EDTA in Dried Blood Spots Leads to False Results in Neonatal Endocrinologic Screening

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BACKGROUND: Blood samples for neonatal screening for inborn errors of metabolism are collected and shipped on standardized filter paper cards. Occasionally these samples are contaminated with EDTA, which is often used for anticoagulation. EDTA may interfere with newborn screening tests based on lanthanide fluorescence and thus lead to false-negative or false-positive results.

METHODS: We used tandem mass spectrometry (MS/MS) to detect EDTA in dried blood spots by use of an extra experiment that was integrated into the standard MS/MS neonatal screening and did not require an additional sample spot, nor extra time or work. We analyzed the influence of different blood sampling procedures on lanthanide fluorescence tests for thyroid-stimulating hormone (TSH) and 17-hydroxyprogesterone (17-OHP).

RESULTS: EDTA was increased in 138 of 190 000 newborn screening samples, 27 of which caused false-positive results in the immunoassay for 17-OHP. No false-negative TSH results were found. False-positive results in the 17-OHP test occurred when EDTA concentrations were >2.0 g/L; the TSH test, however, produced false negatives only when EDTA concentrations were >3.0 g/L. Using EDTA-containing devices the procedure of blood collection significantly influenced the concentration of the anticoagulant.

CONCLUSION: Addition of EDTA quantification into standard MS/MS tests is a simple and useful method to avoid false-positive or false-negative neonatal screening results in lanthanide fluorescence-based tests.

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In many laboratories newborn screening includes the measurement of parameters detected by immunoassays based on lanthanide fluorescence. During the last step of those tests the marker is released for detection by time-resolved fluorescence. EDTA is a chelating agent, so the lanthanides are set free at the beginning of the test and are washed out at undue time (1). To detect contamination of samples with EDTA, we introduced an additional tandem mass spectrometry (MS/MS) experiment (2) into the standard newborn screening analysis for amino acids and acylcarnitines. In addition we analyzed the influence of different procedures of blood collection on EDTA contamination of dried blood spots as well as the influence of EDTA concentrations on screening results for thyroid-stimulating hormone (TSH) and 17-hydroxyprogesterone (17-OHP). Independent results for 17-OHP were obtained by a specific MS/MS method that has been described previously (3).

Materials and Methods

Extraction and butylation of amino acids and acylcarnitines during the standard procedure (2) led to butylation of EDTA in 4 positions. We tested 190 000 newborn samples for EDTA, using the mass transition 517.1–188.1 for EDTA measurement in multiple reaction mode. The limit of detection was 0.1 g/L. In the same samples, TSH and 17-OHP were measured according to the instruction manuals of the dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA®) test kits Neonatal 17α-hydroxyprogesterone and Neonatal hTSH (PerkinElmer). Independent quantification of 17-OHP was achieved by use of an MS/MS method for steroids (3). In addition, 3 types of experiments were performed with whole blood samples from healthy adult donors: (a) The blood was spiked with human TSH (Calbiochem/Merck) and EDTA (Merck) at different concentrations and spotted on Whatman 903 filter paper (Whatman). (b) Blood samples containing 2 different concentrations of TSH were spotted either directly on filter paper or using EDTA-coated plastic capillaries (Microvette® CB 300, Sarstedt). (c) Blood samples (2.7 mL, 1 mL, and 0.5 mL) spiked with human TSH were filled into plastic devices designed for collection of 2.7 mL EDTA-blood for hematological tests (S-Monovette®) and spotted on filter paper.

More detailed information on the methods can be found in the Data Supplement that accompanies the online version of this Brief Communication at http://www.clinchem.org/content/vol54/issue3.

Results

EDTA was found in 138 of 190 000 samples. None of these were false negative for TSH, as demonstrated by the results of control analysis of second samples. In 27 samples, EDTA caused results that were in the patho-
logical range for 17-OHP and were shown to be false positive by analysis of a second sample. In 21 cases MS/MS quantification of 17-OHP and other steroids in the first sample confirmed the negative screening results. Two examples of normal steroid profiles of EDTA-contaminated samples compared with samples from a premature newborn and a patient affected by congenital adrenal hyperplasia are shown in Table 1 in the online Data Supplement.

When EDTA was detected in a sample, information about the blood collection procedure was obtained from the hospital or midwife. This information revealed three reasons for EDTA contamination: (i) Venous blood was collected in devices prepared with EDTA and then spotted on the filter paper. EDTA was evenly distributed within these samples. (ii) Blood was taken by heel prick and transferred to the filter cards via capillary devices coated with EDTA. Irreproducible 17-OHP and EDTA results were found in different spots and even throughout one single spot. (iii) After a hematological microcontainer prepared with EDTA was filled, venous blood was spotted directly onto the filter card. The top of the needle touched the container wall and EDTA was transferred to the filter paper card. Again, irreproducible EDTA results were found in different spots and even throughout one single spot.

