The effects of cooling conditions on EDTA whole canine blood samples

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Background. Preanalytic factors such as time and temperature can have significant effects on laboratory test results. For example, ammonium concentration will increase 31% in blood samples stored at room temperature for 30 minutes before centrifugation. To reduce preanalytic error, blood samples may be placed in precooled tubes and chilled on ice or in ice water baths; however, the effectiveness of these modalities in cooling blood samples has not been formally evaluated. The purpose of this study was to evaluate the effectiveness of various cooling modalities on reducing temperature of EDTA whole blood samples.

Methods. Pooled samples of canine EDTA whole blood were divided into two aliquots. Saline was added to one aliquot to produce a packed cell volume (PCV) of 40% and to the second aliquot to produce a PCV of 20% (simulated anemia). Thirty samples from each aliquot were warmed to 37.7°C and cooled in 2 ml allotments under one of three conditions: in ice, in ice after transfer to a precooled tube, or in an ice water bath. Temperature of each sample was recorded at one minute intervals for 15 minutes.

Results. Within treatment conditions, sample PCV had no significant effect on cooling. Cooling in ice water was significantly faster than cooling in ice only or transferring the sample to a precooled tube and cooling it on ice. Mean temperature of samples cooled in ice water was significantly lower at 15 minutes than mean temperatures of those cooled in ice, whether or not the tube was precooled. By 4 minutes, samples cooled in an ice water bath had reached mean temperatures less than 4°C (refrigeration temperature), while samples cooled in other conditions remained above 4.0°C for at least 11 minutes. For samples with a PCV of 40%, precooling the tube had no significant effect on rate of cooling on ice. For samples with a PCV of 20%, transfer to a precooled tube resulted in a significantly faster rate of cooling than direct placement of the warmed tube onto ice.

Discussion. Canine EDTA whole blood samples cool most rapidly and to a greater degree when placed in an ice-water bath rather than in ice. Samples stored on ice water can rapidly drop below normal refrigeration temperatures; this should be taken into consideration when using this cooling modality.

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14   **Abstract**

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16   laboratory test results. For example, ammonium concentration will increase 31% in blood
17   samples stored at room temperature for 30 minutes before centrifugation. To reduce preanalytic
18   error, blood samples may be placed in precooled tubes and chilled on ice or in ice water baths;
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21   modalities on reducing temperature of EDTA whole blood samples.

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31   significantly lower at 15 minutes than mean temperatures of those cooled in ice, whether or not
32   the tube was precooled. By 4 minutes, samples cooled in an ice water bath had reached mean
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34   remained above 4.0°C for at least 11 minutes. For samples with a PCV of 40%, precooling the
35   tube had no significant effect on rate of cooling on ice. For samples with a PCV of 20%, transfer
36   to a precooled tube resulted in a significantly faster rate of cooling than direct placement of the
37   warmed tube onto ice.

38   **Discussion.** Canine EDTA whole blood samples cool most rapidly and to a greater degree when
39   placed in an ice-water bath rather than in ice. Samples stored on ice water can rapidly drop below
40   normal refrigeration temperatures; this should be taken into consideration when using this
41   cooling modality.

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Introduction

Laboratory error can occur in the pre-, intra-, and postanalytic phases of sample analysis, with 31.6% to 75% of errors occurring in the preanalytic phase.[1] Examples of preanalytic errors include lack of patient fasting, iatrogenic hemolysis, mislabeling of samples, and inappropriate sample storage. Measurement of whole blood and plasma ammonium concentration is useful for diagnosis of portovascular anomalies and hepatic encephalopathy and for monitoring response to treatment.[2-4] Ammonium is very labile, and time and temperature can have significant preanalytic effects on laboratory results.[5,6] For example, storage of whole blood at 4 degrees C and 22 degrees C for 1 hour results in increases in plasma ammonium of 31.7% and 39.9%, respectively.[6] Heparinized whole blood samples stored in ice water for 30 minutes before centrifugation have significantly less increases in plasma ammonium than those stored at room temperature for 30 minutes before centrifugation (13% vs. 31%, respectively).[7] Recommendations for handling samples for ammonium measurement include collecting the blood in a stoppered ammonia-free vacuum tube, placing the tube immediately in an ice or ice water bath, and separating the plasma from the sample within 15 minutes of collection to prevent spontaneous ammonium generation.[2,7-10] Some authors also recommend using a precooled vacuum sample tube to speed the specimen cooling process.[2,10] Effectiveness of precooling tubes and of use of ice versus ice water baths for cooling blood samples has not been formally evaluated and reported in the medical literature. The purpose of this study was to compare different sample cooling protocols to determine which would be most effective for rapidly reducing blood sample temperature before centrifugation and analysis. This work is part of an effort to optimize sample handling protocols for ammonium analysis in our hospital.

