Parathyroid hormone (PTH) plays a crucial role in calcium homeostasis. It is frequently measured during the workup of calcium and bone disorders. However, optimal use of the various PTH assays requires an understanding of both the biology of PTH in health and disease, as well as the performance characteristics of the different assays available. In the following discussion we briefly review both aspects and outline some suggested testing strategies.

The Physiological Role of PTH

Serum calcium concentrations are regulated within a narrow range. This is achieved through the interactions of 2 hormonal regulatory loops (Figure 1).

In a fast, short-term regulatory loop, serum calcium levels are sensed through parathyroid calcium sensing receptors (CASR). CASR signaling upon calcium binding inhibits parathyroid hormone (PTH) release. When serum calcium levels fall, this negative feedback inhibition diminishes and increased PTH secretion occurs. PTH in turn interacts with its specific G-protein coupled receptors (PTHR-1), which are predominately expressed in the kidneys, resulting in increased renal tubular reabsorption of calcium, while at the same time promoting phosphate excretion. This leads to a rapid increase in total and ionized serum calcium, with resultant inhibition of further PTH release as eucalcemia is restored (Figure 1a).

While this fast “calciostat” prevents any significant short-term perturbances of serum calcium levels, it has little effect on long-term calcium balance. Intestinal calcium absorption and storage in bone are the main determinants of the latter. 1,25-OH-VitD$_{2,3}$ plays a central role in both these processes, promoting intestinal calcium absorption and, in concert with PTH,
skeletal calcium deposition, or less commonly, calcium mobilization. Renal calcium and phosphate reabsorption is also promoted. The net result is a positive calcium balance, and increasing serum calcium and phosphate levels (Figure 1b).

The fast and slow calciostat pathways interact at several points. Most importantly, they are coupled at the level of regulation of 1,25-OH-VitD3 production. Many tissues are capable of synthesizing 1,25-OH-VitD3 from its precursor, 25-OH-VitD3, which in turn is derived from dietary ergocalciferol (VitD2) or from cholecalciferol (VitD3), the latter being synthesized in the skin under ultraviolet (UV) light exposure and also absorbed from food. However, the vast majority of circulating 1,25-OH-VitD3 is produced by the kidneys. PTH increases renal conversion of 25-OH-VitD3 to 1,25-OH-VitD3 at the expense of the alternative, biologically inactive hydroxylation product 24,25-OH-VitD3. PTH, therefore, not only leads to short-term increases in serum calcium levels, but also promotes replenishment of calcium stores through increased absorption, mediated by 1,25-OH-VitD3. PTH and 1,25-OH-VitD3 also interact in complex, incompletely understood ways in regulating calcium flux in and out of bone. Sustained, elevated PTH levels, in the presence of at least some 1,25-OH-VitD3, promote calcium mobilization from bone. This maintains constant serum calcium levels, even when short-term regulation of renal reabsorption and 1,25-OH-VitD3-mediated intestinal absorption fail to restore a positive calcium balance. By contrast, brief or intermittent elevations in PTH together with 1,25-OH-VitD3 have the opposite effect, promoting calcium deposition in bone, thereby ensuring that absorbed extra calcium is preserved (Figure 1c).

**Biological Variables Impacting PTH Result Interpretation**

One major biological variable is that the parathyroid glands can respond with altered PTH secretion to even minor differences in serum calcium levels within the reference range. This may be observed, for example, in very mild, subclinical vitamin D deficiency. Consequently, 25-OH-VitD3 stores are correlated with PTH levels. Since a good proportion of 25-OH-VitD3 is derived from UV exposure, population-based PTH upper reference limits are higher the further one gets away from the equator. If the upper limit of a local reference range is used as a cutoff for the diagnosis of hyperparathyroidism, the diagnostic sensitivity is lower in populations residing in regions of low UV exposure.

For example, in our Rochester, MN population, a diagnostic threshold of approximately 34 pg/mL (~3.6 pmol/L) for the new Bio-Intact PTH assay results in 100% sensitivity and 83% specificity for the diagnosis of primary hyperparathyroidism in hypercalcemic individuals with normal renal function. By contrast, if the population reference range-based cutoff of 55 pg/mL (~5.8 pmol/L) is used, sensitivity falls to around 55% with a modest increase in specificity to about 95%.

