CLINICAL PRACTICE GUIDELINE:

Prevention of Blood Specimen Hemolysis in Peripherally-Collected Venous Specimens

Which preanalytic variables related to peripheral venous specimen collection and transportation decrease blood culture hemolysis?
Table of Contents

Background and Significance .................................................. 3
Methodology ............................................................................ 3
Summary of Literature Review .................................................. 5
Description of Decision Options/Interventions and the Level of Recommendation .................................................. 9
References .................................................................................. 11
Authors ..................................................................................... 13
Acknowledgements .................................................................... 13
Appendix 1: Evidence Table ....................................................... 14
Appendix 2: Other Resources Table ............................................ 35
Appendix 3: Study Selection Flowchart and Inclusion/Exclusion Criteria ................................................................. 36
Background and Significance
Collection of peripheral venous blood specimens is a daily practice in many healthcare settings. Hemolysis of blood samples can lead to inaccurate results and repeat draws, causing additional pain, delaying treatment decisions, and increasing length of stay (Tanabe, Kyriacou, & Garland, 2003). Hemolysis accounts for 40% to 60% of blood specimen rejections by the laboratory (Söderberg, Jonsson, Wallin, Grankvist, & Hultdin, 2009). Hemolysis rates from 3.3% to 77% have been reported, and vary depending on the method of blood sample collection (Halm & Gleaves, 2009). No substantiated benchmark for hemolysis rate was found in the literature. This Clinical Practice Guideline (CPG) evaluates the scientific evidence for the prevention of hemolysis in the preanalytic phase (i.e., prior to laboratory analysis).

Methodology
This CPG is based on a thorough review and critical analysis of the literature following ENA’s “Requirements for the Development of Clinical Practice Guidelines.” All articles relevant to the topic were identified via a comprehensive literature search. The following databases were searched: PubMed, Google Scholar, CINAHL, eTBLAST, Ovid, Cochrane Library, Agency for Healthcare Research and Quality (AHRQ; www.ahrq.gov), Specimen Care (www.specimencare.com), and the National Guideline Clearinghouse (www.guidelines.gov). Searches were conducted using various combinations of key words including hemolysis, phlebotomy technique, and blood samples. Initial searches were limited to English language articles from January 2002 to October 2012. This search limit was found to be inadequate, and the time frame was therefore extended to begin with January 1990. A new search was conducted in 2016, following the guidelines used for the initial search, that included October 2012 to June 2016. In addition, the reference lists in the selected articles were scanned for further pertinent research reports. Research articles from emergency department settings, non-emergency department settings, position statements, and guidelines from other sources were also reviewed.

Articles that met the following criteria were chosen to formulate the CPG: research studies, meta-analyses, systematic reviews, and existing guidelines relevant to the topic of blood specimen hemolysis. Articles cited in meta-analyses or systematic reviews were not considered independently unless they addressed additional factors. Other types of reference articles and textbooks also were reviewed and used to provide additional information. The CPG authors used a standardized reference table to collect information and assist with the preparation of tables of evidence, ranking each article in terms of the level of evidence, quality of evidence, and relevance and applicability to practice. Clinical findings and recommendation levels regarding patient management were made by the ENA 2012 Emergency Nursing Resources Development Committee and revised by the ENA 2016 Clinical Practice Guidelines Committee, following ENA’s classification of levels of recommendation for practice. These are: Level A, High; Level B, Moderate; Level C, Weak; and Not recommended for practice (Table 1).
### Table 1. Levels of Recommendation for Practice

<table>
<thead>
<tr>
<th><strong>Level A recommendations: High</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>• Reflects a high degree of clinical certainty</td>
</tr>
<tr>
<td>• Based on availability of high quality level I, II, and/or III evidence rated using the Melnyk and Fineout-Overholt grading system (Melnyk &amp; Fineout-Overholt, 2015)</td>
</tr>
<tr>
<td>• Based on consistent and good quality evidence; has relevance and applicability to emergency nursing practice</td>
</tr>
<tr>
<td>• Is beneficial</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Level B recommendations: Moderate</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>• Reflects moderate clinical certainty</td>
</tr>
<tr>
<td>• Based on availability of Level III and/or Level IV and V evidence rated using the Melnyk and Fineout-Overholt grading system (Melnyk &amp; Fineout-Overholt, 2015)</td>
</tr>
<tr>
<td>• There are some minor inconsistencies in quality evidence; has relevance and applicability to emergency nursing practice</td>
</tr>
<tr>
<td>• Is likely to be beneficial</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Level C recommendations: Weak</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>• Has limited or unknown effectiveness</td>
</tr>
<tr>
<td>• Level V, VI, and/or VII evidence rated using the Melnyk and Fineout-Overholt grading system (Melnyk &amp; Fineout-Overholt, 2015) — Based on consensus, usual practice, evidence, case series for studies of treatment or screening, anecdotal evidence, and/or opinion</td>
</tr>
<tr>
<td>• There is limited or low quality patient-oriented evidence; has relevance and applicability to emergency nursing practice</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Not recommended for practice</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>• No objective evidence or only anecdotal evidence available, or the supportive evidence is from poorly controlled or uncontrolled studies</td>
</tr>
<tr>
<td>• Other indications for not recommending evidence for practice may include:</td>
</tr>
<tr>
<td>◦ Conflicting evidence</td>
</tr>
<tr>
<td>◦ Harmfulness has been demonstrated</td>
</tr>
<tr>
<td>◦ Cost or burden necessary for intervention exceeds anticipated benefit</td>
</tr>
<tr>
<td>◦ Does not have relevance or applicability to emergency nursing practice</td>
</tr>
<tr>
<td>• There are certain circumstances in which the recommendations stemming from a body of evidence should not be rated as highly as the individual studies on which they are based. For example:</td>
</tr>
<tr>
<td>◦ Heterogeneity of results</td>
</tr>
<tr>
<td>◦ Uncertainty about effect magnitude and consequences</td>
</tr>
<tr>
<td>◦ Strength of prior beliefs</td>
</tr>
<tr>
<td>◦ Publication bias</td>
</tr>
</tbody>
</table>
Summary of Literature Review

Reliable laboratory results are dependent on quality throughout the three phases of laboratory testing: preanalytic, analytic, and postanalytic (Plumhoff, Masoner, & Dale, 2008). Of the three phases, most errors occur in the preanalytic phase (i.e., prior to laboratory analysis) and are related to specimen collection, specimen handling, and patient variables (Plumhoff et al., 2008). Owing to the importance of the preanalytic phase on specimen quality, the literature review centered on identifying preanalytical variables associated with hemolysis.

Reliability of laboratory results affects treatment decisions, and blood draws that have to be repeated because of hemolysis increase length of stay and workload, and can affect clinical outcome. A variety of factors have been studied for their association with hemolysis, including anatomic site, tourniquet time, equipment, technique, transport, personnel, education, monitoring and feedback, and phlebotomy workload. There is limited evidence regarding the number of intravenous (IV) or venipuncture attempts or the phlebotomy workload. Only one study was found that evaluated the number of attempts for venipuncture, which found no difference in hemolysis between one attempt and greater than one attempt (Saleem, Mani, Chadwick, Creanor, & Ayling, 2009). One study looked at the total number of venipunctures performed in a setting and found a decreased phlebotomy workload may be associated with increased hemolysis (Hawkins, 2010).

ANATOMIC SITE

The anatomic site used for blood collection can have an impact on hemolysis of blood samples (Fang, Fang, Chung, & Chien, 2008; Halm & Gleaves, 2009; Lippi, Avanzini, Aloe, & Cervellin, 2014; Tanabe, Kyriacou, & Garland, 2003; Wollowitz, Bijur, Esses, & Gallagher, 2013). In a descriptive cross-sectional study, Fang et al. (2008) found the risk of hemolysis was 3.35 times less likely when the antecubital space was used compared with other sites (p = 0.001). Higher hemolysis rates have been linked to sites on the non-dominant extremity or below the antecubital space (Halm & Gleaves, 2009). Lippi et al. (2014) found blood drawn from intravenous catheters placed below the median veins carries a higher risk of hemolysis (hemolyzed specimens: 17% from median cephalic and basilica veins; 29% from the cephalic vein [p = 0.01]; 33% from the basilic vein, [p < 0.01]; 75% from the metacarpal plexus veins, [p < 0.01]). In a prospective cohort study, Tanabe et al. (2003) found when specimens were obtained at sites other than the antecubital site, the risk in hemolysis increased two to three times (RR 2.61; 95% CI [1.39, 4.9]). In a meta-analysis of four studies, Heyer et al. (2012) found a 55% reduction in hemolysis when the antecubital site was used (RR = 0.45, 95% CI [0.35, 0.57]). Wollowitz et al. (2013), in a prospective, observational, cross-sectional study of 4,513 specimens, found the rate of hemolysis was higher when blood was drawn from a site other than the antecubital fossa (OR 2.1, 95% CI [1.7, 2.5]).

TOURNIQUET TIME

Tourniquets are applied before blood draws and a longer tourniquet time can increase the risk of hemolysis. A prospective study by Saleem et al. (2009) found the odds ratio (OR) for hemolysis with a tourniquet time greater than one minute was 19.5 (95% CI [5.6, 67.4]). Wollowitz et al. (2013), in a prospective, observational, cross-sectional study found tourniquet times of one minute or longer increased hemolysis rates (6.8%; OR 1.3; 95% CI [1.0, 1.6]).

EQUIPMENT

Equipment used for blood collection has been associated with hemolysis. The equipment studied included needleless connectors, extension tubing, needles, and IV catheters made of different materials and in different gauges, vacuum tubes, and syringes. In a randomized, single-blinded control trial, Dwyer, Fry, Somerville, and Holdgate (2006) compared hemolysis rates when specimens were drawn directly from the IV or with an Interlink® device and found no significant difference. The subjective determination of ease of aspiration was the only significant predictor of hemolysis (p < 0.0001).

Dietrich (2014) completed a prospective study comparing three methods of sample collection: samples obtained while starting intravenous access, obtained from existing vascular access, and obtained by direct venipuncture. Rates of rejection because of hemolysis were 1.1% when collected during the intravenous catheter start, 0.8% when collected from an existing vascular access, and 0.1% when collected by venipuncture with a steel needle, demonstrating that hemolysis rates below the benchmark of 2% can be achieved by sampling during intravenous line placement.
The type of catheter used has been identified as a potential contributor to hemolysis. Sharp and Mohammad (2003) performed in vitro experiments to evaluate various catheters, connectors, needle sizes, syringes, and vacuum tubes and found the greatest level of cell-free hemoglobin for a sample was 43 mg/dL, with the average less than 12 mg/dL. They concluded no combination of equipment produced hemolysis that would impact common clinical assays. Tanabe et al. (2003) compared the type and gauge of needle or catheter. They found an increased risk of hemolysis for samples drawn from Vialon™ catheters 20-gauge or smaller (RR 7.42; 95% CI [1.8, 30.52]) and no relationship between steel gauge needle size and hemolysis. The hemolysis rate was 10% with Vialon™ catheters and 1.5% for steel needles. In a randomized, prospective study, Raisky, Gauthier, Marchal, and Blum (1994) compared different materials for collecting blood samples from patients in the ED. Blood samples collected with stainless steel needles were 12% less likely to be hemolyzed than those drawn from IV catheters. In a comparison of blood samples drawn with Teflon FEP (Cathlon®) and polyurethane Vialon™ (Insyte™) catheters, hemolysis was less likely with samples drawn from Teflon catheters. Sharp and Mohammad (1998) found no consistent trend in hemolysis related to cannula material.

