Rapid Intraoperative Immunoassay of Parathyroid Hormone and Other Hormones: A New Paradigm for Point-of-Care Testing

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Background: The first description of the use of a rapid assay for the measurement of intact parathyroid hormone (PTH) in patients undergoing parathyroidectomy for hyperparathyroidism was reported in 1988. Subsequent improvements in the analytical performance of the rapid intraoperative PTH assay allowed the establishment of its clinical utility in the surgical management of hyperparathyroidism. These modifications also allowed the assay to be performed in or near the operating suite.

Methods: We searched MEDLINE, using the following key words: intraoperative, rapid, quick, parathyroid hormone, hormone, and immunoassay. Relevant articles that focused on the analytical aspects and clinical utility of rapid intraoperative hormone immunoassays were selected for this review.

Content: On the basis of the positive impact that the rapid intraoperative PTH test has had on both patient outcomes and cost savings, other rapid intraoperative hormone immunoassays for the diagnosis and/or treatment of other endocrine-hormone-secreting tumors have been developed. These hormones share certain characteristics that make them suitable for use as rapid intraoperative tests, i.e., short analyte half-life and/or large analyte concentration gradient, rapid analysis time, and positive clinical utility. Initial studies with cortisol, gastrin, insulin, adrenocorticotropic hormone, and testosterone have shown promising results in preoperative localization studies and/or for assessing the effectiveness of tumor resection during surgery.

Conclusion: The emergence of these rapid intraoperative immunoassays indicates that this test format is likely to provide future opportunities to improve patient care by advances in clinical laboratory testing.

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The first recorded instance of intraoperative testing may be the electromotor test developed by Burge and Vane in 1958 (1) to assess the completeness of vagotomy in patients undergoing antrectomy because of gastric ulcer disease. In a 1989 review of intraoperative tests for the completeness of vagotomy, D.S. Burkitt was perhaps the first to define the criteria applicable to any intraoperative test: “Any intraoperative test must be safe, reproducible, simple to interpret, and preferably rapid. Cost is a consideration, but the cost can be offset against the potential savings of not having to treat so many recurrent ulcers” (2).

Among the first of the newer intraoperative tests that rely on accurate and precise quantitative measurement of a clinical laboratory analyte is the rapid intraoperative test for parathyroid hormone (PTH)4 (3–5). Nussbaum et al. (6) were the first to describe the use of a rapid assay for PTH in patients undergoing neck exploration for hyperparathyroidism. Subsequent improvements in the analytical performance of the rapid intraoperative PTH assay (7, 8) allowed for the establishment of its clinical utility in the surgical management of hyperparathyroidism (9–14). These modifications also allowed the assay to be performed in the vicinity of the operating room, thus improving the turnaround time of the result and communication between the laboratory and the surgical team. Recently, Wians (15) coined the term, “point-of-surgery testing

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4 Nonstandard abbreviations: PTH, parathyroid hormone; ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; and GH, growth hormone.
(POST)” for rapid intraoperative immunoassays and suggested that it represents a new paradigm for reducing the costs of medical care and for improving patient outcome by clinical laboratory testing. This prediction appears to already be coming true with the recent development of other types of rapid intraoperative immunoassays (16–20). This review will focus on the development and use of the rapid intraoperative PTH test, as well as other rapid intraoperative immunoassays, from a clinical laboratory perspective.

Early Development of Intraoperative PTH Assays

Although the success rate for surgical treatment of hyperparathyroidism in most medical centers before the use of the rapid intraoperative PTH test was >90–95% (21, 22), a troubling fraction of patients would have persistent hypercalcemia after surgery and would require additional procedures to be cured. Furthermore, the success of the procedure, before the rapid intraoperative PTH test, was dependent on performing a complete neck dissection and inspection of all four parathyroid glands, which complicates the procedure and occasionally leads to morbidity to the patient, mostly as a result of inadvertent damage to the recurrent laryngeal nerves (23). Numerous studies have established that unexplained persistent hypercalcemia after parathyroidectomy surgery is usually attributable to unrecognized ectopic or multiple adenomas, unrecognized supernumerary glands, insufficient excision of hyperplastic tissue, and difficulty in histologically distinguishing between adenomatous and hyperplastic glands on frozen sections. Unfortunately, repeat surgery to reexplore the neck for missed parathyroid adenomas or hyperplastic tissue is often more challenging because of fibrosis and scarring from the initial surgery. As a consequence, repeat procedures have higher complication rates and lower success rates than do initial explorations.