**The Impact of Different Assay Configurations on PTH Measurements**

Analytical problems, related to assay cross-reactivity with PTH metabolites, cause further problems in the interpretation of PTH results. In the parathyroid glands, PTH is synthesized as a 115-amino acid precursor (pre-pro-PTH), cleaved to pro-PTH, and then to the mature 84-amino acid polypeptide, PTH(1-84); numbering, by universal convention, starting at the amino-terminus. The precursors remain within the parathyroid cells, while PTH(1-84) is secreted, along with small amounts of several different cleavage products of the full-length molecule. Following secretion, PTH(1-84) undergoes rapid cleavage and metabolism to form carboxyl-terminal fragments (PTH-C), amino-terminal fragments (PTH-N), and midmolecule fragments (PTH-M) (Figure 2, page 3). Only those portions of the molecule that carry the aminoterminus (ie, the whole molecule, PTH(1-84) and PTH-N fragments) activate the PTHR-1. The active forms have half-lives of approximately 5 minutes. The inactive PTH-C and PTH-M fragments, with half-lives of 24-36 hours, make up >90% of the total circulating PTH, and, as they are primarily cleared by the kidneys, can accumulate to even higher levels in renal failure. Consequently, the ability of any PTH assay to diagnose PTH(1-84)
hypersecretion or hyposecretion is closely related to its degree of cross-reactivity with the cleavage fragments.

First-generation PTH assays were insensitive and displayed substantial cross-reactivity with various PTH fragments. The intact molecule contributed no more than 20% of the total measured immunoreactivity. Even in patients with normal renal function, changes in PTH secretion had to be profound and prolonged in order to be reflected in elevated, or decreased, first-generation assay PTH measurements. Modification of these assays to target midmolecule epitopes led to some improvements, but were, by today’s standards, still unsatisfactory, particularly in renal failure. These modified first-generation assays are often referred to as “midmolecule” PTH assays.

The second generation of PTH assays, which have come to dominate the market during the last decade, were designed to detect exclusively the intact PTH(1-84) molecule, and are called “intact” or “whole molecule” PTH assays. Generally, these assays have performed substantially better than older assay configurations, for the first time turning PTH measurements into a truly useful diagnostic tool. The Mayo Clinic Endocrine laboratory offered one of the first such assays for many years, created in-house before any major immunoassay manufacturer sold a similar product. However, it has become apparent that this assay, like other second-generation assays, is still not completely free of cross-reactivity with PTH fragments. In particular, all second-generation PTH assays cross-react with a group of fragments dubbed non-(1-84)PTH, which consists of fragments that include most, but not all of the N-terminus of PTH, chiefly, PTH(7-84). These fragments do not interact with the canonical PTH receptor, yet, depending on the second-generation assay used, account for 10-30% of PTH immunoreactivity in patients with normal renal function, and more than 45% in patients with renal failure. Therefore, as measures of PTH(1-84), even second-generation PTH assays perform suboptimal in cases of renal failure. In addition, it has become apparent that PTH(7-84) and some other PTH-C fragments, display unique biological effects, even though they do not activate the PTHR-1. There is evidence that these fragments interact with a unique, yet unidentified, receptor. At high concentrations, such as may be observed in patients with impaired renal function, these non-(1-84)PTH fragments oppose the biochemical and bone metabolic effects of PTH(1-84). This adds a biological dimension to the analytical problem of cross-reactivity with non-(1-84)PTH in patients with renal failure.

To address these problems, a third generation of PTH immunoassays has been developed during the last 4-5 years. These assays are extremely specific for PTH(1-84), with no significant cross-reactivity with the previously identified PTH fragments (Table 3, page 4). In patients with normal renal function, these assays perform at least as well as second-generation assays. In our own patient population, a third-generation PTH assay (Nichols-Institute “Bio-Intact”) performed similar to our in-house second-generation PTH assay in the diagnosis of primary hyperparathyroidism. In the crucial, high-sensitivity and high-specificity portion of the assay Receiver-operator-curves (ROC), which determine where the best diagnostic cutoff levels should be placed, the “Bio-Intact” assay performed better than our in-house assay (Figure 3, page 4). Other investigators have found even clearer evidence for superior performance of third-generation PTH assays in the diagnosis of primary hyperparathyroidism when compared with second-generation assays. However, the main strength of these new assays lies in PTH measurement in renal failure patients. The new assays allow unequivocal measurement of “true” PTH(1-84) in these individuals, resulting in a more accurate assessment of calcium and bone metabolism. Furthermore, when combined with a second-generation PTH assay with 100% cross-reactivity with PTH(7-84), a separate, useful
A ratio >1 predicts a high bone-turnover state, while a ratio of <1 is associated with low bone turnover.8,14

Formula 1:
\[
\text{Ratio} = \frac{\text{3rd-generation PTH}}{\text{2nd-generation PTH} - \text{3rd-generation PTH}}
\]

Because of the various advantages of the third-generation assays, we have replaced our in-house second-generation assay with the Nichols Bio-Intact (third generation) PTH assay, #84213 Parathyroid Hormone, 1-84 Bio-Intact, Serum. In addition, we will be offering the Nichols intact (second-generation) PTH assay sometime in early 2004. Since the Nichols intact PTH assay has 100% cross-reactivity with PTH(7-84), this will allow the calculation of fragment ratios in renal failure patients. Our current in-house assay will be retired, as it has only 30% cross-reactivity with PTH(7-84) and is therefore not suitable for fragment ratio calculations in renal failure.

