Comparison of One-Tier and Two-Tier Newborn Screening Metrics for Congenital Adrenal Hyperplasia

WHAT’S KNOWN ON THIS SUBJECT: The false-positive rate of newborn screening for classic congenital adrenal hyperplasia (CAH) remains high and has not been significantly improved by adjusting 17α-hydroxyprogesterone cutoff values for birth weight and/or gestational age. In response, 4 states have initiated second-tier steroid profile screening.

WHAT THIS STUDY ADDS: Under second-tier screening, the false-positive rate remains high, and classic CAH cases missed by screening (false-negatives) occur more frequently than reported. Physicians are cautioned that a negative screen does not necessarily rule out CAH.

abstract

BACKGROUND: Newborn screening (NBS) for the classic forms of congenital adrenal hyperplasia (CAH) is mandated in all states in the United States. Compared with other NBS disorders, the false-positive rate (FPR) of CAH screening remains high and has not been significantly improved by adjusting 17α-hydroxyprogesterone cutoff values for birth weight and/or gestational age. Minnesota was the first state to initiate, and only 1 of 4 states currently performing, second-tier steroid profiling for CAH. False-negative rates (FNRs) for CAH are not well known.

METHODS: This is a population-based study of all Minnesota infants (769,834) born 1999–2009, grouped by screening protocol (one-tier with repeat screen, January 1999 to May 2004; two-tier with second-tier steroid profiling, June 2004 to December 2009). FPR, FNR, and positive predictive value (PPV) were calculated per infant, rather than per sample, and compared between protocols.

RESULTS: Overall, 15 false-negatives (4 salt-wasting, 11 simple-virilizing) and 45 true-positives were identified from 1999 to 2009. With two-tier screening, FNR was 32%, FPR increased to 0.065%, and PPV decreased to 8%, but these changes were not statistically significant. Second-tier steroid profiling obviated repeat screens of borderline results (355 per year average).

CONCLUSIONS: In comparing the 2 screening protocols, the FPR of CAH NBS remains high, the PPV remains low, and false-negatives occur more frequently than has been reported. Physicians should be cautioned that a negative NBS does not necessarily rule out classic CAH; therefore, any patient for whom there is clinical concern for CAH should receive immediate diagnostic testing. PEDIATRICS 2012;130:e1261–e1268
Congenital adrenal hyperplasia (CAH) due to 21α-hydroxylase (21α-OHase) deficiency is included in all state-sponsored newborn screening (NBS) programs in the United States and in many NBS panels worldwide. Introduced in 1977, first-tier NBS for CAH measures 17α-hydroxyprogesterone (17OHP) from dried blood spots (DBSs) on filter paper. In most US NBS programs, the DBS sample is drawn 24 to 48 hours after birth, and a time-resolved fluoroimmunoassay is used to measure 17OHP.

Compared with nearly all other NBS disorders, the false-positive rate (FPR) of CAH screening is high and the positive predictive value (PPV) is low. Adjusted 17OHP cutoff values by birth weight and/or gestational age have been implemented but have not significantly reduced the FPR. Cases of classic CAH missed by NBS (false-negatives [FNs]) have not been well documented. In June 2004, second-tier steroid profiling by using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to measure 17OHP, androstenedione, and cortisol simultaneously in blood spots was initiated in Minnesota to improve the FPR and PPV. Minnesota was the first state to initiate second-tier testing and is 1 of only 4 states that currently perform routine second-tier testing for CAH (http://nnsis.uthscsa.edu/). Drawing on 5.5 years of data from second-tier testing in Minnesota, this study aimed to measure changes in screening metrics from the previous one-tier NBS. We report on the FPR, FN rate (FNR), and PPV under the previous one-tier NBS. We report results from all 769,834 infants screened in Minnesota from 1999 to 2009. Through a public–private collaboration for the study between the Minnesota Department of Health (MDH) and the largest pediatric endocrinology centers in Minnesota (University of Minnesota, the Mayo Clinic, and Children’s Hospitals of Minnesota), cases of CAH identified and missed by NBS were determined by review of the MDH NBS registry and the medical records of the participating institutions; diagnostic codes for CAH, adrenal insufficiency, salt wasting, clitoromegaly, precocious puberty, and ambiguous genitalia were used to retrieve patients diagnosed with classic CAH during the 11-year study period. Confirmation of classic CAH of those identified and missed by NBS was based on elevated serum 17OHP levels, clinical and biochemical presentation, and in some cases, molecular testing of the CYP21A2 gene by using a common mutation panel or sequencing.

