1 Title: Interference of asfotase alfa in immunoassays employing alkaline phosphatase
2 technology.
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4 Running head: Interference of asfotase alfa in Immunoassays
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22

23 Abbreviations:

24 HPP: paediatric-onset hypophosphatasia

25 TNSALP: nonspecific alkaline phosphatase

26 ALP, ALKP1: Alkaline phosphatase

27 NBT/BCIP: nitroblue tetrazolium salt and 5-bromo-4-chloro-3-indolyl-phosphate

28 NADPH: Nicotinamide adenine dinucleotide phosphate

29 pNPP: para-Nitrophenylphosphate

30 EMIA: Enzyme Mediated Immunometric Assay

31 EIA: Enzyme Immunoassay

32 TnI: cardiac troponin I

33 hCG: β-human chorionic gonadotropin

34 CHO: Chinese Hamster Ovary

35 IL6: Interleukin 6

36 fT4: free T4 (thyroxin)

37 TSH: thyroid stimulating hormone

38 LH: Luteinising hormone

39 FSH: Follicle stimulating hormone

40 BAP: bone alkaline phosphatase

41 HCL: hydrochloric acid

42 HRP: horseradish peroxidase

43 **Abstract**

44 Background: Asfotase alfa (STRENSIQ®, Alexion Pharmaceuticals, Inc.) is the only approved
45 treatment for patients with paediatric-onset hypophosphatasia, a disease caused by a mutation
46 in the tissue-nonspecific alkaline phosphatase (TNSALP) gene. ALP is often used as signalling
47 system in routine immunoassays. Because asfotase alfa contains the active site of the full ALP
48 enzyme, it can catalyse the substrate as the antibody-conjugated ALP would within an assay.
49 Therefore, its presence in a treated patient's sample may generate false positive or false
50 negative results. We investigated whether the presence of asfotase alfa within a sample induced
51 interference in immunoassays that utilise ALP or alternative detection systems.

52 Methods: Asfotase alfa was added to samples at concentrations from 0.08-5 µg/mL. Asfotase
53 alfa was detected in all ALP assays but ALKP1 (RayBiotech). We observed no changes in
54 normetanephrine and noradrenaline (IBL) at any asfotase alfa concentration. However, asfotase
55 alfa significantly interfered in an oxytocin (ENZO) assay in non-extracted samples. Extraction
56 using a C18 column eliminated the interference.

57 Results: No interference was observed on automated analysers using alternative detection
58 system (COBAS fT4 and TSH; Advia Centaur FSH, fT4; Architect LH; FSH). Immulite 2000
59 fT4, TSH, testosterone and hCG (ALP based) showed no interference. However the presence
60 of asfotase alfa resulted in a dose-dependent increase of Troponin I signal.

61 Conclusion: The presence of asfotase alfa must be taken into consideration when analysing
62 blood samples in treated patients to avoid any risk of misinterpretation of false
63 positive/negative results. It is essential that assays be tested for this possible interference.

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67 **Statement**

68 Asfotase alfa is used in the treatment of hypophosphatasia. It contains the active site of alkaline
69 phosphatase (ALP). Here we show that it interferes in immunoassays that utilize ALP-
70 conjugated antibodies (Troponin I, Oxytocin). This can result in false positive or false negative
71 measurements. Asfotase alfa treatment should therefore be disclosed when requesting
72 biochemical analyses of patient samples and should be considered in the interpretation of
73 results.

74 **1. Introduction**

75 Asfotase alfa (Strensiq®, Alexion Pharmaceuticals, Inc.) is the only approved treatment for
76 patients with paediatric-onset hypophosphatasia (HPP) (1). HPP is an inherited metabolic
77 disorder, the result of a loss-of-function mutation, which results in low activity of the tissue-
78 nonspecific alkaline phosphatase (TNSALP), an enzyme that plays a role in bone
79 mineralization. The defect in TNSALP induces altered mineralisation of bones that become
80 prone to fracture and deformity and this can result in functional impairments and pain (2,3). In
81 2011, the prevalence of severe forms of HPP in Europe was estimated at 1 per 300,000 live
82 births (4). However the exact prevalence for the different forms is not clear (5). Asfotase alfa
83 is injected subcutaneously to patients three or six times per week. With a bioavailability of 46-
84 98%, and a half-life of 2.3±0.6 days, asfotase alfa reaches highest blood concentrations
85 between 24 to 48 hours post injection (6).