**Table 1. Influence of filling grade of EDTA-coated blood-collection devices on measured EDTA and 17-OHP concentrations.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>EDTA MS/MS, g/L</th>
<th>Measured 17-OHP concentration, nmol/L&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Measured TSH concentration, mU/L&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood sample without EDTA</td>
<td>0.0</td>
<td>2.5</td>
<td>18.8</td>
</tr>
<tr>
<td>Device (2.7 mL) filled correctly with 2.7 mL blood</td>
<td>0.9</td>
<td>2.7</td>
<td>20.6</td>
</tr>
<tr>
<td>Device (2.7 mL) filled with 1 mL blood</td>
<td>2.6</td>
<td>168.0</td>
<td>12.9</td>
</tr>
<tr>
<td>Device (2.7 mL) filled with 0.5 mL blood</td>
<td>5.5</td>
<td>1675.0</td>
<td>1.4</td>
</tr>
</tbody>
</table>

<sup>a</sup> Cutoff: 60 nmol/L.  
<sup>b</sup> Cutoff: 15 mU/L.

**BLOOD COLLECTION PROCEDURES**

Blood was collected successively in 5 drops of about 50–60 μL in the same EDTA coated capillary and transferred one after the other to filter paper. Most of the EDTA was found in the first spot, resulting in falsely increased 17-OHP. A pathological TSH value in a comparable experiment, however, was not affected. Analyses of multiple punches from the first blood spot in this experiment showed highly variable 17-OHP results. Values from 73 nmol/L to >2000 nmol/L in 5 different punches were obtained, and EDTA concentrations varied from 1.3–2.4 g/L.

Filling the capillary with 300 μL blood at once and transferring it to 5 blood spots on filter paper resulted in a maximum concentration in the last blood spot, for which an extremely high false positive result for 17-OHP was measured.

The same experiment using a sample spiked with TSH in a highly pathological concentration of 180 mU/L again did not show any effect. Only when the blood volume was decreased to about 150 μL did the TSH result become false negative in the last of 3 spots.

For blood from EDTA blood collection devices, the results are summarized in Table 1. In samples from devices filled correctly with 2.7 mL blood, the results for TSH and 17-OHP were not influenced significantly. When only 1 mL of blood was added, a true concentration of 20 mU/L TSH gave results lower than the cutoff of 15.0 mU/L. The true-negative result for 17-OHP was turned into a false positive one. Further reduction to 0.5 mL blood turned highly pathological TSH values into false negatives and true-negative 17-OHP concentrations into extremely pathological values.
Discussion

EDTA is a widely used anticoagulant in blood collection. Therefore contamination of dried blood samples with EDTA can be expected to occur in newborn screening samples.

Our results show that EDTA in concentrations achieved by correct use of devices for hematological testing does not lead to false results in tests for TSH and 17-OHP. These blood samples contain 1.2–2.0 g/L EDTA, according to manufacturer information. No significant influence of anticoagulants on immunofluorometric test results had been reported previously (3). In other reports, the use of EDTA-blood in a time-resolved immunofluorometric assay is explicitly recommended (4), or the addition of EDTA to the sample buffer is reported to decrease unspecific background signals (5). These data and our own results indicate that, if containers for hematological testing are filled with the correct amount of blood, the test results will not be falsified. For some immunofluorometric assays, however, EDTA and other anticoagulants in blood samples have been reported to influence test results (1). Under everyday conditions, many different blood collection procedures and device applications are used. It is common, for example, for blood collection devices not to be filled completely, because only 300–400 μL of blood are needed for newborn screening. Under such conditions very high concentrations of EDTA may occur, leading to false immunofluorometric results. Fortunately, the 17-OHP test is affected more strongly by EDTA than the test for TSH. If EDTA is distributed evenly throughout the whole sample, a false-negative congenital hypothyreosis screening is unlikely to occur without a false-positive congenital adrenal hyperplasia screening.

EDTA may be distributed unevenly throughout the filter paper card, even throughout a single blood spot, especially when EDTA-coated capillary devices are used. In this situation, repetition of immunofluorometric tests with material from a single test card may produce variable results, and MS/MS EDTA measurement may not always detect such contamination. It is always possible that the filter paper sample used for MS/MS analysis will be free of measurable EDTA although the one used for a lanthanide-labeled test is highly contaminated.

In our study, EDTA was detected in 0.07% of test cards. In 27 cases this led to a false newborn screening result and the screening had to be repeated on a control sample. EDTA measurement in dried blood samples can directly decrease the number of invalid samples received; in our study the reporting of EDTA results had a significant educational effect. Obstetrical wards sending contaminated samples repeatedly learned to avoid incorrect application of capillaries or other devices. We therefore recommend newborn screening laboratories which already
use immunofluorometric screening methods and MS/MS to add this inexpensive and simple measurement to their routine. Irreproducible and implausible pathological 17-OHP results can be explained and, most importantly, false screening results for congenital adrenal hyperplasia and for congenital hypothyreosis can be avoided.

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**References**


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