Permanent hypoparathyroidism is a rare but detrimental complication of thyroid and parathyroid surgery, and often requires lifelong high-dose calcium and vitamin D supplements—putting patients at risk for metabolic derangements and nephrolithiasis. But replacement of parathyroid hormone (PTH) using exogenous synthetic forms is not feasible. In patients for whom the normal parathyroid glands are devascularized during surgery, autotransplantation of parathyroid tissue is successful in providing a source of endogenous PTH and improving calcium homeostasis.1

Cryopreservation permits parathyroid tissue storage for autotransplantation without compromising cellular integrity or function.

This article communicates the cryopreservation technique used at Cleveland Clinic since 2002. Although clinical indications for cryopreservation exist, it is estimated that this resource is available to <30% of endocrine surgeons across the United States.2 To our knowledge, infrequent published literature exists defining a practically illustrated technique for cryopreservation. To facilitate efforts by surgeons to establish cryopreservation for autologous parathyroid transplant, we describe our technique and relate the patterns of specimen storage and usage at our institution during the last 10 years.

OVERVIEW OF CRYOPRESERVATION TECHNIQUE

Our technique is based on the programmable freezing process as reported by Walgenbach and colleagues.3 The cryopreservation process begins with the removal of parathyroid tissue from patients undergoing parathyroid or thyroid surgery. Fragments of parathyroid tissue are minced into small pieces and suspended in sterile saline in the operating room. These tissues are then transported on ice to the laboratory. Finally, the specimens are cryopreserved in freshly prepared freezing media and stored in liquid nitrogen. The tissues can be thawed at a later date when required for autotransplantation.

The details of tissue cryopreservation can differ between hospitals, depending on the department performing the task. We will outline the cryopreservation technique currently in place in our Andrology Laboratory and Sperm Bank, a center of expertise for long-term tissue storage.

METHODS

Indications for parathyroid cryopreservation

Initial neck operations that have a high risk for permanent postoperative hypoparathyroidism

These include subtotal (3.5-gland), near-total, or total parathyroidectomy. Patients with multigland parathyroid hyperplasia, especially those with familial or hereditary forms of primary hyperparathyroidism also fall into this category, as do those with secondary and tertiary hyperparathyroidism in the setting of renal disease.4

Reoperative neck procedures

Parathyroid reoperations performed for persistent or recurrent hyperparathyroidism. Successful resolution of hypercalcemia is estimated to be 80% to 90%, but the patient’s postoperative course can be complicated by the new risk of permanent hypoparathyroidism, reported in the literature to be as high as 18%.5

Patient-specific scenarios

Any operative scenario for thyroid or parathyroid disease where the surgeon identifies potential benefit to the patient in having stored parathyroid tissue can be an appropriate indication.

Parathyroid cryopreservation technique: illustrated step-by-step

Step 1

The following is performed intraoperatively using sterile technique. After a subtotal parathyroidectomy (Fig. 1A), the excised tissue is dissected with a #10-blade scalpel into 30 to 40 pieces of 2 × 2 mm within ice-chilled saline (Fig. 1B), making for more uniformly frozen and viable samples. Next, the small pieces are
drawn up into 1-mL tuberculin syringes (Fig. 1C), and each syringe is capped and labeled with appropriate patient identifiers. We aim to do this within 15 minutes of excision, with immediate transport to the laboratory, which is located within 5 to 10 minutes walking distance. The parathyroid tissue is never placed directly onto an ice cube or crushed ice, where it would adhere and be more difficult to process. It remains in the syringes with saline, placed into biohazard bags (sterile or nonsterile) and surrounded by crushed ice, or in the cryovials on ice, as Figure 1 illustrates.

The patient’s blood (approximately 5 to 10 mL) is collected and labeled in tubes that contain no additives (“red-top tubes”), and will be used to prepare the freezing media. Although fetal calf serum can be used for this purpose, our preference is to use autologous serum isolated from the patient’s blood. The blood can be drawn conveniently from the same venipuncture site used for parathyroid hormone measurement.