There thus has been a search over the past several decades for a rapidly responding biochemical measure that would be indicative of a physiologic cure to serve as an adjunct to surgical expertise and to the histologic examination of frozen tissue sections to assess parathyroidectomy success. In 1978, urinary cAMP was investigated as a potential marker, but its concentration did not change rapidly enough to be of use (24). In 1988, after the introduction of a two-site IRMA for intact PTH (25), Nussbaum et al. (6) proposed that a modified version of this assay with a reduced incubation time (from 22 h to 15 min) could be used as an intraoperative test. The theory behind the utility of PTH measurements includes the fact that PTH is produced only in the parathyroid glands, that the intact 84-residue PTH molecule has a half-life of <5 min, and that secretion of PTH is suppressed by properly functioning parathyroid glands. Therefore, blood concentrations of intact PTH should decrease rapidly within a short period of time subsequent to the removal of all hypersecreting parathyroid tissue (26). In the typical protocol, PTH concentrations are measured at baseline, before exploration, and then at 5–10 min post tumor excision, with a 50% decrease in values observed if all hypersecreting tissue has been removed (7, 27).

Subsequently, several other quick or rapid intraoperative PTH assays were developed with incubation times ranging from 10 to 30 min, giving turnaround times of up to 1 h. Increased incubation temperatures, introduction of continuous shaking, and alterations in sample type and sample and reagent volumes were used to alter assay kinetics. However, in general these assays were limited in their practical usefulness because they required radiotracers; they had relatively long turnaround times, with results obtained when the patient was already in the recovery room; and because of low analytical sensitivities. The utility of rapid intraoperative PTH measurements and successful implementation was not realized until several years later, when nonradioactive assays were developed with greatly shortened incubation times, which allowed these assays to be performed in or close to the operating suite (7).

Early reports by an endocrine surgeon, George Irvin, and his coworkers (7, 9, 27), and by others (8, 28), describing the development and clinical applications of rapid intact PTH assays with both radioactive and nonradioactive formats have led to the implementation of commercial rapid intraoperative PTH assays, which are now in widespread use in most medical centers (29). In 1994, Irvin et al. (9), and subsequently other surgeons (30), proposed combining the use of rapid intraoperative PTH monitoring with preoperative tumor localization, using 99mTc-sestamibi (MIBI) scintigraphy. This allowed a directed approach to identifying the affected gland, obviating the need to perform a traditional bilateral neck exploration of all glands. A further refinement of this approach has been the use of the rapid intraoperative PTH test in patients undergoing surgery in an outpatient or ambulatory setting (30, 31).

Description of Commercially Available Rapid Intraoperative PTH Assays

The commercially available assays used for rapid intraoperative PTH testing and their characteristics are described in Table 1. The variety and number of assays available speaks to the current interest in this application of the PTH assay. Assays are now available in both manual/semiautomated (7, 32, 33) and automated formats (12, 28, 34, 35). In addition, some assays for routine intact PTH testing have turnaround times acceptable for use intraoperatively (36, 37). These assays are primarily assays for intact PTH and may measure amino-terminal truncated PTH fragments (such as 7–84); however, a rapid intraoperative bio-intact assay, proposed to measure only the 1–84 amino acid molecule, is available (Table 1).

