

HAEMATOLOGICAL PARAMETERS IN DISEASE DIAGNOSIS

Haematological parameters are very important in determining health and physiological status of the fish. In addition, these parameters reflect the changes in the organism correctly and play an important role in the detection of disease and metabolism of fish living in different ecological environments. Haematological and immunological values are considered important criteria for fish health. Fishes are poikilothermic creatures, in which changes are observed in haematological parameters due to environmental factors such as bacteria, parasites, water temperature, oxygen content, pH and so on. The haematological values in fish changes with the effects of seasonal variations those are associated with changes in water temperature and climatic changes.

Materials required:

1. Syringe
2. EDTA
3. erythrocyte pipette
- 4.

PROCEDURE AND DIFFERENT HEMATOLOGICAL PARAMETERS

Collection of blood from the experimental fish

Blood of the fish were drawn with the help of the sterilized small insulin needles directly from the caudal vein containing EDTA as an anticoagulant.

Total erythrocyte count (EC)

Counting of red blood cell (RBC) was done following the method of Schaperclaus (1991). Blood was drawn in a dry erythrocyte pipette to the 0.5 graduation and then Hayem's solution was drawn up to the 101 mark (dilution 1:200). The RBC pipette was then shaken for 1 minute and counting of the cells was done under trinocular microscope 400x magnifications. The erythrocytes were counted in 5 group squares (1 group square = 16 small squares). All cells lying inside the group squares and also the erythrocytes lying to the left and below the

demarcation line were counted. Final erythrocyte count (EC) was calculated with the formula given below:

$$\text{EC mm}^{-3} = \text{Erythrocytes in 80 small squares} / 80 \times 1/400 \times 1/10 \times 1/200$$

Total leucocytes count (LC)

White blood cells (WBC) were counted following the method of Schaperclaus (1991). Blood was drawn up to the 0.5 mark and then the dilution mixture (WBC diluting fluid) was drawn up to 101 marks (dilution 1:200) into the WBC pipette. The filled WBC pipette was gently revolved for 2-3 minute to mix with the diluting fluid. After efficient mixing, counting chamber was filled with a small drop of diluting blood. The cells were allowed to settle for 3 minute and counting was done in the large square of the four corners of the chamber is demarcated by triple line (1 mm³). Final WBC count or leukocyte count (LC) was calculated with the formula as follows:

$$\text{LC mm}^{-3} = \text{No. of leucocytes in 4 square of 1 mm} / 4 \times 1/10 \times 1/200$$

Packed cell volume (PCV)

Packed cell volume (PCV) was measured following modified method of Schaperclaus (1991) with slight modification. Blood was drawn up to the graduation mark 100 on the heparinised capillary tube. The openings of the tube were closed with wax and the tube was centrifuged at 3000 rpm for 3 minutes. After centrifuging, the capillary tube was placed on a reading device and the volume was recorded. The PCV or haematocrit value was expressed as the percentage fraction of blood cells in the total volume (volume %).

Total haemoglobin

Estimation of haemoglobin in blood was done by using Sahli Haemometer. In this method, the measuring tube was first filled with 0.1N hydrochloric acid up to the mark 2, and then 20 µl of blood was added (Schaperclaus, 1991). After proper mixing, the colour of the sample was compared with the standard following dilution of the sample by adding distilled water drop wise. Reading of the haemoglobin content was determined from the scale given in the measuring tube.

Mean corpuscular volume (MCV)

To calculate the MCV, expressed in femtoliters (fl or 10⁻¹⁵ l), the following formula was used (Dacie and Lewis, 2001).

$$\text{MCV} = (\text{Hematocrit or PCV (\%)} \times 10) / \text{RBC count (EC in million mm}^{-3}\text{)}$$

Mean corpuscular haemoglobin (MCH)

The mean corpuscular haemoglobin or mean cell haemoglobin (MCH) was the average mass of haemoglobin per red blood cell in a sample of blood. This represented the absolute amount of haemoglobin in the average red cell in a sample. Its units are pictograms (pg) per cell. The MCH was calculated from the Hb and the RBC using the following equation (Dacie and Lewis, 2001).

$$\text{MCH} = (\text{Haemoglobin (g dl-1)} \times 10) / \text{RBC count (EC in million mm-3)} \quad 23$$

Mean corpuscular haemoglobin concentration (MCHC)

To calculate the MCHC, expressed as grams of haemoglobin per 100 ml packed cells, the following formula was used (Daice and Lewis, 2001).

$$\text{MCHC} = \text{MCH} / \text{MCV}$$

RESULTS: