**Prins Leopold Instituut voor Tropische Geneeskunde**
**Institut de Médecine Tropicale Prince Léopold**
**Prince Leopold Institute of Tropical Medicine**
**Instituto de Medicina Tropical Principe Leopoldo**

Nationalestraat, 155
B – 2000 Antwerpen

Stichting van Openbaar Nut 0410.057.701

**POSTGRADUATE IN TROPICAL MEDICINE AND INTERNATIONAL HEALTH**

**MODULE 2**
**CLINICAL & BIOMEDICAL SCIENCES OF TROPICAL DISEASES**

# Practical notes

# TROPICAL HAEMATOLOGY

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**Philippe Gillet – Luc Boel - Jan Jacobs**
pgillet@itg.be , lboel@itg.be , jjacobs@itg.be

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# BLOOD COLLECTION

## CAPILLARY BLOOD

Capillary blood is the cheapest and the easiest method for blood collection. Capillary blood is mainly used when the volume of required blood is small (up to 100 µl). Disadvantages in using capillary blood for blood test include:

- Great possibility of sampling errors, particularly when the blood is not free-flowing (dilution of the sample with tissue fluid).
- Difficulty in obtaining sufficient blood (for more than one test).
- Rapid clotting of blood.
- Test cannot be repeated or further tests cannot be performed when results are unexpected.

Capillary blood is obtained by puncturing the skin with a lancet. In adults or children, the best place will be on the 3$^{rd}$ or 4$^{th}$ finger of the left hand, at the side of the finger, which is less sensitive than the tip. In infants, the best place will be the side of the heel or the big toe (The puncture should not be too deep because of the risk of osteomyelitis!). Never collect blood from an infected finger of foot. Never collect blood from an arm in which an intravenous infusion is being given (haemodilution). The puncture should be deep enough to result in free bleeding. A free flow of blood is essential and only the gentlest squeezing is permissible (risk of dilution with tissue fluid resulting in unreliable values).

1. Prepare all supplies in advance : sterile lancet, 2 pieces of cotton wool, one dry, the other soaked with alcohol 70 % and blood collection material (Sahli pipette, slide, capillary tube,…).
2. If possible, ask the patient to clean his hands with soap and hot water (vasodilatation), next dry his hands thoroughly.
3. Slightly massage the place where blood will be taken. Make sure the puncture area is warm enough to allow the blood to flow freely. If necessary, soak the hand or foot of an infant in warm water prior to collecting a sample.
4. Cleanse the puncture area with a cotton swab dipped in 70 % alcohol, let alcohol react for at least 30 seconds, then with a dry cotton to remove any remaining ethanol.
5. Using a sterile lancet, make a rapid puncture, sufficiently deep to allow the free flow of blood. Discard immediately the lancet in a safety container.
6. Execute a slight pressure on the finger to realise a better blood flow.
7. Wipe away the 1$^{st}$ drop of blood with a dry piece of cotton wool since it may contain tissue fluid or disinfectant. (Except for microfilaria detection, since the first drop contains more microfilaria).
8. Press the finger (not too hard) to produce in one time the required amount of blood.
9. When sufficient blood has been collected, press a piece of cotton dipped in 70 % alcohol over the puncture area until bleeding stops.

**For glycaemia determination**: The use of ether or any disinfectant should be avoided as it may interfere with the reagent strip (glucose oxidase peroxidase reaction). To decrease risk of infection and food contamination (fruit juice, ... ) it is therefore important to clean the hands with soap, before blood collection.

## VENOUS BLOOD

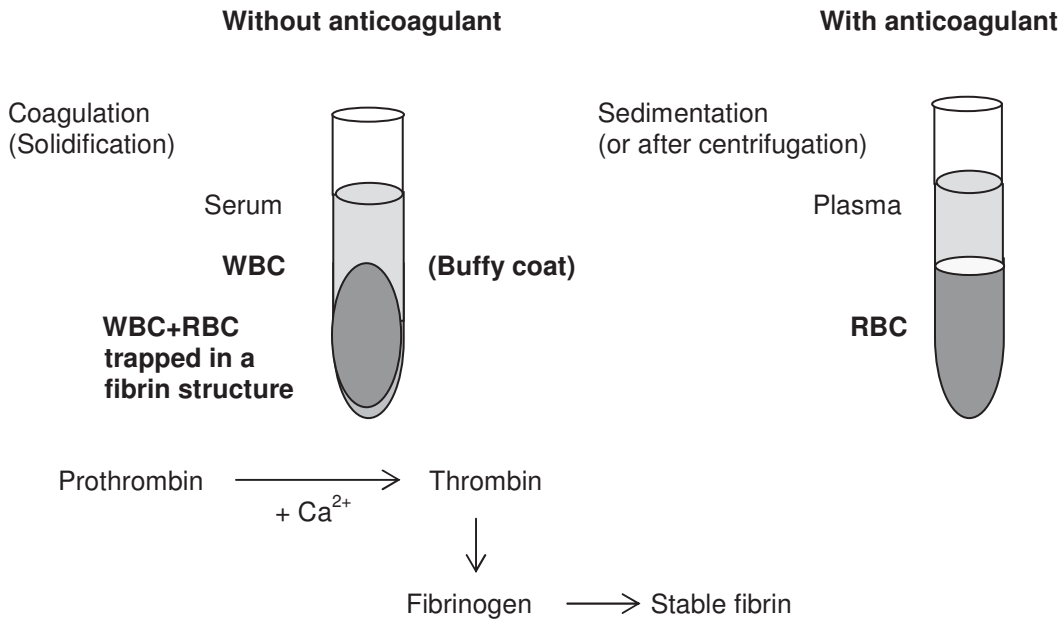
Venous blood is used when more than 100 µl of whole blood is required or when serum from a clotted blood sample is needed. If whole blood is needed, an anticoagulant should be used to prevent clotting and/or morphological blood cell changes. An anticoagulant acts by removing calcium (example : EDTA, trisodium citrate) or by interference with coagulation factors (example : Heparin)

- For most haematological tests (Haemoglobin, PVC, WBC count, blood group determination, …), dipotassium EDTA is recommended.

  Preparation of dipotassium EDTA tubes : Bring 40 µl of a dipotassium EDTA solution (10 g / 100 ml distilled water) in 3 ml tube. Leave the open tubes to dry at room temperature (protect from dust). Close when dry. The correct amount of blood must be added to avoid blood cell changes (2,5 ml). Excess EDTA causes shrinkage and degenerative changes, lack of EDTA will not prevent the coagulation.

- For measuring the ESR, trisodium citrate is used to anticoagulate the blood. 4 volumes of venous blood with 1 volume of trisodium citrate 32 g/l.

## DIFFERENCE BETWEEN SEDIMENTATION AND COAGULATION




**SERUM = PLASMA WITHOUT COAGULATION PROTEINS**

# HAEMOGLOBIN DETERMINATION

## INTRODUCTION

Anaemia is defined as having an amount of haemoglobin below reference values. Anaemia is present when haemoglobin concentration falls below 11 g/100 ml (hematocrit below 33 %). Anaemia is described as severe when the haemoglobin is below 7 g/100 ml (hematocrit below 21 %). Some authors (and maybe the WHO in the future) use 8 g/100 ml as cut-off value for severe anaemia (hematocrit below 24 %).

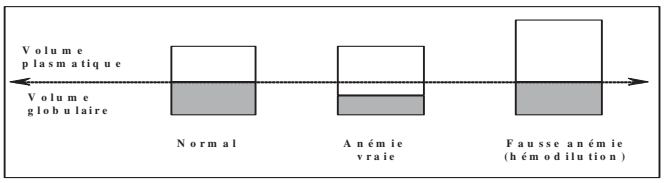
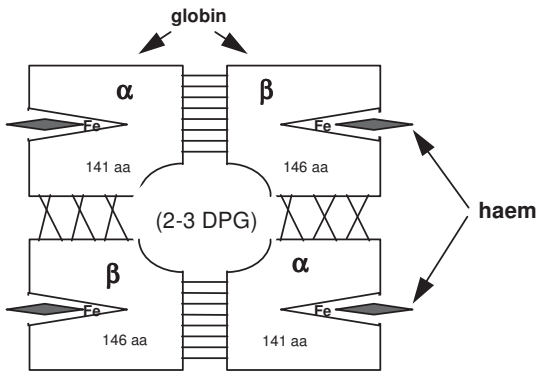



Fig.1 : True and "false" anaemia due to haemodilution. In some circumstances, the reduction of hemogram values is related to a haemodilution caused by plasma excess resulting in a "false anemia": pregnancy, splenomegaly, heart insufficiency, monoclonal immunoglobulines, especially IgM….