In 2001, 47% of respondents (n = 92) to a survey by the College of American Pathologists Point-of-Care Testing resource committee (38) reported using the Immulite
Table 1. Characteristics of PTH assays for intraoperative monitoring.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Quick-IntraOperative Intact PTH</th>
<th>Turbo Intact PTH</th>
<th>STAT-I0-I-PTH</th>
<th>Quick-IntraOperative Bio-Intact PTH(1-84)</th>
<th>Elecsys PTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>Nichols Institute Diagnostics</td>
<td>Diagnostic Products Corporation</td>
<td>Future Diagnostics</td>
<td>Nichols Institute Diagnostics</td>
<td>Roche Diagnostics</td>
</tr>
<tr>
<td>Automated system</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Assay design</td>
<td>Capture antibody</td>
<td>Goat polyclonal anti-PTH(39–84)</td>
<td>Goat polyclonal anti-PTH(44–84)</td>
<td>Goat polyclonal anti-PTH(39–84)</td>
<td>Goat polyclonal anti-PTH(26–32)</td>
</tr>
<tr>
<td>Form of PTH measured</td>
<td>Intact</td>
<td>Intact</td>
<td>Intact</td>
<td>Intact</td>
<td>Intact</td>
</tr>
<tr>
<td>Label</td>
<td>Acridinium ester</td>
<td>Alkaline phosphatase</td>
<td>Isoluminol</td>
<td>Isoluminol</td>
<td>Acridinium ester</td>
</tr>
<tr>
<td>Signal</td>
<td>Chemiluminescence</td>
<td>Chemiluminescence</td>
<td>Chemiluminescence</td>
<td>Chemiluminescence</td>
<td>Electrochemiluminescence</td>
</tr>
<tr>
<td>Solid phase</td>
<td>Bead</td>
<td>Bead</td>
<td>Bead</td>
<td>Bead</td>
<td>Bead</td>
</tr>
<tr>
<td>Single-patient packaging</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Specimen type</td>
<td>EDTA plasma</td>
<td>EDTA plasma; serum</td>
<td>EDTA plasma; serum</td>
<td>EDTA plasma</td>
<td>EDTA plasma; serum</td>
</tr>
<tr>
<td>Specimen volume, µL</td>
<td>200</td>
<td>100</td>
<td>100</td>
<td>250</td>
<td>50</td>
</tr>
<tr>
<td>Time to result (including</td>
<td>~15</td>
<td>~15</td>
<td>~15</td>
<td>~10</td>
<td>~12</td>
</tr>
<tr>
<td>specimen processing), min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incubation conditions</td>
<td>Shake at 400 rpm and 45 °C</td>
<td>Intermittent agitation at 37 °C</td>
<td>Shake at 1400 rpm at room temperature</td>
<td>37 °C</td>
<td>37 °C</td>
</tr>
<tr>
<td>Measuring range, a ng/L</td>
<td>6–1250</td>
<td>4–2500</td>
<td>6–2500</td>
<td>5–1800</td>
<td>1.2–5000</td>
</tr>
<tr>
<td>Calibration curve</td>
<td>Each assay</td>
<td>Readjust every 2 weeks</td>
<td>Each assay</td>
<td>Readjust every 7 days</td>
<td>Readjust after 7 days (same reagent set)</td>
</tr>
<tr>
<td>Intraassay imprecision (CV)*</td>
<td>9.4% (29 ng/L)</td>
<td>6.7% (49 ng/L)</td>
<td>10.7% (41 ng/L)</td>
<td>7.0% (21 ng/L)</td>
<td>5.4% (30 ng/L)</td>
</tr>
<tr>
<td></td>
<td>8.0% (162 ng/L)</td>
<td>6.5% (247 ng/L)</td>
<td>4.9% (255 ng/L)</td>
<td>3.9% (309 ng/L)</td>
<td>4.0% (271 ng/L)</td>
</tr>
<tr>
<td>Reference interval, a ng/L</td>
<td>10–65</td>
<td>12–72</td>
<td>10–65</td>
<td>6–40</td>
<td>15–65</td>
</tr>
</tbody>
</table>

* Manufacturer’s specifications.
Turbo assay to perform intraoperative testing. Thirty-three percent of respondents used the QuiCk-Intraoperative method, 7% used the Elecsys method, 13% used another method, and 2% did not respond. As shown in Table 1, all of these PTH assays have acceptable measuring ranges and precision for their intended purposes as intraoperative monitors. Studies comparing these assays analytically have in general found good correlations between rapid intraoperative assays and standard-length PTH assays (8, 28, 29, 32, 39), as well as with other rapid intraoperative assays (33–36, 39). In addition, in studies directly comparing two intraoperative methods clinically (33–36), there was diagnostic agreement between assays with no advantages with respect to patient outcomes observed for a specific assay. Published studies on the use of rapid PTH assays are limited (33, 35). Whether these second-generation intact assays will be advantageous intraoperatively remains to be determined.