Materials and Methods

Leftover refrigerated canine EDTA whole blood samples were obtained from the University of Tennessee Clinical Pathology Laboratory and used for testing. All samples were 7 days old at the time of entry into the study and had been stored at 4°C. Samples were pooled, and a packed cell volume (PCV) of the resulting pool was measured. The pool was then divided into two aliquots. For aliquot 1 (normal PCV), physiologic saline was added to decrease the PCV to 40%. For aliquot 2 (simulated anemia), physiologic saline was added to decrease the PCV to 20%. Each aliquot was divided into 2 ml samples and 2.5 ml samples that were placed in 7 ml, stoppered, EDTA-free sample tubes (Vacutainer®, Becton Dickinson Company) and kept refrigerated at 4°C until testing. In all test conditions, the cooling rate of 2 mls of blood was to be evaluated; however, placement of a warmed blood sample into a precooled tube required transfer of the blood. For those samples, an extra 0.5ml of blood was included in the storage tubes to ensure a full 2 mls of blood was transferred at the time of testing.

The testing apparatus consisted of a lidded 148 ml plastic specimen cup (Fisherbrand™ multipurpose specimen storage containers, Fisher Scientific), a temperature probe with an accuracy of ±0.3°C (Fisher Scientific™ Traceable™ Hi-Accuracy Thermometer), and a support stand with clamps. A hole the diameter of the sample tube was drilled in the lid of the specimen

89   cup to allow the tube to be inserted through the lid and to be held upright and centered in the
90   specimen cup with the bottom of the tube touching the bottom of the cup and the stopper resting
91   above the surface of the lid. A hole was drilled in a tube stopper, which was secured onto the
92   temperature probe and used for all measurements, so that the temperature probe would maintain
93   the same location and depth in each tube and so that each tube remained stoppered for the
94   entirety of the test.

95   Immediately before testing, stoppered blood tubes were placed on a rack in a 38°C water bath
96   and warmed to 37.7°C. Samples from each aliquot (normal PCV or anemic PCV) were tested
97   under one of three cooling conditions: 1, Ice bath; 2, Precooled (P/C) tube in ice bath; and 3, Ice
98   water bath. For Condition 1 (ice bath), the specimen cup was filled with 70 gm of crushed ice
99   and capped with the prefabricated lid. A 7 ml stoppered sample tube containing 2mls of warmed
100 (37.7°C) blood was inserted through the hole in the lid, and its stopper was replaced with the
101 stopper-temperature probe unit. For Condition 2 (P/C tube in ice bath), an empty 7 ml stoppered
102 sample tube was placed on ice. After 5 minutes, 2 mls of blood were withdrawn from a storage
103 tube containing 2.5 mls of warmed (37.7°C) blood and transferred into the P/C sample tube,
104 which was then positioned in a cup containing 70 g of crushed ice and capped with the stopper-
105 temperature probe unit, as for Condition 1. For Condition 3 (Ice water bath), the specimen cup
106 was filled with 70g of crushed ice and 45g of water. A 7ml sample tube containing 2 mls of
107 warmed blood was capped and positioned in the specimen cup as described for Conditions 1 and
108 2. In each condition, the surface of the blood sample rested several cm below the surface of the
109 cooling agent (ice or ice water). Temperature of each sample was recorded at one-minute
110 intervals for 15 minutes.

111 Six treatments (ice bath/PCV 20%; ice bath/PCV 40%; P/C in ice/PCV 20%; P/C in ice/PCV
112 40%; ice water/PCV 20%; ice water/PCV 40%) were evaluated over 16 time points (0 to 15
113 minutes), with 30 trials per treatment. Temperature data were evaluated using a one-way
114 ANOVA with repeated measures, with treatment as the between subject factor and time as the
115 within subject factor. The rate of cooling data was analyzed using one-way ANOVA. Ranked
116 transformation was applied when data violated ANOVA assumptions such as non-normality and
117 unequal variance. Post hoc multiple comparison was conducted with Tukey's adjust.
118 Significance was set at $p < .05$ and all analysis was processed in SAS9.4 TS1M1 for Windows
119 x64 (Cary, NC).