Haemoglobin is the most important part of the red blood cell. It is the red pigment that gives the colour to the red blood cells. It carries oxygen and carbon dioxide. Each molecule of haemoglobin contains four linked polypeptide (globin) chains and four haem groups. (Fig. 2). Haem is an iron-containing porphyrin pigment, which is the oxygen carrying part of the haemoglobin molecule. Oxygen binds reversibly with ferrous ions ($Fe^{2+}$), contained in each haem group. More than 97 % of normal adult haemoglobin is Hb $A_1$, having 2 *alpha* chains and 2 *beta* chains ($\alpha_2\beta_2$). Up to 3,5 % is $HbA_2$, consisting of 2 *alpha* chains and 2 *delta* chains ($\alpha_2\delta_2$). Less than 1 % is HbF (foetal) composed of 2 *alpha* chains and 2 *gamma* chains ($\alpha_2\gamma_2$). HbF is the predominant haemoglobin in foetus; untill 3-6 months of life.

Fig 2 : Haemoglobin structure
**(Haemoglobin A)**




2-3 DPG = 2-3 diphosphoglycerate

The measurement of haemoglobin is important for the diagnosis of the severity of anaemia.
Different techniques, more or less reliable, are available for haemoglobin determination. They are based on different principles, which can be classified in 3 families:

1. Techniques based on the red colour of blood without dilution nor haemolysis (Talquist, HCS, Lovibond, …)

2. Techniques based on the red colour of blood after haemolysis of the red blood cells (DHT,…).

3. Techniques based on the transformation of the haemoglobin (Sahli, Hemocue, Drabkin, …).

The PCV (Packed cell volume) or hematocrit may also be used to screen for anaemia.

The choice between these techniques will be based on reliability, repeatability, precision, accuracy, price, equipment needed, level of technical difficulty, staff training level, …

## REFERENCE VALUES[1]

The reference ranges for haemoglobin vary by age and sex as shown in the table below.

| Age | Sex | Haemoglobin |
|---|---|---|
| **Units** | | **g/100 ml** |
| 3 months – 12 months | Men and Women | 10,0 – 14,0 |
| 1 year – 12 years | Men and Women | 10,5 – 15,0 |
| 12 years – 100 years | Men (Europe) | 13,2 – 17,3 |
| 12 years – 100 years | Women (Europe) | 11,7 – 15,5 |

[1] Reference ranges vary in different population and in different laboratories (different techniques). District laboratories should check the figures above for the technique in use with their nearest hematology reference laboratory.

# HCS METHOD (HAEMOGLOBIN COLOUR SCALE)

PRINCIPLE :

The intensity of the red colour of blood corresponds with the amount of haemoglobin. The degree of anaemia can be visually assessed by matching the colour of a drop of blood on special filter paper against a standardized colour chart. The colour chart is developed to represent the colour range of normal to anaemic blood on filter paper : 14, 12, 10, 8, 6 and 4 g /100 ml.

EQUIPMENT AND SUPPLIES :

Blood collection equipment and supplies + Kit HCS (booklet of 6 shades of red, instructions for use, dispenser of 200 special absorbent test strips in handy box). Use only the special test-strips that are provided by Copack, since others may give inaccurate results. Keep these test-strips dry, clean and protected from direct sunlight at any time. 70 % Alcohol.

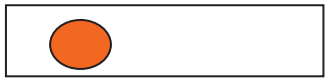
BLOOD COLLECTION :

Capillary or venous blood. For venous blood, dry anticoagulant should be used (to avoid dilution). EDTA di-potassium salt or heparin are recommended.

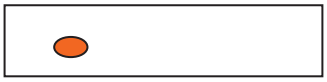
METHOD :

Find a suitable place: a room which is well-lit by daylight and/or artificial light. Avoid direct sunlight and marked shade. Do not read the scale in your own shadow.

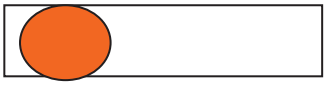
1. Place a drop of blood on one end of the test-strip so that it forms a spot, which is large enough to spread beyond the area of an aperture in the scale (about 1 cm in diameter).



Correct amount of blood : sufficient to spread beyond the area of an aperture in the scale.



Too little blood : The area of an aperture in the scale will not be covered.

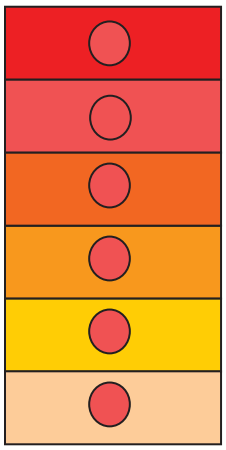


Too much blood : The spread will be too thick and the blood will take too long to dry.

2. Wait about 30 seconds after applying the blood; then read immediately. Any delay in reading the test will cause an error as the blood stain will change colour, becoming lighter and unreliable. Starting from the lightest shade or darkest shade, slide the blood stain up and down behind the apertures in the scale until you find the best colour match. When reading, keep the test-strip close to the back of the scale to prevent any stray of light from entering.

    - If the blood stain matches one of the shades of red exactly, record the haemoglobin value.
    - If the colour lies between two shades on the scale, record the mid-value.
    - If there is any doubt between two shades, record the lower value.

**Example:**



14 g/100 ml :Too light

**12 g/100 ml: Correct.**

10 g/100 ml: Too dark.

8 g/100 ml : Too dark.

6 g/100 ml : Too dark.

4 g/100 ml : Too dark.

MAINTENANCE :

To clean the scale, wipe the back side with a humid tissue (alcohol 70 %), then with a dry tissue. The scale should be cleaned at the end of each session and during the session if the surface becomes soiled during use.
The scale can be used for thousands of tests, but to avoid deterioration of the colours, always keep the booklet closed after use and never leave it exposed to direct sunlight. It should be replaced periodically.

# LOVIBOND® METHOD (Harrison's method)

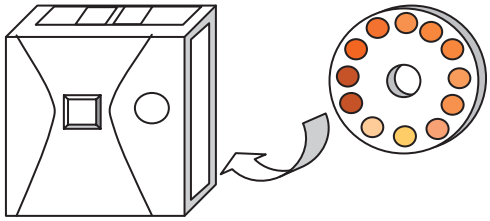
PRINCIPLE :

The Harrison's method is a simplified version of a visual comparative technique for estimating haemoglobin. The red colour of blood corresponds with the amount of haemoglobin. Blood is inserted directly into a special cell (thickness of 0,004") without any preliminary manipulation. The colour is compared with a series of reference glasses in a Lovibond® comparator.

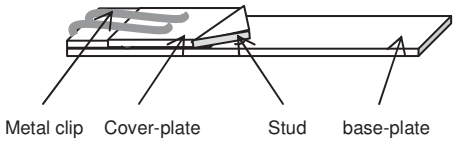
MATERIAL :

Blood collection equipment and supplies + Lovibond 2000 comparator, standard discs Lovibond 5/8 A and 5/8 B, Lovibond cell 0,004", soft paper, Beaker, chlorine solution 1 %, 70 % alcohol.

Lovibond® 2000 comparator Disc



Lovibond® 0,004"[2]




The blood cell consists of a base-plate and a cover-plate constructed from plain white glass. Fused on the cover-plate are three small studs of glass which create, when in position a cell of 0,004" thickness. This cell is filled from the side by capillarity.

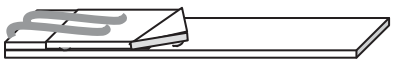
BLOOD COLLECTION :

Capillary blood. [For venous blood, dry anticoagulant should be used (to avoid dilution). EDTA di-potassium salt or heparin are recommended].