Interpretation of Rapid Intraoperative PTH Assay Results
On the basis of the half-life of intact PTH, a >50% decrease in PTH concentrations after removal of the hyperfunctioning parathyroid gland(s) is a generally accepted guideline for the interpretation of PTH concentrations (7), although limited studies have proposed limits of 40% (42), 65% (43), and 75% (40). The intraoperative PTH values in a patient with a single adenoma are illustrated in Fig. 1. In the case of multiglandular disease, it would be expected that the postresection samples would remain increased, indicating the presence of other hyperfunctioning glands, prompting the surgeon to do further exploration to look for additional abnormal glands.

Samples are typically drawn peripherally, although they may also be drawn directly from neck veins. Conditions such as timing and number of samples are less clearly defined. Initial baseline samples are drawn before incision and may be collected in the pre-op area, in the operating room, and before, after, or at introduction of anesthesia. Drawing a second preexcision baseline specimen when the affected gland is identified has been recommended (33, 42) to account for any nonspecific release of PTH from potential tumor manipulation during surgery. The highest baseline value for PTH has been recommended for calculating the percentage change in PTH concentration. Use of preexcision samples has been suggested to reduce the number of false-negative results in patients with a single adenoma (33, 42). Timing of postexcision samples is generally at 5 and/or 10 min, although timings of 7 and 20 min have been used in reported studies (44, 45). A recent protocol has suggested that an immediate post-gland excision sample may also be useful (46). Whether the postexcision sample should also fall below the lowest baseline or the upper limit of the reference interval in addition to a prescribed percentage change has also been debated with a recent study (47) advocating a 50% change from the highest baseline with a result lower than the lowest baseline. A novel approach reported by Libutti et al. (48) to address interindividual variability in half-life and the use of the 50% decrease to indicate cure is a kinetic algorithm that predicts the success of parathyroidectomy surgery based on the rate of PTH decay.

Clinical Utility of Rapid Intraoperative PTH Tests
Numerous studies have shown that rapid intraoperative PTH testing, in the setting of primary hyperparathyroidism, is accurate in predicting surgical success. Cure rates of >95% have been reported in several studies (7, 29, 32, 49, 50), including one study with up to 5 years of follow-up (51). In addition, the rapid PTH assay has been shown to be useful in cases of secondary-tertiary hyperparathyroidism (32, 39, 52), as well as in reoperative cases for failed surgery or recurrent disease (32, 45, 53, 54). The assay has also been reported to predict severe postoperative hypocalcemia in reoperative patients with multiglandular disease (55). It has been suggested in limited reports, however, that intraoperative PTH monitoring does not accurately detect the presence of double adenomas (56) and does not significantly affect the overall success of traditional surgery with bilateral neck exploration (43). As advised in the very first intraoperative study, the assay is intended to provide guidance and should complement, not replace, the judgment and experience of the surgeon to determine surgical cure (6).

A major benefit of the rapid intraoperative PTH assay
in surgery for primary hyperparathyroidism, where 85% of cases have a single adenoma (21), is that in combination with preoperative localization studies, the operation may be performed with a unilaterally directed resection, variously termed limited, concise, directed, minimally invasive, radioguided, and endoscopic parathyroidectomy (57). Components of a minimally invasive surgical approach incorporating rapid intraoperative PTH assays and localization may differ, but in general advantages include use of local or regional, as opposed to general, anesthesia, ability to perform the procedure on an outpatient basis, decreased exploration (unilateral approach), and a smaller incision (12, 31). With this approach patient satisfaction is greater with respect to cosmetic result and postoperative pain (11). This technique has also been shown to minimize costs, such as operating room time and associated fees, frozen section use, and hospital lengths of stay (9–14). In addition, overall hospital charges have been reported to be reduced 50%, reflecting the decreased operating times and significantly shortened lengths of stay resulting from a change from an inpatient to an outpatient procedure (13).