The needle gauge or catheter size is often considered to be related to the level of hemolysis, but studies reveal conflicting results. Sharp and Mohammad (1998) found hemolysis increased with increases in cannula diameter and driving pressure differences between Vacutainer® and syringe draws. In comparing 18-gauge to 22-gauge needles with use of connectors, they found 18-gauge caused the most hemolysis and 22-gauge the least (p = 0.0008). In contrast, an observational study by Schwarzer et al. (2001) found more of the hemolyzed specimens had been drawn through smaller catheters (61.5% for 20-gauge vs. 26.9% for 18-gauge).

A prospective, cross-sectional study by Seguin, McEachrin, and Murphy (2004) found no difference in hemolysis rates between the use of vacuum tubes or syringes, but did find increased hemolysis with smaller sizes of both butterfly and IV catheters. Heyer et al. (2012), in a meta-analysis of three studies that compared hemolysis rates for draws with 21-gauge and smaller needles, found a substantial mean risk ratio for 21-gauge or smaller needles (RR = 0.037; 95% CI [0.27, 0.52]), but the size effect in the individual studies was inconsistent.

A prospective, two-group randomized study was used to compare the presence or absence of extension tubing connected to the IV catheter hub when blood was drawn (Stauss et al., 2012). They found no significant difference in hemolysis (p = 0.84).

Vacuum tubes and syringes are used to collect blood specimens. There is conflicting evidence as to whether one or other of these is more likely to cause hemolysis. Sharp and Mohammad (2003) compared syringe-drawn samples with vacuum-tube-drawn samples and found less hemolysis with vacuum tubes (p = 0.023).

Halm and Gleaves (2009) performed a systematic review, evaluating eight studies of the causes of hemolysis. Incidence of hemolysis was compared in samples drawn from IV catheters, IV catheters into vacuum tubes or syringes, and separate venipuncture sites. They found less hemolysis occurs with collection from a separate venipuncture site using vacuum tubes and straight needles. They also recommended that if vacuum tubes cannot be used, a 3–10 mL syringe should be used. Lippi, Cervellin, and Mattiuzzi (2013) conducted a meta-analysis of 15 studies to compare the risk of hemolysis with the use of intravenous catheters and evacuated tubes vs. straight needles and evacuated tubes. A significantly greater risk of hemolysis was found with the use of catheters and evacuated tubes vs. straight needles and evacuated tubes (random effect OR = 3.4; 95% CI [2.9, 3.9], and random effect RR = 1.07; 95% CI [1.06, 1.08]). Additionally, Lippi, Cervellin et al. (2013) found there was a significant risk of hemolysis in studies assessing catheter and evacuated tubes vs. catheter and manual aspiration of blood (OR 3.7; 95% CI [2.7, 5.1] and RR 1.32; 95% CI [1.24, 1.40]).

In contrast, Ong, Chan, and Lim (2009) found vacuum tube use caused significantly more hemolysis than use of syringe (OR 4.1; 95% CI [1.8, 9.4]). Heiligers-Duckers, Peters, van Dijck, Hoeijmakers, and Janssen (2013) compared the use of vacuum tubes with syringes and found vacuum tubes resulted in more hemolysis (24% vs. 16%; p = 0.008). Other studies found no difference in hemolysis when using a needle and syringe or IV catheter and vacuum tube (Bush, Mueller, Sumwalt, Cox, & Hilfiker, 2010; Ong et al., 2009; Saleem et al., 2009; Seguin et al., 2004).
In a randomized prospective study, Lippi, Avanzini, Aloe, and Cervellin (2013) compared the use of a conventional tube holder (BD Vacutainer® one-use holder, Becton Dickinson, Milan, Italy) with the Holdex® (Greiner Bio-One GmbH, Kremsmünster, Austria) disposable tube holder made of polypropylene with a stainless steel needle inside that is designed to decrease erythrocyte injury during blood draws. Lippi , Avanzini et al. (2013) found the use of the Holdex® vacutainer system may decrease hemolysis when samples are drawn from an existing 20-gauge peripheral intravenous line. The mean cell-free hemoglobin was not statistically significantly different, but the frequency of samples with gross hemolysis (cell-free hemoglobin more than 3.0 g/L) was higher for a conventional tube holder than with the Holdex®.

Specific vacuum tubes have been associated with the risk of hemolysis. Heyer et al. (2012) in a meta-analysis of two studies found the use of low (partial) vacuum tubes in place of full vacuum tubes resulted in an 89% reduction in hemolysis (RR = 0.11; 95% CI [0.02, 0.52]). Schwarzer et al. (2001) also found using “soft draw” tubes was less likely to lead to hemolysis than full draw tubes (2.9% vs. 21.7% incidence).

In an evaluation of tube volumes, Unger, Filippi, and Patsch (2007) found EDTA influences free hemoglobin (fHb) and hemolytic index (HI) in a concentration-dependent manner. They concluded if vacuum tubes were filled adequately with the required amount of blood, no differences were identified for fHb and HI when comparing EDTA- or lithium-heparinate-plasma. In the study by Ong, Chan, & Lim (2008), blood sample volume was not associated with a significant difference in hemolysis rates. Halm et al. (2009) identified that transport of underfilled tubes via a pneumatic tube system is associated with hemolysis. Wollowitz et al. (2013) found increased hemolysis was associated with collection tubes that were no more than half full (OR 1.9; 95% CI [1.5, 2.4]).

Tamechika, Iwatani, Tohyama, and Ichihara (2006) evaluated insufficient filling of tubes and the effect of hemolysis on basic analytes, finding hemolysis was dependent on the length of exposure of the blood to the vacuum in the underfilled tube before centrifugation. They hypothesized that the length of time the red blood cells are exposed to the vacuum remaining in the tube increases fragility of the red blood cell (RBC) membrane, causing increased hemolysis. Tamechika et al. (2006) recommended sample volumes match the draw volume of the vacuum tube. They also recommended if a tube is not completely filled, the cap should be removed as soon as possible to release the vacuum and help prevent hemolysis.

TECHNIQUE

Blood samples are most frequently drawn from either an IV catheter when the IV is started, or from a separate venipuncture. The advantages of drawing blood via an IV catheter are obvious: the patient has fewer needle sticks and therefore less pain and anxiety, staff members have less potential for exposure to blood, and the laboratory specimens are generally obtained more quickly. Berger-Achituv, Budde-Schwartzman, Ellis, Shenkman, and Erez (2010) and Saleem et al. (2009) found no significant difference in hemolysis rates with venipuncture or peripheral intravenous (PIV) catheter. Berger-Achituv et al. (2010) performed a paired, within-subject study on children 1–16 years of age and compared nine hematological and chemical indices to determine if there were differences between samples from venipuncture or IV. Only one venipuncture sample had significant hemolysis. No other samples exceeded their standard for significant hemolysis (HI of 25 or serum hemoglobin [Hgb] 51 mg/dL). There were no significant differences in hemolysis between venipuncture and PIV catheter samples in terms of the serum fHb levels (20.7 + 10 mg/dL vs. 19.6 + 10.7 mg/dL, p = 0.64) or HI. Saleem et al. (2009) evaluated blood sampling and found no significant difference in hemolysis associated with the method used for obtaining the blood sample. In a meta-analysis of 11 studies, Heyer et al. (2012) found the use of straight needle venipuncture reduced hemolysis by 84% (RR = 0.16, 95% CI [0.11, 0.24]).

In other studies, venipuncture was associated with less hemolysis when compared with samples obtained from PIV catheters (Bush et al., 2010; Halm & Gleaves, 2009). Bush et al. (2010) found an increased risk of hemolysis with PIV catheters (16%) compared with venipuncture samples (6%; p = 0.04).

The rate of blood flow was not found to be a factor in hemolysis in a study by Ong et al. (2008). The association of the perceived difficulty of IV catheter insertion with the risk of hemolysis has also been studied. Ong et al. (2008) found no correlation between perceived difficulty of catheter insertion and risk of hemolysis. However, Stauss et al. (2012) found a significant difference in hemolysis when the nurses perceived the IV catheter insertion to be difficult and there were problems with blood flow (p = 0.00021).
CLINICAL PRACTICE GUIDELINE:
Prevention of Blood Specimen Hemolysis in Peripherally-Collected Venous Specimens

SPECIMEN TRANSPORT

Blood samples may be hand-carried to the laboratory for evaluation or sent by pneumatic tube system, and the method of transport can have an impact on hemolysis rates. Some studies have reported no statistically significant difference in hemolysis between hand-carried samples compared with those sent by pneumatic tube system (Saleem et al., 2009; Stair, Howell, Fitzgerald, Bailey, & Bastasch, 1995). Fang et al. (2008) found hemolysis was 8.7 times more likely when the specimen was hand transported by ward assistants compared with hand transport by laboratory personnel (p = 0.01). Ellis (2009) and Fang et al. (2008) studied the impact of the tube system on hemolysis. Ellis (2009) found increased hemolysis was associated with a malfunctioning pneumatic tube system, but causation was not proven. Streichert et al. (2011) found a positive correlation between pneumatic tube speed/acceleration and the degree of hemolysis (p < 0.05), and that the changes were critical for potassium, phosphate, aspartate aminotransferase (ASAT), and lactate dehydrogenase (LDH) measurements, which exceeded minimum standards for deviation. Tiwari, Pandey, Dixit, and Raina (2012) determined the effect of distance and speed on samples transported through a pneumatic tube system. They found no evidence of hemolysis from measurements of supernatant hemoglobin, potassium, and lactate dehydrogenase for short distances and slow speeds (115 m at 2 m/s; p < 0.05). Evliyaoglu, Toprak, Tekin, Basarali, Kilinc, & Colpan (2012) found a positive correlation between hemolysis and the speed and distance traveled in a pneumatic tube system for samples transported at 4.2 m/sec, and 3.1 m/sec for more than 2200 m (r = 0.774 and 0.766, respectively). They tracked potassium (K+) and lactic dehydrogenase (LDH) levels and found a positive correlation with distance for non-centrifuged serum samples transported at 4.2m/sec. When serum samples were centrifuged prior to transport through the pneumatic tube system, however, there was no effect on hematology and coagulation results.