METHOD :

1. Clean and decontaminate the base-plate and the cover-plate, first with water, then with 70 % alcohol.

2. Place the two plates in position and join them with the clip.



[2] 0,004 inch = 1.016 mm

3. Check if the discs are clean. Wipe with a cloth if they become dirty. Select the appropriate disc (evaluation of conjunctival pallor) and insert it into the comparator with the values towards the front of the instrument. Rotate the disc until the lowest or the highest value.

**CHECK THE CELL BEFORE USING IT :**

Decontamination with hydrochloric solution, next with 70 % alcohol?
Is it dry and dust free, and clean, no lines nor finger prints?
Does the cover plate cover properly the base plate ?
Are the figures 004 well readable?

4. Fill the cell by capillarity with capillary blood. If there are air bubbles in the cell, restart from point 1.

5. Clean the blood excess around the cell with a soft paper.

6. Remove the clip.

7. Place the cell, containing the prepared sample in the right compartment. Using a diffuse light (facing southern daylight in the southern hemisphere), rotate the disc until the closest colour matches with the sample. (Be quick to avoid desiccation or coagulation). The Lovibond value will be shown in the window in the bottom right corner of the comparator.

**Find a suitable place for the colour comparison :**

- During the day : Facing a white surface (southern daylight in the southern hemisphere).
- During the night : Facing a white surface illuminated by a white lamp (not fluorescent light).
- Direct sunlight or direct artificial light gives incorrect results.

8. Convert the Lovibond value in g/100 ml (cf. conversion table).

9. Decontaminate the Lovibond cell (contact with 1 % chlorine solution during 30 minutes).

10. To avoid deterioration of the colours, store immediately the disks in a box.

10. Clean and decontaminate the base-plate and the cover-plate, first with water, then with 70 % alcohol.
11. For practical reasons it can be advised to mount the plates in advance so that no time will be lost in case of emergency.

## SMALL PROBLEMS AND SOLUTIONS :

**ALWAYS CHECK THE CONCORDANCE BETWEEN THE HAEMOGLOBIN VALUE AND THE COLOUR OF THE CONJONCTIVES.**

1. **No value appears in the inner opening of the comparator.**

   ⇒ The disk is placed back to front. Take out the disk and turn it round.

2. **No colour corresponds with the colour of the blood of the patient.**

   ⇒ The chosen disk does not correspond with the expected haemoglobin value. Use the other disk.

   ⇒ Thee patient is icteric (jaundice). His upper conjunctives are yellow. Find the closest colour and note the presence of jaundice in the report.

   ⇒ The blood colour is inferior to the minimum of the comparator. Give as result < than 20 % or < than 3,3 g/100 ml.

3. **The measured haemoglobin value seems to be too low.**

   ⇒ Beware that the cover plate is placed in the correct way (figures 004 readable). If this is not the case, restart the measurement with another plate.

⇒ Beware there are no air bubbles between base and cover plate. If this is not the case, restart the measurement with another plate.

⇒ Check the cleanliness of the comparison disk and, if necessary, clean it with a soft tissue.

⇒ Check the cleanliness of the translucent plate of the comparator and, if necessary clean it with a hydrochloric solution of 1 %, next with filtered water.

**If non of these solutions are helping and if the values are systematically too low, make a comparison between the new and the old disks (the colour of the disks is degrading with light).**

**4. Too high haemoglobin values.**

⇒ The upper side of the cover-plate and/or the under side of the base-plate are soiled with blood. Take of the blood on both sides with a soft paper.

⇒ The disinfectant used for the blood taking is stained (e.g.Betadine). Restart the measurement using Ethanol à 70 %.

⇒ Check the cleanliness of the translucent plate of the comparator and, if necessary clean it with a hydrochloric solution of 1 % , next with filtered water.

⇒ Thee patient is icteric (jaundice). His upper conjunctives are yellow. Find the closest colour and note the presence of jaundice in the report.

⇒ The patient is dehydrated (provoking a haemoconcentration). No solution, note the dehydration in the answer.

CONVERSION TABLE % LOVIBOND TO g / 100 ml :

IT IS HELPFUL TO TAKE THE COLOR MATCH UNTIL SLIGHTLY BELOW,
THEN SLIGHTLY ABOVE, IN ORDER TO FIND THE BEST COLOR MATCH.

| DISKS | PERCENTAGE | g / 100 ml |
|---|---|---|
| Disc N° 5/8 A (light colour) For low Haemoglobin values | 20 | 3.3 |
| | 24 | 4.0 |
| | 28 | 4.7 |
| | 32 | 5.3 |
| | 36 | 6.0 |
| | 40 | 6.7 |
| | 46 | 7.3 |
| | 52 | 8.7 |
| | 58 | 9.7 |
| Disc N° 5/8 B (dark colour) For high Haemoglobin values | 64 | 10.7 |
| | 70 | 11.7 |
| | 76 | 12.7 |
| | 84 | 14.0 |
| | 92 | 15.3 |
| | 100 | 16.7 |
| | 110 | 18.3 |
| | 120 | 20.0 |
| | 130 | 21.7 |

# SAHLI METHOD

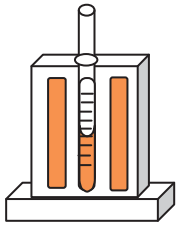
PRINCIPLE :

The Sahli method is based on converting haemoglobin to acid haematin (brown colour) and then visually matching its colour against a solid glass standard. Diluted hydrochloric acid is mixed into a graduated cylinder with an accurate volume of blood sample and distilled water is added until the colour of the diluted blood sample matches the glass standard. The dilution will be determined by the Haemoglobin level of the blood sample.

The Sahli method, still used in a lot of places, is not recommended. The Sahli method is not an accurate way of estimating haemoglobin: Not all the forms of haemoglobin are changed into acid haematin, the colour changes when viewed visually are not very great and the brown colour of the glass standard is not a true match for an acid haematin solution.

MATERIAL :

Blood collection equipment and supplies + Sahli haemoglobinometer, small glass rod, Sahli pipette, safety device for pipetting, Sahli tube and dropping pipette.



The Sahli haemoglobinometer is equipped with two glass colour standards. The Sahli tube graduated until 16 g/100 ml and/or in "percentage" (16g/100 ml = 100 %), is placed in-between.

REAGENTS :

Hydrochloric acid 0,1 N :

| | | |
|---|---|---|
| Hydrochloric acid, concentrated[*] | : | 9,5 ml |
| Distilled water | : | up to 1 litre |

[*] **Caution, hydrochloric acid** is irritant and corrosive. Handle with care in well ventilated area (or in a fume cupboard). Fill until half a 1.000 ml volumetric flask with distilled water. Add slowly 9.5 ml concentrated hydrochloric acid. After cooling, fill until the 1,000 mark with distilled water and mix well. This reagent is stable for at least 1 year at room temperature.

Distilled water (or filtered water).

BLOOD COLLECTION :

Capillary or venous blood. For venous blood, dry anticoagulant should be used (to avoid dilution). EDTA di-potassium salt or heparin are recommended.

METHOD :

1. Fill the graduated measuring tube up to the bottom graduation line with 0.1 N hydrochloric acid. (The mark level should be equal with the bottom of the meniscus formed by the liquid).

2. Check the tip of the Sahli pipette. Discard if broken (volume error). Check if the pipette is dry.

3. Draw the blood a little bit further than the 20 µl mark of the Sahli pipette. Do not allow air bubbles to enter. Wipe the outside of the pipette with absorbent paper and adjust the blood on the 20 µl mark.

4. Blow the blood from the pipette into the graduated tube of the acid solution.

5. Rinse the pipette by drawing in and blowing out the acid solution.

6. Allow to stand for 1 minute. The mixture will become dark brown and clear.

7. Place the graduated tube in the haemoglobinometer, compare the colour in diffused day light. Add water drop by drop and mix with the glass stirrer until the colour of the solution matches the colour of the reference tube.

8. When equal colours are reached, take the glass stirrer out of the graduated tube and read the level of the base of the menisci of the liquid. Hold the instrument about 50 cm away from your eyes, on the same height under diffuse light.

9. Note the reached mark that corresponds with the level on the tube.

# DRABKIN METHOD

PRINCIPLE :

**The haemiglobin cyanide method is the most accurate method of measuring haemoglobin and is considered as the reference standard (« gold standard").** Whole blood is precisely diluted 1 on 201 in a Drabkin solution. The red cells are haemolysed and the haemoglobin is oxidized by the ferricyanide to methaemoglobin. This is converted by the cyanide to stable haemoglobin cyanide.