Institutional review boards at all sites approved the study.

### Methods

All first-tier 17OHP CAH screens used whole blood drawn by heel prick or venous puncture and dried on filter paper collected between 24 and 48 hours after birth. For determination of 17OHP, a time-resolved fluoroimmunoassay (DELFIA, PerkinElmer Life and Analytical Sciences, Turku, Finland) that utilizes weight-based cutoff values was used (Table 1).

First-tier 17OHP cutoff values were adjusted during the study period as listed in Tables 1 and 2 according to the assay and antibody used. In September 2005, the 17OHP reference ranges and weight-based cutoff values for preterm infants were revised because PerkinElmer Life and Analytical Sciences reformulated the 17OHP kit by using a polyclonal antibody with greater cross-reactivity with 11-deoxycortisol and 17α-hydroxyprogrenolone. In March 2006, the same 17OHP kit was moved from a manual assay to an automated platform (AutoDELFIA, PerkinElmer Life and Analytical Sciences). In September 2009, the manufacturer again changed the polyclonal antibody used in the kit to improve specificity and reference ranges needed to be adjusted accordingly. Interassay comparability was determined by testing confirmed positives, many comparison plates, and proficiency testing specimens, and cutoffs were adjusted accordingly.

### NBS CAH Classification During One-Tier Protocol

Before June 1, 2004, first-tier 17OHP results were defined as negative, positive, or equivocal (Fig 1). A first-tier negative result was reported out by the Minnesota NBS program as negative. A first-tier positive result was considered presumed positive but was not classified as true-positive (TP) or false-positive (FP) until the diagnosis was confirmed by an endocrinologist. For an equivocal (mildly elevated) first-tier result, a repeat screen was performed on the infant. Repeat screens require recalling the family, informing them of a possible abnormal NBS, and performing another heel prick on the infant. Diagnostic testing was not performed unless the repeat screen was also elevated. If negative, the result was classified as negative. If positive, or if the mild elevation persisted, the result was considered presumed positive but was not classified as TP or FP until the diagnosis was confirmed by an endocrinologist. The confirmed diagnostic result of each infant, not sample, was used as the final reported NBS result.

<table>
<thead>
<tr>
<th>TABLE 1 17OHP Weight-Based Cutoff Values of One-Tier Screening</th>
<th>Weight, g</th>
<th>Positive (ng/mL)</th>
<th>Equivocal (ng/mL)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1500</td>
<td>&gt;150</td>
<td>80–150</td>
<td></td>
</tr>
<tr>
<td>1500–2500</td>
<td>&gt;130</td>
<td>65–130</td>
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<tr>
<td>&gt;2500</td>
<td>&gt;80</td>
<td>50–80</td>
<td></td>
</tr>
</tbody>
</table>

*Repeat screen performed.
TABLE 2 17OHP Weight-Based Cutoff Values for Tier 1 and Reference Ranges for Tier 2 of Two-Tier Screening for CAH

<table>
<thead>
<tr>
<th>Tier 1 of Two-Tier Screening</th>
<th>Tier 2 of Two-Tier Screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>Positive (ng/mL)b</td>
</tr>
<tr>
<td>≤1500</td>
<td>≥80</td>
</tr>
<tr>
<td>1500–2500</td>
<td>≥65</td>
</tr>
<tr>
<td>&gt;2500</td>
<td>≥50</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>17OHP</td>
<td>17OHP+D4A</td>
</tr>
<tr>
<td>Cortisol (ng/mL)</td>
<td>Cortisol (ng/mL)</td>
</tr>
<tr>
<td>&gt;12.5</td>
<td>&gt;3.75</td>
</tr>
<tr>
<td>21DF</td>
<td>2.5</td>
</tr>
<tr>
<td>&gt;12.5</td>
<td>&gt;3.75</td>
</tr>
<tr>
<td>21DF</td>
<td>2.5</td>
</tr>
</tbody>
</table>

First-tier 17OHP cutoff values are higher than second-tier 17OHP cutoff values due to cross reactivity of other steroid hormones during the time-resolved fluoroimmunoassay measurement. 11Df, 11-deoxycortisol; 21DF, 21-deoxycortisol; D4A, androstenedione.


