



# Mouse Clinical Pathology: Hematology Controlling Variables that Influence Data

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**ABSTRACT** Relatively simple and inexpensive blood tests can provide valuable data for phenotyping and other studies involving rodents. Hematology can contribute important information relevant to a variety of phenotypes including but not limited to infections, anemias, immunodeficiencies and some neoplasms. Small specimen volumes and survival collections of good quality anti-coagulated whole blood samples from mice are a special challenge. Animal, specimen and equipment related variables may affect hematology data, resulting in excessive variability and poor reproducibility, and may also affect artifacts that can be misinterpreted as phenotypes or otherwise significant findings.

To optimize the utility of our Hemavet® 950 (Drew Scientific, Oxford, CT) automated hematology analyzer for mouse phenotyping, we evaluated a number of variables that were suspected of influencing automated hematology results. This instrument generates a complete blood count (CBC) from only 20µl of anti-coagulated blood, and was selected for our phenotyping core clinical pathology laboratory because of its small specimen volume requirement. Terminal specimen collections of mice designated for euthanasia were obtained at euthanasia from barrier-raised and -maintained mice of various stocks, strain, sexes and ages. Collection sites, needle gauges, mixing effects, probe depths, test delays, temperature, and types of anticoagulant are some factors that can influence data generated by automated hematology instrumentation and ultimately can influence published data, statistical analysis, interpretation and conclusions. Based on our results, we developed recommendations for our facility and equipment for standardization of mouse blood collection and handling in order to minimize hematology variables that can result in 'false phenotypes'.

**INTRODUCTION** Blood parameters play a critical role in diagnosis, assessing progression, and in the characterization of disease and phenotypes in clinical and research situations. The accuracy and reliability of the whole blood parameter analysis depends on identification and control or elimination of variables that may affect these results. Different blood collection and handling strategies represent source of variability that can be controlled in many instances.

In order to develop blood collection and handling recommendations for our facility and equipment, we investigated the variables that were recognized to vary among submissions to our laboratory, and might influence data derived from the Hemavet® 950 hematology analyzer. These included collection sites, needle gauge, anticoagulants, specimen mixing (agitation), specimen holding temperatures and time delay, and probe depth.

**STUDY DESIGN** Animal care and use was in compliance with the Guide (ILAR 1996) and approved by Johns Hopkins Institutional Animal and Use Committee (IACUC). The mice were adults of various stock, strains, sexes and ages. Mice were manually restrained for facial blood collection and using 20g needle as lancet, were euthanized with CO<sub>2</sub> inhalation for cardiac blood puncture via 21g or 25g needle and 1 ml syringe (Becton Dickson #309725). Bleeding was performed cage side to limit travel stress, and to parallel research practices in our barrier facility. Microtainer® EDTA coated tubes #36974 were used. For the anticoagulant study, Microtainer® Lithium Heparin coated tubes #36965 were also used.

## STUDY DESIGN (cont)

**Collection Site: Peripheral versus Central**

**Quantity:** 300µl whole blood **Site:** Facial vessels; Cardiac

**n** = 10 x 2 groups **Tubes:** 2 x 150µl/tube

**'Normal' Control:** Facial vessel drip; processed immediately (<30 min)<sup>1</sup>

**Treatment:**

**Site:** Cardiac blood gently aspirated through 21g needle (1ml syringe); needle removed from syringe for ejection into tube.