**$Hb + K_3Fe(CN)_6 \rightarrow MHb$**

**$MHb + KCN \rightarrow HiCN$**

[Hb = haemoglobin, MHb = methaemoglobin, HiCN = haemiglobincyanide]

The chemical reaction takes place at a pH stabilized by a monopotassium phosphate buffer in order to obtain a complete reaction in a reasonable time. Addition of a detergent facilitates haemolysis and prevents turbidity caused by plasmatic proteins. The optical density is in proportion with the haemoglobin quantity that is present in the blood.
Absorbance of the HiCN is read in a spectrophotometer at the wavelength 540 nm or in a colorimeter using a yellow-green filter. The absorbance obtained is compared with that of a reference HiCN standard solution. Haemoglobin values are obtained in the calibration graph.

BLOOD COLLECTION :

Capillary or venous blood. For venous blood, dry anticoagulant should be used (to avoid dilution). EDTA di-potassium salt or heparin are recommended. After thoroughly mixing with anticoagulant, the blood can be frozen for as long as 2 years (and used as control).

MATERIAL :

Blood collection equipment and supplies + spectrophotometer (or colorimeter) that transmits light at 540 nm, Sahli pipette (or 20 µl automatic pipette), 5 ml graduated pipette (or 5,0 ml dispenser), safety device for pipetting, test tubes, cuvettes, different volumetric flasks and different calibrated pipettes (for calibrator's preparation), graph paper.

REAGENTS :

Drabkin's neutral diluting fluid (pH 7,0 – 7,4) (also "ready to use" available example Sigma D 5941) :

| | | |
|---|---|---|
| Potassium hexacyano ferrate ($K_3Fe(CN)_6$) | : | 0,20 g |
| Potassium Cyanide (KCN)☠ | : | 0,05 g |
| Potassium dihydrogen phosphate ($KH_2PO_4$) | : | 0,14 g |
| Tween 20 | : | 1 ml |
| Distilled water | : | to 1 litre |

(Tween20 can be replaced by 0.5 ml Brij-35 at 30 %)

☠ **CAUTION : Potassium cyanide is highly poisonous**. It is a pale yellow clear fluid which may no longer be used if its colour is lost or if it becomes turbid. The prepared Drabkin solution is stable for at least 6 months at room temperature protected from light (amber bottle).

Haemiglobin cyanide (cyanmethaemoglobin) standard for calibration:

HiCN reference standard solutions are stable for long periods and are commercially available as: Haemoglobin standard (SIGMA), HiCN BS 3985 (Merck/BDH), …

Example with Haemoglobin standard from SIGMA :

- Reconstitute one vial of the standard by adding 50,0 ml of the Drabkin solution.
- Mix well.
- Wait at least 30 minutes before use.

The standard must be stored at 2- 8°C in the dark and is stable for at least for 6 months.

Prepare a calibration graph from this HiCN reference standard. Plot the absorbance readings at 540 nm of different standard dilutions against their known concentrations of haemoglobin. The curve is linear, passing through the origin.

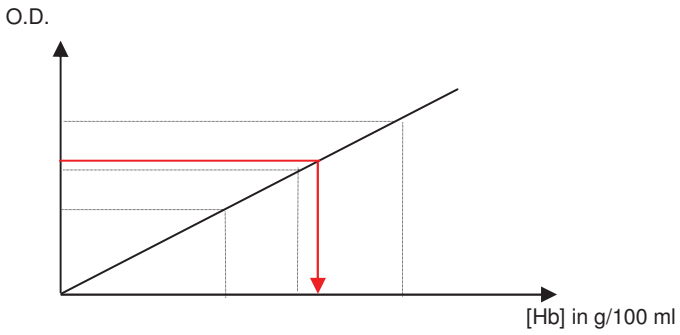
Dilution's example:

| Tube N° | Volume in ml Drabkin solution | Volume in ml diluted standard | final concentration in g % |
|---|---|---|---|
| 1 | 4 | 0 | 0 |
| 2 | 3.5 | 0.5 | 2.25 |
| 3 | 3 | 1 | 4.5 |
| 4 | 2 | 2 | 9 |
| 5 | 1 | 3 | 13.5 |
| 6 | 0 | 4 | 18 |

METHOD :

1. Set the spectrophotometer wavelength at 540 nm.
2. Set up a series of labelled test tubes for blank and tests.
3. Add 4.0 ml of the Drabkin solution to all tubes.
4. Add 20 µl of whole blood sample to each labelled test tube.
5. Rinse the pipette 3 times with the reagent.
6. Mix well and allow to stand for at least 5 minutes at room temperature. (Attention, the reaction can take until 30 minutes for a sample containing an increased proportion of carboxy haemoglobin).
7. Read and record absorbance of each test (or control) versus the blank as the reference at 540 nm in the same instrument used for preparing the calibration curve. The colour is stable for several hours.

Determine the haemoglobin concentration of each test directly from the calibration curve.




**Or**

$$\text{Calculated: } \frac{\text{O.D. test}}{\text{O.D. Calibrator}} \times \text{calibrator's concentration} = \text{sample's Hb concentration}$$

# HEMOCUE® B

PRINCIPLE :

The HemoCue® is an example of a robust, portable and accurate haemoglobinometer readily available for use. Although not affordable by most district laboratories, it may be used for survey. The HemoCue uses calibrated disposable cuvettes that are treated with chemicals (sodium desoxycholate, sodium nitrite and sodium azide) which rupture the red cell wall and combine with the haemoglobin to form a compound (azidemethaemoglobin) which can be measured photometrically (modified Vanzetti reaction : Sodium nitrite converts the haemoglobin iron from the ferrous to the ferric state to form methaemoglobin. The methaemoglobin then combines with azide to form azidemethaemoglobin and is measured photometrically at two wavelengths, 570nm and 880nm). The result in g/100 ml is displayed in digital form on the face of the instrument. Web site : http://www.hemocue.co.uk/

MATERIAL :

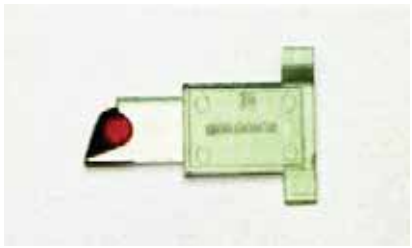
Blood collection equipments and supplies + HemoCue® B disposable cuvettes, HemoCue® B instrument, HemoCue® B standard control cuvette, batteries.

BLOOD COLLECTION :

Capillary or venous blood. For venous blood, dry anticoagulant should be used (to avoid dilution). EDTA di-potassium salt or heparin are recommended.

METHOD :

1. Check the stability of the calibration : Place the control cuvette into the cuvette older and push it into the measuring position. The displayed value should not deviate more from the assigned value on the control cuvette card than + /- 0.3 g/100 ml (for our control cuvette N° 0149-003-037 the value lies between 11.0 and 11.6 g/100 ml).



2. Fill the disposable cuvette with the blood drop by touching the capillary tip of the cuvette with the blood drop.

3. Be sure that the cuvette is entirely filled with blood. If air bubbles are present, discard the cuvette and fill a new disposable cuvette.

4. Wipe off the excess of blood on the outside of the cuvette tip. Make sure that no blood is drawn out of the cuvette in this procedure.

5. Place the filled cuvette into the cuvette holder immediately and push it into the measuring position.

6. After approximately 30 to 50 seconds the result is displayed (in g/100 ml (or g/l or mmol/l). The filled cuvette should be analysed at least 10 minutes after it has been filled (evaporation).

