fficiency. However, serum PTH was not an accurate predictor of post-thyroidectomy hypocalcemia in patients with vitamin D deficiency.

Vitamin D deficiency is associated with increased serum PTH. Therefore the decline in serum PTH to an absolute cutoff value may take longer in these patients. We measured serum PTH at 8–10 h postthyroidectomy. Further studies should assess the value of PTH measured at a later time point in predicting postthyroidectomy hypocalcemia in vitamin D–deficient patients. A limitation of this pilot study was the small number of patients with vitamin D deficiency, which may have had an effect on the area under the ROC curve in this group.

Absolute concentrations of PTH are commonly used to predict postthyroidectomy hypocalcemia. Our results confirm that a serum PTH concentration measured 8–10 h after total thyroidectomy predicts the development of hypocalcemia, but only in the absence of vitamin D deficiency. Further studies are required to investigate whether percentage decrease in serum PTH (rather than absolute concentration) is a better predictor of postthyroidectomy hypocalcemia in vitamin D–deficient patients. A decrease in PTH of 60% coupled with a simultaneous decrease in calcium of 10% that occurs 5–6 h postthyroidectomy has been reported to have a sensitivity and specificity of 100% for predicting hypocalcemia (4).

Serum PTH did not accurately predict the occurrence of hypocalcemia in vitamin D–deficient patients. It is therefore important to recognize this limitation of serum PTH, given the high prevalence of vitamin D deficiency in patients undergoing total thyroidectomy. It has been shown that the Liaison immunoassay is less accurate than liquid chromatography–tandem mass spectrometry for measuring concentrations of 25-hydroxyvitamin D (5). It would therefore be desirable to use liquid chromatography–tandem mass spectrometry for measuring 25-hydroxyvitamin D in future studies. Preoperative measurement of 25-hydroxyvitamin D and vitamin D replacement before surgery, where possible, may increase the reliability of PTH as a predictor of postthyroidectomy hypocalcemia.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

References

Letters to the Editor

condition. The current screening method for SCID is by the T-cell receptor excision circles assay (1), which detects the presence or absence of an excised segment of DNA; however, molecular techniques are not routinely adopted in many newborn-screening laboratories, where an immunoassay would be preferred. As recently reported (2), we developed an immunoassay that quantifies CD3, a component of the T-cell receptor complex (3), to provide an alternative or complementary method to T-cell receptor excision circles testing. Low or absent T cells in the peripheral blood is a common characteristic of T-cell immunodeficiencies and of all but one form of SCID.

We have previously described an immunoassay using the Luminex microsphere technology that displays excellent potential for the detection of CD3 from dry blood spot samples (2). We report the validation of an improved immunoassay with 124 dry blood samples from the Danish Newborn Screening Biobank (4).

In our previous study, we used a pair of commercial antibodies (2); however, because of lot-to-lot variation and availability issues, we felt a more reliable supply of antibody was necessary for a dependable assay. We contracted for Invitrogen to produce antigen-specific avian antibodies to human CD3, because IgY antibodies have been shown to be stable after long-term storage and not to bind to human Fc receptors, rheumatoid factor, or complement. Therefore, nonspecific binding of interfering substances is greatly reduced. IgY antibodies are purified from the yolks of chicken eggs laid by a hen that produces enough eggs in a week to permit the purification of antibody amounts equivalent to the quantity of antibodies in 75–90 mL of mammalian serum (5). Our study demonstrates that the custom IgY antibodies to CD3 have a low background and are highly specific.

When we compared the commercial anti-CD3 detection antibody to the avian anti-CD3 detection antibody, the avian IgY antibody proved to have a more robust performance than the IgG antibody. Less antibody is required per assay, and the IgY antibody has an expanded linear interval (0.2 × 10⁶ to 15 × 10⁶ T cells/mL) compared with that of the commercial antibody (1.0 × 10⁰ to 7.5 × 10⁶ T cells/mL). Other improvements to the protocol over the previously published assay are a reduction in the incubation time of blood eluate with microspheres from 3 h to 2 h and removal of the amplification step and its associated wash steps. This enhanced assay protocol consists of 7 steps instead of the 11 steps in the previous assay. These steps include washes between each incubation, incubation of the eluate with microspheres, incubation with detection antibody, incubation with phycoerythrin-conjugated streptavidin, resuspension of the microspheres, and data collection on the Luminex 100 (Luminex Corporation).