86 Alkaline phosphatase (ALP) is used as the measuring/signalling system in many routine assays
87 for proteins, hormones and other small molecules. ALP is usually linked to an antibody directed
88 against the molecule of interest (analyte) in the immunoassay. After addition of a substrate
89 molecule (nitroblue tetrazolium salt and 5-bromo-4-chloro-3-indolyl-phosphate (NBT/BCIP),
90 Nicotinamide adenine dinucleotide phosphate (NADPH), or para-Nitrophenylphosphate
91 (pNPP)), the ALP converts the substrate to generate a colour change that can be estimated using

92 a spectrophotometer. In a classical "sandwich" assay, Enzyme Mediated Immunometric Assay
93 (EMIA), or non-competitive assay, the colour produced is proportional to the concentration of
94 analyte captured between the capture and the detection antibodies. An alternative type of assay
95 is an Enzyme Immunoassay (EIA), or competitive assay, in which the analyte of interest in the
96 sample competes with an ALP-labelled analyte. In this type of assay the colour produced is
97 inversely proportional to the concentration of the analyte. These assay formats are commonly
98 employed in large multichannel analysers.

99 Asfotase alfa is a recombinant glycoprotein containing the catalytic domain of TNSALP.
100 Because of the presence of this active site, asfotase alfa is able to catalyse the substrate in a
101 similar manner to antibody-conjugated ALP. Depending on the type of assay format
102 (competitive or non-competitive) and the concentration of asfotase alfa present in a patient's
103 sample it is possible to generate a false positive or a false negative result. In competitive assays,
104 the higher the concentration of asfotase alfa, the lower the concentration of the analyte that will
105 be reported, as this will give the impression that little "cold" (unlabelled) analyte is in the
106 specimen. In a non-competitive assay, the amount of signal generated is proportional to the
107 concentration of the analyte in the sample, so the higher the concentration of asfotase alfa the
108 greater the signal produced, and so a higher concentration of the analyte will be reported. A
109 limited number of case studies have shown that asfotase alfa may cause interference with some
110 assays such as testosterone (7), cardiac troponin I (TnI) and β-human chorionic gonadotropin
111 (hCG) (8). In this study we tested a range of assays that used ALP as detection system and
112 assay using a different detection system. We also tested a range of analysers commonly used
113 in clinical laboratories.

114 The consequence of false negative and false positive results may be significant for patients who
115 could experience misdiagnosis of additional conditions. We investigated whether the presence
116 of asfotase alfa within a sample induced interference in immunoassays, i.e. sandwich and

117 competitive assays using base-plate and automated analyser technology with various detection
118 systems.

119

120 **2. Material and methods**

121 2.1 Samples

122 Anonymized urine and blood samples were provided by Department of Laboratory Medicine
123 at the Norwich and Norfolk University Hospital in accordance with local ethical committee
124 approval (9).

125 Samples were pooled according to their matrix. Asfotase alfa was added to the sample pool at
126 final concentrations of 5.0, 2.5, 1.25, 0.63, 0.31, 0.16 and 0.08µg/mL. An aliquot of the original
127 pool without addition of asfotase alfa was also measured as control (asfotase alfa 0µg/mL).
128 Measurements were reproduced a minimum of 3 times (new preparation) for each assay.

