Supplementary Online Content

LI D, Radulescu A, Shrestha RT, et al. Association of biotin ingestion with performance of hormone and nonhormone assays in healthy adults. *JAMA*. doi:10.1001/jama.2017.13705

eFigure 1. Principle of the Roche cobas e602 sandwich immunoassay for measurement of TSH in a blood sample (A and B) and effect of excess biotin in a blood sample (C)

eFigure 2. Principle of the Roche cobas e602 competitive immunoassay for measurement of total T4 in a blood sample (A and B) and effect of excess biotin in a blood sample (C)

eFigure 3. Concentrations of ferritin (A-D), NT-proBNP (E-H), 25-OHD (I-L), PSA (M-N) at baseline without biotin, day 7 with biotin and day 14 without biotin

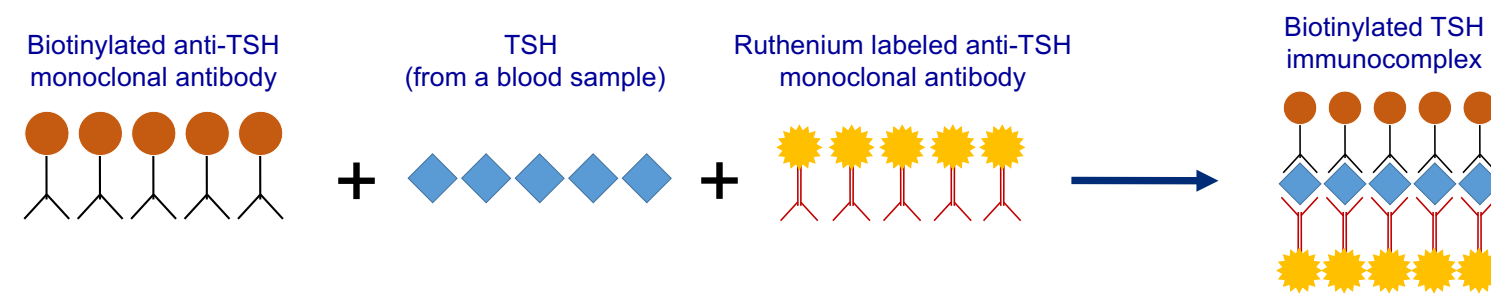**eTable 1.** Summary of the 37 assays evaluated in the study

eTable 2. Assay imprecisions

eTable 3. Concentrations of NT-proBNP, 25-OHD, ferritin and PSA across the study systems at baseline without biotin, day 7 with biotin and day 14 without biotin
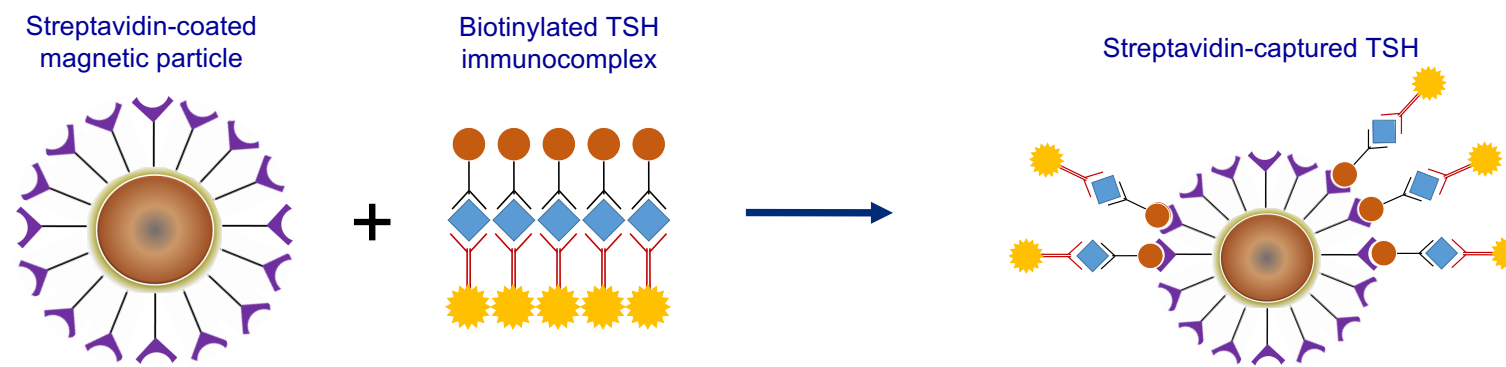
eTable 4. Analyte mean individual comparisons over time between baseline without biotin, day 7 with biotin and day 14 without biotin