To validate this improved assay, we measured the CD3 concentration of 124 coded neonatal dry blood spots obtained from the Danish Newborn Screening Biobank. These results were sent to Drs. B. Nørgaard-Pedersen and C. Heilmann for decoding. The decoded results revealed that samples from healthy infants had T-cell counts that ranged from 2.14 × 10⁶/mL to ≥16 × 10⁶/mL. Eleven samples were from infants with T cell–related immunodeficiencies. Nine of these samples were from infants diagnosed with SCID; one of these infants had 25% engraftment of maternal T cells. The remaining 2 samples were from infants diagnosed with Omenn syndrome and Wiskott–Aldrich syndrome. The affected infants had lower estimated T-cell counts than controls. These counts ranged from below our limit of detection (0.2 × 10⁶/mL) to 1.07 × 10⁶/mL.

To date, the CD3 immunoassay we developed with these antibodies has been able to correctly identify samples with low or undetectable numbers of T cells from infants with various forms of SCID and other immunodeficiencies. Because of its enhanced performance, ease of use, and ability to correctly identify T-cell deficiency, this assay may be considered as a complement or alternative to other T cell–detection techniques for dry blood spots.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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Disclaimer: Use of trade names is purely for discussion purposes and does not constitute a recommendation of, or statement about, the performance of those materials.
Importance of Cystatin C Assay Standardization

To the Editor:

Cystatin C is an alternative blood biomarker of kidney function (1, 2). Relatively little effort has been devoted to standardization of cystatin C measurement until recently, when the IFCC formed a working group to produce an international certified cystatin C reference material (ERM®-DA471/IFCC), which was released in June 2010. In the current study, we evaluated a new cystatin C particle-enhanced turbidimetric assay (PETIA) (3) (Gentian AS) that is traceable to this certified cystatin C reference material (3) and can be routinely run on a chemistry autoanalyzer, therefore potentially increasing availability and decreasing costs (4).

For assay validation, waste patient serum that had been stored for no more than 7 days at 4 °C (n = 102) was obtained from the Mayo Clinic Central Clinical Laboratory. We also studied banked samples for which cystatin C had been measured by particle-enhanced nephelometric assay (PENIA) (1) (Gentian AS) that is traceable to this certified cystatin C reference material (3) and can be routinely run on a chemistry autoanalyzer, therefore potentially increasing availability and decreasing costs (4).

The PENIA and PETIA methods were compared by Passing–Bablok regression analysis; Bland–Altman plots were used for analyzing the difference between these 2 methods. Results were analyzed with the statistical analysis programs Analyse-it® (version 2.12; Analyse-it Software), JMP® (version 8; SAS Institute, www.sas.com), and Microsoft Excel (version 2003; Microsoft Corporation).

PETIA measurements of cystatin C produced imprecision estimates (CVs) of 0.65% to 1.3% at cystatin C concentrations between 0.98 and 1.88 mg/L. The lower limit of quantification was established at 0.35 mg/L with a CV of <2% by measuring 3 serum samples with signals just above those of the lowest calibrator 5 times each. Linearity studies of serum and plasma samples and the international standard diluted with water yielded percentages of the measured cystatin C concentration with respect to the expected concentration of 90%–105% over a signal interval of 0.33–5.97 mg/L, thereby setting the upper end of the analytical measurement interval at 6 mg/L. The response was linear and parallel when high-signal serum (1.87 mg/L) and plasma (3.00 mg/L) samples and the certified reference material (5.97 mg/L) were diluted up to 8-fold. To further assess performance, we carried out a study in which we mixed a high-signal patient serum sample (3.05 mg/L) and 3 low-signal serum samples (0.99–1.29 mg/L) and demonstrated recovery rates of 100%–105% of the predicted value.

Nonstandard abbreviations: PETIA, particle-enhanced turbidimetric assay; PENIA, particle-enhanced nephelometric assay; eGFR, estimated glomerular filtration rate.

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