** First-tier sample automatically sent for second-tier screening.

NBS CAH Classification During Two-Tier Protocol

After June 1, 2004, a repeat screen for equivocal 17OHP elevations was no longer performed (Fig 1). A negative first-tier 17-OHP result under the two-tier protocol was classified as negative. A first-tier positive result was flagged, and the original DBS on filter paper card, without involvement of the parents or newborn, was automatically and immediately analyzed at Mayo Clinic’s Biochemical Genetics Laboratory by using a second-tier method of steroid profiling by LC-MS/MS (Table 2) as described in Lacey et al. A negative second-tier result (either normal 17OHP concentration or normal 17OHP + androstenedione divided by cortisol ratio) was classified as negative. If second-tier was positive (typically both the 17OHP level and ratio were elevated), the result was considered presumed positive but was not classified as TP or FP until the diagnosis was confirmed by an endocrinologist.

Initial reference ranges set by Mayo Clinic in June 2004 for second-tier testing were lowered in February 2006 (Table 2). In February 2008, 11-deoxycortisol and 21-deoxycortisol were added as additional analytes and gender-specific reference ranges were incorporated (Table 2).

**TABLE 2**

17OHP Weight-Based Cutoff Values for Tier 1 and Reference Ranges for Tier 2 of Two-Tier Screening for CAH

**FIGURE 1**

The retrospective analysis of the original DBS filter paper of FN cases missed by first-tier screening during the two-tier protocol was performed by using the most current second-tier hormonal profile shown in Table 2.

A NICU 3-screen system for screening infants weighing ≤1800 g was implemented in January 2006 and performed in conjunction with the two-tier protocol, including using the same 170HP weight-based cutoff values. The Minnesota NBS Program recommended that NICU facilities within the state draw 3 newborn screens on all ≤1800 g infants: 1 at 24 to 48 hours, the second at 2 weeks, and the third at 4 weeks or at discharge. If the screen was positive for CAH, it was automatically sent for second-tier screening. The purpose of the NICU 3-screen system was to capture premature infants with initial FN newborn screens for congenital hypothyroidism due to delayed thyrotropin elevations.11,12 Because Minnesota's NBS Program does not differentiate repeat screens, all analytes, not just thyrotropin, were also screened 3 times, including 170HP.

Statistical Methods

We have used the final diagnostic result after confirmation testing by an endocrinologist to classify the NBS result as TP, true-negative (TN), FP, or FN. All rates were calculated per infant's final confirmatory result rather than per sample. Accuracy of screening results was assessed with the FNR = FN/(TP+FN) = (1 – sensitivity), FPR = FP/(TN+FP) = (1 – specificity), PPV of the screen PPV = TP/(TP+FP), and detection rate (average number that needs to be screened to detect 1 case = 1: number screened/TP). For proportions with denominators <30, Wilson 95% confidence limits are given, and for denominators ≥50 the Agresti-Coull 95% confidence limits are given.13 Rates were compared by $\chi^2$ or Fisher's exact test (2-sided) if the $\chi^2$ approximation was unreliable. Computations were performed with the "binom" package in R (R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org/).

RESULTS

Between 1999 and 2009, a total of 769 834 infants were screened. There was no statistically significant difference in CAH incidence between eras defined by screening protocols (Table 3), over the whole 11-year period, incidence was 7.8 cases per 100 000 infants (95% confidence interval: 6–10). With two-tier screening, FNR doubled to 32%, FPR increased to 0.065%, and PPV decreased to 8%, but these changes were not statistically significant (Table 3).

During the one-tier protocol, there was an average of 355 cases of equivocal first-tier screens yearly that required a repeat first-tier screen. During the two-tier protocol, these repeat first-tier screens for equivocal 170HP elevations were no longer performed.

Cases of Classic CAH Missed by NBS

The 15 FN cases (6 boys, 9 girls; 11 simple-virilizing [SV], 4 salt-wasting [SW], 4 under the one-tier protocol, 11 under the two-tier protocol) are presented in Table 4.

All of the boys with CAH known to have been missed by NBS were diagnosed during the first 2.3 to 5.5 years after birth, except case 11, a patient with SW-CAH, who was diagnosed prenatally because of his affected sibling. Because the fetus was a boy, prenatal treatment with dexamethasone was discontinued early in second trimester.

Of the 9 girls missed by NBS, all had ambiguous genitalia at birth except case 9, cases 5, 7, 14, and 15 had ambiguous genitalia at birth but were not identified with CAH until 3 months to 6.5 years due to growth acceleration and bone age advancement.

Of the 11 FNs during the two-tier protocol, 4 were correctly identified as positive on first-tier screen but determined to be negative on the second-tier screen (cases 6, 8, 9, and 11) as, per protocol, the second-tier result overrides the first-tier screen. The remaining 7 did not have second-tier screening as they were identified as negative by first-tier screening.

