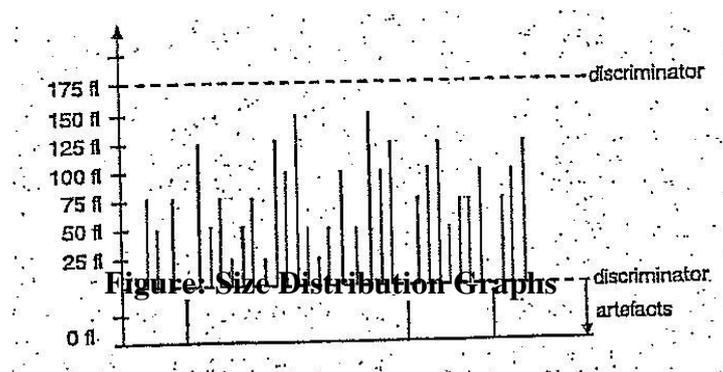




The amplitude of each pulse, that directly corresponds to the cell volume, is measured and accumulated. The AutoCounter has LOW and HIGH discriminators to filter any amplitudes not within the required range.

The size distribution graphs show the size of the counted cells in femtolitre along the x-axis and the relative number of cells along the y-axis. The x-axis is divided in 4096 different channels in varying width depending on the cell type. The AutoCounter reports the number of cells which have been registered in the respective channels. The findings are then presented in a histogram in relation to the number of cells in each channel.

Each RBC, PLT and WBC count is measured on a precise volume of the dilution. The amount measured is determined by the distance between two optical sensors, which are mounted on a precision column called the measuring tube.



During each measurement cycle of RBC/PLT and WBC a vacuum pump pulls the dilute through the measuring tube. When the liquid meniscus passes the optical path of the start sensor, the counting is activated. Detected pulses within the discriminators are accepted and accumulated only when the cycle is in counting mode.

When the liquid meniscus reaches the optical path of the stop sensor, the counting stops. During each measurement, two or more cells can enter the orifice simultaneously. The corresponding change in impedance is detected as a single pulse with a high amplitude, resulting in the loss of one or more pulses (counts). The reduction, referred to as coincidence passage loss, is statistically predictable,

and is related to the effective volume of the orifice and to the concentration of the dilution. The AutoCounter automatically corrects each RBC, PLT and WBC count for coincidence passage loss.

In order for the method to work properly the following is required:

- A correct cell dilution.
- A sufficient and repeated mixing of the cell dilution.
- A constant flow rate through the orifice.
- A constant radius of the orifice.
- A constant measuring volume.

(The orifice radius is influenced by proteins which are concentrated in the transducer, thereby reducing the radius. This results in an imprecise determination of the cell size. Frequent cleaning of the transducer and its orifice is thus important in order to eliminate the proteins).

#### **14.2 RBC – Red Blood Cell Count**

RBC is presented in number of cells per litre or microlitre. For human blood the RBC discriminators are set to minimum 30 and maximum 250 femtolitre.

#### **14.3 MCV – Mean Cell Volume**

MCV is presented in femtolitre or cubic micrometer. Determination is based on statistical methods from size distribution span of counted red blood cells.

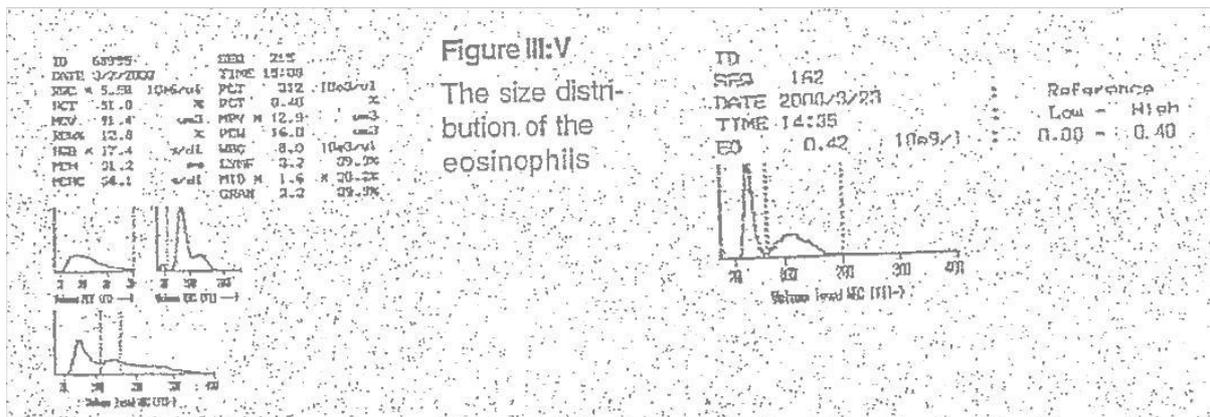
MID region (mid size cells): Ranges from 95 to 120 femtolitre. Cells in this area typically correlate to monocytes, eosinophils and basophils and also degranulated neutrophils, precursor cells, blasts and plasmacytes.  
GRA region (large size cells): Ranges from 120 to 420 femtolitre. Cells in this area typically correlate to neutrophils. In approximately 20% of the samples eosinophils can also locate in this region. Precursor granulocytic

cells, especially bands, have a tendency to locate close to the mid cell region.

#### 14.4 EO-Eosinophils

In the models AC910EO-0, AC920EO+2, AC920EO+0 and AC970EO-0 it is impossible to determine the eosinophils using the Swelab EO kit. EO is presented in number of cells per litre or microlitre. The eosinophils belong to the granulocytes and in normal samples the total amount is low and can not be detected in a 3-part differential. The semi automatic EO measurement is a quantitative method that is performed when a significant high MID cell count is obtained or when a high EO content can be suspected. Detection of DO is accomplished by lysing all cells except the eosinophils using an alkaline non-ionic based surfactant. The remains, activated and non-activated eosinophils, are counted In the AutoCounter.

The EO apper in an intermediate position overlapping the MID and GRA areas in the WBC histogram. After treatment with the lyse reagent the eosinophil nuclei is similar in size to nuclies of monocytes, some abnormal cells and occasionally granulocyees. The presence of elevated MID cells can therefore be an indication of high eosinophils level. The discriminators set in the "6 Set up menu", determine the minimum and maximum size of the eosinophils. The EO discriminators are set to 70 and 200 femtoliter.



**Figure: WBC Histogram with a MID Cell Fraction**

#### 14.5 PLT – Platelet Cell Count

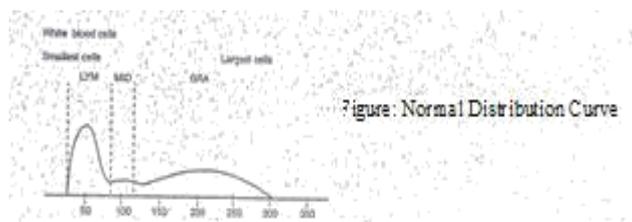
PLT is presented in number of cells per litre or microlitre. The AutoCounter uses floating discriminators for PLT counting. Within the defined limits the software automatically find the minimum concentration of cells and set the discriminator to this point. The range for human samples is from 2 and the upper limit is floating between 15 and 30 fl. This means that the AutoCounter will search for a distinct discrimination point between 15 and 30 fl.

#### 14.6 MPV – Mean Platelet Volume

MPV is presented in femtolitre or cubic micrometer determined on the total number of PLT counted. The histograms describes the size distribution span of the counted cells. When the PLT count is less than  $40 \times 10^9/l$  MPV is not reported.