Summary and Suggested Strategy for Assessment of Hypercalcemia, Hypocalcemia, and Metabolic Bone Disease

1. Serum levels of calcium, phosphate, creatinine, 25-OH-VitD3, 1,25-OH-VitD3, and, depending on the clinical scenario, magnesium, parathyroid-hormone related peptide, and albumin, should be determined in conjunction with PTH.

2. In patients with normal renal function, a second- ("intact" or "whole molecule"), or, preferably a third-generation PTH assay ("Bio-Intact") should be used; there is no place for "midmolecule" first-generation or other older PTH assays, in modern practice.

3. When interpreting results in patients with normal renal function, the population vitamin D status and its effect on the reference ranges should be taken into account. In northern parts of the US, the optimal cutoff for the diagnosis of primary hyperparathyroidism may be significantly lower than the upper reference range limit of PTH and probably lies somewhere around 30-35 pg/mL (~3.2-3.7 pmol/L) when measured with a Bio-Intact assay (10-30% higher than that with "intact/whole molecule" assays).

4. Patients with significant renal impairment (estimated GFR <20% of normal) should have their PTH levels measured with a Bio-Intact PTH (third-generation) assay. Previously, second-generation assays required PTH levels of >3-4 times the upper reference limit to be considered indicative of high bone-turnover disease states in patients with renal failure. With third-generation PTH assays, any level above the normal population reference range is suspicious of secondary or tertiary hyperparathyroidism. A small degree of hyperparathyroidism might be desirable in renal failure,15 but results that exceed the upper reference range limit more than twofold are likely to be associated with pathologically increased bone turnover. Testing with a third-generation assay, #84213 Parathyroid Hormone, 1-84 Bio-Intact, Serum, may be supplemented with additional PTH measurement using a second-generation assay with 100% cross-reactivity with PTH(7-84), eg, the "old" Nichols intact PTH assay. A ratio >1 predicts a high bone-turnover state, while a ratio of <1 is associated with low bone-turnover states (see Formula 1).

If you need assistance selecting the appropriate tests for your patient, please contact Mayo Laboratory Inquiry at 800-533-1710.
### Table 1: Diagnosis of hypercalcemia

<table>
<thead>
<tr>
<th></th>
<th>PHPT¹</th>
<th>FHH²</th>
<th>Hypercalcemia of malignancy</th>
<th>THPT*</th>
<th>Vitamin D intoxication</th>
<th>Autonomous 1,25OH-VitD₂₃ production</th>
<th>Pseudo-hypercalcemia²²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total serum calcium</td>
<td>↔ to ↑↑</td>
<td>↔ to ↑</td>
<td>↑ to ↑↑↑</td>
<td>↔* to ↑↑</td>
<td>↔ to ↑↑</td>
<td>↑ to ↑↑</td>
<td>↑ to ↑↑</td>
</tr>
<tr>
<td>Ionized serum calcium</td>
<td>↑ to ↑↑↑</td>
<td>↑ to ↑↑</td>
<td>↑↑ to ↑↑↑</td>
<td>↔* to ↑↑</td>
<td>↑↑ to ↑↑</td>
<td>↑ to ↑↑</td>
<td>↔</td>
</tr>
<tr>
<td>Serum phosphate</td>
<td>↔ to ↓</td>
<td>↔ to ↓</td>
<td>↓ to ↓↓↓</td>
<td>↑↑* to ↓</td>
<td>↑ to ↑↓</td>
<td>↑ to ↑↑</td>
<td>↔</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>↔ to ↑</td>
<td>↔ to ↑</td>
<td>↑ to ↑↑↑</td>
<td>↔ to ↑↑*</td>
<td>↔ to ↑</td>
<td>↑ to ↑</td>
<td>↔</td>
</tr>
<tr>
<td>Serum PTH</td>
<td>↔ to ↑↑</td>
<td>↔ to ↑</td>
<td>↑↑ to ↓↓</td>
<td>↑↑↑ to ↑</td>
<td>↓ to ↑</td>
<td>↑ to ↑↑</td>
<td>↔</td>
</tr>
<tr>
<td>Serum 25OH-VitD₂₃</td>
<td>↔ to ↓</td>
<td>↔ to ↓</td>
<td>↓ to ↑</td>
<td>↑↑↑ to ↑</td>
<td>↑↑↑ to ↓</td>
<td>↑ to ↑↑</td>
<td>↔</td>
</tr>
<tr>
<td>Serum 1,25OH-VitD₂₃</td>
<td>↔ to ↑</td>
<td>↔ to ↑</td>
<td>↑ to ↑↑</td>
<td>↓* to ↑↑</td>
<td>↔ to ↑↑</td>
<td>↑ to ↑↑</td>
<td>↔</td>
</tr>
<tr>
<td>Other</td>
<td>-</td>
<td>-</td>
<td>PTHRP³↑</td>
<td>-</td>
<td>-</td>
<td>ACE⁵↑</td>
<td>Albumin ↑ to ↑↑</td>
</tr>
</tbody>
</table>