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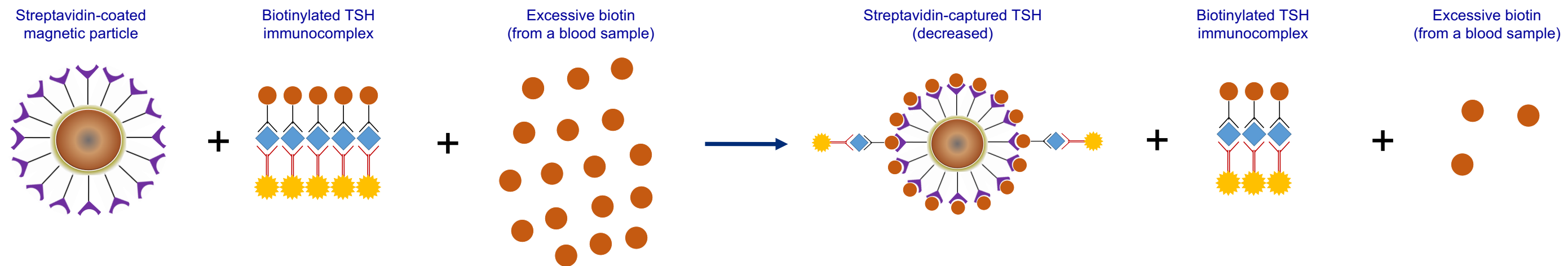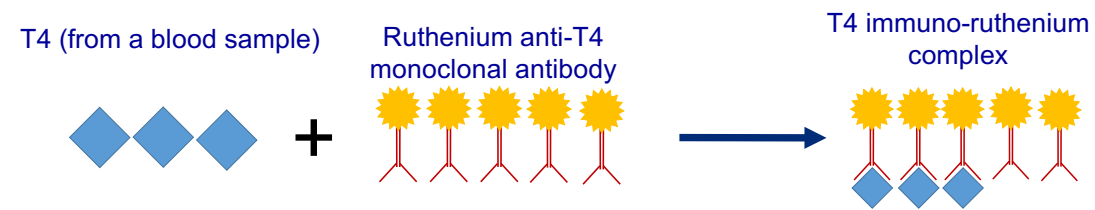
Supplement eFigure 1. Principle of the Roche cobas e602 sandwich immunoassay for measurement of TSH in a blood sample (A and B) and effect of excess biotin in a blood sample (C).

(A). Biotinylated anti-TSH monoclonal antibody and a ruthenium labeled anti-TSH monoclonal antibody form a sandwich immunocomplex with the TSH from a blood sample;

(B). Streptavidin-coated micro-particles are added to magnetically separate, or capture, via biotin and streptavidin interaction, the biotinylated TSH immunocomplex. The amount of streptavidin-captured TSH is directly proportional to the TSH present in the blood sample;

(C). When a blood sample contains excessive biotin, biotin completes with the biotinylated TSH immunocomplex for binding to the streptavidin-coated magnetic particles, resulting in reduced capture of the biotinylated TSH immunocomplex. Since the amount of streptavidin-captured TSH is directly proportional to the TSH present in the blood sample, the excess blood biotin produces a falsely low result for TSH.

A. Formation of T4 immuno-ruthenium complex

T4 (from a blood sample)