#### 14.7 WBC – White Blood cell Count

The differentiation of the WBC cells into lymphocytes, mid-cells and granulocytes is presented in number of cells per litre or microlitre and in percentage of total number of WBC cells. The MID discriminator of WBC WBC is set to 95 and 120 fl. The WBC histogram is automatically adjusted depending on number of cells, i.e. expanded for low values and compressed for high values. The size distribution of non-differential WBC should be seen as a check of the hemolysing process only. A too low concentration of hemolyzer gives a too high number of cells due to presence of only partially hemolyzed red blood cells at 30 femtolitre or just above. A too high concentration of hemolyzer gives a too low number of WBC. The cells will decrease in size to below 30 femtolitre. The WBC differentiation as in the AutoCounter, is a screening method. Les common normal and abnormal cells and cell distribution must be visually investigated in a microscope.



LYM region (small size cells): Ranges from 30 to 95 femtolitre. Cells in this area typically to lymphocytes. Other cell type that could locate in this region are nucleated red blood cells, clumped platelets, macrocyte platelets, variant (atypical) lymphocytes or blasts.

#### **14.8 Calculated Parameters**

##### **HCT-Hematocrit**

The HCT is presented in percent or litre per litre. The HCT is the volume of packed erythrocytes in relation to the total blood volume.

$$\text{HCT} = \text{RBC} \times \text{MCV}$$

#### **14.9 RDW-Red Cell Distribution Width**

The RDW is presented in percentage of the red cell volume distribution. The RDW is an index of the variation in red cell size (anisocytosis). The RDW is calculated directly from the RBC histogram. Not all cells are included in the RDW calculation thus RDW is only measured on a portion of the RBC histogram.

#### **14.10 MCH and MCHC, Indices Calculation**

MCH – Mean Cell Hemoglobin –s presented in pictogram or femtomol.

$$\text{MCH} = \text{HGB} / \text{RBC}$$

MCHC – Mean Cell Hemoglobin Concentration – is presented in grams per litre, grams per deciliter or millimol per litre.  $\text{MCHC} = \frac{\text{HGB}}{\text{HCT}}$

HCT

The red cell indices provide an indication of red cell morphology and can also be used to indicate instrument calibration and stability. The indices are very stable parameters. They do not significantly change from day to day or year to year even though the parameters which are used to calculate them dramatically increase or decrease. The indices are calculated automatically.

#### 14.11 PCT – Plateletcrit

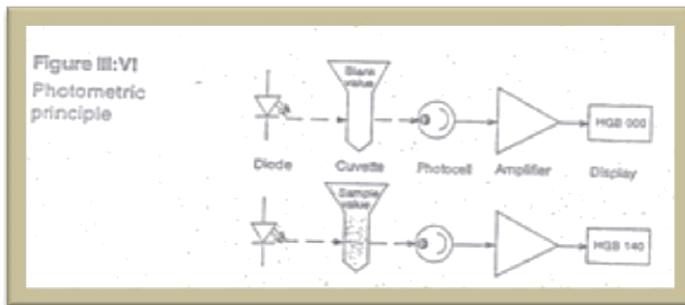
The PCT is presented in percent or litre per litre. The PCT is the volume of packed platelets in relation to the total blood volume.  $PCT = PLT \times MPV$

#### 14.12 PDW – Platelet Distribution Width

The PDW is presented in percentage of the platelet cell volume distribution. The PDW is an index of the variation in platelet cell size. The PDW is calculated directly from the histogram. Not all cells are included in the PDW calculation thus PDW is only measured on a portion of the PLT histogram.