Importantly, any transported and cryopreserved parathyroid tissue is first histologically confirmed by frozen section to be hypercellular parathyroid tissue, so that...
inadvertent storage of cancer or nonparathyroid tissue can be avoided. Also important is to have a consistent size and quantity of minced fragments within each tuberculin syringe. The material in one syringe will be stored in one cryovial (see steps 2 to 8) and allocated to the freezer. We aim to place about 15 to 20 small $2 \times 2$ mm fragments into each syringe or cryovial. This is relevant for later decisions with regard to how many specimen vials to unthaw. An accepted convention is that parathyroid autotransplantation usually implants an amount equal to 2 normal parathyroid glands (normal gland $\sim$15 to 30 mg) or about 15 small $2 \times 2$-mm fragments. This quantity might be larger, depending on patient’s case history, degree of hypocalcemia, prediction of viability of stored
tissue (specimens in storage for 1 to 2 years can have lower viability than those frozen within a few months), or surgeon’s experience. To our knowledge, no precise algorithm exists to ensure survival of implanted parathyroid grafts, but this has been our general approach.

A data sheet with patient identification, diagnosis and histology, type of operation, and native anatomical position of parathyroid tissue is completed and accompanies the specimens to the laboratory.

**Step 2**

Once in the laboratory, patient name and medical record number are verified on the syringes and blood tube. The laboratory aims to keep the total processing time to approximately 1 to 2 hours. We try to schedule parathyroid operations with a high likelihood of needing cryopreservation early in the day and alert the laboratory in time. Step 1 is always performed by the operating surgeon; steps 3 through 8 are performed by laboratory technicians familiar with human tissue storage techniques or tissue culture. Syringes containing parathyroid fragments should remain positioned upright in ice (with cap side down) until processed (Fig. 2), allowing the fragments to settle at the bottom of the syringe. Alternatively, the specimens can be placed in the refrigerator at 2°C to 8°C, if processing is delayed. Labels and a cryopreservation data sheet for recording specimen and process details are prepared.

**Step 3**

Next, all necessary components for cryopreservation are collected under a sterile hood (Fig. 3). These include 2-mL cryovials, 2-mL pipettes, pipet holder, cryocanes, and a rack with a 15-mL tube to hold serum. A cryomarker is used to label a 2-mL cryovial (patient name, medical record number, date, and freeze number) for each tuberculin syringe received from the operating room.

**Step 4**

Now we turn our attention to serum isolation for the freezing media. The blood specimen collected intraoperatively is centrifuged at 1,600 rpm for 5 minutes to separate the serum from the red blood cells. If a serum clot still persists after centrifugation, it should be broken up using sterile application sticks and the sample recentrifuged. The serum (supernatant) is transferred into a sterile 15-mL centrifuge tube and placed on ice, leaving the red blood cells (pellet) behind (Fig. 4). The isolated serum should be enough to cryopreserve up to 9 syringes of tissue from the same patient. Approximately 10% to 30% of the final freezing media is the serum isolated in this step.
Step 5
The freezing media consists of Roswell Park Memorial Institute (RPMI) 1640 solution (Sigma-Aldrich), dimethyl sulfoxide (a cytoplasmic stabilizer), and patient serum (isolated in step 4).

In our laboratory, the freezing media is prepared as follows:
1. 8 mL RPMI-1640 is pipetted into a sterile centrifuge tube and placed on ice;
2. 1 mL autologous serum is added to the same tube and mixed gently;
3. 1 mL dimethyl sulfoxide is added to the tube slowly, mixed gently, and placed on ice; and
4. Any remaining patient serum is transferred to a labeled cryovial and stored in a −50°C freezer.

Step 6
Now all of the components of the cryopreservation process are ready and should be placed under the sterile hood (Fig. 5).

Step 7
The volume in syringe and tissue site of origin are noted on the cryopreservation worksheet for each specimen. Using sterile technique, approximately 10 parathyroid tissue fragments are transferred from each tuberculin syringe to the correspondingly labeled cryovial (Fig. 6A). Subsequently, the freezing media is taken off ice and 1 mL is added dropwise to each cryovial containing parathyroid tissue (Fig. 6B). The maximum total volume per vial should be kept at <1.8 mL.

Step 8
The parathyroid fragments are ready for the freezing process. The goal of this process is to preserve cellular integrity and function through the temperature change. The vials are cooled slowly before being transferred into a liquid nitrogen freezer for long-term storage.

In our laboratory, the cryovials are loaded on the labeled cryocanes with sleeves (Fig. 7A) and are then inserted into a −20°C freezer (Fig. 7B). After 15 minutes, the cryocanes are transferred to a −50°C freezer for an additional
15 minutes. The time of insertion into each freezer is noted on the cryopreservation worksheet. The cryocanes are then transferred to the vapor-phase nitrogen storage tank for two 24-hour cycles (Fig. 7C).