**N.B. :** 3 different models are now commercialized : HemoCue® B et HemoCue® 201+ for « normal working conditions » (temperature < 30°C and low humidity) and The Hb 301 system is optimized for use in primary care and designed for high temperatures and humidity. If the 2 first model (Band and 201) use the principle explained above, the model 301 use a different analytical method : The measurement takes place in the analyzer, which measures the absorbance of whole blood at a Hb/HbO2 isobestic point. The analyzer measures at two wavelengths (506 and 880 nm) in order to compensate for turbidity. In fact, it's an automatised version of the Lovibond technic.[3]


Hemocue® B


Hemocue® 201+

Hemocue® 301+



Microcuvettes B and 201 are to be stored at room temperature (15-30°C). The reagents contained within the HemoCue® microcuvettes B and 201 are moisture sensitive: Recap vial immediately after removing cuvettes and do not remove desiccant from the vial. The microcuvettes 301 are to be stored at 10–40 °C (50–104 °F). Once the seal of the vial is broken, the microcuvettes are stable for 3 months. An unopened vial of microcuvettes can be stored for a shorter period of time (6 weeks) between -18–50 °C. HemoCue Hb 301 Analyzer is only to be used with correspondent HemoCue Hb microcuvettes.

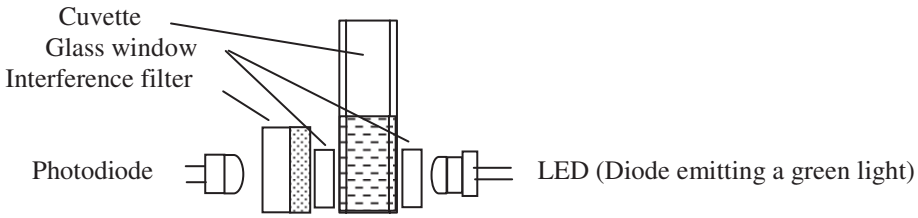

HemoCue® B-HB Photometer Hemoglobin Controls HYC84665 3x3 ml (1 low, 1 normal, 1 high) : HemoCue® B-HB Photometer Hemoglobin Controls. Features a 2-year expiration from date of manufacture at 2 – 8°C, 60-day open vial stability at 2 – 8°C, and a 30-day open vial stability at room temperature. Utilizes stabilized whole human red cells which process like a fresh patient sample.

[3] Evaluation of the utility of the Hemocue 301 haemoglobinometer for blood donor screening. L. D. Morris, A. Ossei-Bimpong, D. McKeown, D. Roper and S.M. Lewis. Vox sanguinis (2007) 93, 64-69.

# DHT HAEMOGLOBINOMETER (Developing Health Technology) (Ammonia technique)

PRINCIPLE :

Whole blood is diluted 1 in 101 in a weak ammonia solution. The red cells are haemolysed and the amount of haemoglobin is measured over a narrow spectral band. The measurement of the optical density is carried out at a wavelength of 523 nm, the crossing point of absorption curves of various haemoglobin forms, which are thus detected with equal sensitivity. This optical density is automatically converted to read-out directly as haemoglobin concentration in g/l on a liquid crystal display. No zero adjustment or calibration is required and no calibrating solutions are needed. Web site : www.dht-online.co.uk




MATERIAL :

Blood collection equipment and supplies + DHT haemoglobinometer, test tubes, Sahli pipette (or 20 µl automatic pipette), 2 ml graduated pipette (or 2,0 ml dispenser), safety device for pipetting, 1.000 ml volumetric flask, 10 mm light path cuvettes.

BLOOD COLLECTION :

Capillary or venous blood. For venous blood, dry anticoagulant should be used (to avoid dilution). EDTA di-potassium salt or heparin are recommended.

REAGENTS :

Ammonia solution 0,04 % (v /v):

Preparation with ammonia 28 % concentrated:

| | |
|---|---|
| Ammonia 28 % concentrated | 1,4 ml |
| Distilled water | up to 1.000,0 ml (volumetric flask) |

**CAUTION : Ammonia solution is a corrosive chemical with an irritating vapour.** Handle with care in well-ventilated area (or in a fume cupboard). **Keep the stock bottle well stoppered.**

This solution is stable when kept in a tightly stoppered bottle. Renew every 4 weeks

Any concentration of any ammonia solution can be made by taking a volume of concentrated solution equivalent to the required % and making this up with distilled or deionised water to a volume equivalent to the % of concentration :

**C1 x V1 = C2 x V2**

C1 = Concentration of the concentrated ammonia solution
V1 = Volume of the concentrated ammonia solution

C2 = Needed ammonia concentration
V2 = Needed volume of the weak ammonia solution

Example : C1 = 28 %
V1 = ?
C2 = 0,04 %
V2 = 1.000 ml

28 % x V1= 0,04 % x 1000 ml ➔ V1 = 1,429 ml

METHOD :

It's vital to understand the factors that influence accuracy of measurement and dilution. The accuracy of haemoglobin measurement depends on accuracy of proportion of blood to diluent. The quality of the used water and ammonia as diluent is also quite important.

INITIAL CHECKS OF OPERATION AND FACTORY CALIBRATION : **(Caution : Used values are only valid for** the **haemoglobinometer N° 0931)** that is used at the ITM.

Do not touch the clear working side areas of the cuvette. Avoid contamination of these, handling the cuvette only by the top of the etched surfaces at front and back (non optical sides). Always ensure the outer surface of the cuvette is completely dry. When filling cuvette, ensure no air bubbles are present to affect measurement.

A. CHECK BLANK :

Install a CLEAN and DRY cuvette into the cuvette aperture. (Use the same kind of cuvette which is used for haemoglobin determination). The value on the display must correspond with the blank value BR1 (+/- 5) [14-24 g/l for our haemoglobinometer 0931]. If this is not the case check for the following cause:

| **PROBABLE CAUSE** | **REQUIRED ACTION** |
|---|---|
| Cuvette not well installed : | Insert the cuvette with the mat etched surface in front of you. Check again the reading for BR1. |
| Scratched cuvette : | Use new cuvette.<br>Check again the reading for BR1. |
| Dirty or wet cuvette surface : | Clean and dry the cuvette.<br>Check again the reading for BR1. |
| Value of scale factor M incorrect : | Press and hold down the « R » button located at the back of the device. The symbol "HHH", and after some seconds the scale factor M will appear. Compare this value with the figure for M shown in "certification" (162 for our device). If there is a difference between these values, then adjust the displayed value by pressing the "L" button to lower the value and the "R" button to raise the value. Check again the reading for BR1. |
| Zero level of the device incorrect | Place a clean cuvette filled with distilled water into the cuvette window. Press and hold down the "L" button. At first the display will show the last reading, followed after several seconds by a "beep". After this beep the LCD turns off and the new zero level is stored in the device. Check again the reading for BR1. |
| Other type of cuvette used for the calibration : | Place a clean cuvette filled with distilled water into the cuvette window. Press and hold down the "L" button. At first the display will show the last reading, followed after several seconds by a "beep". After this beep the LCD turns off and the new zero level is stored in the device. Check again the reading for BR1. |
| Dirty or wet photocell windows : | Clean the glass of the photocell windows with alcohol on cotton swap stick. Check again the reading for BR1. |

B. CHECK ZERO :

Place a cuvette containing 1-2 ml of the weak ammonia solution (0,04 %) into the cuvette aperture. (Use the same kind of cuvette which is used for haemoglobin determination). The value on the display should be zero. If this is not the case remove the cuvette and within 2 seconds press and hold the "L" button until the beep. Zero will automatically be reset. Check again the reading for zero.