In addition to use of the rapid PTH assay intraoperatively to monitor surgical success, the assay has been used both pre- and perioperatively to localize abnormal parathyroid tissue (58–60). In reoperative cases with discordant imaging, angiography combined with selective venous sampling and PTH testing in the angiography suite is a method to identify a potential PTH gradient. The rapid PTH assay allows for real-time analysis and feedback to the angiographer (58). Similarly, venous sampling on either side of the neck and sampling after tissue massage may aid in gland localization in the operating room (59). The assay has also been used to assay tissue, as an alternative to frozen section analysis, to confirm the identification of removed tissue as parathyroid (60). Finally, the intraoperative PTH assay has been used to monitor parathyroid function during thyroidectomy, to identify patients at risk of clinically significant hypocalcemia, and to potentially facilitate use of parathyroid autotransplantation (61). The importance of accurate labeling and reporting of results, with regard to the anatomic location of the sample, is underscored when the rapid PTH test is used during localization procedures.

Implementation of Rapid Intraoperative PTH Tests in Clinical Laboratories
A very interesting statistic revealed by the recent College of American Pathologists survey (38) was the fact that 71% of laboratories performing rapid PTH testing are carrying out testing in the central laboratory, with only 23% performing testing in the operating suite. Six percent perform testing in a satellite laboratory. The first assay developed for rapid intraoperative testing was designed with instrumentation on a portable cart to allow transport to the point of care. Subsequent applications have moved to standard laboratory immunoassay instrumentation, which are not as easily transportable. There are advantages and disadvantages to performing testing in the operating suite (3). The most obvious advantage is turn-around time, although less obvious advantages include the ability to interact with the surgical team, increased visibility for the laboratory, and more involvement in patient care for the technologists. In hospitals with a pneumatic tube system or a laboratory in close proximity to the surgical site, transport of samples through the tube or by courier is an alternative, albeit more time-consuming, option. In a situation where multiple surgeries are performed consecutively in 1 day, using a surgical approach minimizing operative times, the additional transport time may be unacceptable. Alternatively, if surgeries are performed in multiple locations (i.e., inpatient and outpatient surgical suites) simultaneously, central laboratory testing allows for that accommodation.

For the laboratory, the important issues that help determine the optimum assay system and location for performing rapid intraoperative testing are staffing and costs. An on-site approach may necessitate the acquisition of additional instrumentation and additional operator training, whereas testing in a central laboratory is often performed on an analyzer already in use. Operating room testing requires a dedicated technologist and >1 h of set-up time for calibration and instrument checks. Use of an automated system may require less frequent calibrations, and technologists in the central laboratory may be able to perform other laboratory testing concurrently. Costs for central laboratory testing have been estimated to be lower (12), with one study estimating $760 vs $360 for operating room and central laboratory testing, respectively (34). Higher reagent costs may reflect the dedicated nature of the instrumentation and individual patient-use packaging. As in all testing, volume is an important consideration. Sixty-eight percent of sites perform testing five times or less a month (38). There are several factors, therefore, for clinical laboratories to consider, to decide which test format is the most appropriate for their institutions.

Other Rapid Intraoperative Immunoassays
The positive impact that the rapid intraoperative PTH test has had on both patient outcomes and on cost savings has led to great interest in developing other rapid intraoperative immunoassays. A list of other rapid intraoperative hormone immunoassays is shown in Table 2. All of these hormones share certain characteristics that make them suitable for use as a rapid intraoperative test, i.e., short analyte half-life and/or large analyte concentration gradients, rapid analysis time, and positive clinical utility.

Similar to PTH, the half-life of the hormone being measured by these other rapid assays should be short enough so that any change after resection of the tissue hypersecreting the hormone should be detectable within the time frame of the surgery. Fortunately, most peptide hormones are rapidly catabolized and typically have
Typically, there is a marked increase in cortisol in the left (u) and right (h) adrenal samples compared with a peripheral (antecubital vein; ■) sample indicates that the samples were properly collected and are suitable for other adrenal hormone measurements for tumor localization studies.

