

Hematology

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Summary:

The procedure is used to measure hematological parameters for rodents. Hematological assessment of blood will include blood cell counts (white blood cells, red blood cells, hemoglobin, and platelets) and additional hematological parameters (hematocrit, mean cell volume, mean corpuscular hemoglobin, mean cell hemoglobin concentration) can be derived using these indices. These data will indicate abnormalities in the production of blood and its components (blood cells and hemoglobin) as well as in the associated blood-forming organs.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
HemaVet 950	Drew Scientific	
Control Solution	Drew Scientific	
50% bleach solution	Clorox	
2- 50 ml beakers		
1 ml TB syringe	VWR or others	
1.8 ml Cryotube	ThermoScientific or others	
Whole blood samples collected in EDTA tubes		
Aluminum foil		
Lab Coat/Gloves/PPE		
Wet and Dry ice		
Microvette EDTA tubes	Fisher	NC9299309
Disinfectant -Coverage Plus	Steris	

Protocol:

1. RUNNING THE CONTROL

a. Take the Drew Scientifc control solution out of the fridge and mix on a rotary mixer for 15 minutes.

<u>NOTE:</u> Controls are good for two weeks after they are opened. Write the date the control was opened on the cap and the expiration date on the label before opening the control.

- **b.** Press Start on the HemaVet. When the HemaVet flashes "RDY/DMS and Mix Sample", it is ready for analyses.
- **c.** Establish background counts. Press the Other key. The machine will then run its analysis program without sample and print a report. Acceptable background counts are as follows: WBC 0.2 or less, RBC 0.02 or less, Hb 0.2 or less, PLT 20 or less.
- **d.** While the HemaVet runs the start menu, log onto the computer and open the HemaVet DMS program.
- e. Press the green arrow key at the top of the HV DMS capture window. Check that the HemaVet and DMS program are talking to each other by pressing the following on the HemaVet 9 for special menu, 7 for diagnostics, 3 for DMS, 1 to initialize, and 2 to send. The DMS program should say acknowledgement sent.
- **f.** Run the control solution. Make sure that the solution is properly mix. Press 6 for test number, 2 for ID number, press clear and then type CONT and the date (e.g., CONT053012). Press 3 to exit and 0 to end.
- **g.** Holding the thoroughly and gently mixed control under the probe, press Mouse to sample the control. Once the probe retracts, carefully wipe the top of the control and cap including the threads before recapping and placing the control back in the fridge.
- **h.** The control run takes ~ 2 min and will produce a printout. Verify the results against the control's assay reference ranges sheet.

2. RUNNING THE SAMPLES

- a. Blood samples should be run on the HemaVet at least 15 minutes after they have been drawn and not more than 4 hours after being drawn. Add about 100 ul of blood to a microvette EDTA tube. Leave samples at room temperature. Do not place samples on ice. To run a sample, press 6 for test number, 2 for ID number, and then type the animal ID number. Press Enter, 3 to exit, and 0 to end. Mix the sample well but gently, inverting the tube several times.
 - <u>NOTE:</u> Look at the vial carefully to see if any clots have formed. Samples cannot be run if clots are present.
- **b.** Press Mouse to sample and start the analysis (use the tube holder or hold the tube under the probe.
- **c.** After all the samples are run, press the Excel button on the DMS window. Save the file name as the date.
- **d.** The DMS window can then be closed once the Excel file with the day's data is saved.

3. CLEAN-UP & MAINTENANCE

After use

- a. Do a short clean cycle with 50% bleach at the end of each analytical run. On days when more than 25 samples are processed, a short clean cycle needs to be run every 25 samples. To run a short clean cycle, press 9 on the key pad to access the Special Menu, hold a vial of 50% bleach under the probe, then press Clean on the key pad. At the end of the day, do a full clean cycle with 50% bleach by holding a vial of 50% bleach under the probe and simply pressing Clean on the key pad. The full clean cycle will take about 15 minutes.
- **b.** During periods when the Hemavet is not used for longer than a week, the autoclean cycle should be turned on.

c. Clean bench and surroundings with CoveragePlus solution.

<u>Preparing a 50% bleach solution.</u> The dilution ratio for 50% bleach working solution is 1:1 (one part Clorox bleach to one part deionized water).

- **a.** Prepare the 50% bleach working solution in a 1.8 ml Thermo Scientific Cryotube by combining 0.90 mL of Clorox bleach and 0.90 mL of deionized water.
- **b.** Clorox bleach is poured into a 50 mL beaker and 0.90 mL of bleach is drawn up from the beaker with a new BD 1mL TB syringe and transferred to a new 1.8 mL Cryotube.
- **c.** Deionized water is collected into a clean 50 mL beaker. 0.90 mL of deionized water is drawn up from the beaker using the same 1mL TB syringe and transferred to the 1.8 mL Cryotube containing the 0.90 mL of bleach.
- **d.** Gently inverted the Cryotube back and forth several times to mix the solution.
- **e.** Wrap the 1.8 mL Cryotube with aluminum foil and label with the date the solution was made, the date the solution expires, and the initials of the preparer.
- **f.** Store the 50% bleach solution in an air-tight, aluminum foil-wrapped container. A fresh solution needs to be prepared weekly.

IMPORTANT NOTES:

- Bleach is a strong base and is corrosive. To prevent a chemical exposure, wear the appropriate PPE when using this solution.
- Refer to the Clorox Regular Bleach SDS for information about administering first aid and use of PPE required during use of bleach.

Cleaning the air filter

- **a.** The air filter needs to be checked monthly and cleaned as needed.
- **b.** The HemaVet must be turned off (power-down) before checking or removing the air filter.
- **c.** Remove the grille covering the air filter (at the back of the HemaVet) by grasping the cover at the top and pulling straight out.
- **d.** Wipe dust off the grill and wash filter with mild soap and water, rinse well and air dry completely.
- e. Place filter on grille and snap grille into place. Then power up instrument and run start up cycle.