PERSONNEL

The type of personnel drawing the blood specimen has been evaluated for impact on hemolysis rates. A higher incidence of hemolysis was found in blood drawn by ED personnel when compared with that drawn on medical units by trained phlebotomists (Halm and Gleaves, 2009). Bush et al. (2010) found no statistical difference in hemolysis whether a registered nurse or a laboratory technician collected the blood sample (p = 0.07). Harrison, Speroni, Dugan, and Daniel (2010), Ong et al. (2008), and Saleem et al. (2009) found no statistical difference in rates of hemolysis among the types of staff drawing the blood samples. Harrison et al. (2010) compared blood drawn in the field by emergency medical services with blood drawn in the ED and found no statistical difference in the number of redraws required because of hemolysis.

EDUCATION

Education is used to increase awareness, improve techniques, and teach new procedures to emergency staff. Halm and Gleaves (2009) found staff education about phlebotomy decreases hemolysis. Ong et al. (2009) delivered a 15-minute educational program that led to changes in operator behavior (increased use of syringes) and a decrease in hemolysis from 19.8% to 4.9% (p < 0.001). Corkill (2012) found a positive impact from educational posters placed in toilet stalls, observing a reduction of 19.72% in hemolyzed samples during a 12-month study period. Bölénius et al. (2013) evaluated the impact on hemolysis from a two-hour education program in urban and rural primary healthcare centers (PHCs) that emphasized adherence to venous blood specimen collection guidelines. Overall, the rates of hemolysis increased from 10.5% to 11.8% (p = 0.022) following the educational program. Analysis of the urban and rural PHCs separately found the rural PHCs had a significant reduction in hemolysis (p < 0.001; OR = 0.744, 95% CI [0.651, 0.851]) after the intervention, whereas the urban PHCs had a significant increase (p < 0.001; OR = 1.451, 95% CI [1.192, 1.765]).

MONITORING AND FEEDBACK

Monitoring hemolysis rates and providing feedback to staff has been used to decrease hemolysis rates. McGrath, Rankin, and Schendel (2012) posted each staff member’s hemolysis rates on a secure intranet site. The display encouraged staff to review individual techniques and the literature in an attempt to lower rates. Hemolysis rates decreased when individual rates were publicized. No statistical analysis was performed, however, and thus the effectiveness of the intervention is not clear.
## Description of Decision Options/Interventions and the Level of Recommendation

### Before the Draw: Preparation and Equipment Selection

<table>
<thead>
<tr>
<th>Level B recommendations: Moderate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Education of the staff performing phlebotomy may decrease hemolysis. (Bölenius et al., 2013; Corkill, 2012; Halm &amp; Gleaves, 2009; Ong et al., 2009)</td>
</tr>
<tr>
<td>2. Low (partial) vacuum tubes result in less hemolysis (Heyer et al., 2012; Schwartz et al., 2001)</td>
</tr>
<tr>
<td>3. Direct venipuncture with straight needles is less likely to cause hemolysis than blood collection through intravenous catheters (Berger-Achituv et al., 2010; Bush et al., 2010; Dietrich, 2014; Heyer et al., 2012; Ong et al., 2009; Saleem et al., 2009; Tanabe et al., 2003)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Level C recommendations: Weak</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. The type of personnel performing phlebotomy does not influence hemolysis. (Bush et al., 2010; Halm &amp; Gleaves, 2009; Harrison et al., 2010; Ong et al., 2008; Saleem et al., 2009)</td>
</tr>
<tr>
<td>2. Stainless steel needles are less likely to cause hemolysis than intravenous catheters; Teflon catheters are less likely to cause hemolysis than Vialon™ catheters (Raisky et al, 1994; Sharp &amp; Mohammad, 1998)</td>
</tr>
</tbody>
</table>

### Insufficient Evidence

<p>| |</p>
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. There is conflicting evidence regarding the influence of needle or catheter gauge on hemolysis (Heyer et al., 2012; Seguin et al., 2004; Sharp &amp; Mohammad, 1998; Sharp &amp; Mohammad, 2003; Tanabe et al, 2003)</td>
</tr>
</tbody>
</table>
**During and After the Draw**

**Level B recommendations: Moderate**

1. Hemolysis is less likely when blood is drawn from the antecubital fossa (Fang et al., 2008; Heyer et al., 2012; Lippi et al., 2014; Tanabe et al., 2003; Wollowitz et al., 2013)
2. Drawing blood through needleless connectors does not increase hemolysis (Dwyer et al., 2006; Sharp & Mohammad, 2003)

**Level C recommendations: Weak**

1. Minimize tourniquet time by removing the tourniquet after identifying the venipuncture site while preparing equipment and as soon as good blood flow is established (Saleem et al., 2009; Wollowitz et al., 2013)
2. Filling vacuum tubes to their recommended volume decreases hemolysis (Tamechika et al., 2006; Unger et al., 2007; Wollowitz et al., 2013)
3. Properly functioning pneumatic tube systems using short distance and slow speeds do not increase hemolysis (Ellis, 2009; Evliyaoğlu et al., 2012; Fang et al., 2008; Saleem et al., 2009; Stair et al., 1995; Streichert et al., 2011; Tiwari et al., 2012)
4. Drawing blood through extension tubing attached to an intravenous catheter does not increase hemolysis in adults (Stauss et al., 2012)

**Insufficient Evidence**

1. There is insufficient evidence regarding the impact on hemolysis of blood flow rate into the vacuum tube (Ong et al., 2008)
2. There is insufficient evidence to determine if the number of venipuncture attempts affects hemolysis (Saleem et al., 2009)
3. There is insufficient evidence as to whether perceived difficulty of intravenous catheter insertion is associated with an increased risk of hemolysis (Ong et al., 2008; Stauss et al., 2012; Wollowitz et al., 2013)
4. There is insufficient evidence to determine if the volume/frequency of venipunctures performed influences hemolysis (Hawkins, 2010)
5. There is insufficient evidence to determine if monitoring hemolysis rates and providing feedback to the staff performing phlebotomy decreases the incidence of hemolysis (McGrath et al., 2012)
References


CLINICAL PRACTICE GUIDELINE:
Prevention of Blood Specimen Hemolysis in Peripherally-Collected Venous Specimens

Authors

2016 ENA Clinical Practice Guideline Committee

Judith Young Bradford, DNS, MSN, RN, FAEN
Nancy Erin Reeve, MSN, RN, CEN
Marylou Killian, DNP, MS, RN, CEN, FNP-BC, FAEN, Chairperson
Anna Maria Valdez, PhD, MSN, RN, CEN, CFRN, CNE, FAEN, Chairperson-elect
Marsha Cooper, MSN, RN, CEN
Annie Horigan, PhD, RN
Mindi L. Johnson, DNP, RN, CPN
Stephen Stapleton, PhD, MSN, MS, RN, CEN, FAEN
Mary Alice Vanhoy, MSN, RN, CEN, CPEN, NREMT-P, FAEN
Mary Ellen Zaleski, DNP, MSN, RN, CEN

ENA 2016 Board of Directors Liaison

Jean Proehl, MN, RN, CEN, CPEN, FAEN, FAAN

2016 Staff Liaisons

Lisa Wolf, PhD, RN, CEN, FAEN, Director, IENR
Altair Delao, MPH, Senior Research Associate, IENR
Leslie Gates, Sr. Administrative Assistant, IENR

Acknowledgments

ENA would like to acknowledge the work of the 2012 Emergency Nursing Resources Development Committee for the initial development of this document. ENA also acknowledges the following members of the 2016 Institute for Emergency Nursing Research (IENR) Advisory Council for their review of this document:

Paul R. Clark, PhD, MA, RN
Hershaw Davis Jr., MSN, RN
Martha McDonald, PhD, RN, CEN, CCNS, CCRN, CNE
Michael Moon, PhD, MSN, RN, CEN, CNS-CC, FAEN
Anita Smith, PhD, RN, CNS
Kathleen Zavotsky, PhD, RN, CEN, ACNS-BC, CCRN, FAEN

Developed: December 2016


ENA's Clinical Practice Guidelines (CPGs) are developed by ENA members to provide emergency nurses with evidence-based information to utilize and implement in their care of emergency patients and families. Each CPG focuses on a clinical or practice-based issue, and is the result of a review and analysis of current information believed to be reliable. As such, information and recommendations within a particular CPG reflect the current scientific and clinical knowledge at the time of publication, are only current as of their publication date, and are subject to change without notice as advances emerge.

In addition, variations in practice, which take into account the needs of the individual patient and the resources and limitations unique to the institution, may warrant approaches, treatments, and/or procedures that differ from the recommendations outlined in the CPGs. Therefore, these recommendations should not be construed as dictating an exclusive course of management, treatment, or care, nor does the use of such recommendations guarantee a particular outcome. CPGs are never intended to replace a practitioner’s best nursing judgment based on the clinical circumstances of a particular patient or patient population. CPGs are published by ENA for educational and informational purposes only, and ENA does not “approve” or “endorse” any specific methods, practices, or sources of information. ENA assumes no liability for any injury and/or damage to persons or property arising out of or related to the use of or reliance on any CPG.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Research/Purpose Questions/Hypothesis</th>
<th>Design/Sample Setting</th>
<th>Variables/Measures Analysis</th>
<th>Findings/Implications</th>
<th>Quality of Evidence</th>
<th>Level of Evidence</th>
</tr>
</thead>
</table>
**Sample:** 50 phlebotomy episodes with 13 different staff  
**Setting:** Major emergency dept. | **Variables:** 4 ways of taking blood: cannulation and syringe, cannula with evacuated tube and adaptor, syringe and needle, evacuated tube system  
**Measures:** Observation. Measure for hemolysis not identified  
**Analysis:** Chi-square | Findings: 52% taken using syringe; 44% nonstandard tech for transfer of samples between syringe/tube; 10.7% of samples from all areas of ED were hemolysed; 24% from Majors area of ED  
**Implications:** Recommend straightforward protocols and use of phlebotomy staff in training | III | VI |
| Berger-Achituv, S., Budde-Schwartzman, B., Ellis, M. H., Shenkman, Z., & Erez, I. (2010). Blood sampling through peripheral venous catheters is reliable for selected basic analytes in children. *Pediatrics*, 126(1), e179–e186. doi:10.1542/ peds.2009-2920 | To determine the interchangeability of blood samples drawn via VP and PIV | **Design:** Prospective comparative clinical trial. “Within subjects” design (pts served as their own controls). Paired 2 mL samples drawn and analyzed for hematology and chemistry results. VP in arm opposite PIV via 23 G butterfly + 2 mL syringe for pts age 1–5 (or vacuum type for pts age 6–16). PIV samples obtained within 3 min of VP samples, drawn through 20–24 G catheters with a 2 mL syringe (after IV fluid stopped for 30 s, tourniquet applied for 30 s, and waste of 2 mL). All syringe samples transferred into vacuum tube directly (not through a needle). Samples blinded for analysis.  
**Sample:** N = 40 hemodynamically-stable pediatric patients (age 1–16) who had a functioning peripheral IV (less than 72 hr old, at least 100 mL of fluid had been infused, no signs of thrombophlebitis).  
**Setting:** Inpatient peds and peds surgical units | **Variables:** Time, patient distress – pain, crying.  
**Measures:** Hem assessed with H1 and, when it was possible to draw an additional 2 mL sample, by serum Hgb measurement. Findings assessed against CLIA standards for maximum allowable analytical error and a “clinically acceptable variance” determined by panel of 7 pediatricians or pediatric surgeons.  
**Analysis:** Power analysis indicated sample size of 28 would be adequate to determine 90% with p = 0.05. Paired t-test and Bland-Altman limits of agreement analysis. | Findings: Able to draw through PIV for 40 of 47 (85%) pts; success not related to catheter gauge. Except for glucose, hematology and chemistry results were within acceptable range. Glucose not acceptable via PIV shut off for 30 s if patient had been receiving dextrose-containing solutions. Only one VP sample had significant hem; no other samples exceeded H1 of 25 or serum Hgb of 51 mg/dL. No sig. difference in hem for VP or PIV samples (20.7 + 10 mg/dL vs. 19.6 + 10.7 mg/dL, p = 0.64). Drawing from PIV resulted in significantly less time, pt distress, pain, and crying. | I | IV |
# CLINICAL PRACTICE GUIDELINE:
Prevention of Blood Specimen Hemolysis in Peripherally-Collected Venous Specimens