Subsequently, the canes are submerged in the liquid-phase nitrogen storage tank (Fig. 7D) for long-term storage at any of these recommended temperatures: −170°C, −180°C, −190°C, or −196°C.6,8-10

Parathyroid autotransplantation technique: illustrated step-by-step

Step 1
The day before autotransplantation surgery, the cryopreservation laboratory is notified—allowing time to determine and thaw the quantity of parathyroid tissue necessary. The amount of tissue to thaw is specified according to the considerations mentioned here.

Step 2
The thawing process needs to be done gradually. First, prepare a water bath at 37°C. Measure 5 mL RPMI-1640 for each cryovial that will be thawed and transfer it into a centrifuge tube. Also, add Serum Substitute Supplement (SSS; Irvine Scientific) to the centrifuge tube, to a proportion of 10% of the total mixture. The centrifuge tube is then placed in the water bath.

Step 3
Remove the necessary number of cryovials from the liquid nitrogen freezer using cryogloves. Using universal precautions (gloves and goggles), each cryovial is submerged in the 37°C water bath until thawed.

Step 4
Warmed RPMI-SSS mixture from step 1 is added to the thawed tissue fragments, diluting the dimethyl sulfoxide, which can damage cells at room temperature. First, 0.5 mL warm RPMI-1640 media is slowly added to the tissue cryovial and is allowed to sit at room temperature for 5 minutes. Second, 1 mL freezing media is removed from the tissue vial and replaced with 1 mL from the RPMI-SSS vial. This stepwise replacement of the tissue freezing media with 1 mL RPMI-SSS mixture is repeated twice more, each time allowing the cryovial to sit at room temperature for 5 minutes.

Step 5
Next, label a centrifuge tube for each parathyroid specimen (with the patient name, medical record number, freeze number, and specimen origin). The tissue fragments are resuspended in 1 mL RPMI-1640 and transferred to the new labeled centrifuge tube. Based on surgeon preference, the tissue fragments are delivered to the operating room either at room temperature or on ice.

Step 6
The surgeon transfers the sample to a specimen cup and dilutes with sterile saline (Fig. 8). Reimplantation is performed under local anesthesia on the patient’s nondominant forearm. An approximately 3-cm longitudinal incision is made in the forearm, exposing the brachioradialis muscle. Several small pockets are made in the muscle and 1 to 2 parathyroid fragments are transplanted into each pocket (Figs. 9A, 9B). Care must be taken to minimize bleeding and avoid a hematoma that might interfere with revascularization of the implants. The area overlying the implants is marked with surgical clips or nonabsorbable suture, and the incision is closed (Figs. 9C, 9D). Postoperatively, the patient’s serum calcium and PTH levels are monitored weekly at first, then every 1 to 2 months on a case-by-case basis, and eventually every 3 to 6 months. Parathyroid function can take several weeks to manifest as detectable PTH levels on a blood test. Because the autotransplantation site is below the elbow, drawing a blood sample from it would be impractical. The surgeon performs a blood test on the patient’s arm. A chest x-ray may also be obtained to check for any metastases.
each antecubital vein can be a useful way to monitor appearance of PTH secretion (PTH values should be higher from the forearm with the autograft).

RESULTS
In the decade since 2002, our endocrine surgeons have performed >2,000 parathyroid operations, generating 630 cryopreserved specimens (approximately 30%) and 9 parathyroid transplantation procedures (approximately 1.5% of cryopreserved tissue and <1% of all patients). The 31% cryopreservation rate is consistent with our philosophy of using this resource in all patients with multigland parathyroid hyperplasia and those undergoing reoperative parathyroid surgery. Of the 9 patients requiring parathyroid reimplantation 3 to 22 months after the original parathyroid surgery, all achieved correction of hypocalcemia; improvement in detectable PTH levels; and, in the majority of patients, complete symptomatic relief (Table 1). All were able to discontinue taking calcitriol except the patients receiving ongoing hemodialysis, for whom this is part of their dialysis protocol.