The analysis time of rapid intraoperative assays also must be short enough so that the test results can be used during the time frame of the procedure. Parathyroidectomy surgery is relatively short compared with other surgical procedures; therefore, PTH analysis time assay is critical if the assay is going to have a beneficial impact on patient outcome and in possibly reducing the overall procedure time. For more complex and lengthy surgical procedures, the speed of analysis may not be as critical. For example, the first intraoperative test for gastrin took ~1 h to perform (20). Although a more rapid gastrin test has been reported recently (66), the greater length of time needed for resecting gastrinomas makes it less important to have as short of an analysis time. Most of the other rapid intraoperative assays typically take 30 min or less to perform (Table 2). The two most common variables that have been modified to improve the speed of intraoperative tests are the sample size and the reaction temperature. In the case of two-site sandwich type immunoassays, the two antibodies are in molar excess; therefore, the rate of reaction follows pseudo-first-order kinetics with re-

Table 2. Characteristics of other rapid intraoperative immunoassays.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Half-life, min</th>
<th>Assay types</th>
<th>Assay time, min</th>
<th>Uses</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH</td>
<td>15</td>
<td>ICMA; a IRMA</td>
<td>15</td>
<td>DX; TX</td>
<td>(19, 62, 71)</td>
</tr>
<tr>
<td>Cortisol</td>
<td>&gt;90</td>
<td>Fluorescence polarization</td>
<td>8</td>
<td>DX</td>
<td>(16)</td>
</tr>
<tr>
<td>Gastrin</td>
<td>10</td>
<td>IRMA</td>
<td>30–60</td>
<td>TX</td>
<td>(20, 66)</td>
</tr>
<tr>
<td>GH</td>
<td>15</td>
<td>IRMA</td>
<td>15</td>
<td>TX</td>
<td>(18, 63)</td>
</tr>
<tr>
<td>Insulin</td>
<td>9</td>
<td>ICMA; IRMA</td>
<td>8–14</td>
<td>DX; TX</td>
<td>(17, 67, 69)</td>
</tr>
<tr>
<td>Testosterone</td>
<td>&gt;60</td>
<td>ICMA</td>
<td>18</td>
<td>DX</td>
<td>(64)</td>
</tr>
</tbody>
</table>

a ICMA, immunochemiluminometric assay; DX, diagnostic localization; TX, intraoperative monitoring during surgical resection.
pect to the concentration of the analyte. Simply increasing the sample volume, and hence the concentration of the analyte being measured in the reaction, will accelerate the rate of binding of the antibodies to the analyte. Like the rapid PTH tests, some of these other rapid intraoperative tests (Table 2) are also performed at 37 °C or higher, which accelerates the rate of reaction as long as the reaction temperature is kept below the point of denaturation for the antibody or the analyte. Finally, some of the rapid assays use the microparticle assay format (17), which accelerates the rate of the reaction by decreasing diffusion distances and by increasing the effective concentration of the capture antibodies.