129 2.2 Reagents

130 Asfotase alfa was kindly provided by Alexion Pharmaceuticals Inc. as a solution of 100
131 mg/mL. Asfotase alfa was produced by recombinant DNA technology using mammalian
132 Chinese Hamster Ovary (CHO) cell culture. The solution excipients are sodium chloride,
133 sodium phosphate dibasic heptahydrate and sodium phosphate monobasic monohydrate in
134 water.

135 For manual assays, oxytocin (ENZO Life Science Inc., Lausen, Switzerland), Normetanephrine
136 (IBL International GmbH, Hamburg, Germany), and Alkaline Phosphatase (ALKP1,
137 RayBiotech, Inc., Norcross, USA) ELISA kits were purchased from Oxford Biosystems
138 (Milton Park, UK). Interleukin 6 (IL6) was purchased from R&D Systems (Abingdon, UK).
139 COBAS 6000 assays for ALP, free T4 (fT4) and thyroid stimulating hormone (TSH) were
140 purchased from Roche Diagnostics (Burgess Hill, UK). Assays for the Architect c16000

141 analyser, ALP, Luteinising hormone (LH) and Follicle stimulating hormone (FSH) were
142 purchased from Abbott (Maidenhead, UK). Assays for Centaur XPT (Siemens, Camberley,
143 UK) were fT4, TSH (TSH3-UL) and ALP (ALP_2c). Assays for Immulite 2000 (Siemens)
144 were testosterone, TnI, hCG, fT4 and TSH. Liaison XL bone alkaline phosphatase (BAP) assay
145 was purchased from DiaSorin (Dartford, UK). Table 1 details assay characteristics and
146 detection systems.

147 All assays were used following manufacturer's instructions. All assays were performed on the
148 same day as the sample preparation.

149 Statistical Analysis:

150 Statistical analysis was performed using SPSS software 25 (IBM Corporation, Armonk, New
151 York, USA). Normality of data was confirmed by Shapiro-Wilk test. Data were analyzed by
152 one way ANOVA to find potential significant effect of asfotase alfa concentration on
153 specimen parameters. Data of different asfotase alfa concentrations were compared with the
154 control group (no asfotase alfa) using Dunnett (equal variance) and Dunnett T3 (non-equal
155 variance).

156

157 **3. Results**

158 The presence of asfotase alfa was detected in the ALP chemistry assays (COBAS, Centaur and
159 Architect ALP) that measured the activity of the enzyme. ALP showed a significant (ANOVA,
160 $p<0.0001$) linear increase in concentration with increasing asfotase alfa concentration (Fig 1A)
161 for all three assays. Architect ALP was only measured to 1.25µg/mL and a linear projection
162 was estimated. We also observed a stronger increase ($p<0.05$) for the Centaur XPT assay as
163 compared to the other two (COBAS, Architect). We observed a strong cross-reactivity of

164 asfotase alfa with the Liaison XL BAP assay ($p<0.001$; Fig 1B). The signal increased very
165 quickly and a hook effect was observed for concentrations of asfotase alfa > 0.31 µg/mL.

166 The COBAS 6000, Advia Centaur XPT and Architect analyser's immunoassays use acridium
167 ester and ruthenium technology to measure the analyte concentrations. These assays, COBAS
168 fT4 and TSH, Centaur FSH and fT4 and Architect FSH and LH, were not affected by the
169 presence of asfotase alfa, at any concentration (Fig 2A, 2B and 2C, respectively). The
170 concentration of the analytes of interest did not change by more than 2.4%, 9.7% and 7.9%
171 from the original sample without asfotase alfa for assays run on the COBAS 6000, the Advia
172 Centaur XPT and the Architect respectively. We observed no significant change in the
173 concentration of TSH, fT4, testosterone and hCG when using the ALP-detection based assay
174 on the Siemens Immulite 2000 instrument, at any asfotase alfa concentration (Fig 2D and 2E).
175 However, asfotase alfa induced a dose-dependent increase of signal/concentration in TnI (Fig
176 2 F, $p<0.05$). This effect was greatest when using serum as compared to lithium-heparin
177 plasma.

