



## K28 Assessment of *In Vitro* Methemoglobinemia Formation in Infant Samples

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**Learning Overview:** After attending this presentation, attendees will have learned the caveats of postmortem methemoglobin interpretation in adult and fetal postmortem blood and the mechanisms to circumvent these limitations for proper interpretation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing a large dataset from a national reference laboratory for methemoglobin trends in adults and infants with guidance on interpretation of results.

**Hypothesis:** Interpretation of methemoglobin percentage in postmortem samples, specifically infant samples, is complicated by *in vitro* formation and susceptibility of fetal hemoglobin to oxidation in the absence of an exogenous oxidant.

**Content:** The review of historic methemoglobin results in postmortem samples collected from infants illustrated a wide distribution of results and difficulty with interpretation. Redevelopment of an existing forensic method to allow its use in clinical methemoglobinemia determination resulted in the identification of extreme elevations of methemoglobin in postmortem samples previously undetected. Suspected nitrate/nitrite ingestion was indicated for many of the elevated adult methemoglobin results and nitrate/nitrite levels via an enzymatic method were assessed in several specimens to confirm. Elevated methemoglobin results in infant samples did not routinely correlate with case histories and were proposed to be falsely increased due to a spectral interference. However, residual fetal blood samples collected from umbilical cords of otherwise healthy infants had minimal methemoglobin present, decreasing the likelihood of a broad interference due to fetal hemoglobin contributing to the high percentage of elevations observed. Oxidation studies were conducted with stored blood from adult and fetal origin, with and without an oxidizing agent present, to illustrate the relative susceptibility of fetal hemoglobin to oxidize post-collection.

**Results:** Historic data were analyzed to demonstrate the distribution of results before and after redevelopment of an existing methemoglobin method using spectrophotometry and ranged between <1% to >80%. Elevations in methemoglobin percentages in adult postmortem samples consistent with case presentation were confirmed for the presence of nitrate/nitrite using an enzymatic method. Fetal and adult samples were further assessed to evaluate the formation of methemoglobin during storage in the absence and presence of oxidizing agents with studies currently in progress.

**Conclusion:** Interpretation of postmortem methemoglobin results requires caution, specifically in infant samples. However, follow-up testing based on case presentation provides the necessary context to interpretation of the results from postmortem blood.

**Methemoglobin, Pediatric Toxicology, Nitrate/Nitrite**