

Second-tier strategy for newborn screening for isovaleric acidemia: improving specificity and recall rate

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Background:

Currently applied newborn screening for isovaleric acidemia (IVA) using flow injection MS/MS quantifies C5-carnitines (C5) in dried blood spots. However, isovalerylcarnitine, the key C5-ester in IVA, is isobaric to pivaloylcarnitine which can be present in a newborn's blood due to maternal use of pivaloylester-containing substances such as antibiotics like pivmecillinam or nipple care ointments. Since late 2016, when pivmecillinam was explicitly recommended for treatment of urinary tract infections during pregnancy, a dramatic increase of false-positive cases has been observed.

Methods:

From 1/19 to 12/25, all newborns screened in Hamburg with a C5 above cut-off 0.5 μM were tested by a second-tier test, *i.e.*, a chromatographic (UPLC C18 column) MS/MS method developed to differentiate C5 isobars. The samples already analyzed by the NBS method were used after 1:10 dilution; run time 5 min. Purified standards and samples of patients with genetically proven IVA or 2-methylbutyrylglycinuria were used for comparison.

Results:

C5 (valeryl-, isovaleryl-, 2-methylbutyryl- and pivaloylcarnitine) were well separated by our method. Among 337,247 newborns tested, 5 were true-positive while 510 had an elevated C5 due to the presence of pivaloylcarnitine. Cases with false-positive results occurred in varying regional clusters. These cases have continuously increased year by year from 20 in 2019 to 161 in 2025.

Conclusion:

Given these numbers, a second-tier test for IVA screening is indispensable. The method we developed and published (PMID: 36636590) has proven its effectiveness and is now accredited in our laboratory. Thus, additional diagnostic steps are avoided and, most importantly, unnecessary stress and anxiety can be eliminated for parents in a remarkably high number of cases. We recommend that such a test should be included in the recommendations of the German pediatric guidelines (Kinderrichtlinie).