178 All the manual assays were selected because they used an ALP detection system (or measure
179 ALP activity, Raybiotech). We observed no effect of asfotase alfa on the concentration of the
180 analytes (normetanephrine, extracted oxytocin) measured (Fig 2G and 2H respectively). We
181 observed low concentrations of normetanephrine in the urine pool, we therefore spiked the
182 sample with 250µg/L of normetanephrine (Calibrator). The measurement of total
183 normetanephrine required hydrolyzation with HCL 0.1M and acetylation using
184 dimethylformamide. This step is followed by a dilution of the acetylated samples 1:100 with
185 assay buffer. We did not observe any significant effect of asfotase alfa when measuring total
186 normetanephrine in urine samples (Fig 2G). We also tested for free normetanephrine, which
187 does not require the hydrolyzation, but only acetylation and therefore dilution, and found no
188 interference of the asfotase alfa (not shown). Although the imprecision in the assay was
189 relatively high (up to 20%), we observed no effect on IL6 (Fig 2G). We observed a very low

190 concentration of oxytocin in our sample pools and therefore proceeded with spiked (using
191 calibrator at 50, 100 and 150pg/mL) samples. We observed that asfotase alfa significantly
192 interfered in the oxytocin assay (Fig 2H, $p<0.001$), in a concentration-dependant manner when
193 samples were not extracted. Extraction with a C18 cartridge is recommended by the supplier
194 when using samples with high matrix content such as plasma samples. We therefore extracted
195 the plasma pool containing 250pg/mL of oxytocin. We observed no interference of asfotase
196 alfa at any concentration. We observed no significant change in the absorbance (Fig 1B) and
197 therefore the concentration of ALP measured using the RayBiotech assay for ALKP1 at any
198 asfotase alfa concentration. We observed a relatively high imprecision in the assay but no
199 consistent changes in OD or concentration and an effect of asfotase alfa was excluded. The
200 maximum absorbance variation within one experiment was 15%.

201

202 **4. Discussion**

203 Asfotase alfa (STRENSIQ®, Alexion Pharmaceuticals, Inc.) is the first approved ALP
204 replacement therapy for treatment of HPP (1). This glycoprotein contains the catalytic domain
205 of human tissue non-specific ALP (TNSALP) (10) as well as the Fc region of human
206 immunoglobulin G1, and a sequence of ten L-aspartate residues that allows the asfotase alfa to
207 target the bone.

208 Due to the presence of the active site of TNSALP in asfotase alfa, assays that measure the
209 activity of endogenous ALP, such as those employed on the Roche COBAS 6000, Siemens
210 Advia Centaur XP and Abbott Architect c16000 analysers, can react with the asfotase alfa in
211 the blood sample. We found that high concentrations of asfotase alfa in samples required
212 dilution of the samples and we demonstrated that these assays have sufficient linearity to
213 "measure" at least 5µg/mL of asfotase alfa in the sample. In treatment of paediatric-onset HPP,
214 the recommended dosage of asfotase alfa is 6mg/kg per week given as 1 or 2 mg on a single

215 day. A pharmacokinetics study on 38 HPP patients treated with asfotase alfa showed maximum
216 ALP blood concentrations Cmax of 1794 ± 690 ng/mL for patients under the age of 5 and 2108
217 ± 788 ng/mL for patients above the age of 5 (10). We tested concentrations covering the range
218 of observed ALP Cmax observed in both adult and pediatric patients treated with asfotase alfa.
219 It is interesting to note that all COBAS ALP and Architect ALP showed similar increased in
220 measured ALP, however the Centaur XPT ALP was significantly higher at 5µg/mL ($p<0.05$).
221 These results highlight the need of standardized assay for ALP in order to monitor asfotase alfa
222 in an harmonised manner across the laboratories. In the manual immunoassay for ALP from
223 Raybiotech the presence of asfotase alfa did not affect the ALP result obtained. We suggest
224 that at least one of the antibodies against ALP (ALKP-1) in this assay is not directed against
225 the active site of the protein and as a result, this assay was specific for the measurement of
226 endogenous ALP. ALP in this assay is detected using an HRP-labelled antibody. The assay is
227 therefore based on the quantity of protein (mass assay) rather than the activity. In order to test
228 the hypothesis that a mass assay employing antibodies not directed against the active site of
229 ALP would be preferential for measuring endogenous ALP in patients receiving asfotase alfa
230 we measured bone-specific ALP using the Liaison XL assay (mass assay) from DiaSorin. All
231 samples with added Asfotase alfa were above the linear range of the assay (120 ng/L) and we
232 observed a sharp increase in signal and hook-effect at concentration of asfotase alfa of about
233 0.3µg/mL.