**Clinical utility of rapid intraoperative insulin assay**

Even if it is possible to quickly perform an assay on a hormone with a short half-life or large concentration gradient, such a test must also have some clinical utility to be valuable. Except for PTH, most of the other rapid intraoperative tests that are commercially available at present have been described in only a few reports, and their clinical utility has not been fully evaluated. Nevertheless, the initial studies of these other rapid intraoperative tests have shown promising results. Next to PTH, rapid tests for insulin have been the most frequently studied (17, 67–69). Several reports have examined the utility of a rapid intraoperative insulin test for the preoperative localization of pancreatic insulinomas in patients with type 1 multiple endocrine neoplasia. Unlike sporadic insulinomas, which are usually solitary, patients with type 1 multiple endocrine neoplasia often have multiple pancreatic adenomas, but typically only a few of these lesions are actively secreting insulin (70). To intraoperatively identify active insulinomas, a procedure has been described (17) in which insulin is rapidly measured from fine-needle aspirates of adenomas collected under ultrasound guidance (17). Information from the rapid insulin test can then be used to plan a surgical approach that would involve removing a minimum amount of pancreatic tissue, thus possibly avoiding the need for a complete pancreatectomy, which is a potentially morbid and complicated surgical procedure. Intraoperative use of a rapid insulin assay for monitoring decreases in serum insulin after the resection of insulinomas has also been described (67–69). Despite the improved ability to preoperatively locate insulinomas by use of various noninvasive techniques, up to 10% of adenomas are missed during surgery either because of their small size or because of the presence of multiple adenomas (67). Serum insulin concentrations, however, rapidly decrease after the surgical removal of insulinomas (67–69); it thus can be used as a marker of surgical success. In a series of 51 patients (68), a rapid intraoperative insulin test was shown to have an overall sensitivity of 84% and a specificity of 100% for predicting surgical cure when insulin concentrations in patients with increased baseline values decreased to within the reference interval after 20 min postresection. In another study, serum insulin and the ratio of serum insulin to glucose were used to predict surgical success (67).

**Clinical utility of rapid intraoperative assay for adrenocorticotropic hormone**

Several rapid intraoperative tests for adrenocorticotropic hormone (ACTH) have also been described for use during transphenoidal surgery to localize and remove pituitary adenomas (19, 62, 71, 72). The majority of patients with Cushing syndrome have ACTH-secreting adenomas in the pituitary, which can be treated by hemi-resection of the involved part of the pituitary or by enucleation of the adenoma. Because pituitary ACTH lesions are often not apparent radiographically, such lesions are often localized by catheterizing the vessels draining the pituitary and measuring ACTH (73). As shown in Fig. 3, a pituitary adenoma can often be revealed by the marked increase in ACTH concentrations from such samples after corticotropin-releasing hormone (CRH) stimulation. If a rapid ACTH test is used during the catheterization procedure, those patients with increased ACTH in the jugular venous samples do not need to undergo catheterization of the more proximal petrosal veins, which is a more complex and risky procedure (73). A rapid intraoperative ACTH test has also been used for localizing ectopic ACTH-secreting tumors in the lung (73). A rapid test for serum ACTH to monitor surgical success has also been described (19), but serum ACTH values immediately after resection were highly variable, and it was not until at least 2 h post-

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**Fig. 3.** Rapid intraoperative immunoassay for ACTH for testing for jugular vein catheterization samples. Right (■) and left (□) jugular vein samples were collected by catheterization after a CRH stimulation test and were analyzed by a rapid ACTH assay. ACTH results for a peripheral (antecubital vein) sample are also shown ( ). The much greater increase in ACTH after CRH stimulation from the left jugular vein samples is consistent with the presence of an ACTH adenoma in the left side of the pituitary, which was confirmed during surgery.
resection that the assay predicted surgical cure (19). Several other studies have also shown a variable and delayed rate of ACTH decay after complete tumor removal (71, 74), which has been proposed to be attributable to the release of ACTH from healthy pituitary tissue as a result of mechanical manipulation of the gland during surgery (19).

**CLINICAL UTILITY OF RAPID INTRAOPERATIVE GROWTH HORMONE ASSAY**

Similar to ACTH, rapid assays for growth hormone (GH) to assess the removal of GH-secreting pituitary tumors have also been described (18, 62). The surgical success rate of removing GH-secreting pituitary tumors by transphenoidal surgery has been reported to range between 53% and 80% (62); thus a large percentage of patients need to undergo a second procedure. One center routinely uses a rapid intraoperative GH assay during pituitary surgery for acromegaly and has reported on its clinical utility in 78 patients. Using a criterion of a 50% decrease in serum GH after 20 min postresection, they predicted that 51 patients were cured, which was confirmed in 50 of the patients by their postoperative course. The rapid intraoperative GH test also correctly identified all 27 patients by their postoperative course. The rapid intraoperative measurement of GH was estimated to be 88%, although two patients had baseline values for gastrin that were within the reference interval, thus precluding the use of the rapid gastrin test in these patients. All seven patients who were not surgically cured did not show a significant decrease in serum gastrin during surgery. Because of the relatively low success rate in the surgical treatment of these patients, it has been proposed that the intraoperative measurement of gastrin may be useful for identifying those patients who may benefit by more extensive surgery, such as duodenopancreatectomy (20). Because of the mortality and morbidity associated with this procedure, there has been reluctance in using it without first establishing whether a patient is likely to benefit, which may now be possible to do during the initial surgery with the rapid intraoperative gastrin test.