**Note:** PCT and PDW are for laboratory use only.

#### 14.13 Photometric Method



#### 14.14 HGB – Hemoglobin

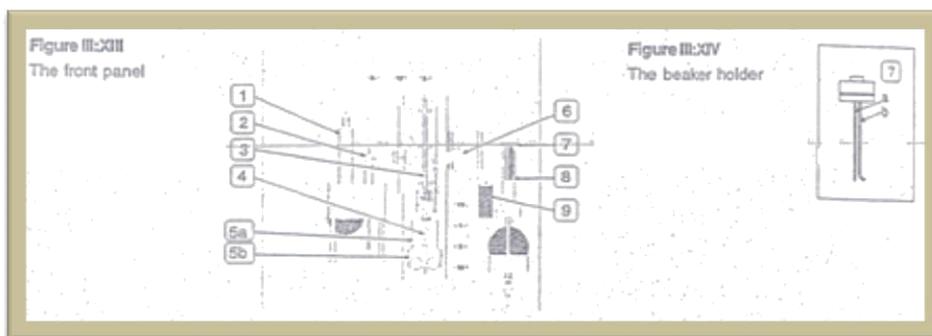
The quantitative determination of the prepared sample is obtained by measuring the light absorption. Light from a diode is passing through the cuvette. First only with the reagents as a zero reference known as a blank value. The zero reference value for each sample is obtained from the RBC/PLT dilution immediately before this dilution is drained from the counting beaker. The light transmission is measured by a photocell.

The light transmission is measured once again the WBC.HGB dilutin to absorb light at 555 nm and is converted to a digital value. HGB – the hemoglobin concentration in blood is measured by the photometer and is presented in grams per litre, grams per deciliter or millimol per litre. The hemolysing reagent is lysing the RBC-membranes and the hemoglobin molecule are released. The  $Fe^{2+}$  is oxidated to  $Fe^{3+}$  and a stable hemoglobin complex is formed. The photometer measures the absorption and calculate the concentration of hemoglobin.

### AC910EO+

#### 14.15 Components

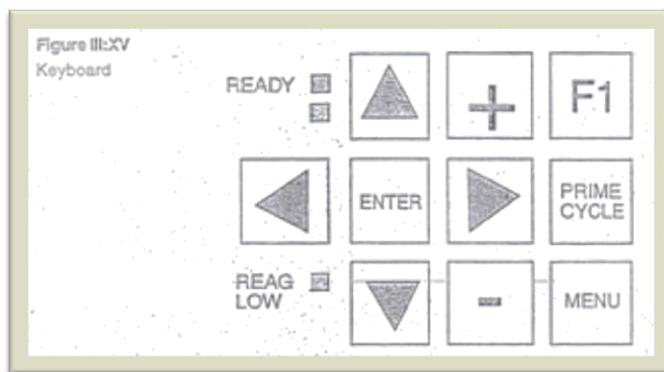
##### The Front Panel



- 1- **Isotonic Diluent Syringe**. The syringe is set to approx. 4 ml.
- 2- The blood volume is determined by the Blood Volume Syringe and is 20  $\mu$ l.
- 3- The dilution is pulled into the **Measuring Tube** by vacuum pump.
- 4- The cells are counted when passing the orifice in the **Transducer**.
- 5- The 5a **Counting Beaker** has nozzles for delivery of the RBC/PLT dilution and the hemolyzed WBC/HGB dilution. The air used for mixing the secondary RBC/PLT dilution in the counting beaker enters via the bottom nozzle which also is the drain. The lower part of the counting beaker is the HGB cuvette, fitted into the 5b **HGB Photometer**.

- 6- After aspiration of a sample the **Pipette Arm** moves down to the counting beaker and the AutoCounter dilutes the sample with diluent.
- 7- The **Beaker Holder** consists of two tubes: Figure:
  - a. Tube for transfer the dilution to the counting beaker.
  - b. Tube for delivery of the hemolysing reagent
- 8- **Hemolysing Reagent Syringe**. The syringe is set to approx. 3 ml.
- 9- The drain from the measuring is transferred into the **Drain Cup**.

## Keyboard



The **ENTER** key is used to:

- Enter into a selected menu
- Enter options within a menu



- The arrow keys are used to:
- Step forwards or backwards within a menu
  - Step sideways within a menu.
  - Change digital position.

The + (Plus) and – (minus) keys are used to:

- Switch on or off a function
- Increase (+) or decrease (-) a numerical value.

The **F1** key is not in use

The **PRIME CYCLE** key is used to:

Flush or fill up the AutoCounter with reagent in the “1 Measurement” The **MENU** key is used to return to the previous menu.