C. CHECK CALIBRATION :

The permanent calibration control standard (checker), supplied with each instrument, is numbered and matched uniquely to one instrument. Place the checker into the cuvette aperture. The value of the displayed reading must correspond to the value CR1 (+/- 5) [133-143 g/l for our haemoglobinometer 0931]. If this is not the case check for the following causes:

| PROBABLE CAUSE | REQUIRED ACTION |
|---|---|
| Cuvette not well installed : | Insert the cuvette with the plastic surface in front of you. Check again the reading for CR1. |
| Scratched control cuvette : | Buy a new cuvette.<br>Check again the reading for CR1. |
| Dirty or wet control cuvette surface : | Clean and dry the cuvette.<br>Check again the reading for CR1. |
| Value of scale factor M incorrect : | Press and hold down the « R » button located at the back of the device. The symbol "HHH", and after some seconds the scale factor M will appear. Compare this value with the figure for M shown in "certification" (162 for our device). If there is a difference between these values, then adjust the displayed value by pressing the "L" button to lower the value and the "R" button to raise the value. Check again the reading for BR1 |
| Zero level of the device incorrect | Place a clean cuvette filled with distilled water into the cuvette window. Press and hold down the "L" button. At first the display will show the last reading, followed after several seconds by a "beep". After this beep the LCD turns off and the new zero level is stored in the device. Check again the reading for BR1. |
| Other type of cuvette used for the calibration : | Place a clean cuvette filled with distilled water into the cuvette window. Press and hold down the "L" button. At first the display will show the last reading, followed after several seconds by a "beep". After this beep the LCD turns off and the new zero level is stored in the device. Check again the reading for BR1. |
| Dirty or wet photocell windows : | Clean the glass of the photocell windows with alcohol on cotton swap stick. Check again the reading for BR1. |

TEST METHOD :

**Attention: The precision of the measurement depends for a great part on the skill and proficiency of the technician in preparing the right concentration and volume of ammonia and blood and of the quality of the distilled water.**

1. Bring 2,0 ml of the ammonia reagent in a test tube for each sample to be measured
2. Check the pipette on its cleanliness, if it's dry and if the point is not broken.
3. Measure 20 µl of capillary blood or well-mixed venous blood
4. Aspirate the blood a bit higher than the 20 µl line of the pipette. Clean the blood on the outside of the pipette and adjust the volume with a clean absorbent paper.
5. Dispense this volume into the 2 ml of the ammonia diluting fluid by rinsing the pipette 3 times (aspirate and blow out the pipette).
6. Stop the tube and mix. The solution can be read immediately. The colour is stable for 6-8 hours.
7. Transfer the patient's sample to a clean 10 mm light-path cuvette.
8. Place the cuvette into the cuvette holder, wait for the audible signal and read the displayed haemoglobin value.
9. Return the sample to its tube and allow the cuvette to drain, e.g. invert it on a paper towel.

Caution, if the audible signal, accompanying the photometry process, ends before the cuvette is fully seated into the device, the result may be wrong. Wait a few seconds for the next measurement cycle to complete.

# PACKED CELL VOLUME BY CENTRIFUGATION or HEMATOCRIT

PRINCIPLE :

The hematocrit level, or packed cell volume, is a measure of the ratio of red cells to the total volume of whole blood (plasma, white blood cells and red blood cells) and is expressed as a percentage.

$$PCV = \frac{\text{Volume of red blood cells}}{\text{Volume of whole blood}} \times 100 = x\ \%$$

In the new units, the PCV is expressed as a ratio (litre/litre), the same formula but without a multiplication by 100.

The blood is placed in a standard size capillary tube and centrifuged at high speed. After centrifugation, the volume occupied by the red cells is measured. Because of a uniform bore of the capillary, the length is directly proportional to the volume.

$$PCV = \frac{\text{Length of red cell column (mm)}}{\text{Length of total column (mm)}} \times 100 = x\ \%$$

EQUIPMENT AND SUPPLIES :

Blood collection equipments and supplies + micro hematocrit centrifuge (radius greater than 8 cm, able to achieve maximum speed within 30 seconds and to maintain a centrifugal force of at least 10.000*g* for 5 minutes without exceeding a temperature of 45°C) , Disposable heparinised capillary tubes (length 75 mm diameter 1.5 mm), spirit lamp (or clay sealant or plasticine), reference chart.

BLOOD COLLECTION :

Capillary or venous blood. For venous blood, dry anticoagulant should be used (to avoid dilution). EDTA di-potassium salt or heparin is recommended. In this case, plain capillary tubes should be used. Since the hematocrit increases in function of the conservation time, the examination must be executed within 6 hours.

METHOD :

1. Fill about three quarters of either:

   A plain capillary with well mixed EDTA anticoagulated blood or
   A heparinised capillary with capillary blood.

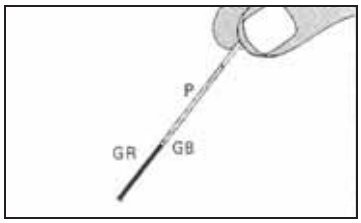
2. Seal by heath the unfilled end using a small flame from a spirit lamp (or seal the unfilled end using a sealant material).

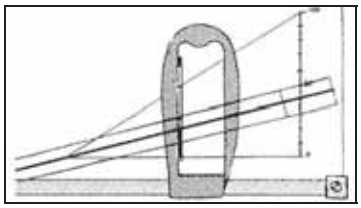
3. Carefully locate the filled capillary in one of the numbered slots of the micro hematocrit rotor with the sealed end against the rim gasket. Write the number of the slot on the patient's form.

4. Balance the diametrically opposite slot with a capillary and centrifuge at high speed for 5 minutes.

5. After centrifugation, the capillary tube will show 3 layers:

- At the top, a column of plasma (P).
- In the middle, a very thin white layer (Buffy coat) of white cells and platelets (GB).
- At the bottom, a column of red cells (GR).



Using a reference chart : Line up the bottom of the red cells at the zero mark. Slide the capillary tube along the scale until the top level of plasma reaches the 100 mark. The line passing through the top of the red cell column will indicate the packed cell volume. Do not include the buffy coat as part of the red cell level in the reading. After reading discard safety the capillary tube.

REMARKS :

Other information from the PCV : Plasma from normal blood appears straw-coloured. In iron deficiency it appears colourless. When it contains an increased amount of bilirubin, it will appear abnormally yellow. If the plasma is pink-red, this indicates a haemolysed sample (a new blood sample should be tested). In thalassaemia major, the red cells column appears dark-red. When white cell numbers are significantly increased (> 20.000/ $mm^3$), this will be reflected in an increase in the volume of the buffy coat layer. The microscopical examination of the dividing line between the blood cells and the plasma, is used for microfilaria or trypanosome detection (Woo technique).

REFERENCE VALUES [4] :

In a similar way to haemoglobin levels, PCV values vary according to age, gender and altitude.

| Age | PCV %<br>(international units) |
|---|---|
| Children at birth | 50 to 58 (0.50 to 0.58) |
| Children (3 months) | 35 to 40 (0.35 to 0.40) |
| Children (1 year) | 31 to 36 (0.31 to 0.36) |
| 5 years | 33 to 37 (0.33 to 0.37) |
| Adult women | 36 to 45 (0.36 to 0.45) |
| Adult men | 42 to 49 (0.42 to 0.49) |

[4] Reference ranges vary in different population and in different laboratories (different techniques). District laboratories should check the above figures for the technique in use with their nearest hematology reference laboratory.

INTERPRETATION OF PCV:

PCV is an easy, simple but indirect technique to detect anaemia. It is of diagnostic value in patients suffering from anaemia, dehydration, shock or burns. The number of red blood cells, the size of red blood cells and plasma volume influence the PCV. If the number of red blood cell stays in the normal range, 1% PCV represents more or less 110.000 red blood cells/mm³ of blood.

As the ratio between the haemoglobin concentration in the red blood cells and the red blood cell volume is quite stable, there is normally a linear relationship between PCV and haemoglobin concentration. The formula [PCV (in %) x 0,3] + 2 gives roughly an idea of the haemoglobin concentration. This is only true in case of normocytic or normochromic anaemia. This formula will not substitute the haemoglobin determination.

> Example : the estimation of the haemoglobin concentration for a PCV of 33 % is 10.1 g/100 ml [(33 x 0.3) + 0.2].

In case of a normocytic or normochromic anaemia, the formula stays equal, since hematocrit and haemoglobin are equally reduced.

> Example: For a hematocrit of 27 %, the haemoglobin is estimated on:
> (27 x 0.3) + 0.2 or 8.4 g/100 ml.

**In case of a macrocytic or hypochromic or megaloblastic anaemia, but also in case of a microcytic anaemia, this estimation does not work.**

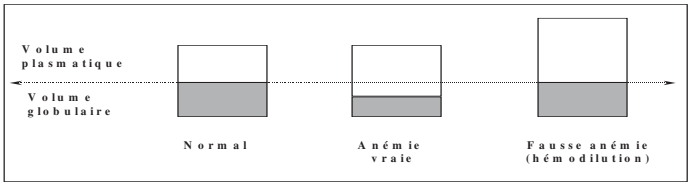



Fig.3 : True and “false” anaemia due to haemodilution.

PCV is decreased in anaemia. PCV values are increased in case of loss of plasma (dehydration, cholera, diarrhoea, severe burns, …), in dengue haemorrhagic fever, and (rarely) in all forms of polycythaemia.

