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Diiodothyropropionic acid (DITPA) cross-reacts with thyroid function assays on different immunoassay platforms

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Dear Editor

Monocarboxylate transporter 8 (MCT8) is a thyroid hormone-specific cell membrane transporter that is expressed in numerous human tissues, including the brain, skeletal muscle, heart, lung, and kidney [1]. Loss-of-function mutations to the *MCT8* gene, located on chromosome Xq13.2, producing severe psychomotor deficits and an unusual combination of thyroid function test abnormalities were identified in 2004 [2,3]. A year later it was demonstrated that subjects with Allan–Herndon–Dudley syndrome with X-chromosome linked mental retardation [4] also had the same thyroid hormone test abnormalities and mutations in the *MCT8* gene [5]. The defects manifested in boys at infancy are hypotonia and poor head control. The neurodevelopmental defect progresses to result in spastic quadriplegia and inability to walk and talk [2,3,5]. Affected individuals have high serum triiodothyronine (TT3), very low reverse T3 (rT3), slight reduction in thyroxine (TT4) and low or mildly elevated thyrotropin (TSH) [2,3,5].

Genetically engineered *MCT8* deficient mice demonstrated that the absence of *MCT8* produces thyroid hormone deprivation in the brain and the T3 excess results in hypermetabolism in peripheral tissue explaining the combination of tissue specific

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hypothyroidism (brain) and hypermetabolism leading to the inability to gain weight [6]. A thyroid hormone analogue, diiodothyropropionic acid (DITPA) was found to enter cells independently of MTC8 [7]. Therefore, this compound was tested in children with MCT8 deficiency and found to normalize their thyroid function tests, reduce the T3-induced hypermetabolism and, thus, improve their nutritional status [8].

A problem in the follow-up of DITPA treatment is the cross-reactivity of DITPA in the routine laboratory measurements of T3 (total and free). This necessitates the measurement of DITPA by LC–MS/MS to correct for its interference in T3 determination by immunoassay. However, the degree of cross-reactivity of DITPA with TT3 assays on different platforms has not been explored. To investigate this, we studied the possible cross-reactivity of DITPA on commercial thyroid hormone immunoassays available from four in-vitro diagnostic companies.

A 1000 µg/dl DITPA (Sigma Aldrich) stock solution was prepared in ethanol (Decon Labs) and then further diluted 100-fold using PBS. Varying amounts of the DITPA stock solution were added to pooled serum to create a series with final DITPA concentrations ranging from 3–75 µg/dl. These samples were then assayed for TT3, TT4, free T3, free T4, and TSH on the E170 (Roche Diagnostics), Immulite, Centaur, and Dimension Vista and EXL (all Siemens Healthcare Diagnostics), Access (Beckman Coulter Inc.) and Architect (Abbott Diagnostics) platforms. All samples were analyzed in duplicate and the respective assay values in the DITPA-treated samples were expressed as the difference above the baseline sample. Further investigation was done to determine if the cross-reactivity by DITPA could be reversed by excess TT3. DITPA was added to pooled serum samples to a final constant concentration of 20 µg/dl and increasing amounts of TT3 in PBS was added. These samples were analyzed by the Roche Elecsys TT3 method.

Results showed that TT4, FT4, and TSH tests were not affected by DITPA on all the immunoassay platforms tested. However, with increasing amounts of DITPA, significant overestimations for TT3 and FT3 (Table 1) above the baseline endogenous values were observed for all immunoassay platforms except for the Centaur. The Access TT3 assay cross-reacted with DITPA the most, followed by the Elecsys, Immulite, and Architect TT3 assays. TT3 assays are not available on the Dimension EXL and the Dimension Vista. The Centaur TT3 assay showed significant DITPA cross-reactivity only at the two highest concentration of DITPA. The DITPA cross-reactivity in the Architect TT3 assay was not dose dependent. Again, very minimal cross-reactivity with DITPA was observed on Centaur FT3 assay, whereas the second and third generation Elecsys FT3 assays cross-reacted the most with DITPA, followed by the Access, Dimension Vista, Dimension EXL, Immulite, and Architect FT3 assays. Similar to the TT3 assay, the cross-reactivity of DITPA in the Architect FT3 assay was not dose dependent. When DITPA was held at a constant the DITPA cross-reactivity on the Elecsys TT3 declined from 208% to 114% recovery with increasing T3 concentrations (Table 2).

The structural difference between T3 and DITPA is the absence of an iodine at the 3' position and the amino group at the α -carbon in DITPA. The high degree of structural similarity between these two molecules can be challenging for immunoassays to distinguish

and selectively detect TT3/FT3 only. Because of this, patients undergoing DITPA treatment requires the measurement of DITPA by LC–MS/MS to correct for any cross-reactivity of TT3 with DITPA. Our results show that DITPA can cross-react significantly in all TT3 assays except for the Centaur assay. The interference is of two types; dose and non-dose related. The dose related cross-reactivity of DITPA suggests that the reagent antibody used in the Elecsys, Access, and Immulite assays binds an epitope that is common to T3 and DITPA whereas the reagent antibody used in the Centaur assay binds an epitope that includes the 3' iodine and/or the amino group on the α -carbon. The nature of DITPA cross-reactivity in the Architect assay (both total and free) is open to speculation. With DITPA held at a constant concentration, the amount of DITPA cross-reacting decreased with increasing amounts of TT3 present in the sample and suggests that DITPA competes with TT3 for binding to the reagent antibody.

To conclude, our studies showed DITPA significantly cross-reacts with several commercial TT3 and FT3 assays with the exception of the Centaur assays. The dose dependent results suggest that the TT3 and FT3 antibody reagents used cross-reacts significantly with DITPA. From our investigation, patients undergoing DIPTA therapy should have their thyroid hormone response be monitored using the Centaur assays.

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Table 1

DITPA interference on different TT3 and FT3 assays.

TT3 (ng/dl)									
DITPA (µg/dl)	Architect	Access	Elecsys	Centaur	Dimension EXL	Dimension Vista	Immulite		
0	0.0	0.0	0.0	0.0	-	-	0.0		
3	30.0	50.0	43.0	3.5	-	-	0.5		
6	29.0	95.0	61.0	1.0	-	-	8.9		
12	27.0	160.0	86.0	1.5	-	-	23.0		
25	27.0	300.0	117.0	3.0	-	-	42.0		
50	33.0	725.0	158.0	8.5	-	-	87.0		
75	33.0	745.0	193.0	12.5	-	-	146.0		

FT3 (pg/dl)									
DITPA (µg/dl)	Architect	Access	Elecsys Gen 2	Elecsys Gen 3	Centaur	Dimension EXL	Dimension Vista	Immulite	
0	0	0	0	0	0	0	0	0	0
3	81	335	404	407	-2	102	79	40	40
6	78	670	691	663	-17	250	204	105	105
12	93	1000	1216	1078	-15	462	427	173	173
25	91	1415	2244	1828	-17	826	802	339	339
50	94	1650	3041	2965	-34	1275	1296	697	697
75	96	2405	3041	3051	-38	1601	1612	1047	1047

Table 2

Increasing TT3 with a constant DITPA concentration assayed on the Roche Elecsys.

DITPA (µg/dl)	Control Sample TT3 (ng/dl)	TT3 with DITPA (ng/dl)	Recovery (%)
20	88	183	208
20	134	226	169
20	252	330	131
20	356	437	122
20	478	545	114