## Appendix 1: Evidence Table

<table>
<thead>
<tr>
<th>Reference</th>
<th>Research/Purpose Questions/Hypothesis</th>
<th>Design/Sample Setting</th>
<th>Variables/Measures Analysis</th>
<th>Findings/Implications</th>
<th>Quality of Evidence</th>
<th>Level of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bölenius, K., Söderberg, J., Hultdin, J., Lindkvist, M., Brulin, C., &amp; Granqvist, K. (2013). Minor improvement of venous blood specimen collection practices in primary health care after a large-scale educational intervention. <em>Clinical Chemistry and Laboratory Medicine</em>, 51(2), 303–310. doi:10.1515/cclm-2012-0159</td>
<td>To determine if venous blood specimen collection practices were improved after a large-scale educational intervention</td>
<td>Design: Follow-up study; the research plan was reviewed and approved by the Regional Ethical Review Board. Sample: Pre-education, N = 6,652; post-education, N = 6,121. Two 3-month reviews were done on all lab specimens in local primary health centers, both rural and urban, in Sweden. Measures: Needle gauge was between 19 and 23; tubes were 3.5 mL evacuated serum separator test tubes with an inert polymer gel barrier and a clot activator (Becton Dickinson). After 30-minute clotting time, tubes were centrifuged locally or in a lab near a PHC. Test tubes were transported in cooled insulated boxes twice daily from the urban centers, and daily from the rural PHCs.</td>
<td>Findings: Combined results of all primary health centers had an increase in hemolysis from 10.5% to 11.8% after education. A higher incidence of hemolysis occurred post-education in the urban centers compared to rural centers. The percentage of specimens drawn by men had a higher hemolysis rate (14%) compared to those drawn by women (9.6%).</td>
<td>II</td>
<td>IV</td>
<td></td>
</tr>
<tr>
<td>Bush, R. A., Mueller, T., Sunwalt, B., Cox, S. A., &amp; Hilfiker, M. L. (2010). Assessing pediatric trauma specimen integrity. <em>Clinical Laboratory Science</em>, 23(4), 219–222.</td>
<td>To compare the quality of specimens drawn from PIV with those drawn by VP</td>
<td>Design: Prospective. Blood drawn via IV or needle (21–25 G) by RN (N = 70) or lab technician (N = 17) with syringes (3–10 mL) and transferred to vacuum tube with transfer device or directly into micro-containers. 18 months of data collected in two separate intervals. Sample: N = 221 pediatric (younger than 14 years) trauma patients. For PIV, 30% were newly inserted and 70% were inserted by EMS. Convenience. Setting: 248-bed tertiary-care pediatric hospital, Level 1 trauma center Variables: Method, type of container, personnel Measures: Hem assessed by lab techs with standardized visual hem chart Analysis: Chi-square and cross tabs analysis. Statistics via SPSS 17.</td>
<td>Findings: Overall hem = 13%. Hem more likely for PIV (16%) than VP (6%) samples (p = 0.04). No statistical association between hem and who collected sample (p = 0.07) or type of container (p = 1).</td>
<td>II Pediatric only, IV gauge not analyzed, small N</td>
<td>VI</td>
<td></td>
</tr>
</tbody>
</table>
## CLINICAL PRACTICE GUIDELINE:
Prevention of Blood Specimen Hemolysis in Peripherally-Collected Venous Specimens

### Appendix 1: Evidence Table

<table>
<thead>
<tr>
<th>Reference</th>
<th>Research/Purpose Questions/Hypothesis</th>
<th>Design/Sample Setting</th>
<th>Variables/Measures Analysis</th>
<th>Findings/Implications</th>
<th>Quality of Evidence</th>
<th>Level of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corkill, D. (2012). Testing the effects of educational toilet posters: A novel way of reducing haemolysis of blood samples within ED. Australasian Emergency Nursing Journal, 15(1), 31–36. doi:10.1016/j.aenj.2011.11.001</td>
<td><strong>Question:</strong> Does the use of educational toilet posters have an impact on the outcomes of clinical care? The aim was to investigate the effectiveness of educational posters placed in staff toilet cubicles on the rates of hemolyzed blood samples collected from patients in ED.</td>
<td><strong>Design:</strong> Quasi-experimental design/time series study/prospective</td>
<td><strong>Intervention:</strong> Poster placed in toilet stalls; hemolysis rates posted for staff</td>
<td>Findings: Pre-intervention: Hem rate 4.93% (SD = 1.05). Statistically significant ($t = 3.56$, $df = 50$, $p = 0.001$) from median post intervention data of 3.95% (SD = 0.84). Difference of 0.97% (95% CI [0.42, 1.52]) represents a 19.72% reduction in hemolysed samples over study period. Educational posters had positive impact and are efficient way of delivering ongoing education to large group.</td>
<td>II</td>
<td>IV</td>
</tr>
<tr>
<td>Dietrich, H. (2014). One poke or two: Can intravenous catheters provide an acceptable blood sample? A data set presentation, review of previous data sets, and discussion. Journal of Emergency Nursing, 40(6), 575–578. doi:10.1016/j.jen.2012.11.002</td>
<td>To show that acceptable rejection rates for hemolysis can be achieved using blood samples collected from intravenous (IV) samples</td>
<td><strong>Design/Method:</strong> Prospective over 4 months</td>
<td><strong>Measures:</strong> Samples obtained by: 1) ED staff while starting IV access; 2) Critical care, medical, or surgical nurses drawing from existing vascular access; 3) Laboratory technicians or phlebotomists by venipuncture with steel needle. Hemolysis measured by automated spectrophotometric process. Acceptable rate of sample rejections for the study defined as 2%. Took total samples collected and number rejected and presented to staff monthly for staff awareness.</td>
<td>Findings: Rejection rates for hemolysis: 1.1% collected from IV catheter start, 0.8% collected from existing vascular access, 0.1% collected by venipuncture with steel needle</td>
<td>I</td>
<td>III</td>
</tr>
</tbody>
</table>
### Appendix 1: Evidence Table

<table>
<thead>
<tr>
<th>Reference</th>
<th>Research/Purpose Questions/Hypothesis</th>
<th>Design/Sample Setting</th>
<th>Variables/Measures Analysis</th>
<th>Findings/Implications</th>
<th>Quality of Evidence</th>
<th>Level of Evidence</th>
</tr>
</thead>
</table>
| Dwyer, D. G., Fry, M., Somerville, A., & Holdgate, A. (2006). Randomized, single blinded control trial comparing haemolysis rate between two cannula aspiration techniques. *Emergency Medicine Australasia*, 18(5–6), 484–488. doi:10.1111/j.1742-6723.2006.00895.x | To compare hemolysis rates with two techniques for obtaining blood from IV catheters | **Design:** Prospective, randomized, single blinded. All samples obtained with 10 mL syringe + 18 or 20 G cannula + 21 G needle to transfer blood into vacuum tubes and sent to lab via pneumatic tube.  
**Sample:** Total N = 1,390  
Method 1: N = 694 (directly from IV);  
**Setting:** Urban tertiary hospital | **Variables:** Age, sex, cannula size, insertion site, personnel category, and ease of aspiration  
**Measures:** Spectrophotometric analysis to detect hem, graded on a 10-point scale. Grade above 2 considered hemolyzed.  
**Analysis:** Chi-square, univariate analysis on each variable, multivariate logistic regression model, SAS (8) | **Findings:** Overall hem rate: 6.8%. Ease of aspiration (p < 0.0001) was the only predictor of hem. Technique used to draw blood did not produce a statistically significant difference in hem rates. | II | II |
| Ellis, G. (2009). An episode of increased hemolysis due to a defective pneumatic air tube delivery system. *Clinical Biochemistry*, 42(12), 1265–1269. doi:10.1016/j.clinbiochem.2009.05.002 | To investigate the reason for increased hem in ED specimens compared with those from inpatient units | **Design:** Prospective and retrospective audit  
**Sample:** N = 22,363 (14,340 ED and 8,023 inpatient)  
**Setting:** Hospital — not otherwise specified | **Variables:** Status of pneumatic tube system, specimen packaging  
**Measures:** Mean hem rate; HI more than 6 on the Abbott Aeroset® analyzer  
**Analysis:** T-test, Fisher’s exact test | **Findings:** Hem specimens: ED 11.2% and inpatient 5.2% (p < 0.0001). Prior to pneumatic tube installation, ED hem rate was 3.3%. Problems found with tube and corrected (hem rate fell to 9%) and then added bubble wrap to cushion specimens during transport (rate fell to 7.1%). | II | VI |
**Sample:** 40 healthy volunteers, each giving 10 vacutainer tubes of blood via single venipuncture by phlebotomist.  
**Setting:** Large university hospital in Turkey | **Variables:** Samples from four different locations (three hospitals and one ED) sent via PTS and porter.  
**Measures:** Distance transported and speed  
**Analysis:** Nonparametric Mann-Whitney U test, correlations with Spearman’s coefficient | **Findings:** Positive correlation between distance and hemolysis in samples transported at 4.2 m/sec, and at 3.1 m/sec for more than 2,200 m (r = 0.774 and r = 0.766, respectively). Positive correlation between distance and levels of K and LDH in non-centrifuged serum samples transported at 4.2 m/sec. After centrifugation of gel-containing tubes, hemolysis rates same as reference values independent of length of PTS.  
**Conclusions:** PTS had no effect on hematolgy and coagulation results when serum was centrifuged prior to transport. | II | III |
## Appendix 1: Evidence Table