Ruthenium anti-T4 monoclonal antibody

T4 immuno-ruthenium complex

B. Separation of biotinylated complex using streptavidin coated beads without excess biotin present in a blood sample

Streptavidin-coated magnetic particle

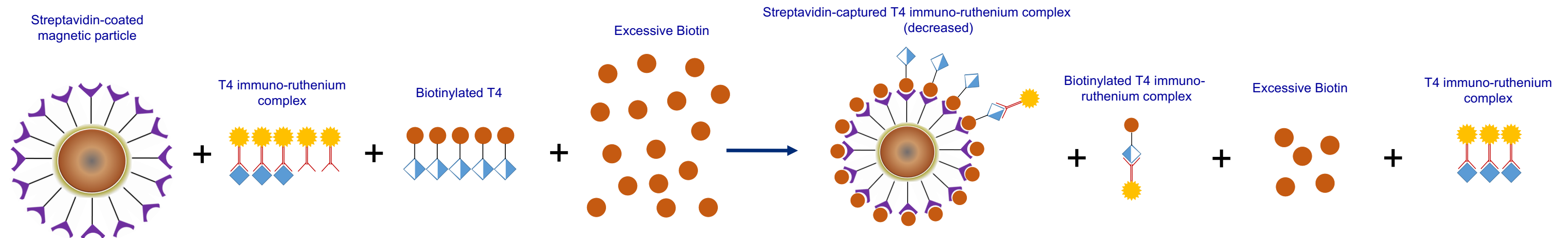T4 immuno-ruthenium complex

Biotinylated T4

Streptavidin-captured T4 immuno-ruthenium complex

T4 immuno-ruthenium complex
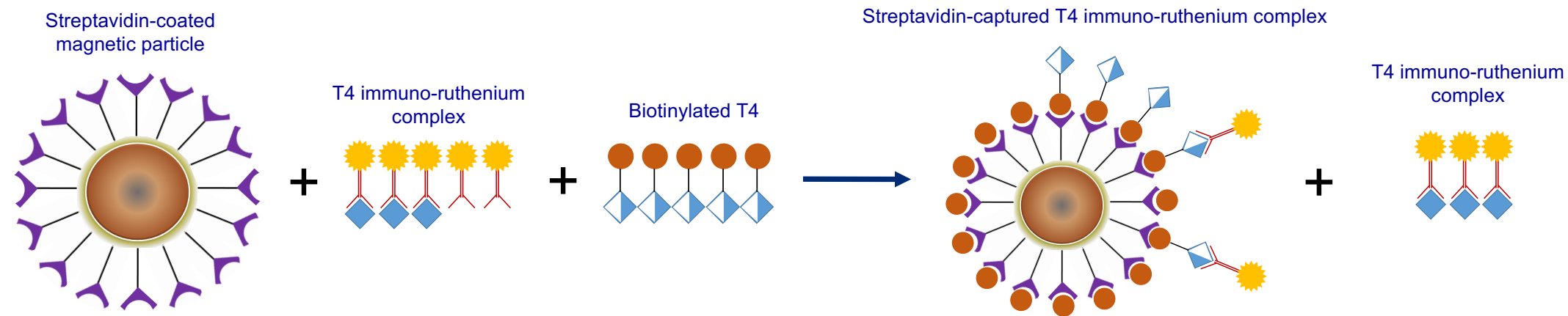
C. Mechanism of Interference when excessive biotin is present in a blood sample that results in decreased streptavidin-capture T4, which produces a falsely high T4 result in a T4 competitive immunoassay

Streptavidin-coated magnetic particle

T4 immuno-ruthenium complex

Biotinylated T4

Excessive Biotin

Streptavidin-captured T4 immuno-ruthenium complex (decreased)

Biotinylated T4 immuno-ruthenium complex

Excessive Biotin

T4 immuno-ruthenium complex

Supplement eFigure 2. Principle of the Roche cobas e602 competitive immunoassay for Total T4 in a blood sample (A and B) and effect of excess biotin in a blood sample (C).
(A). Total T4, released from binding proteins, forms a T4 immuno-ruthenium complex with ruthenium labeled anti-T4 monoclonal antibody;
(B). Streptavidin-coated magnetic particles and a fixed amount of biotinylated T4 are then added; T4 from the blood sample competes with the added biotinylated T4 for binding the unoccupied ruthenium anti-T4 monoclonal antibody. Biotinylated T4 complexes (both ruthenium bound and unbound), are captured on the streptavidin-coated magnetic particles. Streptavidin-captured T4 immuno-ruthenium complexes have an inverse relationship to T4 from the blood sample;
(C). Excessive biotin competes with biotinylated T4 for binding to the streptavidin-coated magnetic beads, and therefore decreases streptavidin captured T4 immuno-ruthenium complex. Because of the negative relationship between streptavidin-captured T4 immuno-ruthenium complex and the competitive assay's ruthenium signal readout, excessive biotin in a blood sample produces a falsely increased T4 result for the blood sample.

(A) Architect (Non-biotinylated, Sandwich Assay) — Ferritin (ng/ml)
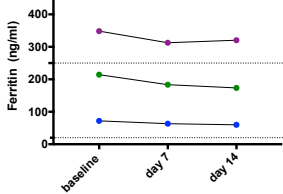
(B) Cobas (Biotinylated, Sandwich Assay) — Ferritin (ng/ml)
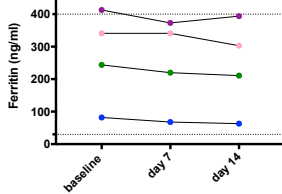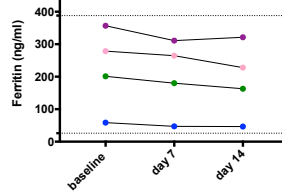
(C) Vista (Biotinylated, Sandwich Assay) — Ferritin (ng/ml)

(D) Vitros (Biotinylated, Sandwich Assay) — Ferritin (ng/ml)

(E) Cobas (Biotinylated, Sandwich Assay) — NT-proBNP(pg/ml)
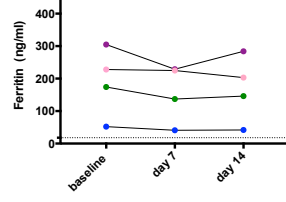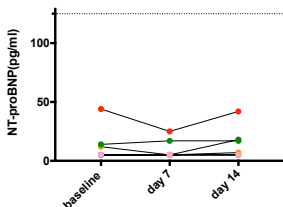
(F) Vitros (Biotinylated, Sandwich Assay) — NT-proBNP(pg/ml)
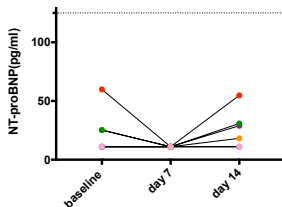
(G) Vista (Biotinylated, Sandwich Assay) — NT-proBNP(pg/ml)
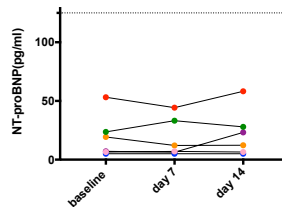
(H) Architect (Biotinylated, Competitive Assay) — 25-OH Vitamin D (ng/ml)
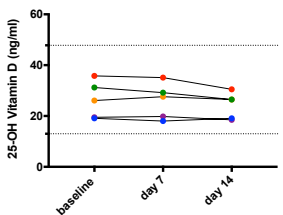
(I) Cobas (Biotinylated, Competitive Assay) — 25-OH Vitamin D (ng/ml)
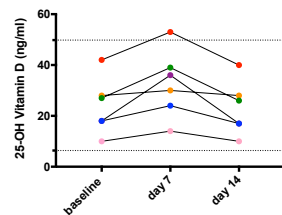
(J) Architect (Non-biotinylated, Sandwich Assay) — PSA (ng/ml)
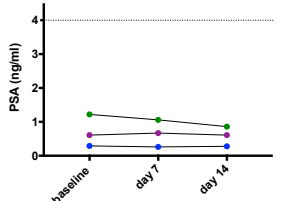
(K) Vista (Biotinylated, Sandwich Assay) — PSA (ng/ml)
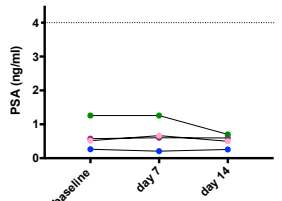
Supplement eFigure 3. **Concentrations of ferritin (A-D), NT-proBNP (E-G), 25-OHD (H-I), PSA (J-K) in the 6 study participants at baseline, day 7 on biotin and day 14 off biotin.**

