost RIA	IGFI-RIACT
LIAISON XL	- 94.86%	0.49 94.86%	0.50 94.83%	0.47 94.95%	0.38 94.05%	0.48 95.22%
iSYS	0.49 94.86%	- 96.46%	0.64 96.08%	0.61 96.11%	0.70 97.00%	0.64 96.46%
IMMULITE 2000	0.50 94.83%	0.64 96.08%	- 96.32%	0.61 95.95%	0.58 95.73%	0.64 96.32%
Mediagnost ELISA	0.47 94.95%	0.61 96.11%	0.61 95.95%	- 95.66%	0.59 96.00%	0.53 95.66%
Mediagnost RIA	0.38 94.05%	0.70 97.00%	0.58 95.73%	0.59 96.00%	- 95.22%	0.48 95.22%
IGFI-RIACT	0.48 95.22%	0.64 96.46%	0.64 96.32%	0.53 95.66%	0.48 95.22%	- 95.22%

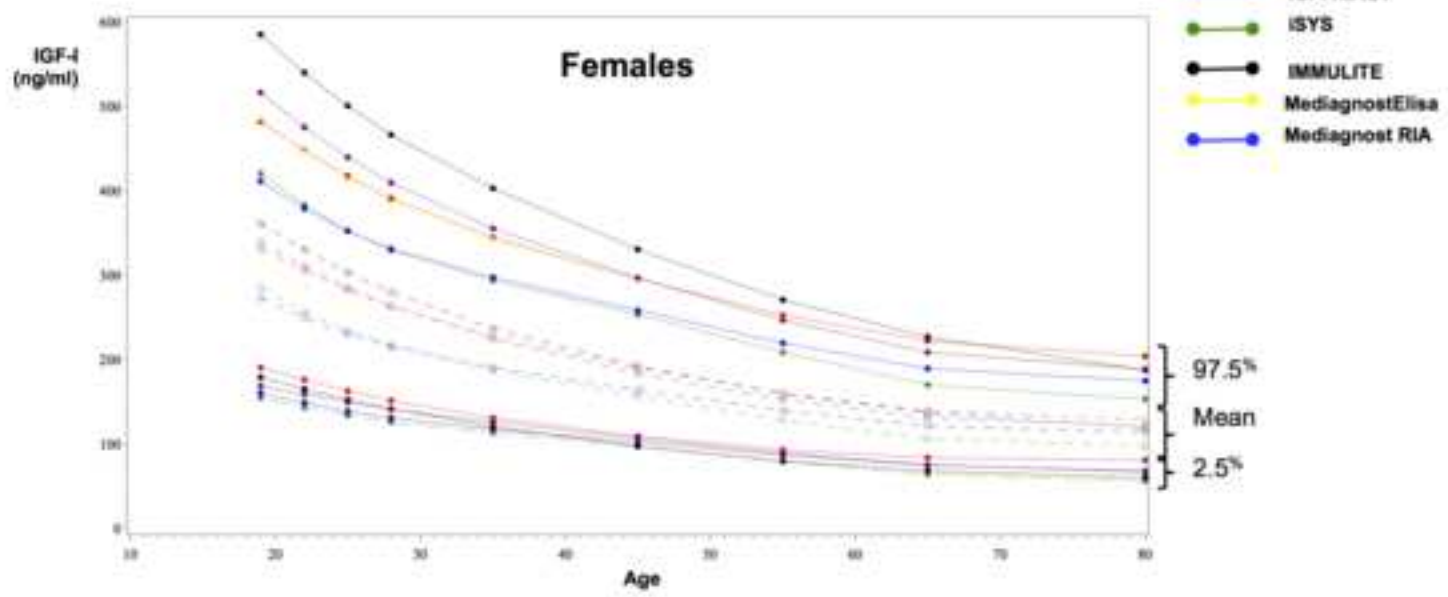
Table 4. Concordance between VARIETE cohort reference intervals and reference intervals provided by each manufacturer, expressed as Kappa and percentages of observed agreement

	LIAISON XL	iSYS	IMMULITE 2000	Mediagnost ELISA	Mediagnost RIA	IGFI - RIACT
Weighted Kappa	0.19	0.35	0.38	0.18	0.17	0.22
% of agreement	83.28	93.36	86.97	93.55	94.77	88.21