¹ PHPT: Primary hyperparathyroidism
² FHH: Familial hypocalcuric hypercalcemia
³ PTHRP: Parathyroid hormone related peptide, a peptide with N-terminal homology to PTH, secreted by many tumors.
⁴ THPT: Tertiary hyperparathyroidism; parathyroid autonomy on top of long-standing secondary hyperparathyroidism. Most commonly seen in renal failure patients, when normal renal function is restored by transplantation.
⁵ ACE: Angiotensin converting enzyme; elevated in 2/3 of cases of sarcoidosis, a main cause of autonomous 1,25OH-VitD₂₃ production (in granulomatous tissues).

* The findings observed commonly in patients with impaired renal function are indicated by the asterisk. Following restoration of normal renal function after transplantation the non-asterixed findings are more common.
** Pseudohypercalcemia is a laboratory artefact caused by elevation in total calcium due to a high albumin. The ionized (=bioavailable) calcium is normal. It has no disease value.
# PTH is only very rarely within the lower portion of the reference range, but mostly low or undetectable.

### Table 2: Diagnosis of hypocalcemia

<table>
<thead>
<tr>
<th></th>
<th>Hypoparathyroidism</th>
<th>Hypomagnesimia</th>
<th>PTH-resistance</th>
<th>Renal failure with secondary hyperparathyroidism</th>
<th>Vitamin D deficiency with secondary hyperparathyroidism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total serum calcium</td>
<td>↓ to ↓↓↓</td>
<td>↓ to ↓↓↓</td>
<td>↔ to ↓↓↓</td>
<td>↓ to ↓</td>
<td>↔ to ↓↓↓</td>
</tr>
<tr>
<td>Ionized serum calcium</td>
<td>↓ to ↓↓↓</td>
<td>↓ to ↓↓↓</td>
<td>↔ to ↓↓↓</td>
<td>↔ to ↓</td>
<td>↔ to ↓↓↓</td>
</tr>
<tr>
<td>Serum phosphate</td>
<td>↑ to ↑↑</td>
<td>↑ to ↑↑</td>
<td>↑ to ↑↑</td>
<td>↑↑↑ to ↑↑↑</td>
<td>↑ to ↑↑↑</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>↔</td>
<td>↔ to ↑↑*</td>
<td>↔ to ↑</td>
<td>↑↑↑ to ↑↑↑</td>
<td>↔</td>
</tr>
<tr>
<td>Serum PTH</td>
<td>↔ to ↓</td>
<td>↔ to ↓</td>
<td>↑ to ↑↑↑</td>
<td>↑ to ↑↑↑</td>
<td>↑ to ↑↑↑</td>
</tr>
<tr>
<td>Serum 25OH-VitD₂₃</td>
<td>↓ to ↑</td>
<td>↓ to ↑</td>
<td>↓ to ↑</td>
<td>↓ to ↑</td>
<td>↓ to ↑</td>
</tr>
<tr>
<td>Serum 1,25OH-VitD₂₃</td>
<td>↔ to ↓</td>
<td>↔ to ↓</td>
<td>↔ to ↓</td>
<td>↓ to ↓</td>
<td>↓ to ↓</td>
</tr>
<tr>
<td>Magnesium</td>
<td>↔ to ↓</td>
<td>↔ to ↓</td>
<td>↔ to ↓</td>
<td>↔ to ↓</td>
<td>↔ to ↓</td>
</tr>
</tbody>
</table>

* Hypomagnesimia is often due to renal tubular magnesium loss and may be associated with impaired renal function, due to underlying short- or long-term kidney disease.
Table 3: Crossreactivity and interference of PTH-N, PTH-M and PTH-C fragments with PTH(1-84) measurement with the Nichols bio-intact PTH assay

<table>
<thead>
<tr>
<th>PTH fragments</th>
<th>Fragment concentrations spiked into patient samples (pg/mL)</th>
<th>Change in bio-intact PTH assay results compared to unspiked samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH(1-31)*</td>
<td>10,000</td>
<td>-80</td>
</tr>
<tr>
<td>PTH(1-34)*</td>
<td>10,000</td>
<td>-80</td>
</tr>
<tr>
<td>PTH(2-38)*</td>
<td>10,000</td>
<td>-80</td>
</tr>
<tr>
<td>PTH(7-84)</td>
<td>10,000</td>
<td>0</td>
</tr>
<tr>
<td>PTH(13-34)</td>
<td>10,000</td>
<td>0</td>
</tr>
<tr>
<td>PTH(18-48)</td>
<td>10,000</td>
<td>0</td>
</tr>
<tr>
<td>PTH(53-84)</td>
<td>10,000</td>
<td>0</td>
</tr>
<tr>
<td>PTH(64-84)</td>
<td>10,000</td>
<td>0</td>
</tr>
</tbody>
</table>

* Endogenous levels of these fragments contribute a minute proportion to circulating PTH under all known conditions (<1%). The quenching of measured PTH signal that occurs at extremely high spiked concentrations of these fragments (~200 fold above the upper limit of the PTH reference range and ~200,000 fold above their endogenous concentrations) can therefore only be observed under experimental conditions. However, these data suggest that in patients who are treated for osteoporosis with synthetic PTH(1-34), PTH(1-84) should probably not be measured immediately after an injection.