Ferritin was measured on Abbott Architect 2000 (A), Roche cobas e602 (B), Siemens Vista Dimension 1500 (C), and OCD Vitros 5600 (D); NT-proBNP on Roche cobas e602 (E), OCD Vitros 5600 (F), and Siemens Vista Dimension 1500 (G); 25-OH Vitamin D on Abbott Architect 2000 (H), Roche cobas e602 (I); and PSA on Abbott Architect 2000 (J) and Siemens Vista Dimension 1500 (K). All tests were performed on 6 participants with the following two exceptions: (i) PSA and ferritin were performed on 4 men, except n= 3 on the Abbott Architect; and (ii) Architect 25-OHD was performed on 5 participants. Dotted lines represent the lower and the upper reference range for the assay. Reference range is not shown for NT-proBNP, which does not have lower reference and where the diagnosis of congestive heart failure may be defined by levels > 125 pg/mL in individuals < age 75. Biotin ingestion of 10 mg/day for 7 days was associated with significantly decreased OCD Vitros NT-proBNP results (p=.03) and significantly increased Roche cobas e602 25-OHD results (p<.001). A unique color is used for each participant across all panels.

Abbreviations: NT-proBNP = N-terminal pro brain natriuretic peptide; 25-OHD= 25 hydroxyvitamin D; PSA= prostate specific antigen; CI = confidence intervals.

Supplement eTable 1. Summary of the 37 assays evaluated in the study.

Immuno-assay Principle	Analyte	Manufacturer/ Analyzer	Testing location	Biotin used in the assay?	Predicted direction of biotin interference	Reference Range
Sandwich	TSH	Roche Cobas e602	JHMI	yes	Falsely Low	0.27-4.20 mIU/L
		OCD Vitros 5600	CMH	yes		0.47-4.68 mIU/L
		Siemens Vista Dimension 1500	UMMC	yes		0.36—3.74 mIU/L
		Abbott Architect	BMC	No	Not affected	0.35-4.94 mIU/L
	Intact PTH	Roche Cobas e602	JHMI	Yes	Falsely Low	15-65 pg/mL
		OCD Vitros 5600	CMH	Yes		7.5-53.5 pg/mL
		Siemens Advia Centaur XP	UMMC	Yes		14-72 pg/mL
		Abbott Architect	BMC	No	Not affected	8.5-72.5 pg/mL
	Prolactin	Roche Cobas e602	JHMI	Yes	Falsely Low	Female 4.79-23.3 ng/mL Male: 4.04-15.2 ng/mL
		Siemens Immulite 2000	CMH	No	Not affected	Female 1.9-25 ng/mL Male: 2.5-17 ng/mL
		Siemens Vista Dimension 1500	UMMC	Yes	Falsely Low	Female 2.2-30.3 ng/mL Male: 2.5-17.4 ng/mL
		Abbott Architect	BMC	No	Not affected	Female 5.18-26.5 ng/mL Male: 3.46-19.4 ng/mL
	NT-proBNP	Roche Cobas e602	JHMI	yes	Falsely Low	< 125 pg/mL
		OCD Vitros	CMH	yes	Falsely Low	< 125 pg/mL
		Siemens Vista Dimension 1500	UMMC	yes	Falsely Low	< 125 pg/mL
	PSA	Siemens Dimension Vista	UMMC	Yes	Falsely Low	< 4 ng/mL
		Abbott Architect	BMC	No	Not affected	< 4 ng/mL
	Ferritin	Roche Cobas e602	JHMI	Yes	Falsely Low	Male: 30-400 ng/mL
		OCD Vitros	CMH	Yes	Falsely Low	Male: 17.9-464 ng/mL
		Siemens Vista Dimension	UMMC	Yes	Falsely Low	Male: 26-388 ng/mL
Sandwich*		Abbott Architect Quantia	BMC	No	Not affected	Male: 20-250 ng/mL

Supplement eTable 1. Summary of the 37 assays evaluated in the study (Continued).