A

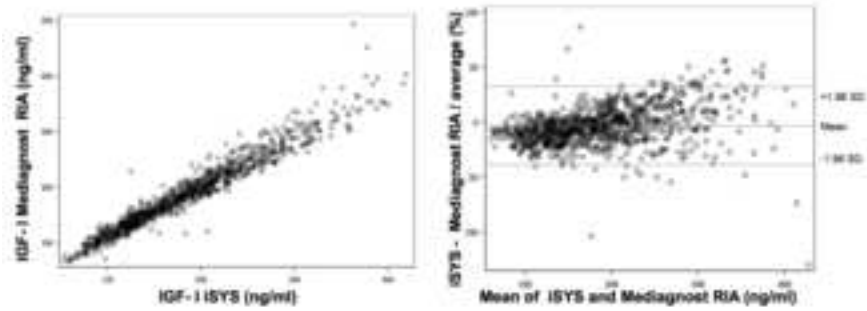


B



iSYS vs Mediagnost RIA

Raw data

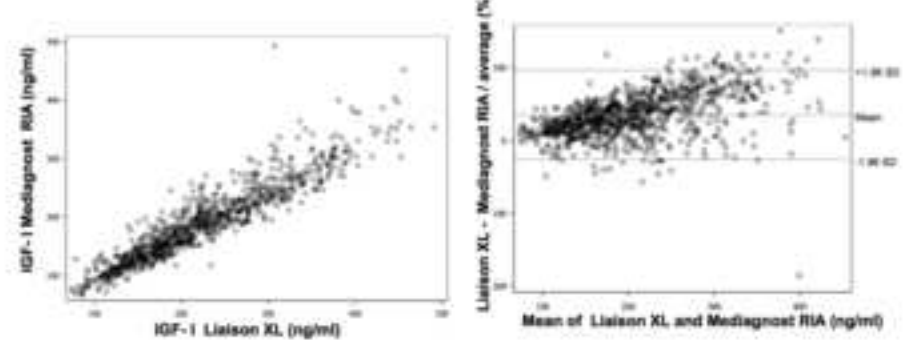


A

B

Liaison XL vs Mediagnost RIA

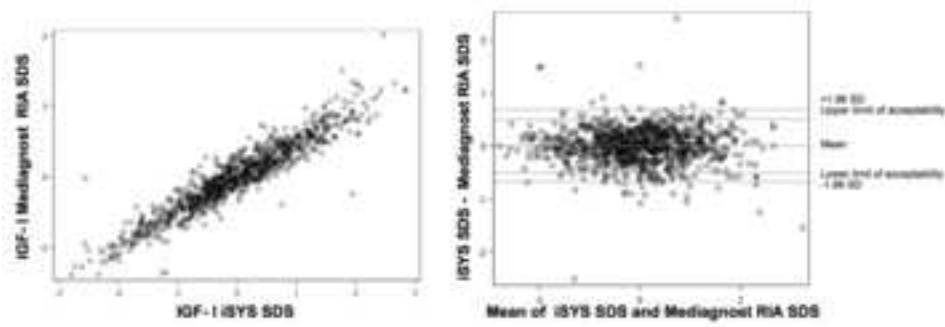
Raw data



E

F

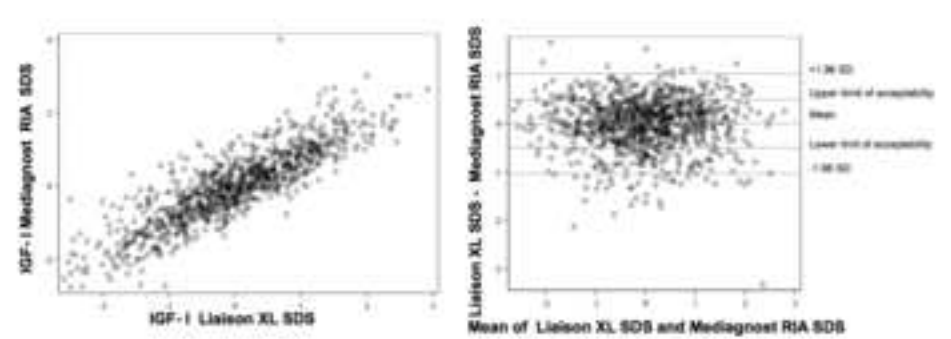
SDS



C

D

SDS



G

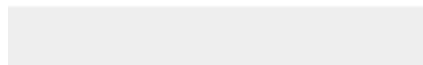
H



Click here to access/download

Supplemental Material

Supplemental material VF .docx





Click here to access/download
Supplemental Material
Suppl Table 1.docx



Click here to access/download
Supplemental Material
Suppl Figure 1VF.docx



Click here to access/download
Supplemental Material
Suppl Figure 2VF.docx