#### **14.15.1 READY Lamp:**

Green light = home position, ready to start next analysis.

Red light = sample aspiration.

Red flashing light = aspirating completed, waiting for next move.

No light = the time between aspiration and home position

#### **14.15.2 REAG LOW Lamp:**

Is flashing red when the reagent level is low in any of the reagent containers.

The reagent which is too low is indicated on the display when entering the “1 Measurement”.

### **14.16 Sample Collection**

#### **14.16.1 Venous Blood**

Collect the blood by venepuncture in a tube containing tripotassium ethylenediaminetetra-acetic acid (K<sub>3</sub>EDTA) as anticoagulant (0.07 mol/ml blood). After blood collection the test tubes should immediately be gently mixed by reversing them approx. 10 times and there after rest of 15 minutes prior to analysis in order for the cells to stabilize. If the sample is analysed immediately, the MVC and WBC differential can be affected.

#### **14.16.2 Stability**

For whole blood cell counts which include WBC differential, the best results are obtained when the samples are analysed within 8 hours after drawing. These samples shall be kept at room temperature.

**Note:** For good quality results it is recommended that hematology samples are analysed as quick as possible after 15 minutes rest.

The blood count, except WBC differential can be analysed up to 24 hours after drawing if the specimens are stored in refrigerator. Make sure that the samples are brought to room temperature and well mixed before analyzing.

### 14.16.3 Capillary Blood

Use the Swelab dispenser calibrated for the AutoCounter. Dispense isotonic diluent into a sample beaker. In the C910EO+ Dispense-function is used. Collect 20  $\mu$ l capillary blood using a micro capillary tube and immediately transfer the blood into the sample beaker with 4 ml diluent. Rinse the capillary tube carefully with the isotonic diluent. Seal the sample beaker and ix gently.

### 14.16.4 Stability

The analysis of the prediluted sample should be performed as soon as possible but no later than within 60 minutes after collection and the sample dilution shall be kept at room temperature.

**Note:** The MVC value in prediluted control blood may decrease up to 5 femtolitre if not measured immediately, due to the dilution effect.

## 14.17 Analysis Process

### 14.17.1 Whole Blood

- A. At green READY light, 20  $\mu$ l blood is aspirated from the whole

blood sample via the pipette when the ▲-key is pressed. The aspiration is indicated by red light and when the aspiration is finished a flashing red light is shown. An unused beaker is placed

under the pipette and the ▼-key is pressed, the primary dilution is performed.

B. 20  $\mu$ l of the primary dilution is aspirated via the pipette when the

▲-key is pressed. The remaining of the primary dilution is placed in the WBC/HGB position and when the start lever is pulled the analysis process starts.

The 20  $\mu$ l of the primary dilution is mixed with 4ml isotonic diluent delivered from the diluent syringe directly into the counting beaker. The RBC/PLT dilution is mixed using air. The dilution is pulled into the measuring tube by the vacuum pump and the RBC/PLT count starts.

While RBC and PLT are counted the primary dilution in WBC/HGB position is hemolysed. When the RBC/PLT counting is ready the HGB blank is measured, the orifice is cleaned and the dilution is drained. The WBC/HGB dilution is transferred to the counting beaker and the WBC is counted. When the WBC counting is ready HGB is measured and the orifice is cleaned. The dilution is drained and the counting beaker is rinsed twice with isolation diluent.

The results are displayed. The READY lamp shows green light when the analysis process is ready and a new sample can be aspirated.

#### **14.17.2 Prediluted Blood**

The pre-diluted blood sample is prepared by adding 20  $\mu$ l of blood to 4 ml diluent. The 4 ml diluent is dispensed using the Dispense-function of AC910EO+. The 20  $\mu$ l blood is added to the 4 ml diluent using e.g. micro capillary tube. The description of the analysis process of the prediluted sample is the same as above from step B.