<table>
<thead>
<tr>
<th>Reference</th>
<th>Research/Purpose Questions/Hypothesis</th>
<th>Design/Sample Setting</th>
<th>Variables/Measures Analysis</th>
<th>Findings/Implications</th>
<th>Quality of Evidence</th>
<th>Level of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alamadi, Y. M., Aldeyab, M. A., McElney, J. C., Scott, M. G., Darwish Elhaggi, F. W., Magee, F. A., . . . Kearney, M. P. (2011). Clinical and economic impact of contaminated blood cultures within the hospital setting. <em>Journal of Hospital Infection, 77</em>(3), 233–236. doi:10.1016/j.jhin.2010.09.033</td>
<td>To identify factors associated with hemolysis. Design: Descriptive, cross-sectional Sample: N = 274 samples from 154 inpatients or ED patients, convenience sample Setting: Regional hospital in southern Taiwan</td>
<td>Variables: Site, tube transfer method, mixing of tubes, transport, patient sex and age, vaporization of alcohol before VP, phlebotomist skill, needle gauge, tourniquet time Measures: Subjective grading of visible hemolysis Analysis: Chi-square, Fisher’s exact, logistic regression, multiple regression, SPSS (10)</td>
<td>Findings: Sites other than antecubital fossa were 3.35 times more likely to be hemolyzed (p = 0.001); blood collected into vacuum tubes through steel needles was 8.7 times more likely to be hemolyzed than blood collected into a syringe without a needle (p = 0.01); tubes shaken or mixed 8–12 times were at higher risk of hem (p = 0.05); specimens transported by ward assistants rather than lab assistants were 8.7 times more likely to be hemolyzed (p = 0.01). Factors not significantly associated with hem: patient gender (p = 0.47), patient age (p = 0.34), vaporization of alcohol prior to venipuncture (p = 1), phlebotomist skill (p = 1; all phlebotomists were skilled), time used for tourniquet (p = 0.337), needle.</td>
<td>I</td>
<td>VI</td>
<td></td>
</tr>
</tbody>
</table>
## CLINICAL PRACTICE GUIDELINE:
Prevention of Blood Specimen Hemolysis in Peripherally-Collected Venous Specimens

### Appendix 1: Evidence Table

<table>
<thead>
<tr>
<th>Reference</th>
<th>Research/Purpose Questions/Hypothesis</th>
<th>Design/Sample Setting</th>
<th>Variables/Measures Analysis</th>
<th>Findings/Implications</th>
<th>Quality of Evidence</th>
<th>Level of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halm, M. A., &amp; Gleaves, M. (2009). Obtaining blood samples from peripheral intravenous catheters: Best practice? <em>American Journal of Critical Care, 18</em>(5), 474–478. doi:10.1111/j.1365-2702.2006.02057.x</td>
<td>Synthesize evidence related to the effect of collecting laboratory specimens from intravenous catheters</td>
<td>Design: Systematic review included observational, descriptive, comparative, and experimental studies in the review. Included research and manufacturers’ evidence. Sample: N = 8 studies</td>
<td>Variables: Four studies compared specimens collected from PIV to VP, one examined size of specimen collection tube, and three studies investigated the reason for hemolysis. Measures: In most studies, visual inspection was the method used to detect hem. Analysis: Not applicable</td>
<td>Findings: Rates of hemolysis varied considerably depending on sample collection method: collected from IV lines (3%–77%); collected from the IV using a vacuum tube (5.6%–77%); collected from an IV using a syringe (12.8%–49%); collected from a new IV (12.8%–49%), and 24% from established catheters. Samples collected by VP had less variability (0%–3.8%). Hem was higher in ED compared to medical units (used trained phlebotomists). Factors associated with less hemolysis: collecting from patient’s dominant extremity, antecubital space. Results from studies examining tube size were mixed and no conclusion could be drawn. Partial catheter obstructions were thought to increase hemolysis. Education decreased hemolysis. Under-filled tubes transported by pneumatic tube delivery system were associated with higher rates of hemolysis. The antecubital fossa was the preferred site for specimen collection.</td>
<td>I</td>
<td>V</td>
</tr>
</tbody>
</table>

Note: There is a large crossover with the articles analyzed in Heyer et al. (2012). However, Heyer et al. (2012) do not address education as a factor. Therefore, this can be considered as a source of evidence for education.
### Appendix 1: Evidence Table

<table>
<thead>
<tr>
<th>Reference</th>
<th>Research/Purpose Questions/Hypothesis</th>
<th>Design/Sample Setting</th>
<th>Variables/Measures Analysis</th>
<th>Findings/Implications</th>
<th>Quality of Evidence</th>
<th>Level of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harrison, G., Speroni, K. G., Dugan, L., &amp; Daniel, M. G. (2010). A comparison of the quality of blood specimens drawn in the field by EMS versus specimens obtained in the emergency department. <em>Journal of Emergency Nursing</em>, 36(1), 16–20. doi:10.1016/j-jen.2008.11.001</td>
<td>To compare rates of specimen redraw for EMS- and ED-obtained specimens</td>
<td>Design: Prospective, observational</td>
<td><strong>Variables</strong>: Phlebotomist type, IV site, IV size, number of IV attempts, redraw reason, phlebotomist exposure to blood, patient’s diagnosis and length of stay, patient’s dominant arm</td>
<td><strong>Findings</strong>: Redraw rates were lower for EMS (9.5%) compared with ED (11.5%), but this was not statistically significant (p = 0.8837). The majority of EMS redraws were for insufficient amount (52.6%, ED = 8.7%, p = 0.0133). The majority of the redraws in the ED were for hem (52.2%, EMS = 31.6%, p = 0.1828). Redraws for clotted specimens, critical values were not significant. Phlebotomist type, number of attempts to collect specimen, reason specimen not drawn by EMS, reason EMS specimen discarded, and reason for redraw were not statistically significant. EMS drew the majority of specimens from the left antecubital (52.5%, ED = 23.4%, p &lt; 0.0001), ED used the right antecubital (34.5%). EMS used 18 G IV catheters most often (70%, ED = 35.9%, p = 0.0001). The ED used 20 G IV catheters (46.9%, EMS = 25.5%, p = 0.0799). The majority of the patients were right-hand dominant in both groups (EMS = 90%, ED = 62.5%, p = 0.0001). Patient hand dominance was not related to specimen quality. There was no statistical difference in hemolysis rates for EMS and ED. Specimens drawn by EMS were associated with a faster throughput time (17 min shorter).</td>
<td>II</td>
<td>VI</td>
</tr>
</tbody>
</table>

*Did not include patients younger than 18, patients who died, or those not arriving by EMS*
<table>
<thead>
<tr>
<th>Reference</th>
<th>Research/Purpose Questions/Hypothesis</th>
<th>Design/Sample Setting</th>
<th>Variables/Measures Analysis</th>
<th>Findings/Implications</th>
<th>Quality of Evidence</th>
<th>Level of Evidence</th>
</tr>
</thead>
</table>
**Sample:** N = 111,780 (43% inpts, 26% outpts, 9% ED pts)  
**Setting:** 1,400-bed acute care hospital | **Variables:** HI, age, gender, location of specimen acquisition  
**Measures:** HI measured by spectrophotometry  
**Analysis:** Logistic regression for age and gender analysis. SPSS v. 12 | Findings: Hem (HI more than 3) = 2.4 % overall; ED = 8.2%, inpatient = 3.3%; outpatient = 0.6%. Volume of specimens drawn in outpatient settings higher for sites collecting more than 500 specimens/month except in infectious disease sites, Heme/onc or infusion sites, and private-pay-status pts. No relationship between hem and age or sex. | III | VI |
**Sample:** Serum collected using syringe and vacuum with reported 154–297 samples for each intervention that lasted one week  
**Setting:** Emergency department of VieCuri Medical Centre (Venlo, The Netherlands) | **Measures:** First week, serum collected with syringe (7.5 mL serum gel, ref 01.1602, Sarstedt, Nürnberg, Germany); Second week, serum collected with vacuum tubes (5 mL serum gel, reference 367955, Becton, Dickinson & Co, Franklin Lakes, NY). After switching, two-month acquaintance period allowed before analyzing hemolysis.  
**Statistical Analysis:** Hemolysis measured spectrophotometrically; three hemolysis index cut-off values used to express results: 0.5, 1.0, and 2.0. A bias of less than 10% as a result of hemolysis is accepted without notification (5% in case of K). Deviation greater than 20% resulted in sample rejection (10% with K). Fisher’s exact test (1 tailed) compared hemolysis rates between groups with hemolysis index cut-off value of 0.5. (p < 0.05) | Findings: 16% had substantial hemolysis, rates increased significantly after introducing vacuum tubes (5 mL Lur-slip, 24%, p = 0.045) or low vacuum tube (12%, p < 0.001). No significant difference between use of discard tube and use of low vacuum tube (p = 0.07). None could lower the hemolysis rate to level observed with only straight needle venipuncture (average 3%, p = 0.02 or less). | II | III |
<table>
<thead>
<tr>
<th>Reference</th>
<th>Research/Purpose Questions/Hypothesis</th>
<th>Design/Sample Setting</th>
<th>Variables/Measures Analysis</th>
<th>Findings/Implications</th>
<th>Quality of Evidence</th>
<th>Level of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heyer, N. J., Derzon, J. H., Winges, L., Shaw, C., Mass, D., Snyder, S. R., . . Liebow, E. B. (2012). Effectiveness of practices to reduce blood sample hemolysis in EDs: A laboratory medicine best practices systematic review and meta-analysis. <em>Clinical Biochemistry, 45</em>(13–14), 1012–1032. doi:10.1016/j.clinbiochem.2012.08.002</td>
<td>Review of ED practices for reducing hemolysis in blood samples sent to lab</td>
<td>Design: Evidence review followed CDC-sponsored Laboratory Medicine Best Practices Initiative’s “A 6-cycle prospective systematic review method” for evaluating quality improvement practices</td>
<td>Meta-analysis</td>
<td>Findings: 11 studies used straight needle venipuncture and achieved 84% reduction in hemolysis (RR = 0.16, 95% CI [0.11, 0.24]); 4 studies used antecubital site and reduced hemolysis 55% (RR = 0.45, 95% CI [0.35, 0.57]); 3 studies used syringe vs. vacuum tubes — data insufficient. The meta-analysis of 3 studies with use of 21 G needles and below showed mean risk was substantial (RR = 0.37, 95% CI [0.27, 0.52]), equal to approx. 63% reduction in hemolysis, individual study effect size inconsistent, overall strength of evidence insufficient. Two studies used low (partial) vacuum tubes: meta-analysis reduction in hemolysis of approx. 89% (RR = 0.11, 95% CI [0.02, 0.52]) results consistent and suggestive that use of partial vacuum tubes reduces hemolysis. Evidence of tourniquet time: no studies found in ED — withdrawn from analysis. Implications: Use straight needles for venipuncture, drawing blood from IV start — place at antecubital site rather than distal site. Use of low vacuum tubes is suggestive. Insufficient data for use of 21 G or smaller needles.</td>
<td>I</td>
<td>I</td>
</tr>
</tbody>
</table>
## Appendix 1: Evidence Table