DISCUSSION
First described in 1976, parathyroid tissue cryopreservation with autotransplantation today is performed primarily in patients undergoing surgery for multigland hyperplasia or recurrent hyperparathyroidism, and its routine use is advocated in the management of complex parathyroid disease. It is also valuable in centers with organ transplantation treating patients with secondary hyperparathyroidism and tertiary hyperparathyroidism. Although it might be reasonable to expect this resource to be available in centers that treat a substantial number of patients with thyroid and parathyroid diseases, this is not the current situation. Providing a guide for parathyroid cryopreservation will enable individuals to establish this resource and improve patient postoperative outcomes.

At the Cleveland Clinic, we have maintained continuous parathyroid tissue banking since 2002. We use autologous serum because it is conveniently obtained and minimizes any theoretical adverse reactions from fetal calf serum, a traditional option in experimental tissue cultures. We have not had any contamination-related complications during this decade; parathyroid fragments are not screened for contaminants before freezing or on dethawing. Parathyroid hormone release is not measured on dethawing. The amount of parathyroid tissue to reimplant is gauged according to surgeon experience and traditional conventions advising at least an amount equal to 2 normal glands. The surgeon can opt for more, given a patient’s case history or if using material stored longer than a year, although these estimations are purely empirical.
Studies have shown that cellular viability and function are negatively affected with prolonged cryopreservation times, with the maximum period of viability within the first 2 years at −80°C. Parathyroid cryopreservation, specimen preparation, and storage fees all have identified billing codes and receive reimbursement (Table 2). Patients are not billed for specimen storage beyond 2 years. However, we have found that the space and cost requirements of maintaining tissue are minimal and occasional need for cryopreserved tissue can manifest itself beyond the traditional storage period of 6 months. In addition, the limited source of autologous parathyroid tissue has led to the use of allogeneic transplants, which are covered under different billing codes.

### Table 1. Nine Patients with Parathyroid Transplantation during a Decade

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/sex</th>
<th>Diagnosis</th>
<th>Nadir PTH (maximum Ca)</th>
<th>Nadir PTH</th>
<th>Resulting symptoms</th>
<th>Months between operations</th>
<th>1 Month</th>
<th>Most recent</th>
<th>No. of months</th>
<th>Summary</th>
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<tbody>
<tr>
<td>76/F</td>
<td>PHPT</td>
<td>&lt;4 (11)</td>
<td>7.5</td>
<td>Persistent fatigue</td>
<td>22</td>
<td>4</td>
<td>9.5</td>
<td>59</td>
<td>8.4</td>
<td>20</td>
</tr>
<tr>
<td>25/M</td>
<td>PHPT</td>
<td>&lt;4</td>
<td>4.8</td>
<td>Tingling, decreased quality of life</td>
<td>3</td>
<td>4</td>
<td>7.9</td>
<td>6</td>
<td>9.3</td>
<td>10</td>
</tr>
<tr>
<td>49/M</td>
<td>SHPT</td>
<td>&lt;4</td>
<td>5</td>
<td>Muscle cramps, interferes with work</td>
<td>14</td>
<td>4</td>
<td>6.5</td>
<td>5</td>
<td>9.3</td>
<td>36</td>
</tr>
<tr>
<td>37/M</td>
<td>THPT</td>
<td>5 (12)</td>
<td>3.2</td>
<td>Arrhythmia, hospitalized for tetany</td>
<td>15</td>
<td>26</td>
<td>9</td>
<td>5</td>
<td>9.5</td>
<td>24</td>
</tr>
<tr>
<td>57/F</td>
<td>PHPT</td>
<td>&lt;4 (9)</td>
<td>8</td>
<td>Tingling, decreased quality of life</td>
<td>12</td>
<td>21</td>
<td>8.9</td>
<td>29</td>
<td>9.6</td>
<td>60</td>
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<tr>
<td>65/F</td>
<td>PHPT</td>
<td>&lt;4 (7)</td>
<td>6.1</td>
<td>Anxiety, tingling, fatigue</td>
<td>7</td>
<td>7</td>
<td>8.4</td>
<td>11</td>
<td>9.2</td>
<td>12</td>
</tr>
<tr>
<td>67/M</td>
<td>THPT</td>
<td>&lt;4</td>
<td>4.7</td>
<td>Tingling, exacerbation of chronic CHF</td>
<td>1</td>
<td>4</td>
<td>10</td>
<td>4</td>
<td>8.9</td>
<td>5</td>
</tr>
<tr>
<td>52/F</td>
<td>PHPT</td>
<td>&lt;4</td>
<td>7.7</td>
<td>Fatigue, tingling</td>
<td>3</td>
<td>5</td>
<td>8.8</td>
<td>6</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>21/F</td>
<td>PHPT</td>
<td>&lt;4</td>
<td>7.5</td>
<td>Tingling, muscle cramps</td>
<td>3</td>
<td>6</td>
<td>9.3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Reference ranges: PTH (10–60 pg/mL), Ca (8.5–10.5 mg/dL).

