CPAL
Central **P**ennsylvania **A**lliance **L**aboratory

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Immunoassays
- Testing System Change –

Contact: Steph Frey, 717.812.4768
Operations Manager, Clinical Pathology, CPAL

Dr. Jennifer Thebo, 717.851.3210
Director, Technical Operations and Scientific Affairs, CPAL

Affected Tests:

Mnemonics:	Test Name:	Test Number:	Specimen:	Processing/Storage:
DHEAS	DHEA Sulfate	1750222	-Serum -Plasma (K-EDTA, Na citrate, Na heparin)	Remove from gel/cells within 8 days Refrigerate up to 8 days
Estradiol	Estradiol	1750031	-Serum -Plasma (Li heparin including plasma separator tubes, K-EDTA)	Remove from gel/cells within 7 days Refrigerate up to 7 days
CEA	CEA	1750040	-Serum -Plasma (Na heparin, Li heparin, K-EDTA)	Remove from gel/cells within 72 hrs Refrigerate up to 7 days
CA125	CA125	1750111	-Serum -Plasma (K3-EDTA, Na heparin, Li heparin)	**Remove from gel/cells within 24 hours** Refrigerate up to 7 days
HCYST	Homocysteine	1750113	-Serum -Plasma (Li heparin, K-EDTA)	** All specimens must be collected on ice and removed from the gel or cells within 6 hours** Refrigerate up to 14 days
HCYSTP	Homocysteine Post	1750114	-Serum -Plasma (Li heparin, K-EDTA)	
TTESTOST	Testosterone, Total	1750075	-Serum -Plasma (K_2-EDTA)	Remove from gel/cells within 72 hrs Refrigerate up to 7 days
GR TESTOST	Testosterone, Total and Free	1750074	-Serum -Plasma (K_2-EDTA)	Remove from gel/cells within 72 hrs Refrigerate up to 7 days
.FR TEST	Testosterone, Free	1750094	-Serum -Plasma (K_2-EDTA)	Remove from gel/cells within 72 hrs Refrigerate up to 7 days

Effective Date: Testing will begin March 2, 2015 with samples received in the afternoon on Saturday, February 28, 2015.

Performed: Monday through Saturday

Method Change:
Testing for the above tests will be moved from the Siemens Immulite to the Abbott Architect, as the Immulite is being phased out.

Adjustment to Patient Baseline for CEA and CA125:
For the period of March 2, 2015 through June 2, 2015, CPAL will offer concurrent testing with the old and new methods (at no additional charge) to allow for determining a new baseline of tumor markers (CEA, CA125) for each patient with the new method. The test result reported will be that of the new method. The result on the same sample utilizing the old method will be added in a result comment field for comparative purposes. ***All laboratories should announce this to their respective physicians so their patients can be notified to be tested during this window.***

Results Interpretation:

Test	Old Reference Range	New Reference Range
CA 125	0 - 21 U/mL	0 – 35 U/mL
CEA	0.0 – 3.1 ng/mL	0.0 – 3.0 ng/mL
DHEA-S	Females: 1 month to 1 year: <100 μg/dl 1 to 4 years: <30 μg/dl 4 to 8 years: <60 μg/dl Prepubertal > 8 years: <95 μg/dl Pubertal: 40-300 μg/dl Adult females: 35-430 μg/dL Post-menopausal: 10-190 μg/dl Adult Males: 80-560 μg/dL	Females: 11-14 years: 9 – 170 ug/dL 15-19 years: 61 - 494 ug/dL 20-24 years: 134 - 408 ug/dL 25-34 years: 96 - 512 ug/dL 35-44 years: 75 - 410 ug/dL 45-54 years: 56 - 283 ug/dL ≥55 years: 30 - 182 ug/dL Males: 11-14 years: 17 - 243 ug/dL 15-19 years: 45 - 385 ug/dL 20-24 years: 238 - 539 ug/dL 25-34 years: 168 - 592 ug/dL 35-44 years: 140 - 484 ug/dL 45-54 years: 136 - 448 ug/dL ≥ 55 years: 49 - 362 ug/dL Children: <1 week: 25 - 303 ug/dL 1-4 weeks: 9 - 317 ug/dL 1-12 months: 32 - 214 ug/dL 1-4 years: 33 - 276 ug/dL 5-10 years: 24 - 210 ug/dL
Estradiol	Pre-pubertal females: up to 70 pg/mL Adult females (Ovulating): Follicular: up to 160 pg/mL Follicular (days 2-3): up to 84 pg/mL Periovulatory (+/- 3 days):34 to 400 pg/mL Luteal Phase: 27 to 246 pg/mL Oral contraceptives: up to 102 pg/mL Untreated postmenopausal: up to 30 pg/mL Treated postmenopausal: up to 93 pg/mL Males: up to 56 pg/mL	Females: Follicular Phase: 21-251 pg/mL Mid-Cycle Phase: 38-649 pg/mL Luteal Phase: 21-312 pg/mL Postmenopausal females not on HRT:<10-28 pg/mL Postmenopausal females on HRT:<10-144 pg/mL Males: 11-44 pg/mL
HCY	4 – 15 μmol/L	Males: 5 – 16 umol/L Females: 4 – 14 umol/L
SHBG	Males: 13-71 nmol/L Females (non pregnant): 18-114 nmol/L	Males: 11-78 nmol/L Females: 12-137 nmol/L
Testos	Males: 20 – 49 years: 160 - 726 ng/dL ≥ 50 years: 129 - 767 ng/dL Females: Ovulating: < 73 ng/dL Post-menopausal:< 43 ng/dL	Males: 21-49 years of age: 240 – 871 ng/dL ≥50 years of age: 221 – 716 ng/dL Females: 21-49 years of age: 14 – 53 ng/dL ≥50 years of age: 12 – 36 ng/dL

Validation Data:

Precision

Two levels of control were run ten times each within the same run for each assay to determine within run precision. Two levels of control were run ten times each on two different days for each assay to determine between run precision. Precision met manufacturer's claims for all analytes. (Table 1)

Table 1: Precision

Test	Within Run Precision				Between Run Precision				Accept?
	Mean	CV	Mean	CV	Mean	CV	Mean	CV	
CA 125	41.0 U/mL	3.02%	301.2 U/mL	1.75%	41.1 U/mL	2.75%	293.2 U/mL	3.39%	Yes
CEA	2.8 ng/mL	3.19%	44.7 ng/mL	2.42%	2.8 ng/mL	2.84%	44.3 ng/mL	2.49%	Yes
DHEA-S	85.8 µg/dL	3.43%	584.0 µg/dL	1.44%	87.1 µg/dL	3.20%	587.0 µg/dL	1.40%	Yes
Estradiol	49.6 pg/mL	5.72%	501.7 pg/mL	1.26%	49.6 pg/mL	4.91%	500.9 pg/mL	1.30%	Yes
Homocysteine	11.0 µmol/L	1.52%	28.0 µmol/L	3.98%	11.0 µmol/L	1.63%	28.0 µmol/L	3.03%	Yes
SHBG	20.3 nmol/L	3.65%	48.7 nmol/L	4.81%	20.1 nmol/L	4.11%	48.9 nmol/L	3.59%	Yes
Testosterone	89.2 ng/dL	2.44%	1078.6 ng/dL	2.85%	89.9 ng/dL	2.83%	1075.5 ng/dL	2.26%	Yes

Linearity

To validate linearity, standardized materials for each assay were run in replicates of three and the results entered into EP Evaluator. All assays were within allowable error limits. (Table 2)

Table 2: Linearity

Assay	Slope	Intercept	Observed Error	Allowable Error	Acceptable?
CA 125 II	0.976	0.01	3.6%	12%	Yes
CEA	0.963	0.59	8.2%	12%	Yes
DHEA-S	1.015	-0.56	0.4%	12%	Yes
Estradiol	0.969	0.6	7.0%	15%	Yes
Homocysteine	1.015	0.03	0.9%	10%	Yes
SHBG	1.036	0.00	2.9%	12%	Yes
Testosterone	0.985	0.077	4.0%	15%	Yes

Method Comparison

The assays were initially validated on the Architect *i*2000 in 2014. The assays were then validated on the i1000 using the protocol for between instrument validation. Please see the summary in Table 3.