Immuno-assay Principle	Analyte	Manufacturer/ Analyzer	Testing location	Biotin used in the assay?	Predicted direction of biotin interference	Reference Range
Competitive	Total T4	Roche Cobas e602	JHMI	Yes	Falsely High	4.5-11.7 mcg/dL
		OCD Vitros 5600	CMH	No	Not affected	5.53-11 mcg/dL
		Siemens Vista Dimension 1500	UMMC	No		4.5-13.9 mcg/dL
		Abbott Architect	BMC	No		4.87-11.7 mcg/dL
	Total T3	Roche Cobas e602	JHMI	Yes	Falsely High	0.8-2.0 ng/mL
		OCD Vitros 5600	CMH	No	Not affected	0.97-1.7 ng/mL
		Siemens Advia Centaur XP	UMMC	No		0.6-1.8 ng/mL
		Abbott Architect	BMC	No		0.58-1.6 ng/mL
	Free T4	Roche Cobas e602	JHMI	Yes	Falsely High	0.93-1.7 ng/dL
		OCD Vitros 5600	CMH	No	Not affected	0.78-2.2 ng/dL
		Siemens Vista Dimension 1500	UMMC	Yes	Falsely High	0.76-1.5 ng/dL
		Abbott Architect	BMC	No	Not affected	0.70-1.5 ng/dL
	Free T3	Roche Cobas e602	JHMI	Yes		2.0-4.4 pg/mL
		Siemens Vista Dimension 1500	UMMC	Yes	Falsely High	2.2-4.0 pg/mL
	25-OHD	Roche Cobas e602	JHMI	Yes	Falsely High	6.36-49.5 ng/mL
		Abbott Architect	BMC	Yes	Falsely high	13.0-47.8 ng/mL

Assays evaluated in the study are indicated by immunoassay format, type of analyte measured, manufacturer/analyzer, testing location, reference range as defined on the manufacturer's package insert for the assay, whether or not the assay has biotinylated components and the predicated effect and direction of biotin interference.

Abbreviations: TSH =thyroid stimulating hormone; PTH = parathyroid hormone; NT-ProBNP= N-terminal pro-brain natriuretic peptide; PSA=prostate specific antigen; total T4 = total thyroxine; Total T3 = total triiodothyronine; Free T4 = free thyroxine; Free T3= free triiodothyronine; 25-OHD= 25 hydroxyvitamin D, JHMI = Johns Hopkins Medical Institutions; CMH= Children's Mercy Hospital; UMMC= University of Minnesota Medical Center; BMC= Boston Medical Center.

*Abbott Architect Quantia used a polyclonal antibody immuno-agglutination method.

To convert PTH pg/ml to ng/L, multiply by 1.

To convert prolactin ng/ml to pM, multiply by 43.478.

To convert NT-proBNP pg/ml to pM multiply by 0.118

To convert PSA ng/ml to mcg/L, multiply by 1

To convert ferritin ng/ml to pM, multiply by 2.247

To convert Total T4 mcg/dl to nM, multiply by

12.871. To convert Total T3 ng/ml to nM, multiply by

1.54. To convert Free T4 ng/dl to pM, multiply by

12.871. To convert Free T3 pg/ml to pM, multiply by

1.54. To convert 25-OHD ng/ml to nM, multiply by

2.496

Supplement eTable 2. Assay Imprecisions.

Testing Site	Assay	Analyte	QC Level (Unit)	SD	%CV
JHMI	Roche cobas e602	TSH	0.85 µIU/mL	0.02	1.86
		Total T4	8.86 µg/dL	0.32	3.6
		Free T4	1.49 ng/dL	0.04	2.93
		Total T3	1.28 ng/mL	0.05	3.63
		Free T3	2.89 ng/dL	0.1	3.57
		PTH	19.33 pg/mL	1.37	7.11
		Prolactin	10.3 ng/mL	0.32	3.07
		NT-proBNP	113.18 pg/mL	4.44	3.92
		25-OHD	17.71 ng/mL	1.19	6.74
		Ferritin	32.1 ng/mL	0.87	2.7
CMH	OCD Vitros 5600	TSH	0.64 mIU/L	0.03	4.75
		Total T4	9.09 µg/dL	0.40	4.4
		Free T4	1.720 ng/dL	0.13	7.76
		Total T3	1.444 ng/mL	0.05	3.52
		PTH	19.66 pg/mL	1.85	9.43
		NT-proBNP	144.37 pg/mL	6.28	4.35
	OCD Vitros 5600	Ferritin	21.90 ng/mL	1.32	6.0
	Siemens Immulite 2000	Prolactin	7.04 ng/mL	0.80	5.37
BMC	Abbott Architect	TSH	4.3 µIU/mL	0.3	6.98
		Total T4	8.3 µg/dL	0.5	6.0
		Free T4	1.15 ng/dL	0.06	5.22
		Total T3	93 ng/dL	5.5	5.91
		PTH	26 pg/mL	1	3.85
		Prolactin	18 ng/mL	1.5	8.33
		PSA	0.28 ng/mL	0.04	14.29
		25-OHD	29.9 ng/mL	2.9	9.70
		Ferritin	121 ng/mL	10	8.3

Supplement eTable 2. Assay Imprecisions (Continued).