234 Immunoassays we tested that employed analyte-specific antibodies labelled with acridium ester
235 or rutenium detection systems (fT4, FSH, LH, and TSH) showed no effect of asfotase alfa on
236 the concentrations measured. The oxytocin and normetanephrine assays are immunoassays that
237 are based on the use of detection antibodies that are linked to an ALP detection system. In the
238 final step of the assay, the substrate for ALP, pNPP or NAPDH, respectively, is added and the
239 colour develops depending on the concentration of the specific analyte present.

240 The measurements of oxytocin (ENZO) in urine samples showed an interference effect of
241 asfotase alfa within the assay when the samples were not extracted. It is a competitive
242 immunoassay, where the more oxytocin present in the sample the lower the absorbance
243 measured. As the concentration of asfotase alfa increased, the absorbance increased
244 significantly, resulting in a false negative reading of the concentration of oxytocin. The
245 estimated ODs for oxytocin were quickly above the lower limit of detection for the lowest
246 calibrator, asfotase alfa brought the absorbance to saturation, and all the samples containing
247 asfotase alfa resulted in undetectable oxytocin concentrations.

248 The normetanephrine assay is also a competitive immunoassay. This test necessitates the
249 acylation of the samples with prior hydrolysation (for total normetanephrine) or no
250 hydrolysation (for free normetanephrine). We tested both conditions, and spiked
251 normetanephrine in some samples to ensure the presence of the analyte. We did not detect any
252 interference of asfotase alfa in this assay. The volume of sample used was very small (10µL)
253 and highly diluted (1:100) before being analysed. The conditions of use for detection of
254 normetanephrine may be sufficient to decrease the asfotase alfa concentration resulting in no
255 effect in the assay.

256 A case of false positivity due to AA for cardiac TnI and β-hCG has been described previously
257 (8). Analytes were measured on a Beckman Coulter DxI 800 and both assays used ALP as the
258 detection system. Most recently, Sofronescu *et al.* (7) described the case of a patient whose
259 blood testosterone showed falsely decreased results post treatment with asfotase alfa. The
260 patient suffered from HPP and was treated with testosterone for his secondary hypogonadism.
261 After starting treatment with asfotase alfa, his testosterone concentrations were undetectable
262 while his free testosterone was normal. Testosterone was measured on a Coulter 800 DXI using
263 ALP as detection system. The author also suggested the interference could be due to the 62%
264 sequence homology between the Fc region of the immunoglobulin G1 present in asfotase alfa
265 and the Fc fragment of the mouse anti-testosterone antibody used in the assay. We did not have