Table 3: Method Comparison

Assay	Architect i2000 vs Immulite 2000		Architect i1000 vs Architect i2000		
	Correlation Coefficient (R)	N	Correlation Coefficient (R)	N	Acceptable?
CA 125	0.9755	40	0.9992	37	Yes
CEA	0.9950	41	0.9947	52	Yes
DHEA-S	0.9748	40	0.9992	36	Yes
Estradiol	0.9819	35	0.9997	36	Yes
Homocysteine	0.9759	35	0.9947	34	Yes
SHBG	0.9800	39	0.9962	39	Yes
Testosterone	0.9387	36	0.9989	41	Yes

Reference Range Verification

Manufacturer's published reference ranges were verified using separate studies of no less than 20 normal samples and following CLSI EP28-A3C, section 11.2 guidelines, which describes transference "using small number of reference individuals." Specimens were either randomly selected from apparently normal previously tested specimens or from blood donor specimens. Abbott's published range for each assay was verified. (Table 4)

Table 4: Reference Range Verification

Assay	Number of Specimens Run	Agreement	Verified?
AUSAB	41	90% (37/41)	Yes
CA 125	31	100% (31/31)	Yes
CEA	22	100% (22/22)	Yes
DHEA-S	20	100% (20/20)	Yes
Estradiol	24	96% (23/24)	Yes
Homocysteine	23	100% (23/23)	Yes
SHBG	29	100% (29/29)	Yes
Testosterone	20	90% (18/20)	Yes

Limitations/Cautions:

1. The presence of heterophilic (anti-animal) antibodies in human serum (whether due to repeated animal exposure or through therapies) can interfere with immunoassays. Result should be interpreted taking the entire clinical setting into account. Should unexpected results be obtained, please contact the laboratory within seven days of specimen collection for further testing to rule out heterophilic antibody interference.
2. The CEA and CA125 assays should not be used as cancer screening tests.
3. Patients with confirmed carcinoma frequently have a pretreatment CEA level in the same range as healthy individuals. Elevations in circulating CEA levels may be observed in smokers as well as patients with nonmalignant disease. For these reasons, a serum or plasma CEA level, regardless of value, should not be interpreted as absolute evidence for the presence or absence of malignant disease. The CEA level should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.
4. Patients with confirmed ovarian carcinoma may have pretreatment CA125 assay values in the same range as healthy individuals. Elevations in circulating CA125 defined antigen may be observed in patients with nonmalignant disease. For these reasons, a CA125 assay value, regardless of level, should not be interpreted as absolute evidence for the presence or absence of malignant disease. The CA125 assay value should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.
5. The Architect Testosterone assay shows strong interaction with D-(-) Norgestrel (1000 ng/mL), 19-nortestosterone (Nandrolone), Ethisterone, 11b-Hydroxytestosterone, and 11-Ketotestosterone was found. Do not use samples from patients receiving these compounds.
6. The following drugs may elevate levels of homocysteine: methotrexate, carbamazepine, phenytoin, nitrous oxide, anticonvulsants, and 6-azauridine triacetate. The mechanism of action of these drugs affects different parts of the metabolic pathway of homocysteine. S-adenosyl-methionine is an antidepressant whose molecular form is similar to S-adenosyl-homocysteine. This drug may interfere with the Architect Homocysteine assay.
7. Specimens from patients with adrenal tumors or congenital adrenal hyperplasia may exhibit elevated levels of DHEA-S.
8. It cannot be excluded that rheumatoid factor present in human serum can interfere with any *in vitro* immunoassay.

References:

1. Architect System CA 125 II package insert; 05/2009.
2. Architect System CEA package insert; 6/12.
3. Architect System DHEA-S package insert; 10/05.

4. Architect System Estradiol package insert; 7/13.
5. Architect System Homocysteine package insert; 06/2013.
6. Architect System SHBG package insert; 09/07.
7. Architect System 2nd Generation Testosterone package insert; 11/13.
8. Ricos C, et al. *Desirable Specifications for Total Error, Imprecision, and Bias, derived from intra- and inter-individual biologic variation.* From: "Current databases on biologic variation: pros, cons and progress." Scand J Clin Lab Invest 1999;59:491-500. Databases updated 2014.
9. CLSI. *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory*; *Approved Guideline—Third Edition.* CLSI document EP28-A3c. Wayne, PA: Clinical and Laboratory Standards Instititute; Nov 2008. Corrected Version Oct 2010.