Testing Site	Assay	Analyte	QC Level (Unit)	SD	%CV
UMMC	Siemens Vista Dimension 1500	TSH	0.674 mIU/L	0.02	2.63
		Total T4	10.72 µg/dL	0.67	6.3
		Free T4	1.365 ng/dL	0.02	1.76
		Free T3	2.06 pg/mL	0.10	4.80
		Prolactin	6.67 ng/mL	0.17	2.52
		PSA	0.0289 µg/L	0.01	3.04
		NT-proBNP	108.52 pg/mL	2.83	2.61
		Ferritin	23.58	0.66	2.8
	Siemens Advia Centaur XP	PTH	366.19 pg/mL	37.19	5.96
	Siemens Advia Centaur XP	Total T3	106.19 ng/dL	11.73	5.94

Imprecision data are shown for assay systems and individual analytes including the quality control analyte concentration at which the precision analysis was performed (QC level), SD (standard deviation), and percent intra-assay coefficient of variation (%CV).

Abbreviations: TSH =thyroid stimulating hormone; PTH = parathyroid hormone; NT-proBNP= N-terminal pro-brain natriuretic peptide; PSA=prostate specific antigen; Total T4 = total thyroxine; Total T3 = total triiodothyronine; Free T4 = Free thyroxine; Free T3 = Free triiodothyronine; 25-OHD= 25 hydroxyvitamin D, JHMI = Johns Hopkins Medical Institutions; CMH= Children's Mercy Hospital; UMMC= University of Minnesota Medical Center; BMC= Boston Medical Center.

Supplement eTable 3. Concentrations of NT-proBNP, 25-OHD, ferritin, and PSA across the study systems at baseline without biotin, day 7 with biotin and day 14 without biotin.

System	Time	Mean of analyte concentrations (95% CI)	Mean of day 7 differences from the mean of baseline and day 14 (95% CI)	Day 7 difference p-value	Mean of analyte concentrations (95% CI)	Mean of day 7 differences from the mean of baseline and day 14 (95% CI)	Day 7 difference p-value
		NT-proBNP pg/mL	pg/mL		**Ferritin ng/mL**	ng/mL	
Cobas	Baseline without	13.7 (-2.6; 30)	-3.67 (-8.06; 0.72)	.13	270 (42; 498)	-5.9 (-21; 9.3)	.48
	Day 7 with	9.7 (0.1; 19)			251 (30; 471)		
	Day 14 without	13.0 (-2.8; 29)			243 (18; 467)		
Vista	Baseline without	19.0 (-0.5; 38)	-2.66 (-8.07; 2.75)	.36	224 (22; 426)	-6.0 (-21; 8.9)	.46
	Day 7 with	17.8 (0.2; 35)			201 (16; 385)		
	Day 14 without	22.0 (1.0; 43)			190 (5.7; 374)		
Vitros	Baseline without	23.9 (4.0;44)	-13.9 (-24.7; -3.12)	.03	190 (20; 359)	-21 (-44; 1.9)	.12
	Day 7 with	< 11.1 (11;11)			158 (17; 299)		
	Day 14 without	25.8 (8.4;43)			169 (6.9; 331)		
Architect	Baseline without				212 (-131; 555)	-12 (6.35)	.14
	Day 7 with				186 (-123; 496)		
	Day 14 without				185 (-140; 509)		
		25-OHD ng/mL	ng/mL		**PSA ng/mL**		
Cobas	Baseline without	23.8 (12; 35)	9.25 (5.72; 12.8)	< .001			
	Day 7 with	32.7 (19; 47)					
	14 without	23.0 (12; 34)					
Architect	Baseline without	26.3 (17; 35)	0.66 (-0.95; 2.27)	.44	0.71 (-0.5; 1.9)	0.02 (-0.12; 0.16)	.82
	Day 7 with	25.9 (17; 35)			0.66 (-0.3; 1.7)		
	Day 14 without	24.2 (18; 31)			0.58 (-0.1; 1.3)		
Vista	Baseline without				0.65 (-0.02;1.3)	0.1 (-0.10; 0.30)	.36
	Day 7 with				0.68 (-0.01; 1.4)		
	Day 14 without				0.51 (0.2; 0.8)		

Analyte concentrations are expressed in columns 3 (NT-proBNP and 25-OHD)) and 6 (ferritin, PSA) as mean (95% CI). Each analysis was a

repeated measures ANOVA (mixed linear model) where the random effect was a participant and the within-participant fixed effect was time (day of

study). The primary comparison was day 7 (with biotin) versus the mean of days 0 and 14 (without biotin) using a contrast in the ANOVA, i.e., (day 7- (½ (baseline [day 0] + day 14)). Columns 4 and 7 show the difference data, presented as the absolute mean difference from combined baseline (95% CI of the difference). The units for the day 7 difference are the same as those of analyte concentrations.

All the tests were performed on 6 participants with the following two exceptions: (i) PSA and ferritin were performed on 4 men, except n= 3 on the Abbott Architect; and (ii) Architect 25-OHD was performed on 5 participants.