**CLINICAL UTILITY OF RAPID INTRAOPERATIVE TESTS FOR STEROID HORMONES**

As already described for cortisol (Fig. 2), the concentration gradient for an analyte may also be used to localize endocrine hormone-secreting tumors. Such a strategy has been used for testosterone, in a case report of a woman with an ovarian virilizing tumor (64). Preoperative radiologic tests did not reveal the location of the tumor, but blood collected from the right and left ovaries during surgery was used to rapidly measure testosterone, which led to the localization of a Leydig cell tumor. Similar to cortisol, testosterone has a relatively long half-life (Table 2), but there was a >20-fold difference between the testosterone concentrations secreted by the healthy ovary and those secreted by the affected ovary, making it relatively easy to intraoperatively localize the tumor.

**CLINICAL UTILITY OF RAPID INTRAOPERATIVE GASTRIN ASSAY**

Despite the recent improvement in the preoperative localizations of gastrinomas, the surgical resection of such tumors is still difficult, and only a minority of patients are cured by surgery (75). Unlike many of the other endocrine hormone-secreting tumors, gastrinomas are often diffuse and can be present in a relatively wide tissue distribution, such as the stomach, pancreas, duodenum, and periaortic lymph nodes (75). In one study involving 20 patients with Zollinger–Ellison syndrome and gastrinomas (20), a rapid intraoperative test for gastrin was evaluated as a measure for monitoring the resection of gastrinomas. The intraoperative gastrin test was considered to be indicative of a surgical cure if the serum gastrin decreased to within the reference interval 20 min postresection and/or if the patient had a normal secretin stimulation test for gastrin. The sensitivity for predicting surgical cure based on the intraoperative gastrin test was estimated to be 88%, and the use of the rapid gastrin test in these patients. All seven patients who were not surgically cured did not show a significant decrease in serum gastrin during surgery. Because of the relatively low success rate in the surgical treatment of these patients, it has been proposed that the intraoperative measurement of gastrin may be useful for identifying those patients who may benefit by more extensive surgery, such as duodenopancreatectomy (20). Because of the mortality and morbidity associated with this procedure, there has been reluctance in using it without first establishing whether a patient is likely to benefit, which may now be possible to do during the initial surgery with the rapid intraoperative gastrin test.

**Summary**

It has been 25 years since the original concept of monitoring cAMP as an intraoperative marker for parathyroidectomy surgery. The early efforts of Nussbaum et al. (6) and Irvin and Deserio (7) established PTH as the ideal marker for monitoring parathyroidectomy surgery and laid the foundation for the eventual development of automated commercial assays for the rapid intraoperative PTH test. The rapid intraoperative PTH test has been shown to meet all of the criteria first described by Burkitt (2) for an intraoperative test: it is safe, reproducible, simple to interpret, rapid, and cost-effective. In addition to the positive impact that the rapid intraoperative PTH test has had on the surgical treatment of hyperparathyroidism, it is also relevant for laboratorians because it represents a new opportunity for future laboratory test innovation. The clinical laboratory has experienced steady improvements in the turnaround time for most assays, but this effort has largely been driven by improvements in laboratory efficiency. Rapid intraoperative immunoassays represent a new paradigm for how the delivery of rapid results can provide information critical to deciding the optimum surgical treatment of patients. Like other point-of-care tests, it also provides an avenue for laboratorians to get more directly involved in patient care. Finally, the emergence of other rapid intraoperative immunoassays indicates that this test format is likely to provide future
opportunities to improve patient care by advances in clinical laboratory testing.

References

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