References

Lactate Dehydrogenase Isoenzymes Reference Value Change

Test #8679 Lactate Dehydrogenase (LD) Isoenzymes, Serum, includes determination of the total LD level and the isoenzyme fractions. The reference values for the total LD assay were changed because of a change in calibrator set point. In addition, a normal value study and literature review were performed, which resulted in a consolidated reference value for males and females, and expanded the reference values for pediatric patients. There were no changes to the isoenzyme portion of the test.

New Reference Values
TOTAL
1-30 days: 135-750 U/L
31 days - 11 months: 180-435 U/L
1-3 years: 160-370 U/L
4-6 years: 145-345 U/L
7-9 years: 143-290 U/L
10-12 years: 120-293 U/L
13-15 years: 110-293 U/L
16-17 years: 105-233 U/L
≥18 years: 122-222 U/L
ISOENZYMES (no change)
I (fast band): 17.5-28.3%
II: 30.4-36.4%
III: 19.2-24.8%
IV: 9.6-15.6%
V (slow band): 5.5-12.7%

Previous Reference Values
Males
0-5 years: not established
6-13 years: 120-312 U/L
14-17 years: 106-290 U/L
18-25 years: 94-213 U/L
26-30 years: 92-202 U/L
31-35 years: 91-196 U/L
36-40 years: 91-198 U/L
41-45 years: 92-202 U/L
46-50 years: 94-210 U/L
51-55 years: 98-221 U/L
56-60 years: 104-236 U/L
≥61 years: 112-257 U/L

Females
0-5 years: not established
6-13 years: 120-280 U/L
14-17 years: 100-275 U/L
18-25 years: 94-213 U/L
26-30 years: 92-202 U/L
31-35 years: 91-196 U/L
36-40 years: 91-198 U/L
41-45 years: 92-202 U/L
46-50 years: 94-210 U/L
51-55 years: 98-221 U/L
56-60 years: 104-236 U/L
≥61 years: 112-257 U/L

Phospholipid (Cardiolipin) Reference Value Changes
The reference values for the phospholipid antibody tests have been reevaluated and now report an additional range for weakly positive. This change affects the following tests:

#8093 Phospholipid Antibodies (Cardiolipin Antibodies) IgG, Serum
#81900 Phospholipid Antibodies (Cardiolipin) IgM, Serum
#82976 Phospholipid Antibodies (Cardiolipin Antibodies), IgG and IgM, Serum

New Reference Values
IgG Phospholipid (Cardiolipin) Antibodies
negative ≤15.0 GPL
weakly positive 15.1-29.9 GPL
positive 30.0-79.9 GPL
strongly positive ≥80.0 GPL

IgM Phospholipid (Cardiolipin) Antibodies
negative ≤10.0 MPL
weakly positive 10.1-29.9 MPL
positive 30.0-79.9 MPL
strongly positive ≥80.0 MPL

GPL and MPL units are used widely to express the results of antiphospholipid antibody tests performed by enzyme immunoassays. The terminology refers to arbitrary units, and the abbreviations "GPL" and "MPL" denote whether the result is for the IgG or the IgM isotype, respectively. The letters "PL" denote specificity for phospholipid antigens.

Previous Reference Values
IgG Phospholipid (Cardiolipin) Antibodies
negative ≤15.0 GPL
positive 15.1-79.9 GPL
strongly positive ≥80.0 GPL

IgM Phospholipid (Cardiolipin) Antibodies
negative ≤10.0 MPL
positive 10.1-79.9 MPL
strongly positive ≥80.0 MPL
C-Peptide Reporting Change
The report format for #8804 C-Peptide, Serum has been changed to provide results in both pmol/L and ng/mL.

New Reference Value
0.9-4.3 ng/mL; 297-1419 pmol/L
Previous Reference Value
0.9-4.3 ng/mL

In addition, the interpretive information has been expanded and includes the following discussion.

Interpretive Information for Utilizing the Insulin/C-Peptide Ratio
To compare insulin and C-peptide concentrations (ie, insulin to C-peptide ratio), convert insulin to pmol/L: insulin concentration (in µIU/mL) x 7.18 = insulin concentration (in pmol/L). The insulin conversion is based on our assay, which is calibrated against the 1st IRP 66/304.