<table>
<thead>
<tr>
<th>Reference</th>
<th>Research/Purpose Questions/Hypothesis</th>
<th>Design/Sample Setting</th>
<th>Variables/Measures Analysis</th>
<th>Findings/Implications</th>
<th>Quality of Evidence</th>
<th>Level of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lippi, G., Avanzini, P., Aloe, R., &amp; Cervellin, G. (2013). Reduction of gross hemolysis in catheter-drawn blood using Greiner Holdex® tube holder. Biochemia Medica, 23(3), 303–307. doi:10.11613/BM.2013.036</td>
<td>Does use of the Holdex phlebotomy system decrease hemolysis when blood is drawn from an existing intravenous line?</td>
<td><strong>Design:</strong> Randomized prospective study; informed consent by each participant; IRB approval  <strong>Setting:</strong> Large urban ED</td>
<td>Same two nurses drew all lab samples through an inserted 20 G IV catheter. For even-numbered patients, first and second tubes were drawn with a BD vacutainer, the third tube was drawn with a Holdex; for odd-numbered patients, the first two tubes were drawn with Holdex, and the third was drawn with BD.</td>
<td>All tests performed in duplicate. CI = 95%; p = 0.05. Use of Holdex may be mildly effective in decreasing hemolysis when samples are drawn from an indwelling IV catheter.</td>
<td>I</td>
<td>IV</td>
</tr>
<tr>
<td>Lippi, G., Cervellin, G., &amp; Mattiuzzi, C. (2013). Critical review and meta-analysis of spurious hemolysis in blood samples collected from intravenous catheters. Biochemia Medica, 23(2), 193–200. doi:<a href="http://dx.doi.org/10.11613/BM.2013.022">http://dx.doi.org/10.11613/BM.2013.022</a></td>
<td>To estimate the risk of spurious hemolysis in blood samples collected from intravenous catheters</td>
<td><strong>Design:</strong> Meta-analysis with calculation of odds ratio (OR) and relative risk (RR) along with 95% confidence interval using random effect mode</td>
<td>Included 17 studies: 14,796 patients in 13 studies assessing catheters and evacuated tubes vs. straight needle and evacuated tubes; 1,251 patients in four studies assessing catheter and evacuated tubes vs. catheter and manual aspiration of blood</td>
<td>Significant risk of hemolysis found in studies assessing catheter and evacuated tubes vs. straight needle and evacuated tubes (random effect OR 3.4; 95% CI [2.9, 3.9] and random effect RR 1.07; 95% CI [1.06, 1.08]). In studies assessing catheter and evacuated tubes vs. catheter and manual aspiration of blood (OR 3.7; 95% CI [2.7, 5.1] and RR 1.32; 95% CI [1.24, 1.40]).  <strong>Conclusion:</strong> Higher risk of hemolysis when sample collected through intravenous catheter compared with standard blood drawn by straight needle.</td>
<td>I</td>
<td>I</td>
</tr>
</tbody>
</table>
### Appendix 1: Evidence Table

<table>
<thead>
<tr>
<th>Reference</th>
<th>Research/Purpose Questions/Hypothesis</th>
<th>Design/Sample Setting</th>
<th>Variables/Measures Analysis</th>
<th>Findings/Implications</th>
<th>Quality of Evidence</th>
<th>Level of Evidence</th>
</tr>
</thead>
</table>
| Lippi, G., Avanzini, P., Aloe, R., & Cervellin, G. (2014). Blood collection from intravenous lines: Is one drawing site better than others? Laboratory Medicine, 45(2), 172–175. doi:10.1309/LM2XCV5SQMLION™ | Establish whether hemolysis in samples collected from intravenous lines is influenced by catheterization site | **Design**: Prospective. IRB: Followed state ethical standards as established by institution  
**Sample**: N = 67  
**Setting**: ED, Academic Hospital of Parma, Italy | **Measures**: Blood collected by 20 G catheter into an evacuated blood tube. Hemolysis index measured by multi-wavelength photometric readings. Sample collection: first tube discarded, second tube sent to lab. Hemolysis defined as cell-free hemoglobin greater than 0.5 g/L.  
**Statistical Analysis**: Wilcoxon-Mann-Whitney test (continuous variables), Chi-square test (categorical variables). Relative risk calculated using MedCalc Version. | **Findings**: Overall frequency of hemolysis: 30% (20/67). Concentration of cell-free hemoglobin in samples from median cephalic and basilic veins did not differ significantly but was significantly lower than samples from basilic and metacarpal plexus. Median cephalic vein cell-free hemoglobin 0.1 g/L (95% CI [0.0–0.2 g/L]); median basilic vein 0.1 g/L (95% CI [0.0–0.3 g/L]); median antebrachial vein 0.2 g/L (95% CI [0.0–0.5 g/L]); cephalic vein 0.1 g/L (95% CI [0.0–0.3 g/L]); basilic vein 0.8 g/L (95% CI [0.0–2.5 g/L]); and metacarpal plexus 1.5 g/L (95% CI [0.2–2.8 g/L]).  
**Conclusions**: “Drawing blood from catheters placed distally from median veins carries higher hemolysis risk” (p. 172). | II | III |
**Sample**: N = 80 ED staff  
**Setting**: 29-bed ED, Midwestern academic medical center, Level I adult/pediatric | **Variables**: Individual staff members  
**Measures**: Calculation of hemolysis rates of each staff member | **Findings**: Rates dropped from 14% to 7%; publicizing performance rates by individual nurses led to decreased hem. Rates climbed after posting stopped, but decreased within a month of reposting rates. Display of rates encouraged staff to review technique and literature to lower individual hem rates. | III | VI |
## CLINICAL PRACTICE GUIDELINE:
Prevention of Blood Specimen Hemolysis in Peripherally-Collected Venous Specimens

### Appendix 1: Evidence Table

<table>
<thead>
<tr>
<th>Reference</th>
<th>Research/Purpose Questions/Hypothesis</th>
<th>Design/Sample Setting</th>
<th>Variables/Measures Analysis</th>
<th>Findings/Implications</th>
<th>Quality of Evidence</th>
<th>Level of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ong, M. E. H., Chan, Y. H., &amp; Lim, C. S. (2008). Observational study to determine factors associated with blood sample haemolysis in the emergency department. <em>Annals of the Academy of Medicine, Singapore, 37</em>(9), 745–748.</td>
<td>To determine which factors in blood sampling were associated with increased rates of hem</td>
<td><strong>Design</strong>: Prospective. Questionnaires distributed to phlebotomists (nurses, physicians, nursing and medical students) about method used to collect samples; outcome data from laboratory.</td>
<td><strong>Variables</strong>: Operator, rate of blood flow, difficulty of cannulation or VP, arterial vs. venous sample, specimen volume, processing interval, needle size, phlebotomist category, steel needle vs. IV catheter, vacuum tube vs. syringe</td>
<td><strong>Findings</strong>: 19.8% (45 of 227) samples hem. The following factors did not significantly increase hem (p &gt; 0.05): operator, rate of blood flow, difficulty of cannulation or VP, arterial vs. venous sample, specimen volume, processing interval, needle size, phlebotomist category. Vacuum tube system highest rates of hem (OR = 6, 95% CI [2.3, 15.1]). Does not address steel needle vs. IV catheter in logistic regression; probably means the vacuum tube is responsible for the difference seen there.</td>
<td>III</td>
<td>VI</td>
</tr>
<tr>
<td>Ong, M. E. H., Chan, Y. H., &amp; Lim, C. S. (2009). Reducing blood sample hemolysis at a tertiary hospital emergency department. <em>The American Journal of Medicine, 122</em>(11), 1054.e1–1054.e6. doi:10.1016/j.amjmed.2009.04.024</td>
<td>To determine causes for hemolysis and measure the effect of an intervention to decrease hemolysis</td>
<td><strong>Design</strong>: Phase I — 2008 study. Phase II — 15-min educational program. After Phase I, questionnaires distributed to phlebotomists (physicians and medical students who do most of phlebotomy in ED); outcome data from lab. Educational program emphasized a preference for syringes instead of vacuum tubes, direct VP instead of PIV or a-line, sufficient sample volume, quickly sending of sample to lab, larger bore needles.</td>
<td><strong>Variables</strong>: Method, system, needle gauge, personnel, blood flow, difficulty of VP, source, sample volume, time sample obtained, time sample processed</td>
<td><strong>Findings</strong>: Educational program led to changes in operator behavior (increased use of syringes) and a decrease in hem from 19.8% to 4.9% (p &lt; 0.001). Adjusted OR for Phase I (2008 study) shows only vacuum tube use (OR 4.1; 95% CI [1.8, 9.4]) caused significantly more hem. Adjusted OR for IV catheters not sig. (OR 3.5, 95% CI [0.9, 13.2]).</td>
<td>III</td>
<td>VI</td>
</tr>
</tbody>
</table>
### Reference


### Appendix 1: Evidence Table

<table>
<thead>
<tr>
<th>Reference</th>
<th>Research/Purpose Questions/Hypothesis</th>
<th>Design/Sample Setting</th>
<th>Variables/Measures Analysis</th>
<th>Findings/Implications</th>
<th>Quality of Evidence</th>
<th>Level of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raisky, F., Gauthier, C., Marchal, A., &amp; Blum, D. (1994). Haemolyzed samples: Responsibility of short catheters. <em>Annales de Biologie Clinique, 52</em>(7–8), 523–527.</td>
<td>To compare rates of hem for stainless steel needles, Teflon FEP catheters, and polyurethane Vialon™ catheters</td>
<td>Design: Randomized, prospective Sample: N = 295 (ages 1–95 years); 95 samples collected by Terumo Venoject needle, 100 collected by Teflon FEP, 100 collected by Vialon™ Insyte catheter Setting: ED</td>
<td>Variables: Catheter material Measures: Hemolysis measured by photometry; laboratory personnel blinded to collection method Analysis: One-way ANOVA by ranks, Kruskal-Wallis test</td>
<td>Findings: “Highly significant relationship (p &lt; 0.000) between the occurrence and degree of hemolysis … and the material used ….” (p.526). Hem less likely (12%) when blood collected with needle vs. short IV catheter. Between the two IV catheters, less hem occurred with the Teflon FEP (42%) than with the Vialon™ (Insyte) catheter (55%) (p &lt; 10⁻⁶). When limited to hem greater than 1.5 g/dL (threshold for assay error), differences were even larger: needle = 4.2%, Teflon = 9%, Vialon = 30%</td>
<td>I</td>
<td>II Note: Included in Heyer et al. (2012) systematic review. However, Heyer et al. (2012) did not address needle composition, only straight needle vs. IV start.</td>
</tr>
<tr>
<td>Saleem, S., Mani, V., Chadwick, M. A., Creanor, S., &amp; Ayling, R. M. (2009). A prospective study of causes of haemolysis during venipuncture: Tourniquet time should be kept to a minimum. <em>Annals of Clinical Biochemistry, 46</em>(Pt. 3), 244–246. doi:10.1258/acb.2009.008228</td>
<td>To assess incidence of hem and any factors associated with it</td>
<td>Design: Prospective; pro forma used to gather data about each phlebotomy; hem data from hospital computer system Sample: N = 353 blood samples Setting: 26 locations within a hospital</td>
<td>Variables: Tourniquet time, person collecting, method of collection, number of attempts, transport method Measures: Automated HI determination Analysis: Chi-square via StatXact 8; logistic regression via SPSS v 15, odds ratios</td>
<td>Findings: 6.5% hem overall; increased risk of hem with tourniquet time greater than 1 min (p &lt; 0.001); OR for hem with tourniquet time greater than 1 min = 19.5 (95% CI [5.6, 67.4%]). Not significant: person collecting, method of collection, number of attempts, transport method.</td>
<td>VI</td>
<td></td>
</tr>
</tbody>
</table>
## Evidence Table