As part of this study, second-tier testing was performed retrospectively on 5 of these 7 FN specimens (cases 5, 10, 12, 13, and 14). Second-tier correctly identified 1 FN specimen as positive for CAH (case 12) but incorrectly diagnosed the other 4 as negative (5, 10, 13, and 14) as both the 170HP and the ratio should be abnormal to be identified as positive (Table 5). Time between original first-tier testing of these 5 samples and the second-tier testing performed for this study ranged from 9 days to 4 years.

We did not identify any FNs in newborns weighing <1800 g over the entire study period, despite this population being more likely to receive antenatal and/or

| TABLE 3 Screening Results Divided by Screening Protocols (One-Tier Versus Two-Tier) |
|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| Incidence (per 100 000)           | 7.3 (5–11)                        | 8.5 (6–12)                        | .678                               |
| Detection Rate                    | 1.16704                           | 1.17493                           | —                                  |
| TPs                               | 22                                | 23                                | —                                  |
| FPs                               | 209                               | 261                               | —                                  |
| TNs                               | 367251                            | 402053                            | —                                  |
| FNs                               | 4                                 | 11                                | —                                  |
| FNR (%)                           | 15.4 (6–34)                       | 32.4 (19–49)                      | .229                               |
| PPV (%)                           | 9.5 (6–14)                        | 8.1 (5–12)                        | .680                               |
| FPR (%)                           | 0.057 (0.05–0.065)                | 0.065 (0.06–0.07)                 | .170                               |

Values are counts of infants, or rates (95% confidence interval). FNR (%) = 100% – sensitivity, FPR (%) = 100% – specificity.
### TABLE 4 FNs During One-Tier and Two-Tier Screening Periods

<table>
<thead>
<tr>
<th>Case</th>
<th>Gender</th>
<th>Birth Weight, g</th>
<th>First Tier 17OHP (ng/mL)</th>
<th>First Tier Cutoff</th>
<th>Second Tier 17OHP (ng/mL)</th>
<th>Second Tier Cutoff</th>
<th>Second Tier Ratio 17OHP + D4A Cortisol</th>
<th>Second Tier Ratio Cutoff</th>
<th>CAH Subtype</th>
<th>Serum 17OHP at Dx (ng/dL)</th>
<th>Age at Dx (y)</th>
<th>Molecular Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cutoff</td>
<td></td>
<td>Cutoff</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>1</td>
<td>Girl</td>
<td>3487</td>
<td>15.5</td>
<td>50.0–80.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>SW</td>
<td>5514</td>
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<td>g.655C/A&gt;G (Int 2 splice)</td>
</tr>
<tr>
<td>2</td>
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<td>39.3</td>
<td>50.0–80.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>SV</td>
<td>10 500</td>
<td>4.0</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>Boy</td>
<td>3333</td>
<td>44.0</td>
<td>50.0–80.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>SV</td>
<td>9864</td>
<td>5.0</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>Boy</td>
<td>4454</td>
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<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>SV</td>
<td>8650</td>
<td>5.5</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>Girl</td>
<td>3005</td>
<td>20.0</td>
<td>50.0</td>
<td>N/P</td>
<td>N/P</td>
<td>—</td>
<td>—</td>
<td>SV</td>
<td>11 000</td>
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<tr>
<td>6</td>
<td>Girl</td>
<td>3657</td>
<td>52.7</td>
<td>7.5'</td>
<td>&gt;12.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>SW</td>
<td>7210</td>
<td>6.5</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>Girl</td>
<td>3740</td>
<td>18.0</td>
<td>50.0</td>
<td>N/P</td>
<td>N/P</td>
<td>—</td>
<td>—</td>
<td>SV</td>
<td>45 100</td>
<td>4.3</td>
<td>30 kb del</td>
</tr>
<tr>
<td>8</td>
<td>Boy</td>
<td>4111</td>
<td>120.9</td>
<td>65.0</td>
<td>51.2'</td>
<td>&gt;12.5</td>
<td>1.83'</td>
<td>&gt;3.75</td>
<td>SV</td>
<td>30 kb del</td>
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</tr>
<tr>
<td>9</td>
<td>Girl</td>
<td>3177</td>
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<td>24.5'</td>
<td>&gt;10.2</td>
<td>1.40'</td>
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<td>Not done</td>
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</tr>
<tr>
<td>10</td>
<td>Boy</td>
<td>2890</td>
<td>44.0</td>
<td>65.0</td>
<td>N/P</td>
<td>N/P</td>
<td>—</td>
<td>—</td>
<td>SV</td>
<td>5920</td>
<td>2.3</td>
<td>g.655C/A&gt;G (In2 splice)</td>
</tr>
<tr>
<td>11</td>
<td>Boy</td>
<td>3527</td>
<td>204.5</td>
<td>65.0</td>
<td>47.2'</td>
<td>&gt;10.2</td>
<td>0.21'</td>
<td>&gt;2.5</td>
<td>SW</td>
<td>812</td>
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<td>N/P</td>
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<td>N/P</td>
<td>—</td>
<td>—</td>
<td>SV</td>
<td>14 900</td>
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<td>N/P</td>
<td>—</td>
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<td>N/P</td>
<td>—</td>
<td>—</td>
<td>SV</td>
<td>24 300</td>
<td>2.0</td>
<td>g.655C/A&gt;G (In2 splice)</td>
</tr>
</tbody>
</table>