120 **Results**
121
122 Results are summarized in Tables 1 and 2 and Figure 1. Within treatment conditions (ice, ice
123 water, and P/C tube on ice), sample PCV had no significant effect on cooling. Cooling in ice
124 water was significantly faster than cooling on ice only or P/C tube on ice (Figure 2). Regardless
125 of PCV, mean temperatures of samples cooled in ice water were significantly lower at 15
126 minutes than mean temperatures of samples cooled on ice only or in P/C tube on ice ($p < .0001$).
127 By 4 minutes, samples cooled in ice water had reached mean temperatures less than 4°C (that of
128 refrigeration), regardless of PCV.
129

130 For samples with a 20% PCV, mean temperature at 4 minutes of cooling in ice water was
131 significantly lower than mean temperature at 14 minutes for samples cooled on ice only
132 (p=0.0148) or at 11 minutes for samples in P/C tube on ice (P<.0001). Of samples with a 40%
133 PCV, mean temperature at 4 minutes of samples cooled in ice water was significantly cooler than
134 mean temperature at 13 minutes for samples cooled on ice only (p=0.0335) or at 12 minutes for
135 samples in P/C tube on ice (p<.0001).
136
137 For samples with a PCV of 40%, there was no difference in mean temperatures or rate of cooling
138 of samples placed on ice and those placed in P/C tubes on ice. For samples with a 20% packed
139 cell volume, however, rate of cooling was significantly faster for samples in a P/C tube on ice,
140 versus those placed on ice. From 4 to 13 minutes, mean temperatures of samples with a 20%
141 PCV that were placed in a P/C tube on ice were significantly lower than that of samples placed
142 on ice only.
143

Discussion

145
146 In this study we determined blood sample cooling occurred more rapidly and to a greater extent
147 when samples were placed in an ice water bath versus in a cup of crushed ice. With a closed
148 system that lacks motion, as described in this study, cooling of blood samples relies primarily on
149 conduction. Conduction is the flow of heat that reduces the temperature difference between two
150 materials.[11] Transfer of heat via conduction relies on exchange of kinetic energy between
151 microscopic particles within substances: in this case, between the blood and the tube containing
152 it, and between the tube and its external environment (ice or ice water). Variables important to
153 heat transfer include the material's innate ability to conduct heat (thermal conductivity), the
154 thickness of the materials involved, the surface area available for heat transfer, and the
155 temperature difference across these surfaces.[11,12] In our study, sample volume and tube size and
156 thickness were standardized; therefore, differences in rates of cooling were primarily dependent
157 on the thermal conductivity of the materials used for cooling, the amount of contact each of these
158 substances has with the tube, and the temperature of the cooling condition.
159
160 In our study, cooling was performed using closed systems containing crushed ice or ice water.
161 Ice water consists of two major components, ice and water, with thermal conductivity
162 coefficients of 2.1 W/(m K) at 0°C for ice and 0.6W/(m K) at 25°C for water. Crushed ice
163 contains a small amount of liquid water but primarily consists of ice and air, the latter of which
164 has a thermal conductivity coefficient of 0.024W/(m K) at 0°C. Because thermal conductivity of
165 water is much greater than air, it serves as a better conductor of heat and thus increases the rate
166 of heat loss from the sample.[12]
167
168 Surface areas of the cooling materials used in this study cannot be calculated, since pieces of
169 crushed ice varied greatly in size and shape and thus in their ability to contact the surface of the
170 tube. Therefore, a variable amount of air was present within the crushed ice condition. Addition
171 of water to the ice increased the surface area for diffusion of heat via a material with higher
172 conductivity, thereby speeding heat transfer. Temperature of the condition (ice or ice water) was
173 not measured in this experiment. Temperature difference was likely greater between the sample

174 and the ice water, however, since pockets of air within the crushed ice could have maintained
175 higher temperatures than the ice water.

176

177 Between conditions, packed cell volume had no significant effect on rates of cooling. This is not
178 surprising, since the coefficients of thermal conductivity for EDTA blood and EDTA plasma are
179 approximately the same as that of water.[13] However, while no significant difference was noted
180 between cooling on ice and use of a precooled tube plus cooling on ice for samples with a PCV
181 of 40%, use of a precooled tube produced a significantly greater reduction in temperature for
182 samples with a PCV of 20%. Rather than being an effect of PCV per se, a possible cause for this
183 finding is an effect of tube handling. To mimic clinic conditions used in sample collection for
184 ammonia analysis, precooling was performed by placement of a stoppered tube on ice. Leaving
185 the stopper in place prevents inadvertent contamination of the tube with environmental factors,
186 such as cigarette smoke or cleaning agents, that can result in erroneously increases in measured
187 ammonium.[14] However, in our system the stoppered tubes were manually removed from the ice
188 bath to have their caps removed, which could have increased temperature of the tube by
189 introduction of warmer environmental air and by heat transfer from the hand to the tube surface.
190 Although it is unlikely, we cannot rule out that tubes containing blood with a 40% PCV were
191 handled more or kept out of the ice bath longer during cap removal and tube filling, resulting in a
192 smaller temperature difference between the sample and the tube and a slower rate of cooling.

193

194 One concern with use of an ice water bath for cooling of diagnostic whole blood specimens is
195 whether the rapid rate of cooling and attainment of "near-freezing" temperatures will adversely
196 affect samples. In terms of ammonium concentration, sample storage at 0°C to -80°C will reduce,
197 but not halt, spontaneous ammonia generation.[6,15,16] Both rapid increases in temperature and
198 intracellular formation of ice crystals can increase red cell fragility, which can result in
199 hemolysis and increased plasma ammonium concentrations.[7,10,17,18] In one study, rate of
200 ammonia increase in whole blood significantly correlated with erythrocyte count and plasma
201 alanine aminotransferase and gamma glutamyltransferase concentrations when blood was stored
202 at 20°C but not when stored at 0°C. [16] Although hemolysis was not specifically investigated in
203 that study, those findings argue that RBC-related increases in ammonium concentration may be
204 related to cell metabolism rather than hemolysis. Hemolysis was not evaluated in our study, and
205 further study of whether rapid specimen cooling in an ice water bath induces hemolysis, and to
206 what magnitude, is warranted.

207

208 **Conclusions**

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210 Canine EDTA whole blood samples cool most rapidly and to a greater degree when placed in an
211 ice water bath rather than in ice. Use of ice water baths is critical for reducing preanalytical error
212 in the measurement of ammonium and other analytes altered by metabolic processes that
213 continue within blood samples after they are collected. Samples stored on ice water can rapidly
214 drop below normal refrigeration temperatures; this should be taken into consideration when
215 using this cooling modality.

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218 **References**

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259   hemolysis caused by inappropriate storage of canine red blood cell products. J Vet Intern Med
260   2011;25:927-933.

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263   Table 1. Summary of treatment comparisons (treatment 1 vs. treatment 2) showing effect of
264   cooling conditions and packed cell volume on rate of blood sample cooling.

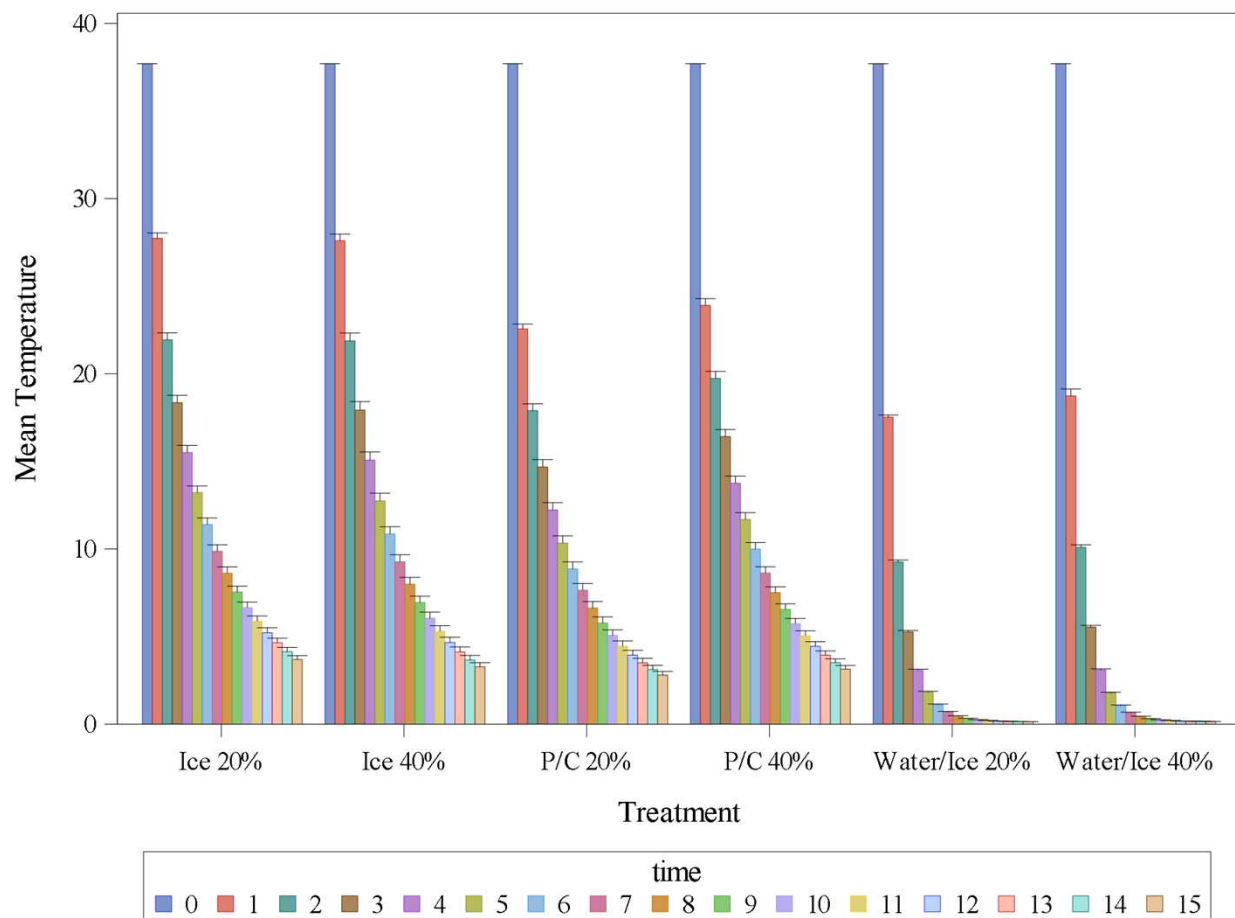| Treatment 1	Treatment 2	t Value	P value
Ice 20%	P/C 20%	5.20	<.0001
Ice 20%	Water/Ice 20%	27.38	<.0001
Ice 40%	P/C 40%	1.28	0.7974
Ice 40%	Water/Ice 40%	25.42	<.0001
P/C 20%	Water/Ice 20%	22.18	<.0001
P/C 40%	Water/Ice 40%	24.15	<.0001
P/C 20%	P/C 40%	-2.38	0.1697
Water/Ice 20%	Water/Ice 40%	-0.42	0.9983
Ice 20%	Ice 40%	1.54	0.6405

265   Ice = ice bath; P/C=precooled tube in ice bath; water/ice = ice water bath.

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268 Table 2: Mean temperature (ºC) per minute of normal and simulated anemic blood samples for 3
269 cooling conditions.

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TIME (MIN)	ICE 20% PCV	ICE 40% PCV	PRECOOLED 20% PCV	PRECOOLED 40% PCV	WATER/ICE 20% PCV	WATER/ICE 40% PCV
0	37.7	37.7	37.7	37.7	37.7	37.7
1	27.74	27.6	22.57	23.91	17.53	19.07
2	21.94	21.88	17.9	19.74	9..26	10.09
3	18.35	17.93	14.68	16.41	5.26	5.53
4	15.5	15.07	12.23	13.76	3.07	3.09
5	13.22	12.75	10.36	11.68	1.84	1.78
6	11.4	10.85	8.86	9.99	1.12	1.06
7	9.88	9.27	7.65	8.64	0.71	0.66
8	8.62	7.99	6.63	7.51	0.47	0.44
9	7.54	6.94	5.78	6.56	0.33	0.31
10	6.65	6.05	5.06	5.74	0.24	0.24
11	5.88	5.3	4.46	5.05	0.20	0.2
12	5.22	4.67	3.98	4.45	0.17	0.18
13	4.65	4.12	3.5	3.94	0.16	0.16
14	4.14	3.66	3.12	3.51	0.15	0.16
15	3.69	3.27	2.8	3.14	0.14	0.15

271 PCV=Packed cell volume

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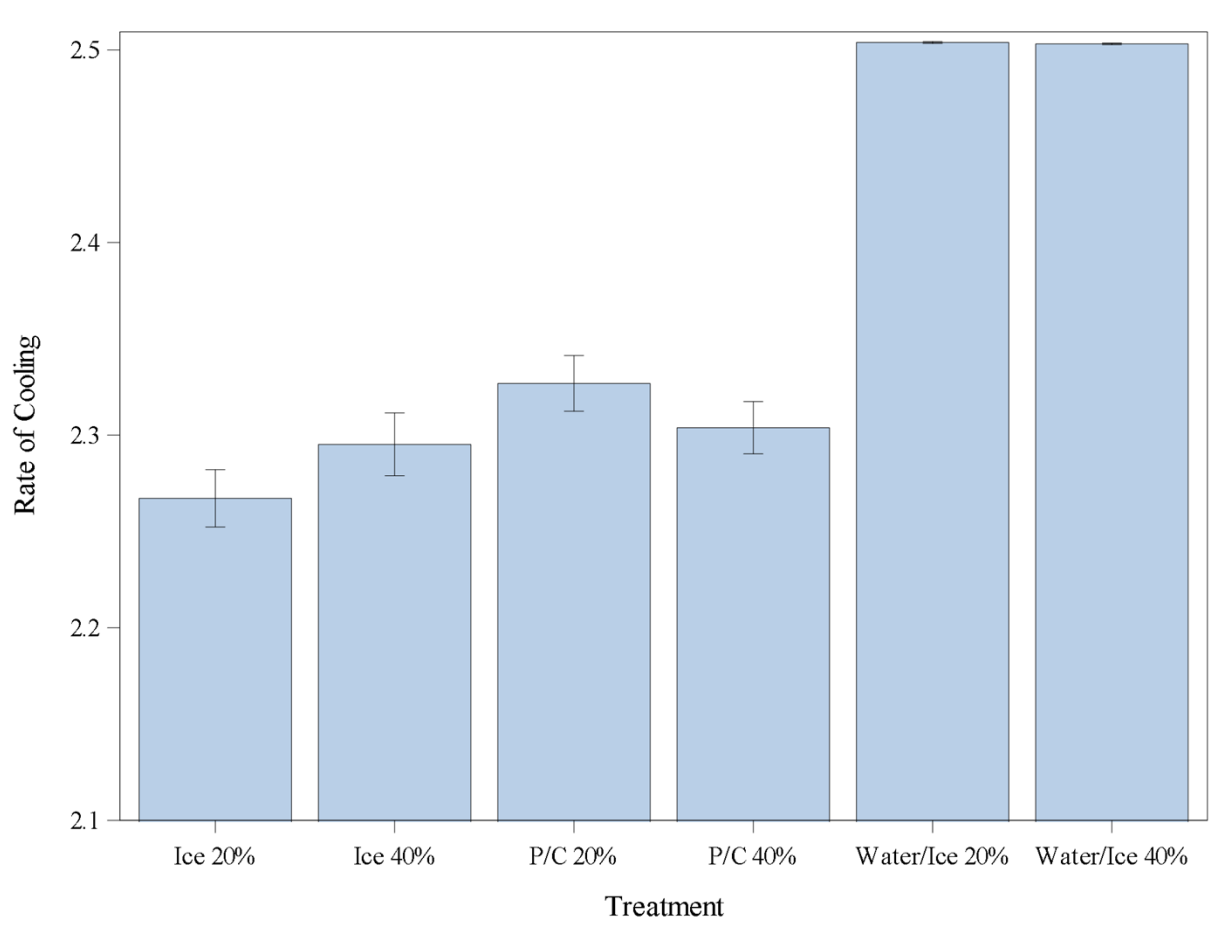284 Figure 1: Cooling of normal and anemic blood samples under different conditions.

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288 Ice = ice bath; P/C=precooled tube in ice bath; Water/ice = ice water bath.

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298   Figure 2. Temperature decrease (degrees C per minute) of samples over 15 minutes.

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301   Ice = ice bath; P/C=precooled tube in ice bath; Water/Ice = ice water bath.

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