#### **14.17.3 Dispense (in AC910EO+)**

The menu is only available in the AC910EO+ software and is used to dispense 4 ml diluent for preparation of prediluted samples.

- 1- From the MAIN MENU step to “3 Dispense” with the ▼ key and press ENTER.
- 2- Place an unused beaker under the pipette and press the ▼ key to dispense 4 ml diluent into the beaker.
- 3- Exit with the MENU key.

#### 14.17.4 Prediluted Blood

The pre-diluted blood sample is prepared by adding 20 µl of blood to 4 ml diluent. The 4 ml diluent is dispensed using the Dispense-function AC910EO+. The 20 µl is added to the 4 ml diluent using e.g. micro capillary tube. The description of the analysis process of the prediluted sample is the same as above from step B.

B      20 µl of the primary dilution is aspirated via the pipette when the ▼-key is pressed. The remaining of the primary dilution is placed in the WBC/HGB position and when the start lever is pulled the analysis process starts.

The 20 µl of the primary dilution is mixed with 4ml isotonic diluent delivered from the diluid syringe directly into the counting beaker. The RBC/PLT dilution is mixed using air. The dilution is pulled into the measuring tube by the vacuum pump and the RBC/PLT count starts.

While RBC and PLT are counted the primary dilution in WBC/HGB position is hemolysed. When the RBC/PLT counting is ready the HGB blank is measured, the orifice is cleaned and the dilution is drained. The WBC/HGB dilution is transferred to the counting beaker and the WBC is counted. When the WBC counting is ready HGB is measured and the orifice is cleaned. The dilution is drained and the counting beaker is rinsed twice with isolation diluent.

The results are displayed. The READY lamp shows green light when the analysis process is ready and a new sample can be aspirated.

## 14.18 Measurement in AC910EO+

### 14.18.1 Background Count

- 1- In the Main Menu step to “1 Measurement” and press ENTER.
- 2- Aspirate with the  $\blacktriangle$ -key.
- 3- Dispense the blank dilution into an unused beaker with the  $\blacktriangledown$ -key.
- 4- Aspirate diluent from the beaker with the  $\blacktriangle$ -key.
- 5- Place the beaker in the WBC/HGB position. Pull the start lever towards the beaker. The analysis starts.
- 6- Repeat the background count until the values do not exceed the recommended level.

<u>RBC</u>	<u><math>\leq 0.02 \times 10^{12}/l</math></u>	<u>PLT</u>	<u><math>\leq 10 \times 10^9/l</math></u>
HGB	00 g/l	WBC	$\leq 0.2 \times 10^9/l$

**Note:** Always start and finish a measurement serie with a background count.

### 14.18.2 Blood Count of Whole Blood

- 2- In the Main Menu step to “1 Measurement” and press ENTER.
- 3- Mix the blood sample carefully and aspirate it through the pipette with the  $\blacktriangle$ -key. When the READY lamp shows red flashing light, the aspiration is completed. Wipe the pipette carefully.

- 4- Dispense the primary dilution into an unused beaker with the -key and continue with step 2 in the below section “Blood Count of Prediluted Samples”.

### 14.18.3 Blood Count of Prediluted Blood

- 1- In the Main Menu step to “1 Measurement” and press ENTER.
- 2- Mix the prediluted sample by gentle swirling and aspirate via the pipette with the -key. When the READY lamp shows red flashing light, the aspiration is completed. Wipe the outside of the pipette carefully.
- 3- Place the beaker in the WBC/HGB position. Pull the start lever towards the beaker. The analysis starts.
- 4- Press ENTER to enter the ID-number with the + (plus) or – (minus) keys. It is possible to enter the ID-number during the total counting time. The AutoCounter measures the prediluted blood sample. The measurement is completed when the READY lamp shows green light. The measurement results remain on the display until start of next analysis. To view the histogram press the -key. Press the -key to return to the results of the analysis.
- 5- Repeat from step 2 for all prediluted samples.