<table>
<thead>
<tr>
<th>Reference</th>
<th>Research/Purpose Questions/Hypothesis</th>
<th>Design/Sample Setting</th>
<th>Variables/Measures Analysis</th>
<th>Findings/Implications</th>
<th>Quality of Evidence</th>
<th>Level of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seguin, D., McEachrin, C., &amp; Murphy, T. (2004). Venipuncture equipment, technique, and hemolysis of laboratory blood samples obtained in the emergency department. <em>Journal of Emergency Nursing, 30</em>(5), 418. doi:10.1016/j.jen.2004.07.036</td>
<td>To identify venipuncture equipment and techniques associated with hemolysis of blood samples obtained in ED</td>
<td>Design: Prospective cross-sectional</td>
<td>Variables: Technique type and equipment size used</td>
<td>Findings: Overall hem rate = 6.1%. No statistical difference (p = 0.645) between syringe and vacuum tube technique. Drip method of filling tubes removed due to small sample size. Significant difference between smaller (23 and 25 G) and larger (less than 23 G) butterfly needles (p &lt; 0.001). Significant differences (p &lt; 0.001) between smaller (22 and 24 G) and larger (less than 22 G) IV catheters. Excluded hem samples done by straight needle draw.</td>
<td>II Limited information available; abstract only</td>
<td>VI</td>
</tr>
<tr>
<td>Schwarzer, B. A., McWilliams, L., Devine, K., &amp; Sesok-Pizzini, D. A. (2001). Increased number of hemolyzed specimens from the emergency department and labor and delivery with use of IV safety catheters. <em>Transfusion, 41</em>, 138S-139S.</td>
<td>To determine the effect of IV safety catheters on hemolysis</td>
<td>Design: Prospective, survey submitted with each sample sent to lab</td>
<td>Variables: Type of tube (full vs. soft draw), method of draw, gauge of catheter (18 vs. 20)</td>
<td>Findings: Overall 9.5% hem. Of the hem specimens, 88.5% had been drawn via 18 G (26.9%) or 20 G (61.5%) IV. Increased hem in specimens drawn from IV, from 20 G catheters vs. 18 G catheters, full draw red-top tubes were more likely to hem (21.7 %) than heparinized soft draw tubes (2.9 %).</td>
<td>III Abstract only, limited information available; small N; statistical analysis not described</td>
<td>VI</td>
</tr>
<tr>
<td>Reference</td>
<td>Research/Purpose Questions/Hypothesis</td>
<td>Design/Sample Setting</td>
<td>Variables/Measures Analysis</td>
<td>Findings/Implications</td>
<td>Quality of Evidence</td>
<td>Level of Evidence</td>
</tr>
<tr>
<td>-----------</td>
<td>--------------------------------------</td>
<td>-----------------------</td>
<td>-----------------------------</td>
<td>-----------------------</td>
<td>---------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Sharp, M. K., &amp; Mohammad, S. F. (1998). Scaling of hemolysis in needles and catheters. <em>Annals of Biomedical Engineering, 26</em>(5), 788–797. doi:10.1114/165</td>
<td>To identify needle and cannula factors associated with shear which causes hem</td>
<td><strong>Design</strong>: In vitro study with fresh human blood forced through a syringe inside a pressure chamber through 14, 18, and 22 G catheters (both Teflon and polyurethane) and 304 stainless steel needles (all needles 40 mm long). Samples tested in random order. <strong>Sample</strong>: N = 5 volunteers donated the blood; 270 hem measurements for pressure difference; testing each gauge with three factors (54 total measurements) <strong>Setting</strong>: Research laboratory</td>
<td><strong>Variables</strong>: Needle gauge, catheter/needle composition <strong>Measures</strong>: Hemolysis measured by spectrophotometer. <strong>Analysis</strong>: ANOVA</td>
<td><strong>Findings</strong>: Hem increased with pressure difference and cannula diameter; no consistent trend re cannula material. However, the pressure differences required to produce hemolysis were higher than typical for clinical venipunctures. There is substantial variability in risk of hemolysis between individual subjects.</td>
<td>III</td>
<td>III</td>
</tr>
<tr>
<td>Sharp, M. K., &amp; Mohammad, S. F. (2003). Hemolysis in needleless connectors for phlebotomy. <em>American Society for Artificial Internal Organs Journal, 49</em>(1), 128–130. doi:10.1097/00002480-200301000-00021</td>
<td>To evaluate hemolysis associated with needleless connectors</td>
<td><strong>Design</strong>: In vitro study, fresh human blood drawn first into vacuum tubes (10 mL red top) and then into syringes (10 mL) without a connector and also through two needleless connectors using 16, 18, and 22 G catheters (all needles 40 mm long). Samples tested in random order. <strong>Sample</strong>: N = 5 volunteers <strong>Setting</strong>: Research laboratory</td>
<td><strong>Variables</strong>: Brand of needleless connector, needle gauge, vacuum tube vs. syringe <strong>Measures</strong>: Hem determined with spectrophotometer <strong>Analysis</strong>: ANOVA</td>
<td><strong>Findings</strong>: Increased hemolysis with needleless connectors; hemolysis greater with Posi-Flow than with Clave (p = 0.0008). 18 G caused the most hemolysis and 22 G the least (p = 0.008). Vacuum tubes caused less hemolysis than syringes (p = 0.023). Substantial variability between subjects (p &lt; 0.0001). No combination of connector, needle size, or device produced clinically significant hemolysis.</td>
<td>III</td>
<td>III</td>
</tr>
</tbody>
</table>
### Reference


### Research/Purpose Questions/Hypothesis

To evaluate the effect of unit age, gender on the prevalence of hem, using HI as an indicator of hem; identify differences in preanalytical quality

To determine if transport via pneumatic tube system was associated with hemology

### Design/Sample Setting

**Design:** Retrospective

**Sample:** N = 9,504 (primary care centers, 8,849; nursing homes, 208; ED, 447)

**Setting:** Various healthcare settings in Sweden

**Design:** Two tubes drawn, one hand-carried to the lab (HC), one sent via pneumatic tube (PT). Hem (visible discoloration of supernatant) determined by technologist blinded to specimen transport method.

**Sample:** N = 291 specimens

**Setting:** ED, urban academic medical center

### Variables/Measures Analysis

**Variables:** Age, sex, unit location

**Measures:** Vitros 5.1 automated analyzer (Ortho-Clinical Diagnostics Inc., Rochester, NY, USA). HI greater than 15 was considered hem.

**Analysis:** Chi-square, multivariate logistic regression

**Variables:** Method of transport, underlying medical condition

**Measures:** Hem determined by visual discoloration of supernatant by blinded laboratory technologist

**Analysis:** McNemar’s test for paired samples or Pearson’s chi-square; study powered to detect a 15% difference in hem rates

### Findings/Implications

**Findings:** Hem 1.7 times more often in primary health centers (PHCs) outside the urban area than in those close to the lab (95% CI [4.9, 2]). The PHCs had fewer hem specimens compared to ED (10.4% vs. 31.1%, p < 0.001). Within the ED, samples from the section staffed by an emergency medicine physician were hem 4.3 times more often (95% CI [2, 9.4]) than the section with the PHC physician. For all samples (N = 9,504), pts older or younger than the median age (63) were associated with higher rates of hem (p < 0.004). Men more likely to have hem sample (13.1% vs. 10.1%; p < 0.001). For ED samples (N = 447), no sig. difference with regard to sex or age.

**Findings:** 87 hem samples, 47 HC and 40 PT; 26 pts with both HC and PT samples hem; 21 pts with HC but not PT; 14 pts with PT but not HC. Pts with both tubes hem had higher incidence of sig. med condition (4/26) than those who only had one tube hem (1/35). No statistically significant difference in hem between HC and PT specimens.

### Quality of Evidence

**Quality of Evidence:** II

**Level of Evidence:** VI

**ED collection by IV, venipuncture, other areas by venipuncture alone; far more samples from non-ED locations than ED

**Relatively small N; no specifics about tube system given (connection points, etc.); tubes were from same phlebotomy — does not account for possibility that only some tubes from same phlebotomy may be hem
## CLINICAL PRACTICE GUIDELINE:
Prevention of Blood Specimen Hemolysis in Peripherally-Collected Venous Specimens

### Appendix 1: Evidence Table

<table>
<thead>
<tr>
<th>Reference</th>
<th>Research/Purpose Questions/Hypothesis</th>
<th>Design/Sample Setting</th>
<th>Variables/Measures Analysis</th>
<th>Findings/Implications</th>
<th>Quality of Evidence</th>
<th>Level of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stauss, M., Sherman, B., Pugh, L., Parone, D., Looby-Rodriguez, K., Bell, A., &amp; Reed, C. R. (2012). Hemolysis of coagulation specimens: A comparative study of intravenous draw methods, <em>Journal of Emergency Nursing</em>, 38(1), 15–21. doi:10.1016/j-jen.2010.08.011</td>
<td>To compare hem rates of samples from IV catheter hub with draw from extension tubing, and to determine if investigators could predict whether a coagulation sample was hemolyzed based on visual observation during withdrawal process</td>
<td>Design: Prospective, two-group randomized comparative: Group 1 obtained after insertion of IV cath; Group 2 with extension tubing connected to IV cath hub and Clave®; 20 G IV catheter for all</td>
<td>Variables: Draw from hub vs. through extension tubing, difficulty of insertion, ease of draw, blood flow</td>
<td>Findings: No significant difference in hem rates of two groups (31.67% vs. 30%; p = 0.84). Nurses more likely to predict sample hemolyzed when it was not and think not hemolyzed when was (p &lt; 0.001). Significant difference in nurse perception of difficulty of IV catheter insertion, blood flow associated with hem (p = 0.00621).</td>
<td>II</td>
<td>II</td>
</tr>
<tr>
<td>Streichert, T., Otto, B., Schnabel, C., Nordholt, G., Haddad, M., Maric, M., . . . Wagener, C. (2011). Determination of hemolysis thresholds by the use of data loggers in pneumatic tube systems. <em>Clinical Chemistry</em>, 57(10), 1390–1397. doi:10.1373/clinchem.2011.167932</td>
<td>To compare hem rates between specimens transported by pneumatic tube and those that were hand-carried</td>
<td>Design: Prospective; two samples from same venipuncture by same physician, one hand-carried, one sent via pneumatic tube; samples drawn in randomized sequence, with mini-data logger for temperature, humidity, pressure, and acceleration in combination with hematological parameters, standard clinical chemistry analyses, blood coagulation, erythrocyte sedimentation rate, and blood gas analysis</td>
<td>Variables: Method of transport</td>
<td>Findings: No significant differences in temperature, humidity, or pressure between methods of transport; major differences in three-axis accelerations; positive correlation between pneumatic tube speed/accelerations and degree of hem; p values critical for K, phosphate, ASAT, and LDH</td>
<td>III</td>
<td>VI</td>
</tr>
</tbody>
</table>