**Needle Gauge versus Drip**

**Quantity:** 300µl whole blood **Site:** Facial vessels

**n** = 10 x 3 groups **Tubes:** 3 x 100µl/tube

**'Normal' Control:** Facial vessel drip; processed immediately (<30 min)<sup>1</sup>

**Treatment:**

**Needle Gauge:** 21g & 25g needle/1ml syringe; Blood aspirated/ejected vigorously; processed immediately (<30 min)

**Anticoagulants: EDTA versus Lithium Heparin**

**Quantity:** 300µl whole blood **Site:** Facial vessels

**n** = 10 x 3 groups **Tubes** 1 ea x 150µl/tube

**Treatment:**

**Anticoagulant:** EDTA; Lithium Heparin processed immediately (<30 min)<sup>1</sup>

**Testing delay to processing, holding temperature, probe depth / specimen mixing**

**Quantity:** 300µl whole blood **Site:** Facial vessels

**n** = 10 x 6 groups **Tubes:** 2 x 150µl/tube

**'Normal' Control:** facial vessel drip; processed immediately (<30 min)<sup>1</sup>

**Treatment:**

**Analyzer probe depth:** probe at surface, probe deep, no mixing

**Time delay to processing:** 50-60 minutes; 5-6 hours of collection

**Temperature:** Room temperature (70oF / 21oC) ; Refrigerated (40oF / 4oC)

**Specimen Mixing by Rolling (Easy Mixer)**

**Quantity:** 300µl whole blood **Site:** Facial vessels

**n** = 10 x 3 groups **Tubes:** 3 x 100µl/tube

**'Normal' Control:** Facial vessel drip; processed immediately (<30 min)<sup>1</sup>

**Treatment:**

**Mixing:** Roller x 3 min; roller x 5-6 hours

**Specimen Mixing by Vortex**

**Quantity:** 600µl whole blood **Site:** Cardiac

**n** = 10 x 3 groups **Tubes:** 3 x 200µl/tube

**Control:** Moderately agitated and processed immediately (<30 min)<sup>1</sup>

**Treatment:**

**Mixing:** Vortex x 5 seconds; Vortex x 10 seconds

**Statistics:** Excel Mean, Standard Deviation and Student T-test- 2 tailed-paired.

<sup>1</sup> Designated as "Normal" Control handling for this study were manufacturer's suggestions of immediate processing (<30min), EDTA anticoagulant, no refrigeration, no rolling, gentle mixing by flicking tube, and probe at mid tube.

## RESULTS (Continued)

**Collection Site: Peripheral versus Central** *Small but statistically significant differences:*

▲ Higher WBC from cardiac puncture, compared to facial vessels.

▼ Lower RBC, hemoglobin, hematocrit from cardiac puncture, compared to facial vessels.

**Needle Gauge Versus Drip** Statistically significant differences were *not* detected in specimens dripped from facial vessels compared to specimens vigorously aspirated and ejected through 22g or 25g needle with 1 ml syringe.

**Anticoagulants** *Small but statistically significant differences:*

▲ Higher WBC with lithium heparin anticoagulant, compared to EDTA (dry) anticoagulant.

**Time delay to processing, holding temperature, analyzer probe depth:** *Small but statistically significant differences:*

**Immediate processing versus 50-60 minutes delay at room temperature:**

▲ Higher hemoglobin, RBC, hematocrit with deep probe depth in unmixed specimens, compared to immediate 'normal' control processing –or to immediate processing with deep probe too???

▼ Lower platelet count with deep & mid tube probe depth, compared to immediate 'normal' control processing.

**Immediate processing versus 5-6 hours delay at room temperature:**

▲ Higher WBC, RBC, hematocrit with deep probe depth in unmixed specimens, compared to mid tube probe depth in gently mixed specimens, or to immediate 'normal' control processing.

**Immediate processing versus 5-6 hours delay refrigerated:**

▲ Higher WBC, RBC, Hematocrit with deep probe depth in unmixed specimens, compared to mid tube probe depth in gently mixed specimens, or to immediate 'normal' control processing ???

▼ Lower hemoglobin, hematocrit from deep and mid tube depth in unmixed specimens, compared to immediate 'normal' control processing ???

**Mixed by Roller (Roller name brand???)** *Small but statistically significant differences:*

**Rolling for 3 minutes:**

▼ Lower RBC, hematocrit, hemoglobin in specimens rolled for 3min, compared to unrolled blood.

**Rolled for 5-6 hours:**

▼ Lower WBC, RBC, hematocrit, hemoglobin, platelets in specimens rolled 5-6 hrs, compared to unrolled blood.

**Mixing by Vortex** There were *no* statistically significant differences, but a trend toward lower values for all five investigated parameters (WBC, RBC, hemoglobin, hematocrit, platelets) was detected.