* Number of months between parathyroidectomy and autologous parathyroid implantation.

1 Number of months of follow-up after autologous parathyroid implantation.

2 Patient received allogeneic parathyroid transplant.

3 Additional patient follow-up will likely result in decreased Ca dose.

Ca, calcium; CHF, congestive heart failure; F, female; M, male; PHPT, primary hyperparathyroidism; PTH, parathyroid hormone; SHPT, secondary hyperparathyroidism; THPT, tertiary hyperparathyroidism.

### Table 2. Billing and Reimbursement Information for Parathyroid Cryopreservation

<table>
<thead>
<tr>
<th>Procedure</th>
<th>CPT code or TMID code</th>
<th>Professional (P) or technical (T) fee, $</th>
<th>Medicare reimbursement, $</th>
<th>Commercial payors reimbursement, $</th>
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<td>Parathyroid cryopreservation (operating room portion)</td>
<td>60669</td>
<td>574 (P)*</td>
<td>82</td>
<td>195–401†</td>
</tr>
<tr>
<td>Parathyroid cryopreservation (laboratory processing and storage portion)</td>
<td>88399099</td>
<td>1,000 (T)†</td>
<td>None</td>
<td>300–500†</td>
</tr>
</tbody>
</table>

* There is no technical fee for the procedure in the operating room.

† Some commercial payors bundle this procedure with the main parathyroidectomy procedure (CPT 60500 series) and do not issue separate reimbursement. Those payors who do reimburse usually require that the hospital’s billing specialist sends additional information, such as the operative report, before payment.

‡ This fee covers processing by the laboratory and indefinite storage time. The same fee is also applied for the process of parathyroid thawing for reimplantation. TMID, transaction master charge ID.
tissue and lack of other options for parathyroid replacement motivate us to maintain the tissue bank beyond the 2-year window. Although such tissue can be later designated for research purposes, this has not been the case at our institution. Ultimately, what service or laboratory performs the cryopreservation, for how long, and for what purpose can be determined according to local and institutional needs.

As stated earlier, in the decade since establishing cryopreservation, 9 parathyroid transplantations have been performed at our institution. By definition, all have been from patients with multigland hyperplasia and all had hypercellular histology. Some experts have reported intraoperative PTH to predict hypocalcemia after parathyroid surgery. In our experience, that tool is not reliable enough to ensure that cryopreservation would be an unnecessary precaution. Parathyroid reimplantation is offered to the patient at the earliest time point when deemed medically reasonable. Patients in our series who had reimplantation 1 to 2 years after initial surgery did so electively later and because of the intermittent nature and severity of their symptoms. An interesting but difficult to explain observation is that a few patients have lower than expected measurable PTH levels after reimplantation, but achieve normal or improved calcium balance. In these patients, bilateral antecubital vein sampling to detect differential PTH levels (higher on side of autotransplant) was not particularly helpful.

An interesting case included a 37-year-old male patient with a history of subtotal parathyroidectomy for tertiary hyperparathyroidism who was evaluated after hospitalization for hypocalcemia-induced tetany and cardiac arrhythmias. Unfortunately, autologous parathyroid tissue cryopreservation was not performed at the referring institution. To correct the profound hypocalcemic symptoms, an allogeneic parathyroid transplantation was performed. During the past 2 years, the patient has had complete symptom resolution and maintains a normal calcium level without high-dose exogenous calcium supplements. Although a rare application, allogeneic parathyroid transplantation was critical to this patient, highlighting another reason to maintain a parathyroid cryopreservation bank.

Cryopreservation of human parathyroid tissue can occur with a simplified technique that offers durable cure to a minority of patients who would otherwise have limited improvement of permanent hypocalcemia. The technique reported here is streamlined, provides useful reference to earlier key literature, is easy to adapt in a typical hospital-based setting, and achieves viable parathyroid tissue storage with successful reimplantation rates.

Author Contributions
Drafting of manuscript: Agarwal, Waghray, Gupta, Sharma, Milas
Critical revision: Agarwal, Waghray, Gupta, Sharma, Milas

REFERENCES