266 access to a DxI to reproduce these results. We therefore tested the interference of these analytes
267 on the Siemens Immulite 2000, using ALP as the detection system. We did not observe any
268 effect of asfotase alfa for testosterone or hCG suggesting an alternative way of measuring these
269 analytes for patients treated with asfotase alfa. However, we did observe an interference when
270 measuring TnI. Interference of ALP in assays for cardiac TnI has been observed by several
271 groups on various analytical platforms using ALP/pNPP as detection system (8,12–15),
272 whether from elevated ALP in patients with suspected myocardial infarction, or from carry-
273 over from an elevated sample. Asfotase alfa is used in patients with HPP to reduce the
274 accumulation of extracellular TNSALP substrates such as inorganic pyrophosphate (PPi),
275 pyridoxal-5'-phosphate (PLP) and phosphoethanolamine (PEA). It has been suggested that
276 asfotase alfa may hydrolyse TNSALP substrates to a higher extend than anticipated due to its
277 continuous activity post blood collection (11). Although not tested in this study, this effect
278 must not be ignored. It may be of consideration to implement the use of tubes containing
279 phosphatase inhibitors (such as levamisole or Bromotetramisole oxalate) when collecting blood
280 samples from a patient treated with asfotase alfa.

281

282 **5. Conclusion**

283 Most assays tested using ALP detection systems were sufficiently specific not to show
284 interference with asfotase alfa up to a serum concentration of 5µg/mL (upper Cmax 2108 ±
285 788 ng/mL for patients with HPP). Oxytocin required extraction of samples in that assay type
286 to demonstrate this specificity. The effects observed depended on the method of ALP detection
287 used. When high sensitivity is required, some manufacturers still rely on the use of ALP
288 detection systems, although use of ALP as a detection system has been decreasing over recent
289 years and being replaced with horseradish peroxidase (HRP) or acridium ester in many

290 automated assays. Alternative assays can be found that lack interference, i.e. Siemens
291 testosterone or hCG instead of Beckman Coulter DxI.

292 When measuring TnI, the likelihood of misdiagnosing myocardial infarction in patients treated
293 with asfotase alfa remains low, but cannot be excluded, and consultants should ensure
294 laboratories are aware of the presence of asfotase alfa in samples sent for analysis. The presence
295 of asfotase alfa must be taken into consideration when analysing blood samples using ALP-
296 based technology to avoid any risk of misinterpretation of false positive/negative results.
297 Various methodologies such as addition of a blocking reagent, or dilution of the sample in non-
298 immune serum, can be used to confirm interference (16,17). It is vital for the clinicians and
299 laboratories to communicate the presence of potential interfering substances to avoid any
300 misdiagnosis.