## REFERENCES

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		WBC (K/ul)	RBC (M/ul)	Hb (g/dL)	HCT (%)	PLT (K/ul)
Central v Peripheral						
	Facial	5.28	10.14	13.44	51.6	851.8
	Cardiac	<b>8.81</b>	<b>9.32</b>	<b>12.38</b>	<b>47.5</b>	<b>1074.2</b>
Needle Gauge						
	Control	5.41	10.24	12.76	51.54	831.7
	21 Gauge	5.40	10.23	12.84	51.84	860.2
	25 Gauge	5.56	10.34	12.90	52.24	908.1
Anticoagulants						
	EDTA	11.82	9.083	13.18	48.14	760.2
	Lithium Heparin	<b>13.71</b>	9.168	13.55	48.56	747.4
Time Delay 50-60 Minutes						
	Control	7.33	10.67	14.82	61.85	688.1
	Surface	7.11	10.52	14.59	61.85	626.9
	Mid	7.14	10.38	14.51	59.70	<b>617.1</b>
	Deep	7.30	<b>10.77</b>	<b>15.21</b>	<b>62.67</b>	<b>591.0</b>
Time Delay 5-6 Hrs. Room Temp.						
	Control	8.54	9.43	14.20	53.92	751.3
	Surface	8.25	9.21	14.01	51.04	712.8
	Mid	8.49	9.28	14.07	51.84	860.2
	Deep	<b>9.27</b>	<b>10.27</b>	<b>15.66</b>	<b>57.44</b>	739.9
Time Delay 5-6 Hrs. Refrigerated						
	Control	9.01	9.50	14.34	50.46	768.7
	Surface	9.62	9.54	<b>13.78</b>	50.24	775.8
	Mid	9.34	9.34	<b>13.80</b>	49.04	805.5
	Deep	<b>11.5</b>	<b>11.98</b>	<b>17.72</b>	<b>62.74</b>	756.1
Mixing – Rolling						
	Control	7.11	10.59	14.72	61.03	672.8
	Roller for 3 minutes	6.88	<b>10.23</b>	<b>14.29</b>	<b>58.60</b>	622.5
	Control	7.59	9.43	13.98	51.45	794.5
	Roller for 5-6 hours	<b>7.23</b>	<b>9.25</b>	<b>13.7</b>	<b>49.14</b>	<b>745.1</b>
Mixing – Vortex						
	Control	7.49	9.13	13.62	45.40	1067
	Vortex 5 sec	7.62	9.06	13.61	44.50	1113
	Vortex 10 sec.	6.96	9.00	13.54	<b>43.66</b>	975.18

**DISCUSSION/RECOMMENDATIONS** Statistically significant differences were achieved by common variations in specimen handling seen in submissions to our laboratory. While these differences may have little or no significance in a clinical setting, they could be expected to complicate data analysis in a research setting. These represent only a few variations in specimen collection and handling that could be expected to influence hematology results, but they emphasize the importance of consistent specimen collection and handling practices within and between studies.

### Collection Site: Peripheral versus Central

- ▶ The same blood collection site should be used for comparisons within and between studies.

Other studies (2,3) have detected lower WBC counts in central or cardiac blood compared to peripheral (tail or retroorbital). Differences between these studies and ours, include the use of anesthesia and differences in peripheral collection sites.

### Needle Gauge versus Drip

- ▶ The same collection method and equipment should be used for comparisons within and between studies.

Although we detected no significant differences using gentle handling of blood with small gauge needle and a 1 ml syringe, unusually fragile blood cells could be expected to be damaged (lysed) by rougher handling (e.g. small needles, stronger suction).

### Anticoagulant: EDTA versus Lithium Heparin

- ▶ The same anticoagulant should be used for comparisons with and between studies.

Lithium heparin may be useful if clinical chemistry evaluations are to be performed on the same specimen. When liquid anticoagulant are used a dilution effect on small blood specimen should be considered.

### Time delay to processing, holding temperature

- ▶ Time delay to processing should be minimized.

- ▶ Room temperature is recommended over refrigeration for delays up to 6 hr.

Delays in processing are inevitable in most situations. This small study found that refrigeration of specimens resulted in significant differences compared to room temperature holding. Specimens refrigerated for 24 hours were found to be acceptable.

### Analyzer Probe depth / Specimen mixing

- ▶ Similar specimen mixing practices and analyzer probe depth should be used for all specimens.

The Hemavet® 950 aspirates 20µl of specimen for analysis. This volume may constitute most of a small specimen (<50µl). With a larger specimens, it is important that the specimen is adequately mixed and that mixing practices do not cause cell damage (lysis).

### Mixing by Roller or vortex

- ▶ Gentle mixing by digitally flicking or gently inverting blood tubes prior to processing is recommended.

Roller mixing resulted in significant differences in several values. Although vortexing gently did not result in significant differences in this small study, unusually fragile blood cells could be expected to be damaged (lysed) by rougher handling.