Factitious hypoglycemia due to surreptitious insulin administration results in elevated serum insulin levels and low or undetectable C-peptide levels, with a clear reversal of the physiological molar insulin to C-peptide ratio (≤1) to an insulin to C-peptide ratio of >1. By contrast, insulin and C-peptide levels are both elevated in insulinoma and the insulin to C-peptide molar ratio is ≤1. Sulfonylurea ingestion also is associated with preservation of the insulin to C-peptide molar ratio of ≤1.

In patients with insulin autoantibodies, the insulin to C-peptide ratio may be reversed to >1, because of the prolonged half-life of autoantibody-bound insulin.

Phosphoglycerate Kinase Test Changes
Phosphoglycerate kinase analysis is one of the possible reflex tests for:

#84157 Hemolytic Anemia Evaluation
#84161 Red Blood Cell Enzyme Evaluation
#84162 Neurologic Enzyme Evaluation

The Metabolic Hematology laboratory has validated an improved specimen-preparation process using a cellulose column. The new method has driven a change in the reference values.

New Reference Value
131-205 IU/g Hbg
Previous Reference Value
141-179 IU/g Hbg

Cyclic Citrullinated Peptide Antibodies Report Change
The report for #84182 Cyclic Citrullinated Peptide Antibodies, Serum was changed to include the established reference values for this test. The technical performance of this test has not changed.

Reference Values
≤5 U/mL is negative
>5 U/mL is positive

Adenylate Kinase Test Changes
Adenylate kinase analysis is one of the possible reflex tests for both #84157 Hemolytic Anemia Evaluation and #84161 Red Blood Cell Enzyme Evaluation. The Metabolic Hematology laboratory has validated an improved specimen-preparation process using a cellulose column. The new method has driven a change in the reference values.

New Reference Value
210-335 IU/g Hbg
Previous Reference Value
144.7-254.3 IU/g Hbg

Autoimmune Liver Disease Evaluation Test Number Change
The test number for Autoimmune Liver Disease Evaluation, Serum has been changed from #80801 to #84248. No other aspect of the test has changed.

HIV-1 Genotyping Test Changes
The test number for Human Immunodeficiency Virus Type 1 (HIV-1) Genotyping, Plasma has been changed from #82951 to #82340. In addition to the test number change, the report has been reformatted to be more concise and allows for future antivirals to be added to the report. An interpretation legend also has been added to the report.
Estrone and Estradiol Test Changes
In conjunction with the launch of the new fractionation test (#84230 Estrogens, Estrone (E1) + Estradiol (E2), Fractionated, Serum) the methodology for the following tests has changed to high-performance liquid chromatography/tandem mass spectrometry (LC-MS/MS):

- #81418 Estrone, Serum
- #81816 Estradiol, Enhanced, Serum

As a result of the method change, the reference values and specimen required for these tests have also changed. Previous reference values and specimen requirements are not listed.

New Specimen Required (for either test)
Draw blood in a plain, red-top tube(s) or a serum gel tube(s). Spin down and send 1.2 mL of serum refrigerated.

Note: Serum must be separated from gel within 24 hours of collection.

#81418 Estrone, Serum
New Reference Value

Children*
1-14 days: Estrone levels in newborns are very elevated at birth but will fall to prepubertal levels within a few days.

<table>
<thead>
<tr>
<th>Tanner Stage</th>
<th>Mean Age</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I#:</td>
<td>7.1</td>
<td>undetectable-16 pg/mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(&gt;14 days and prepubertal)</td>
</tr>
<tr>
<td>Stage II:</td>
<td>11.5</td>
<td>undetectable-22 pg/mL</td>
</tr>
<tr>
<td>Stage III:</td>
<td>13.6</td>
<td>10-25 pg/mL</td>
</tr>
<tr>
<td>Stage IV:</td>
<td>15.1</td>
<td>10-46 pg/mL</td>
</tr>
<tr>
<td>Stage V:</td>
<td>18</td>
<td>10-60 pg/mL</td>
</tr>
</tbody>
</table>

#Puberty onset (transition from Tanner stage I to Tanner stage II) occurs for boys at a median age of 11.5 (±2) years. For boys there is no proven relationship between puberty onset and body weight or ethnic origin. Progression through Tanner stages is variable. Tanner stage V (adult) should be reached by age 18.

Females
Tanner Stage | Mean Age | Reference Range               |
-------------|----------|-------------------------------|
Stage I#:    | 7.1      | undetectable-29 pg/mL         |
|              |          | (>14 days and prepubertal)    |
| Stage II:    | 10.5     | 10-33 pg/mL                   |
| Stage III:   | 11.6     | 15-43 pg/mL                   |
| Stage IV:    | 12.3     | 16-77 pg/mL                   |
| Stage V:     | 14.5     | 17-200 pg/mL                  |

#Puberty onset (transition from Tanner stage I to Tanner stage II) occurs for girls at a median age of 10.5 (±2) years. There is evidence that it may occur up to 1 year earlier in obese girls and in African-American girls. Progression through Tanner stages is variable. Tanner stage V (adult) should be reached by age 18.

