the Bio-Rad, Recipe, and Chromsystems calibrators for metanephrines. The increase in sources of calibrators did not cause a decrease in the accuracy of the results during external quality control program in the 2004–2006 period. The observed differences in published reference values for urinary metanephrines are not mainly because of analytical reasons but most probably are due to differences in the characteristics of reference groups. The present availability of consistent calibrators should prompt efforts to define and validate reference values based on a large pool of observations, which then could be used in multiple laboratories.

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References

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Maple Syrup Urine Disease: Newborn Screening Fails to Discriminate between Classic and Variant Forms

To the Editor:
Maple syrup urine disease (MSUD) of metabolism detectable by newborn screening (NBS). Deficiency of the branched-chain 2-keto acid dehydrogenase leads to the accumulation of branched-chain amino acids (BCAA) leucine, valine, isoleucine, and alloisoleucine. About 75% of MSUD patients have the severe classic form (≤2% enzyme activity). They develop a severe encephalopathic crisis with deep coma, mostly during the second week of life, owing to very high concentrations of BCAAs (>1000 μmol/L leucine). The remainder have milder variant forms (2%–40% enzyme activity) with lower concentrations of BCAAs (<1500 μmol/L leucine) and later onset or absence of cerebral symptoms.

Severely ill newborns require urgent urgent treatment of branched-chain compounds followed by lifelong semisynthetic diet with reduced intake of BCAAs. Because even subjects with a mild form of MSUD are at risk of acute metabolic decompensation during stressful situations, they also may benefit from early presymptomatic diagnosis by NBS. Whereas newborns with classic MSUD need emergency management including intensive care and occasionally extracorporeal detoxification, this expensive treatment is not necessary in newborns with variant MSUD. We performed the present study to find out if classic and variant MSUD can be discriminated by NBS so that adequate treatment may be initiated when receiving the result of a tentative diagnosis of MSUD in NBS.

Since 2002, electrospray ionization–tandem mass spectrometry (ESI-MS/MS)–based NBS has been available in Germany for every newborn. NBS for MSUD is performed by measuring the concentration of “total leucine” (leucine + isoleucine + alloisoleucine + hydroxyproline) in dried blood spots. These 4 isobaric amino acids cannot be separated by routine screening methods. The
tentative diagnosis has to be confirmed by measuring BCAA concentrations in plasma. The recall rate for MSUD in Germany is \( \approx \) 0.01% (calculated from \( >1.5 \) million newborns in the Bavarian and Berlin screening laboratory).

We compared total leucine concentrations in dried blood and leucine concentrations in plasma during confirmation diagnosis between subjects attributed retrospectively to classic or variant MSUD. Data on the patients with classic MSUD have been published recently (2). Data are presented as ranges and/or mean (SD). Data analysis was performed by SPSS-12 (SPSS Inc). The Wilcoxon rank sum test (Mann–Whitney U-test) was used to compare differences between the 2 groups of patients. \( P < 0.05 \) was considered statistically significant.

In Table 1, we present laboratory data of 19 newborns who screened positive for MSUD in Germany and Austria from 1999 to 2005. MSUD was suspected by detecting increased total leucine concentrations in dried blood spots collected between the second and eighth day of life. Total leucine concentrations in newborns suffering from classic MSUD (subjects C1–C10) were significantly higher than concentrations in newborns who were later attributed to a variant form (subjects V1–V9) \( [1240 (557) \mu\text{mol/L} \text{ vs } 549 (267) \mu\text{mol/L}, P = 0.003] \). Because of high variance, however, it was impossible to reli-