# RED BLOOD CELL INDICE (MCHC)

Red cell indices are frequently used in the investigation of anaemia. If a laboratory is able to measure a PCV and to perform an accurate haemoglobin determination, an MCHC (Mean Corpuscular Haemoglobin Concentration) can be calculated. The MCHC gives the concentration of haemoglobin in red blood cells.

| MCHC (g/100 ml) = Haemoglobin concentration (g/100 ml) / PCV (%) x 100 |
|---|

**Example :** A patient with a haemoglobin of 16 g/100 ml and PCV de 45 %.
MCHC = 16/45 x 100 = 36 g %.

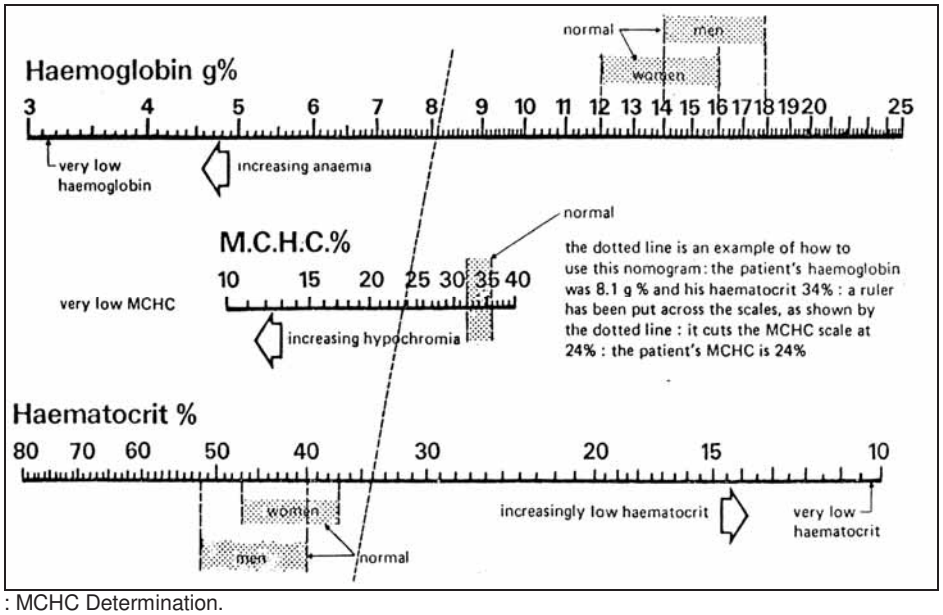


Fig. 4 : MCHC Determination.

A guideline reference range for MCHC in health is 30 - 36 g%. These figures should be checked locally. The MCHC is used in the classification of anaemia :

| | |
|---|---|
| **MCHC < 30 g%** | **Hypochromic anaemia.** |
| **MCHC between 30 and 36 g%** | **Normochromic anaemia**. |

Hyperchromic anaemia does not really exist : A red blood cell cannot contain more haemoglobin than the maximal continence (with one exception for the megaloblastic anaemia).

| Hypochrome anaemia < 30 normochrome anaemia > 36 " *hyperchrome* anaemia " |
|---|

SOURCES OF ERRORS :

**Erroneously high PCV may be due to :**

- Patient's position during the blood collection (10 % of difference between lying or standing).
- Storing the specimen beyond 6-8 hours before performing the test.
- Delay of reading after centrifugation (plasma evaporation).
- Prolonged stasis caused by constriction with a tourniquet for more than 1 minute (haemoconcentration).
- …

**Erroneously low PCV may be due to :**

- Leakage from the tube during centrifugation due to insufficient sealing of the capillary tubes.
- Heparin degradation in hot climate (Tubes should be stored in a cool place).
- Dilution by interstitial fluid, especially where there has been difficulty in venous puncture or failure to obtain free flow of capillary blood
- Blood coagulation if the blood is not immediately mixed with anticoagulant.
- EDTA in excess of 2 mg/ml (diminution of the red cells volume).
- Secondary haemolysis to forcible passage through a fine bore needle.
- Haemolysis caused by heating of the blood, during the sealing of the capillary.
- Inadequate centrifugation (too short or with a too low centrifugal force.
- Blood not properly oxygenated: the blood must be sufficiently, but gently mixed before performing the test.
- Haemolysis of the sample during centrifugation by an overheated centrifuge.
- …

**Erroneously high or low PCV may be due to :**

- Using an inappropriate anticoagulant.
- Reading error (parallax error).
- Poor quality tubes which are not uniform bored.
- Inadequate mixing of the blood prior to filling the micro hematocrit tube.
- …

QUALITY CONTROL :

Specimens run in duplicate must agree within 3 %.

The complete packing of the red blood cells should be verified. After reading the PCV, re-centrifuge the tube for 2, 3 and 5 minutes more. No decreasing of the PCV should be found. In case of PCV value decreasing, choose the centrifugation time which give a constant PCV value.

# CELL NUMBER CONCENTRATION

GENERAL PRINCIPLE :

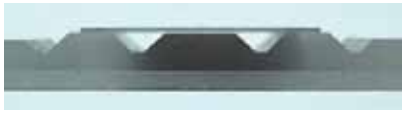
The count of cells per volume unit is quite useful as diagnostic tool. Electronic counter systems are often not available (or affordable) in district laboratories. The only realistic alternative will be to use a counting chamber in which the cells are counted under the microscope. Depending on the kind of liquid to be analysed (expected cell number), a dilution and/or destruction of undesirable cells must be done. A simple calculation taking in account the volume in which the cells are counted and the dilution, will give the number of cells per $mm^3$ or per µl, in the initial biological liquid.

GENERAL MATERIALS :

Collection material + Sahli pipette (or automatic pipette), 1 ml graduated pipette, safety device for pipetting, test tubes, absorbent paper, diluting liquid, pencil, counting chamber, counting chamber cover glasses, plastic Pasteur pipette, hand tally counter, lens paper, microscope (objective 10 X and 40 x), chlorine solution.

COUNTING CHAMBERS :


C B A B C
Neubauer counting chamber



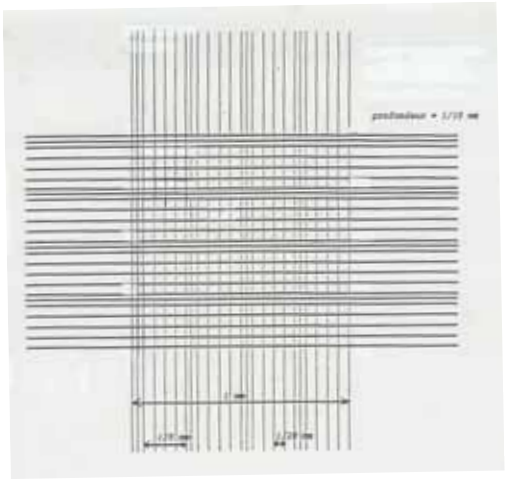
C B A B C
Neubauer counting chamber, sectional view


B A B
Neubauer counting chamber, sectional view

The upperside of the counting chamber is divided in 5 parts by moats or wells, (C, B, A, B and C). The central part A is also divided in 2 by a transversal channel. Each central part of the chamber contains a specially grid area with dimensions as shown in the figures (depending on the type of counting chamber). Counting chambers are so constructed that the distance between the underside of the cover glass and the surface of the chamber is constant (= depth of the chamber). The area and the depth of the counting will define a precise volume.

Characteristics of the most common counting chambers :

**Small volume counting chamber :**



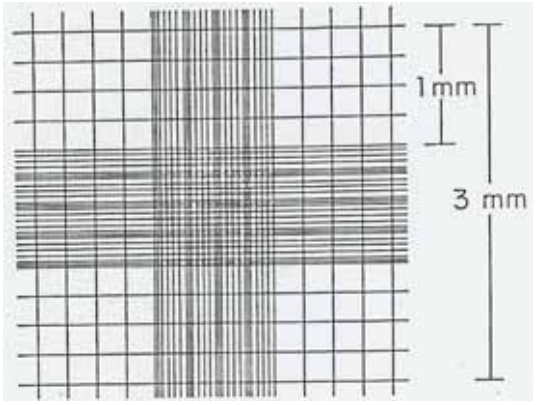
**Thoma counting chamber:**

Area : 1 mm x 1 mm : 1 mm².