To convert NT-proBNP pg/ml to pM multiply by 0.118

To convert PSA ng/ml to mcg/L, multiply by 1

To convert ferritin ng/ml to pM, multiply by 2.247

To convert 25-OHD ng/ml to nM, multiply by 2.496

Abbreviations: NT-ProBNP= N-terminal pro-brain natriuretic peptide; PSA=prostate specific antigen; 25-OH vitamin D= 25 hydroxyvitamin D; CI = confidence interval

Supplement eTable 4. Analyte mean individual comparisons over time between baseline without biotin, day 7 with biotin and day 14 without biotin.

Test (units for the difference result)	System	Overall time effect F-test p-value	LS Mean Difference between Day 7-0 (95% CI)	LS means TUKEY HSD Individual pairs p-value Day 0 vs 7	LS Mean Difference between Day 7-14 (95% CI)	LS means TUKEY HSD Individual pairs p-value Day 7 vs 14	LS Mean Difference between Day 0 -14 (95% CI)	LS means TUKEY HSD Individual pairs p-value Day 0 vs 14
Biotin pg/mL		< .001	2826 (2457; 3197)	<.001	2510 (2141; 2880)	< .001	-317 (-53; 686)	.09
TSH mIU/mL	Cobas	.01	-0.59 (-0.06; 1.2)	.08	-0.85 (0.19; 1.5)	.01	-0.26 (-0.40; 0.91)	.55
	Vitros	< .001	-1.5 (0.89; 2.2)	<.001	-1.8 (1.1; 2.5)	<.001	-0.25 (-0.42; 0.91)	.58
	Vista	.62	-0.03 (-0.58; 0.63)	.99	-0.20 (-0.40; 0.81)	.64	0.18 (-0.43; 0.78)	.71
	Architect	.57	0.05 (-0.55; 0.65)	.97	-0.18 (-0.42; 0.78)	.70	0.23 (-0.37; 0.83)	.57
PTH (pg/mL)	Cobas	.34	-5.2 (-3.9; 14)	.31	-2.5 (-6.6; 12)	.74	2.7 (-6.4; 12)	.71
	Vitros	<.001	-27 (12; 42)	.001	-25 (10; 39)	.002	2.4 (-12; 17)	.89
	Centaur	.89	2.8 (-14; 19)	.89	1.9 (-15; 18)	.95	-0.93 (-16; 17)	.99
	Architect	.69	2.5 (-9.3; 14)	.83	3.8 (-8.1; 16)	.67	1.2 (-11; 13)	.96
Total T4 (mcg/dL)	Cobas	.43	0.10 (-0.48; 0.68)	.89	0.28 (-0.30; 0.86)	.41	0.18 (-0.40; 0.76)	.67
	Vitros	.68	-0.04 (-0.58; 0.65)	.99	0.15 (-0.46; 0.77)	.78	0.19 (-0.43; 0.81)	.68
	Vista	.25	-0.24 (-0.42; 0.90)	.59	0.19 (-0.47; 0.85)	.72	0.43 (-0.23; 1.1)	.22
	Architect	.86	-0.03 (-0.40; 0.45)	.99	0.06 (-0.37; 0.48)	.93	0.08 (-0.34; 0.51)	.85
Total T3 (ng/mL)	Cobas	.004	0.85 (0.26; 1.4)	.007	0.86 (0.27; 1.4)	.007	0.01 (-0.58; 0.60)	1.0
	Vitros	.23	0.04 (-0.03; 0.11)	.31	0.04 (-0.03; 0.11)	.28	0.002 (-0.07; 0.07)	1.0
	Centaur	.37	0.05 (-0.04; 0.14)	.36	0.04 (-0.06; 0.13)	.54	-0.01 (-0.08; 0.10)	.94
	Architect	.39	3.7 (-4.7; 12)	.48	3.9 (-4.5; 12)	.44	0.22 (-8.2; 8.7)	1.0

Supplement eTable 4. Analyte mean individual comparisons over time between baseline without biotin, day 7 with biotin and day 14 without biotin (Continued).