Cases 1 to 4 missed during one-tier screening protocol; cases 5 to 15 missed during two-tier screening protocol. 11DF, 11-deoxycortisol; 21DF, 21-deoxycortisol; D4A, androstenedione; N/P, second-tier testing not performed because first-tier was negative for CAH; PN, prenatal.

- g.655C/A>G (Int 2 splice)
- g.2108C>T (p.R356W)
- g.2494G>A (p.G424S)
- g.1683>T (p.V281L); g.929C>T (p.R149C)
- g.655C/A>G (In2 splice)
- g.994C>T (p.G318X)
- g.994C>T (p.G318X)
- g.994C>T (p.G318X)
- g.994C>T (p.G318X)
- g.994C>T (p.G318X)

- Cases 11 and 12 have older brother with SW-CAH and the same genotype. Parental molecular testing was also performed.
- A cosyntropin stimulation test at 4 weeks of age revealed a baseline and stimulated 17OHOP of 2970 and 15 500 ng/dL, respectively, as well as a stimulated cortisol level <1 µg/dL. An additional patient missed by NBS was not added to the table or calculation because the mother received prenatal therapy with dexamethasone, which normalizes 17-OHP levels in affected infants. None of the mothers received glucocorticoids during pregnancy.
postnatal glucocorticoid therapy and having reduced 17OHP levels during the initial screening.14

DISCUSSION

The goal of second-tier testing has been twofold: to increase the specificity and PPV of CAH screening by identifying those infants with elevated 17OHP values secondary to other causes, thereby reducing the number of FP screens; and to eradicate the need for repeat first-tier screens due to equivocal results. Transient elevated 17OHP values that lead to first-tier FP screening results are due to several confounding factors including the following: birth stress; timing of collection of the newborn screen; physiologically lower enzymatic activity of the 21α-OHase and/or 11β-hydroxylase enzyme in premature newborns; severe illness; poor kidney function; and assay cross-reactivity with other 17-hydroxylated steroids, particularly 17α-hydroxyprogrenolonone, which tends to be elevated in newborns.15–17

With the introduction of second-tier testing, 17OHP levels and the (17OHP + androstenedione)/cortisol ratio were measured by using a more accurate assay (LC-MS/MS). The rationale for second-tier testing was that the biochemical phenotype of classic CAH is characterized not only by elevation of 17OHP, but also of other steroids such as androstenedione, and low cortisol. The analytes 11-deoxycortisol (decreased in 21α-OHase deficiency) and 21-deoxycortisol (elevated in 21α-OHase deficiency) were later added (2008) to the second-tier assay but do not play a formal role in CAH NBS classification because their utility is currently being analyzed. Additionally, gender-specific reference ranges were added to account for potential differences in 17OHP levels between the genders, because girls have been reported to have lower 17OHP levels.2,18

Limitations of this study include changes in the first-tier time-resolved fluororimmunoassay and the second-tier steroid profiling assay throughout the study period, the effects of which are difficult to quantify. There were also changes in the reference ranges and analytes used in the second-tier steroid profiling assay. During the course of the research, we found a number of infants with classic CAH born in other states that were not included in our study. Similarly, we acknowledge the possibility that there could be infants with classic CAH who moved out of the state with their condition never reported to the MDH, that there may be patients with CAH that are still undiagnosed, or unidentified infants with CAH who died. By examining NBS metrics over an extended period of time and across 2 distinct screening protocols (one-tier with repeat screen versus two-tier with second tier steroid profiling), this study illustrates what many in the NBS community suspected: current CAH screening continues to have a high FPR and low PPV. FP NBS results remain a large concern for the NBS community as they cause unnecessary additional testing and can engender long-lasting parental anxiety or dysfunctional parent–child relationships.19,20 Parents may be more overprotective and more focused on physical symptoms of their child with a FP result, which may lead to increased hospitalizations during the first 6 months after birth and continued unjustifiable anxiety about the child’s vulnerability to future events.21