### Table 1. Laboratory data for NBS and confirmation diagnosis in newborns who screened positive for MSUD.\(^a\)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Confirmation of diagnosis, ( \text{day of life} )</th>
<th>Leu, ( \mu\text{mol/L} )</th>
<th>Val, ( \mu\text{mol/L} )</th>
<th>Xle/Phe, molar ratio</th>
<th>Xle/Ala, molar ratio</th>
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<tr>
<td>V1</td>
<td>3</td>
<td>835</td>
<td>830</td>
<td>12.3</td>
<td>5.4</td>
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<td>V2</td>
<td>8</td>
<td>1030</td>
<td>1030</td>
<td>7.2</td>
<td>1.1</td>
</tr>
<tr>
<td>V3</td>
<td>3</td>
<td>485</td>
<td>485</td>
<td>4.4</td>
<td>1.1</td>
</tr>
<tr>
<td>V4</td>
<td>4</td>
<td>405</td>
<td>412</td>
<td>7.2</td>
<td>1.1</td>
</tr>
<tr>
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<td>3</td>
<td>483</td>
<td>469</td>
<td>4.4</td>
<td>1.1</td>
</tr>
<tr>
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<td>288</td>
<td>261</td>
<td>4.4</td>
<td>1.1</td>
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<tr>
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<td>3</td>
<td>298</td>
<td>298</td>
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<td>1.1</td>
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<td>1.1</td>
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<tr>
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<td>586</td>
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<td>900</td>
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<td>7.2</td>
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<tr>
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<td>1080</td>
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<td>4</td>
<td>524</td>
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<tr>
<td>C6</td>
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<td>28.6</td>
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<tr>
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<td>572</td>
<td>572</td>
<td>8.9</td>
<td>3.9</td>
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</tbody>
</table>

\(^a\) Patient diagnosis was confirmed by measuring elevated plasma alloisoleucine for classic MSUD (C) and variant MSUD (V); elevated excretion of branched-chain oxo-acids in urine (C); strongly elevated leucine levels in plasma (C); leucine tolerance (C); enzyme activity in fibroblasts (C and V); and mutation analysis (C and V).

The cutoff for total leucine concentration indicating a positive screening result that requires confirmation diagnosis ranged for the different screening laboratories from 275 to 393 \( \mu\text{mol/L} \). Reference values for plasma BCAA concentrations during confirmation procedure were for leucine \( <230 \), isoleucine \( <105 \), and valine \( <480 \mu\text{mol/L} \).

b Values for valine (Val), total leucine/phenylalanine (Xle/Phe), and total leucine/alanine (Xle/Ala) were not always reported from the screening laboratory.

c Control sample (dried blood spots (DBS)) before confirmation diagnosis by amino acid analysis.

d Control sample (DBS) had normal BCAA; however, ratios to phenylalanine were still elevated. Confirmation of diagnosis was made by amino acid analysis on day 714 of life. Data excluded from statistics. Patient V8 was referred to a metabolic clinic only after multiple requests of the screening laboratory.

e Control sample (DBS) had normal BCAA (in a laboratory not specialized in newborn or selective screening). Confirmation of diagnosis was made by amino acid analysis on day 268 of life. Data excluded from statistics. Patient V9 was referred to a metabolic clinic only after multiple requests of the screening laboratory.

Letters to the Editor

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ably discriminate between classic and variant MSUD by NBS results. In confirmation diagnosis by amino acid analysis performed on day 4–23, differentiation was unambiguous, with a mean plasma leucine concentration of 447 (161) μmol/L in variant MSUD in contrast to 2100 (791) μmol/L in classic MSUD (P = 0.001). Importantly, no newborn with variant MSUD needed emergency management.

Our data show that an unambiguous discrimination between variant and classic MSUD is impossible by NBS. Therefore the detection of any elevation of total leucine in NBS requires immediate referral to a specialized metabolic unit or a local pediatrician/physician (in consultation with a metabolic specialist) to confirm the diagnosis by quantitative determination of plasma amino acids and to institute treatment. Measurement of plasma leucine concentrations by amino acid analysis clearly differentiates between classic and variant MSUD, which is important for choosing the proper treatment. Most likely, leucine and isoleucine concentrations in early postnatal life are crucially influenced by the extent of neonatal protein catabolism, which might differ considerably between individuals, and may account for the reported missed cases of variant MSUD (3).

Oglesbee et al. (4) recently described a method to quantify the isobaric amino acids leucine, isoleucine, alloisoleucine, and hydroxyproline as a second-tier test from the initial NBS dried blood spots. Only samples with both increased total leucine and increased alloisoleucine would lead to further metabolic workup. This approach appears promising for further reducing false-positive rates. Even with this improvement, however, discrimination between classic and variant MSUD may not be possible from the NBS results (5).

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