Test (units for the difference result)	System	Overall time effect F-test p-value	LS Mean Difference between Day 7-0 (95% CI)	LS means TUKEY HSD Individual pairs p-value Day 0 vs 7	LS Mean Difference between Day 7-14 (95% CI)	LS means TUKEY HSD Individual pairs p-value Day 7 vs 14	LS Mean Difference between Day 0 -14 (95% CI)	LS means TUKEY HSD Individual pairs p-value Day 0 vs 14
Free T4 (ng/dL)	Cobas	.03	0.12 (-0.009; 0.24)	.07	0.13 (0.007; 0.26)	.04	0.02 (-0.11; 0.14)	.93
	Vitros	.40	0.02 (-0.08; 0.12)	.85	0.05 (-0.05; 0.15)	.38	0.03 (-0.07; 0.13)	.68
	Vista	.39	0.01 (-0.05; 0.08)	.89	0.03 (-0.03; 0.10)	.37	0.02 (-0.04; 0.09)	.63
	Architect	.63	0.02 (-0.05; 0.08)	.79	0.02 (-0.04; 0.08)	.62	0.007 (-0.06; 0.07)	.95
Free T3 (pg/mL)	Cobas	.01	0.35 (0.04; 0.66)	.03	0.37 (0.06; 0.68)	.02	0.02 (-0.29; 0.33)	.99
	Vista	.001	0.76 (0.32; 1.2)	.002	0.80 (0.35; 1.2)	.002	0.03 (-0.42; 0.48)	1.0
Prolactin (ng/mL)	Cobas	.09	-0.43 (-2.1; 2.9)	.88	-2.1 (-0.36; 4.6)	.1	-1.7 (-0.8; 4.2)	.20
	Vista	.11	-0.42 (-1.6; 2.4)	.84	-1.7 (-0.33; 3.6)	.1	-1.2 (-0.75; 3.2)	.25
	Immulite	.12	-0.33 (-1.5; 2.2)	.88	-1.5 (-0.36; 3.3)	.12	-1.1 (-0.69; 3.0)	.25
	Architect	.07	-0.55 (-1.4; 2.5)	.72	-1.8 (-0.12; 3.8)	.07	-1.3 (-0.67; 3.2)	.22
NT proBNP (pg/mL)	Cobas	.30	-4.0 (-3.1; 11)	.31	-3.3 (-3.8; 10)	.44	0.67 (-6.4; 7.8)	.96
	Vista	.43	-1.1 (-7.6; 9.9)	.93	-4.2 (-4.5; 13)	.42	-3.0 (-5.7; 12)	.62
	Vitros	.08	-13 (-4.4; 30)	.15	-15 (-2.5; 32)	.09	-1.9 (-15.4; 19.2)	.95
Ferritin (ng/mL)	Cobas	.05	-20 (-8.0; 47)	.15	7.8 (-20; 35)	.68	27 (-0.2; 55)	.05
	Vitros	.13	-32 (-9.7; 73)	.12	-11 (-31; 52)	.72	21 (-20; 62)	.33
	Vista	.02	-23 (-3.8; 50)	.09	11 (-16; 38)	.46	34 (7.3; 61)	.02
	Architect	.04	-25 (-1.1; 51)	.06	1.9 (-24; 28)	.96	27 (0.8; 53)	.05

Supplement eTable 4. Analyte mean individual comparisons over time between baseline without biotin, day 7 with biotin and day 14 without biotin (Continued).

Test (units for the difference result)	System	Overall time effect F-test p-value	LS Mean Difference between Day 7-0 (95% CI)	LS means TUKEY HSD Individual pairs p-value Day 0 vs 7	LS Mean Difference between Day 7-14 (95% CI)	LS means TUKEY HSD Individual pairs p-value Day 7 vs 14	LS Mean Difference between Day 0 -14 (95% CI)	LS means TUKEY HSD Individual pairs p-value Day 0 vs 14
25 OHD (ng/mL)	Cobas	.002	8.8 (3.1; 15)	.004	9.7 (4.0; 15)	.002	0.83 (-4.9; 6.5)	.92
	Architect	.12	-0.40 (-2.3; 3.1)	.91	1.7 (-0.99; 4.4)	.23	2.1 (-0.59; 4.8)	.12
PSA (ng/mL)	Vista	.36	0.03 (-0.33; 0.38)	.96	0.17 (-0.19; 0.53)	.37	0.14 (-0.22; 0.50)	.49
	Architect	.43	-0.04 (-0.26; 0.35)	.87	0.08 (-0.23; 0.39)	.65	0.12 (-0.18; 0.43)	.41

Each analysis was a repeated measures ANOVA (mixed linear model) where the subject effect was a person and the within subject effect was time (day of study). The table shows comparisons of pairs of times using Tukey's HSD post hoc test. Least Squares (LS) mean difference data are derived from subtracting LS mean day 7 - 0, day 7 – 14, or day 0 - 14. The units for each analyte difference are listed in column 1.

All tests were performed on 6 participants, except PSA and ferritin, which were performed on 4 men; n=4 for all ferritin and PSA assays except n=3 for Abbott Architect ferritin and PSA, while n for all other tests is 6 except Architect 25-OHD (n=5).

The numerator degrees of freedom (DF) is 2 for all tests. The denominator DF is 4 for Architect PSA and ferritin, 6 for the other male-only tests, and 8 for Architect 25-OHD. For all other tests the denominator DF is 10.

Abbreviations: TSH =thyroid stimulating hormone; PTH = parathyroid hormone; NT-proBNP= N-terminal pro-brain natriuretic peptide; PSA=prostate specific antigen; Total T4 = total thyroxine; Total T3 = total triiodothyronine; Free T4 = Free thyroxine; Free T3 = Free triiodothyronine; 25-OHD= 25 hydroxyvitamin D.

To convert PTH pg/ml to ng/L, multiply by 1.

To convert prolactin ng/ml to pM, multiply by 43.478.

To convert NT-proBNP pg/ml to pM multiply by 0.118

To convert PSA ng/ml to mcg/L, multiply by 1

To convert ferritin ng/ml to pM, multiply by 2.247

To convert Total T4 mcg/dl to nM, multiply by 12.871.

To convert Total T3 ng/ml to nM, multiply by 1.54. To

convert Free T4 ng/dl to pM, multiply by 12.871. To

convert Free T3 pg/ml to pM, multiply by 1.54. To

convert 25-OHD ng/ml to nM, multiply by 2.496