Although there was no statistical difference in FPR between the one-tier and two-tier protocols, a substantial benefit of second-tier testing for CAH was the elimination of repeat first-tier screens due to equivocal 17OHP results. Before second-tier testing, an average of 355 families per year were recalled for the infant to have another heel prick due to equivocal 17OHP results, a number far higher than the average of 30 FPs per year who underwent additional diagnostic serum testing during the two-tier system.

Whether using first-tier screening only or first- and second-tier screening, FNs are a problem (Table 4). During the study period, for every 3 newborns correctly identified with classic CAH, 1 was missed (45 identified by NBS, 15 missed by NBS). Our report also highlights that patients with both classic SW and SV-CAH can be missed by NBS. With 1 exception,22 sensitivity of NBS for detecting SW-CAH is generally reported to be 100%.2,3,24 The reason for the high percentage of FNs is unclear. An unexplained delayed rise in 17OHP in certain patients with classic CAH18 and/or the timing and sensitivity of the assay are possibilities.

Because most states draw the NBS sample at the same time (24–48 hours) and use the same assay to measure 17OHP, Minnesota’s NBS program serves as a microcosm, which suggests that FNs are underestimated nationwide. That more missed cases of classic CAH by NBS have not been previously reported in the

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**TABLE 5** Results of Second-Tier Testing Performed Retrospectively on Samples Originally Identified as FN by First-Tier Screening

<table>
<thead>
<tr>
<th>Case</th>
<th>Gender</th>
<th>Second Tier 17OHP (ng/mL)</th>
<th>Second Tier Cutoff</th>
<th>Second Tier Ratio 17OHP+D4A Cortisol</th>
<th>Second Tier Ratio Cutoff</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>Girl</td>
<td>0.2</td>
<td>&gt;12.5</td>
<td>0.36</td>
<td>&gt;3.75</td>
</tr>
<tr>
<td>10a</td>
<td>Boy</td>
<td>12.0</td>
<td>&gt;10.2</td>
<td>1.11</td>
<td>&gt;2.5</td>
</tr>
<tr>
<td>12a</td>
<td>Girl</td>
<td>5.2</td>
<td>&gt;4.0</td>
<td>11.30</td>
<td>&gt;2.5</td>
</tr>
<tr>
<td>13a</td>
<td>Girl</td>
<td>3.49</td>
<td>&gt;4.0</td>
<td>0.91</td>
<td>&gt;2.5</td>
</tr>
<tr>
<td>14a</td>
<td>Girl</td>
<td>7.13</td>
<td>&gt;4.0</td>
<td>0.55</td>
<td>&gt;2.5</td>
</tr>
</tbody>
</table>

D4A, androstenedione.

a Incorrectly identified as negative on retrospective second-tier testing.

b Correctly identified as positive on retrospective second-tier testing.
literature is probably due to the lack of a consistent follow-up reporting system for physicians to alert their state’s health department when a patient with classic CAH is diagnosed later in infancy or childhood.

It has been suggested that molecular testing may have a role in NBS for CAH as the common mutation panel identifies 90% to 95% of alleles. As a second-tier screen utilizing the same first-tier cutoffs, molecular testing would not identify FNs missed by the first-tier screening because samples are not forwarded for repeat or second-tier screening, but it would have confirmed cases 8, 9, and 11 that were found positive on first-tier and subsequently were overturned by second-tier. Molecular testing as a second-tier screen could potentially decrease FPs, because those that test negative for mutations are most likely not affected. The overall efficacy and cost-effectiveness of molecular testing as a first-tier or second-tier test has not yet been established but warrants further study. Until then, it is pivotal that state screening programs educate providers that NBS does not identify all patients with classic CAH (SW and SV). Thus, any patient for whom there is clinical concern for CAH should receive immediate diagnostic testing, and results of NBS should not be regarded as definitive.

REFERENCES


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Pediatrics 2012;130;e1261; originally published online October 15, 2012; DOI: 10.1542/peds.2012-1219

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