*The reference ranges for children are based on the published literature;1,2 cross-correlation of our assay with assays used to generate the literature data and on our data for young adults.

Adults
Males
Premenopausal: 10-60 pg/mL
Females
Premenopausal: 17-200 pg/mL
Postmenopausal: 7-40 pg/mL

Conversion factor: E1: pg/mL x 3.704 = pmol/L (molecular wt = 270)

#81816 Estradiol, Enhanced, Serum
New Reference Value

Children*
1-14 days: Estradiol levels in newborns are very elevated at birth but will fall to prepubertal levels within a few days.

<table>
<thead>
<tr>
<th>Tanner Stage</th>
<th>Mean Age</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I#:</td>
<td>7.1</td>
<td>undetectable-13 pg/mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(&gt;14 days and prepubertal)</td>
</tr>
<tr>
<td>Stage II:</td>
<td>12.1</td>
<td>undetectable-16 pg/mL</td>
</tr>
<tr>
<td>Stage III:</td>
<td>13.6</td>
<td>undetectable-26 pg/mL</td>
</tr>
<tr>
<td>Stage IV:</td>
<td>15.1</td>
<td>undetectable-38 pg/mL</td>
</tr>
<tr>
<td>Stage V:</td>
<td>18</td>
<td>10-40 pg/mL</td>
</tr>
</tbody>
</table>

#Puberty onset (transition from Tanner stage I to Tanner stage II) occurs for boys at a median age of 11.5 (±2) years. For boys there is no proven relationship between puberty onset and body weight or ethnic origin. Progression through Tanner stages is variable. Tanner stage V (adult) should be reached by age 18.

Females
Tanner Stage | Mean Age | Reference Range               |
-------------|----------|-------------------------------|
Stage I#:    | 7.1      | undetectable-20 pg/mL         |
|              |          | (>14 days and prepubertal)    |
| Stage II:    | 10.5     | undetectable-24 pg/mL         |
| Stage III:   | 11.6     | undetectable-60 pg/mL         |
| Stage IV:    | 12.3     | 15-85 pg/mL                   |
| Stage V:     | 14.5     | 15-350 pg/mL                  |

#Puberty onset (transition from Tanner stage I to Tanner stage II) occurs for girls at a median age of 10.5 (±2) years. There is evidence that it may occur up to 1 year earlier in obese girls and in African-American girls. Progression through Tanner stages is variable. Tanner stage V (adult) should be reached by age 18.

*The reference ranges for children are based on the published literature, cross-correlation of our assay with assays used to generate the literature data and on our data for young adults.

Adults
Males
Premenopausal: 15-350 pg/mL**
Postmenopausal: <10 pg/mL

Conversion factor: E2: pg/mL x 3.676 = pmol/L (molecular wt = 272)

**E2 levels vary widely through the menstrual cycle.
2004 Education Calendar

Interactive Satellite Programs . . .
Genomics, Genes, and the Future of Health Care
March 16, 2004
Presenter: David Schowalter, MD, PhD
Moderator: Robert M. Kisabeth, MD

Osteoporosis Management in 2004: Transition to Anabolic Therapy
April 20, 2004
Presenter: Bart Clarke, MD
Moderator: Robert M. Kisabeth, MD

Heart Failure – Standard and Advanced Management Strategies
May 18, 2004
Presenters: Margaret Redfield, MD; Brooks Edwards, MD; and Robert Frantz, MD
Moderator: Margaret Redfield, MD

Herbal Therapy 2004: Snakes in the Grass (Herb-Drug Interactions Clinicians Need to Know)
September 16, 2004
Presenter: Brent Bauer, MD
Moderator: Robert M. Kisabeth, MD

Markers of Inflammatory Bowel Disease
October 19, 2004
Presenters: Henry Homburger, MD and Edward Loftus, MD
Moderator: Robert M. Kisabeth, MD

The Use of Diagnostic Tests in the Pediatric Age Group
November 2, 2004
Presenter: Robert Jacobson, MD
Moderator: Robert M. Kisabeth, MD

Thyroid Disease – Laboratory Support For Diagnosis and Management
December 7, 2004
Presenter: George Klee, MD, PhD
Moderator: Robert M. Kisabeth, MD

Upcoming Education Conferences . . .
The Phlebotomist...The Competent Professional
March 18-19, 2004
Mayo Clinic, Siebens Building
Rochester, Minnesota

Practical Spirometry
March 31-April 1, 2004
Mayo Clinic, Siebens Building
Rochester, Minnesota

11th Annual International Surgical Pathology Symposium
May 4-7, 2004
Radisson SAS Bay Point Resort
St. Julians, Malta