301

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308

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361

362

Analyte	Analyser/Assay Provider		Detection System	Sample type	Unit	Analyte concentration in original pool (mean ± SEM)	Max change (% ± SD)	Interference	Significance (ANOVA)
ALP	COBAS6000	Roche Diagnostics	ALP/pNPP	Serum	U/L	81.7 ± 3.0	17831 ± 1107	**POS**	**<0.001**
	Architect c16000	Abbott			U/L	199 ± 0.0	14700 ± 6789	**POS**	**<0.001**
	ADVIA Centaur XPT	Siemens			U/L	73.9 ± 4.2	4443.9 ± 284.3[a]	**POS**	**<0.001**
	Liaison XL	Diasorin			U/L	13 .0 ± 1.2	4073.4 ± 799.4	**POS**	**<0.001**
ALKP	Manual	RayBiotech Inc.	HRP	Serum	pg/mL	54.0 ± 11.0	17.7 ± 10.6	no	0.249
TSH	COBAS6000	Roche Diagnostics	Ruthenium	Serum	mIU/L	3.7 ± 0.1	1.0 ± 1.0	no	0.990
	ADVIA Centaur XPT	Siemens	acridinium ester	Serum	mIU/L	1.9 ± 0.0	7.4 ± 3.2	no	1.000
	Immulite 2000	Siemens	ALP/pNPP	Serum	mIU/L	1.9 ± 0.1	11.6 ± 1.0	no	0.756
free T4	COBAS6000	Roche Diagnostics	Ruthenium	Serum	pmol/L	15.4 ± 0.1	1.5 ± 0.7	no	0.384
	ADVIA Centaur XPT	Siemens	Ruthenium	Serum	pmol/L	17.9 ± 0.4	6.7 ± 0.8	no	0.890
	Immulite 2000	Siemens	ALP/pNPP	Serum	pmol/L	17.1 ± 0.4	8.4 ± 3.3	no	0.089
Testosterone	Immulite 2000	Siemens	ALP/pNPP	Serum	ng/dL	136.3 ± 1.2 33.5 ± 3	25.2 ± 13.9	no	0.395
				Li-Hep		175.4 ± 8.2	20.9 ± 4.1	no	0.937
hHCG	Immulite 2000	Siemens	ALP/pNPP	Serum	ng/mL	<2.0 ± 0.0 46.2 ± 2.0	14.2 ± 5.3	no	0.487
				Li-Hep		<2.0 ± 0.0	13.5 ± 4.7		0.460
Troponin I	Immulite 2000	Siemens	ALP/pNPP	Serum	ng/mL	<0.2 ± 0.0	4545.0 ± 1457.7	**POS**	**0.001**
				Li-Hep		<0.2 ± 0.0	1643.0 ± 1281.7	**POS**	**<0.001**
LH	Architect c16000	Abbott	acridinium ester	EDTA	IU/L	10.5 ± 0.3	6.0 ± 1.8	no	0.933
FSH	Architect c16000	Abbott	acridinium ester	EDTA	IU/L	26.5 ± 3.5	4.8 ± 2.3	no	0.945
IL6	Manual	R&D Systems	ALP/pNPP	Serum	pg/mL	6.2 ± 1.4	19.9 ± 10.0	no	0.210
Normetanephrine[b]	Manual	IBL International GmbH	ALP/pNPP	Urine	µg/L	581.4 ± 169.4	14.7 ± 8.9	no	0.413
Oxytocin[c] non-extracted	Manual	ENZO life Science Inc.	ALP/pNPP	Urine	pg/mL	130.5 ± 69.6	360.3 ± 88.3	**NEG**	**0.001**
Oxytocin extracted				EDTA†		70.7 ± 5.4	14.8 ± 5.8	no	0.121

Table 1: Table showing the various assays tested for interference with asfotase alfa and their characteristics. The table also include the original analyte concentration in the pool tested (before addition of asfotase alfa) and the p value from the one-way ANOVA test. [a] measured at 1.25 µg/mL of asfotase

366 alfa, samples having not been diluted for values above the assay range; [b] Normetanephrine added at 250, 324 and 612 µg/L; original urine pool <18µg/L;
367 [c] non spiked [Oxytocin] <15pg/mL- spiked with 50, 100, 150pg/mL. LH = Li-Heparin plasma; † containing Aprotinin, tested extracted and non-
368 extracted; PNPP = p-nitrophenyl phosphate; HRP = horseradish peroxidase

369 Figure 1: Graph showing the effect of asfotase alfa ALP neasurement using various platforms.

370 Asfotase alfa can be detected on automated platforms for measurement of ALP. (A) Assays on
371 Centaur XPT (Siemens) and Architect (Abbott) and COBAS 6000 (Roche) showed linear
372 increase of ALP concentration with increased asfotase alfa concentrations up to 5µg/mL. A
373 hook effect was observed in the Liaison XL (DiaSorin) assay for Bone ALP (BAP). (B) Manual
374 assay ALPK1 (Raybiotech) showed no interference of asfotase alfa.

375

376 Figure 2: Graph showing the effect of asfotase alfa on various immunoassay.

377 Automated assays (A) COBAS 6000 fT4 and TSH (B) Advia Centaur fT4 and TSH and (C)
378 Architect LH asnd FSH and Immulite 2000 (D) fT4 and TSH (E) testosterone and hCG showed
379 no interference from the presence of asfotase alfa. Immulite 2000 troponin I (F) showed a dose-
380 dependent interference in both serum and lithium-heparin plasma samples. In manual assays
381 (G) IL6 and normetanephrine and (H) oxytocin showed no interference. When samples were
382 not extracted, asfotase alfa causes interference (false negative) in the oxytocin measurement.