#### **Set Next Seq. No.**

If the sequence number has to be changed see “6.3 Set next sequence number.”

## 14.19 EO Menu

### Measurement EO

### Sample Separation

- 1- Switch on the AutoHeater. The red light diode marked with POWER is switched ON during warm up of the AutoHeater. When the AutoHeater has reached the right temperature, after approx. 10 minutes, the green light diode marked TEMP is switched ON.
- 2- Dispense 4.5 ml of the EO reagent with SWELAB's EO-dispenser into a sample beaker.
- 3- Preheat the EO reagent in the position 1-5, approx. 10 minutes. If more than 5 EO samples shall be analysed, load the AutoHeater during the measurement process.
- 4- Prepare the AutoCounter for an EO measurement:
  - In the Main Menu step to "2 EO menu" and press ENTER. Step to "2.1 Measurement EO" and press ENTER.
  - Start to measure a background count with pre-heated EO-reagent.

**14.19.1 In AC 970EO+/Ac920EO+:**

- b. Take one beaker of preheated EO reagent and place it in the prediluted position.
- c. Press "start PreDilute". The READY lamp switches from green to red light and at the same time the dilution is aspirated.

**14.19.2 In AC910EO+:**

Take one beaker of preheated EO reagent and place it in the WBC/HGB position and pull the start lever towards the beaker.

**14.20 Measurement of EO Dilution in the AutoCounter**

- 1- Remove the beaker from position 1 in the AutoHeater and turn the beaker wheel clockwise one step.
- 2- Add 20 µl of blood using the micro capillary tubes and mix the dilution gently by swirling the beaker. Put the beaker lid on.

**Note:** Do not turn the beaker upside down. The reagent can leak out due to the surface active ingredient in the EO reagent.

- 3- Place the EO dilution in the position marked green and press the beaker to the bottom. An alarm sounds and the timer starts.
- 4- Fill position 5 in the beaker wheel with a new beaker if necessary.
- 5- After 90 seconds the lysing of all cells, except EO, is completed and an alarm sounds. Press once again the beaker to the bottom to switch off the alarm. Measure the sample within 30 seconds.
- 6- In AC970EO+/AC920EO+:**
  - a. Swirl the EO dilution carefully and place it in the prediluted position.
  - b. Press "START PreDilute". The READY lamp switches from green to red light and at the same time the sample is aspirated.
  - c. Press ENTER to enter ID-number with the + (plus) or – (minus) keys. It is possible to enter the ID-number during the total counting time.

**.1 In AC910EO+:**

- a. Swirl the EO dilution carefully and place the dilution in the WBC/HGB position.
  - b. Pull the start lever towards the beaker. The READY lamp for the AutoCounter switches from green to red light and start the analysis.
  - c. Press ENTER to enter the ID-number with the + (plus) or – (minus) keys. It is possible to enter the ID-number during the total counting time.
- 7- The AutoCounter measures the EO sample and presents the results on the display. When measurement is completed the READY lamp shows green light. The results and the histogram remain on the display until start of next analysis.

Note: Results below 0.10 should be reported as  $< 0.10 \times 10^9/l$ .

- 8- Repeat from step 1 for all EO samples.

- 9- Clean and restore the system when all EO samples are measured. Run a background count in an unused beaker with 4 ml diluent.
- 10- After the background count the instrument is ready for measurement of routine blood samples.

#### **14.21 EO Memory**

The “2.2 EO Memory” is designed in the same way as for the „4 Sample memory” but the EO memory only contains the eosinophil results including the histograms.

When the memory is “full” the first sample entered is automatically deleted. In the EO memory a search of ID-number, DATE or SEQ - number can be performed.

2. Select the different ways of search conditions ID, DATE and/or SEQ-number.
3. Select one of the different options below, and press ENTER.
  - a. View selected EO samples.
  - b. Statistical calculation
  - c. Print selected samples
  - d. Send selected samples
  - e. Delete selected samples
4. Exit with the MENU key.