Integration Through Community Laboratory Insourcing: Implementing a Successful Laboratory Outreach Program
June 17-19, 2004
Mayo Clinic, Siebens Building
Rochester, Minnesota

Bleeding and Thrombosing Diseases: The Basics and Beyond
Coagulation Conference and Wet Workshop
August 5-7, 2004
Mayo Clinic, Siebens Building
Rochester, Minnesota

Practical Surgical Pathology
September 16-18, 2004
Mayo Clinic, Siebens Building
Rochester, Minnesota

Practical Spirometry
November 2-3, 2004
Mayo Clinic, Siebens Building
Rochester, Minnesota

Introductory Clinical Mycology
November 18-19, 2004
Mayo Clinic, Siebens Building
Rochester, Minnesota

For additional information, contact Mayo Reference Services Education Department at 800-533-1710. Visit us under “Education” at www.mayoreferenceservices.org.
Please e-mail your questions to mml@mayo.edu.

Q: Does “Estrogens, E1+E2, Fractionated” replace “Estrogens, Total?”

A: Yes, #84230 Estrogens, E1+E2, Fractionated, Serum does replace #80249 Estrogens, Total since the 2 major biologically active estrogens in nonpregnant humans are estrone (E1) and estradiol (E2). The third bioactive estrogen, estriol (E3), is the main pregnancy estrogen, but plays no significant role in nonpregnant women or men.

Q: If the new test “Estrogens, E1+E2, fractionated, S” is equivalent to the old test “Estrogens, total,” why does the report now include 2 separate values, 1 for E1 and 1 for E2, when the old “Estrogens, total” report only contained a single value?

A: The old “Estrogens, total” measured the same estrogen compounds, E1 and E2, like the new test, but could not distinguish between the 2 analytes. Hence, a single total value was reported. Since there are significant differences in biological role and in estrogenic potency between E1 and E2 (eg, E2 is between 1.25 and 5 times as potent as E1), #84230 Estrogens, E1+E2, Fractionated, Serum reports the 2 analytes separately, which is clinically more meaningful.

Q: Should SCA 2 or 7 Large Expansion testing be ordered in addition to the SCA panel if infantile or juvenile SCA is suspected?

A: No, the SCA panel, #80941 Spinocerebellar Ataxia (SCA) Panel (type 1, 2, 3, 6, and 7), will automatically reflex to #81042 Spinocerebellar Ataxia (SCA) Types 2 or 7 Large Expansions if a patient appears to be homozygous for a normal SCA 2 or 7 allele and infantile or juvenile onset is suspected.

Q: Which spinocerebellar ataxia test should be ordered if there is a family history of SCA 2 or 7 mutation?

A: #81097 Spinocerebellar Ataxia Known Mutation (SCA 1, 2, 3, 6, or 7), should be ordered if there is a known expansion in any one of the 5 SCA genes (1, 2, 3, 6, or 7). If the sample is from a fetus, infant, or small child and large expansion testing is indicated, the lab will add it on to confirm the presence or absence of a large expansion within SCA 2 or 7.
Clinical Spectrum and Laboratory Characteristics Associated With Detection of Herpes Simplex Virus DNA in Cerebrospinal Fluid

Cathal E. O’Sullivan, MD; Allen J. Aksamit, MD; Jeffrey R. Harrington, MA; W. Scott Harmsen, MS; P. Shawn Mitchell; and Robin Patel, MD

• Objective: To determine the clinical, neurologic, and laboratory characteristics of patients with herpes simplex virus (HSV) type 1 (HSV-1) or HSV type 2 (HSV-2) DNA detected in cerebrospinal fluid (CSF) with use of polymerase chain reaction.

• Patients and Methods: Clinical, laboratory, and demographic data were determined from 249 CSF specimens (collected from 247 patients >10 years of age) that tested positive for HSV-1 or HSV-2 DNA at the Mayo Clinic from January 1999 to August 2000.

• Results: The median age of the 200 patients whose age was available was 70 years vs 40 years for those with HSV-1 or HSV-2 DNA in CSF, respectively. Detailed data were available for 39 and 78 patients with positive polymerase chain reaction results for HSV-1 and HSV-2, respectively. Of those with HSV-1 DNA detected in CSF, 89% had encephalitis, whereas most patients with HSV-2 DNA detected in CSF had findings compatible with meningitis. Only 5 (7%) of 69 patients in whom HSV-2 was detected in CSF had genital lesions at presentation, and none of the assessable patients with HSV-2 who had recurrent meningitis had active genital lesions at presentation.

• Conclusion: The vast majority (82%) of patients with HSV-2 detected in CSF had no history of genital herpes and no lesions at the time of presentation. Polymerase chain reaction assays designed to detect HSV in CSF should detect HSV-1 and HSV-2 and differentiate between HSV-1 and HSV-2.

Mayo Clinic Proceedings 2003;78:1347-1352

The complete article is available online at URL: http://www.mayo.edu/proceedings/.