**Note:**
- Insertion sites not specified; draw techniques, number of IV insertion attempts not controlled; visual scale for hemolysis not routinely used by laboratory.
- Did not actually measure hem, used changes in K, LDH, and ASAT as markers of hem.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Research/Purpose Questions/Hypothesis</th>
<th>Design/Sample Setting</th>
<th>Variables/Measures Analysis</th>
<th>Findings/Implications</th>
<th>Quality of Evidence</th>
<th>Level of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamechika, Y., Iwatani, Y., Tohyama, K., &amp; Ichihara, K.</td>
<td>To assess the effect of vacuum persistence on elevations in LDH</td>
<td>Design: Experimental. Experiment 1: 8 mL blood from 10 subjects, 6 mL placed tube with serum separator gel and volume of 6 mL drawn; remaining 2 mL placed in another serum separator vacuum tube with draw volume 9 mL (7 mL unfilled). Experiment 2: Time between sampling and centrifugation varied between 60 and 240 min; tubes caps removed or left on; matched pairs centrifuged after 60, 120, 240 min with or without rubber caps</td>
<td>Variables: Fill volume, time, +/- tube cap during centrifugation Measures: Hem determined by urine sticks Analysis: Paired t-test for quantitative assays, Wilcoxon signed rank test for non-quantitative measurement, three-way ANOVA for influence of vacuum exposure and centrifugation</td>
<td>Findings: Experiment 1: LDH, AST, K significantly elevated (p &lt; 0.01). Experiment 2: level of hem depends on length of exposure to vacuum before centrifugation. No statistical difference in level of hem with or without tube cap during centrifugation. Conclusions: Sample volume should match draw volume of vacuum tube; length of exposure increases fragility of red blood cell membranes, increasing hem when tube not fully filled; for partially filled tubes, the cap should be removed as soon as possible</td>
<td>IV</td>
<td>VI</td>
</tr>
<tr>
<td>Tanabe, P., Kyriacou, D. N., &amp; Garland, F. (2003).</td>
<td>To determine the effect of various risk factors on the hem of blood bank specimens</td>
<td>Design: Prospective cohort; RN or tech determined method of draw, gauge of needle or catheter, anatomic location of draw Sample: N = 605; ED and labor and delivery patients needing type and cross tube drawn; convenience sample Setting: Urban university medical center</td>
<td>Variables: Patient demographics, type and gauge of needle or catheter, vacuum tube vs. syringe, venipuncture site, patient care area Measures: Blood bank technicians visually determined hem Analysis: Cox proportional hazards multivariate regression modeling</td>
<td>Findings: Overall hem = 7%; Vialon catheter = 10%; steel needle = 1.5%; increased risk of hem for samples drawn from Vialon catheters (RR 6.73; 95% CI [2.08, 21.8]; p = 0.001); Vialon catheter 20 G or smaller (RR 7.42; 95% CI [1.8, 30.52]; p = 0.005) or at sites other than AC (RR 2.61; 95% CI [1.39, 4.9]; p = 0.003).</td>
<td>II</td>
<td>IV</td>
</tr>
</tbody>
</table>

**Reference**
# CLINICAL PRACTICE GUIDELINE:
Prevention of Blood Specimen Hemolysis in Peripherally-Collected Venous Specimens

## Appendix 1: Evidence Table

<table>
<thead>
<tr>
<th>Reference</th>
<th>Research/Purpose Questions/Hypothesis</th>
<th>Design/Sample Setting</th>
<th>Variables/Measures Analysis</th>
<th>Findings/Implications</th>
<th>Quality of Evidence</th>
<th>Level of Evidence</th>
</tr>
</thead>
</table>
| Tiwari, A. K., Pandey, S., Dixit, S., & Raina, V. (2012). Speed of sample transportation by a pneumatic tube system can influence the degree of hemolysis. Clinical Chemistry and Laboratory Medicine, 50(3), 471–474. doi:10.1515/CCLM.2011.779 | To find the incidence of hemolysis in samples transported through a pneumatic tube system (PTS) at different speeds | Design: Prospective observational study. IRB: Yes  
Sample: Short distance, 52; Long distance and high speed, 215; short distance and slow speed, 45  
Setting: Medanta, The Medicity Hospital, National Capital Region, New Delhi, India | Measures: All indices of hemolysis analyzed using paired t-test (95% confidence interval) to compare samples transported via PTS with those transported by human courier | Findings: Short distance and high speed: LD elevated in PTS arm and not in human courier. Long distance and high speed: all three indices of hemolysis — supernatant Hb, K, and LD — statistically significantly elevated in the PTS arm and not in the human courier arm. Short distance and slow speed: no hemolysis in any of three indices of hemolysis (p < 0.05).  
Conclusions: Each hospital should validate the PTS at the center and avoid hemolysis by interventions like reducing speed. | I | III |
Sample: N = 49  
Setting: Academic medical center, Austria | Variables: Sample type  
Measures: Laboratory equipment measured HI, fHb  
Analysis: Univariate ANOVA, correlation coefficients | Findings: Mean (SD) HI did not differ between the two materials (153 [173] for EDTA and 173 [225] for Li-heparinate). R values between fHb and HI were 0.939 in EDTA and 0.967 in Li-heparinate. EDTA influences fHb and HI in a concentration-dependent manner, but the effect can be disregarded when collection tubes are correctly filled.  
Letter to the editor, limited information available, small N | III | VI |
### Reference

### Research/Purpose Questions/Hypothesis
To identify the smallest number of remediable factors that independently increase the risk of hemolysis and to design an effective strategy to address the issue. Hypothesis: There is a strong independent association between hemolysis rate and phlebotomy device.

### Design/Sample Setting
**Design/Method:** Prospective, observational, cross-sectional study
**IRB:** Yes — expedited review
**Sample:** N = 4,513
**Setting:** Urban, academic ED, Albert Einstein Medical Center, Bronx, NY

### Variables/Measures Analysis
**Variables**:
- Rate of hemolysis was determined by measurement of free serum hemoglobin levels. A hemolysis index of 150 or higher used to define hemolysis for the study.
- Characteristics of blood draw recorded on standardized collection sheet by person drawing blood: device, bore size of needle or catheter, site of blood draw, tourniquet time, difficult stick, amount of blood in collection tube. Latter three characteristics estimate by person who performed phlebotomy.

**Data Analysis**:
- Rate of hemolysis and 95% confidence intervals (CI) around difference between rates; multivariable logistic regression to assess independent association between each remediable characteristic.

### Findings/Implications
**Findings**: Hemolysis rate 12.5% (95% CI [11.6, 13.5]), 14.6% in blood drawn from IV catheters, 2.7% from butterfly needles (difference = 11.9%; 95% CI [10.2, 13]). Device was strongest independent predictor (OR = 7.7; 95% CI [4.9, 12.0]). For IV catheter use, hemolysis significantly higher when blood drawn from locations other than antecubital fossa, with small-gauge catheters, collection tubes half full or less, tourniquet time 1 min or more, and difficult venipuncture. None of factors associated with hemolysis when butterfly needle used.

### Quality of Evidence Level of Evidence
I
III
List of Acronyms Used in Table:

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>HgB</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>G</td>
<td>Gauge</td>
</tr>
<tr>
<td>PIV</td>
<td>Peripheral Intravenous Catheter</td>
</tr>
<tr>
<td>Hem</td>
<td>Hemolysis</td>
</tr>
<tr>
<td>HI</td>
<td>Hemolysis Index</td>
</tr>
<tr>
<td>vs.</td>
<td>Versus</td>
</tr>
<tr>
<td>VP</td>
<td>Venipuncture</td>
</tr>
</tbody>
</table>

GRADING THE QUALITY OF THE EVIDENCE

I. Acceptable Quality: No concerns
II. Limitations in Quality: Minor flaws or inconsistencies in the evidence
III. Major Limitations in Quality: Many flaws and inconsistencies in the evidence
IV. Not Acceptable: Major flaws in the evidence

GRADING THE LEVELS OF THE EVIDENCE (MELNYK & FINEOUT-OVERHOLT, 2015)

I. Evidence from a systematic review or meta-analysis of all relevant randomized controlled trials or evidence-based clinical practice guidelines based on systematic reviews of RCTs
II. Evidence obtained from at least one properly designed randomized controlled trial
III. Evidence obtained from well-designed controlled trials without randomization
IV. Evidence obtained from well-designed case control and cohort studies
V. Evidence from systematic reviews of descriptive and qualitative studies
VI. Evidence from a single descriptive or qualitative study
VII. Evidence from opinion of authorities and/or reports of expert committees
**Appendix 2: Other Resources Table**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Research Purpose</th>
<th>Conclusions</th>
</tr>
</thead>
</table>
Appendix 3: Study Selection Flowchart and Inclusion/Exclusion Criteria

### Inclusion Criteria

- Studies published in English
- Studies involving human subjects
- October 2011–October 2015
- Studies addressing the PICOT question

### Exclusion Criteria

- Studies not published in English
- Non-human studies
- Studies not in the timeframe listed
- Studies not addressing the PICOT questions

The following databases were searched: PubMed, Google Scholar, CINAHL, Cochrane - British Medical Journal, Agency for Healthcare Research and Quality (AHRQ; www.ahrq.gov), and the National Guideline Clearinghouse (www.guidelines.gov).

Search terms included: “difficult intravenous access,” “tools intravenous access,” “heat,” “nitroglycerin,” “tourniquet,” “ultrasound,” “light,” “illumination,” “subcutaneous rehydration therapy,” and “hypodermoclysis,” “interosseous,” “infrared”, and “ultrasound guided”, using a variety of different search combinations.