Depth : 0,1 mm.

Total volume: 0,1 mm³ or µl.

**Medium volume counting chambers :**

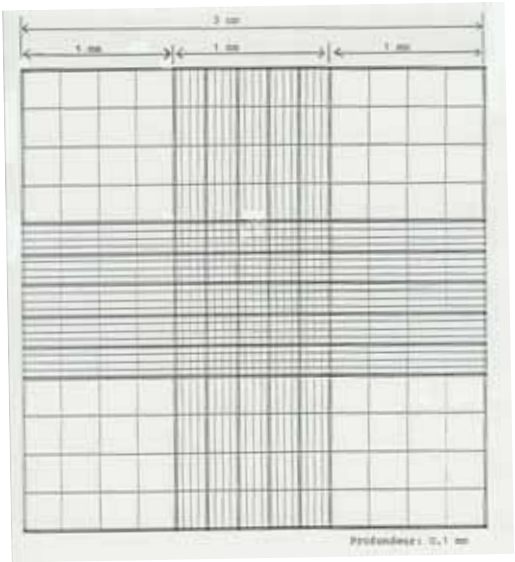



**Neubauer counting chamber :**

Area : 3 mm x 3 mm = 9 mm².

Depth : 0,1 mm.

Total volume: 0,9 mm³ or µl.

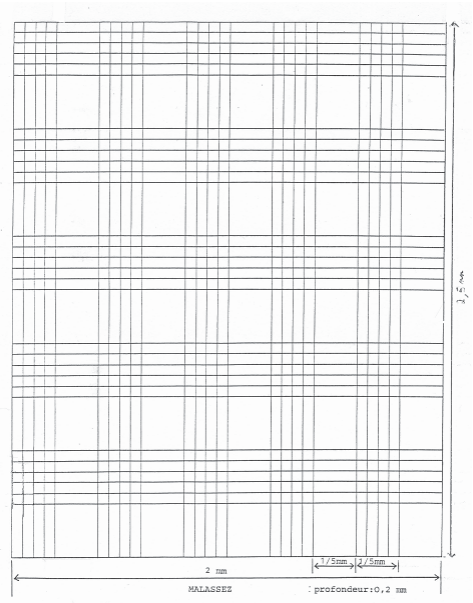


**Neubauer (double improved) counting chamber:**

Area : 3 mm x 3 mm = 9 mm².

Depth : 0,1 mm.

Total volume: 0,9 mm³ or µl.



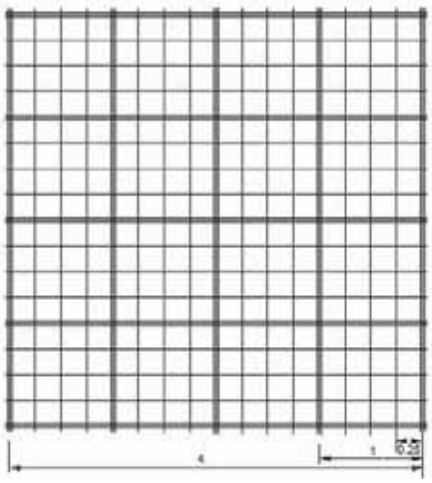

**Malassez counting chamber :**

Area : 2 mm x 2,5 mm = 5 mm².

Depth : 0,2 mm.

Total volume : 1 mm³ or µl.

**Big volume counting chambers :**



**Fuchs-Rosenthal counting chamber :**

Area : 4 mm x 4 mm = 16 mm².

Depth : 0.2 mm.

Total volume : 3,2 mm³ or µl.

**Nageotte counting chamber:**

Area : 10 mm x 10 mm = 100 mm².

Depth : 0,25 mm ;

0,50 mm ;

or 1mm.

Total volume : 25 mm³ or µl ;

50 mm³ or µl ;

100 mm³ or µl.

The used type of counting chamber is a matter of availability. Small volume counting chambers are better for liquids with a lot of cells (red blood cells count in blood for example). Big volume counting chambers are better for liquids with few cells (white blood cells in CSF for example). Medium volume counting chambers may be used for all kind of liquids. The Neubauer double improved counting chamber is the most common type.

# WHITE CELL COUNT IN BLOOD

REAGENT :

Turck solution :

Fill a bottle with 96 ml of distilled water
Add 3 ml concentrated (glacial) acetic acid ($CH_3COOH$) and mix
Add 1 ml gentian violet 1 % (w/v).

- **Caution: acetic acid** is a corrosive chemical with an irritating vapour. Handle with care in well ventilated area (or in a fume cupboard). **Never pour water in pure acetic acid.** Addition of a small quantity of water in acid produces enough heath to cause an explosion of the bottle.
- This reagent is stable for at least 3 months in a fridge.

BLOOD COLLECTION :

Capillary or venous blood. For venous blood, dry anticoagulant should be used (to avoid dilution). EDTA di-potassium salt or heparin are recommended.

METHOD :

1. Check the tip of the Sahli pipette. Discard if broken (volume error). Check if the pipette is clean and dry.

2. Pipette 0,38 ml of Turck solution into a test tube, using the 1 ml graduated pipette.

3. Label the tube with the patient's name and/or number. Draw venous or capillary blood at the 0.020 mark of Sahli pipette. Do not allow air bubbles to enter.

4. Wipe the outside of the pipette with absorbent paper. Check that the blood is still on the 0.02 ml mark.

5. Expel the blood into the test tube. Rinse the pipette by drawing in and discharging the fluid from the test tube 3 times. The dilution of blood is 1 on 20.

6. Mix the diluted blood well and wait 3 minutes before filling the counting chamber (red blood cells lysis).

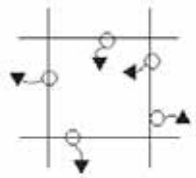
7. Assemble the counting chamber : Prior moistening of the chamber surface on each side of the grid areas (« b »), is necessary for the cover glass to adhere to the chamber. Slide the cover glass into position over the grid areas and press down on each side until rainbow colour (Newton's rings) are seen.

Neubauer counting chamber

8. Mix the diluted blood well. Use a Pasteur pipette (or a Sahli pipette) to fill the counting chamber completely. Take care not to overfill beyond the ruled area. **Caution** : if the liquid overflows into the channel between the two chambers, you must start again : remove and clean the coverslip, clean the counting chamber and refill with another drop.

9. Leave the counting chamber on the bench for 3 minutes to allow the cells to settle.

10. Place the counting chamber on the microscope stage.



11. Using the 10 x objective with the condenser iris sufficiently closed to give good contrast, focus the rulings of the chamber. Then, using the 40 x objective, count the leukocytes in the four large corner squares of the chamber which have a surface of 1 mm². Leukocytes appear as small, transparent cells, with light blue nucleus. Do not take dust or unlysed red cells for leukocytes. Include the cells, lying on the lines of two sides of each square in the count (use all the time the same lines) and exclude the cells on the two other sides.

12. Calculate the number of leukocytes in 1 mm³ of blood by multiplying the number of leukocytes counted in the four large squares by 50. Explanation of calculation :

    - **Dilution factor** : 20 µl of blood + 380 µl Turck solution, gives a **20** x blood dilution [(20+380)/20].
    - **Volume factor** : 4 large squares counted, or 4 x 0.1 mm³. Thus division by 4 and multiplication by 10 will give the number of leukocytes in 1 mm³ of diluted blood [10/4 = **2,5**].

    **Global factor 50 [20 x 2,5]**.

| | | |
|---|---|---|
| **Example :** | Large square n° 1 | 25 leukocytes |
| | Large square n° 2 | 26 leukocytes |
| | Large square n° 3 | 24 leukocytes |
| | Large square n° 4 | 26 leukocytes |
| | **Total for 4 larges squares :** | **101 leukocytes** |

Number of leukocytes par mm³ (or µl) of blood = 101 x 50 = 5.050

REFERENCE RANGES:

| **Normal leukocyte number concentration by age group (per mm³ of blood)** [5] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **AGE** | **1 day** | **15 days** | **2 months** | **6 months** | **2 years** | **6 years** | **12 years** | **Adults** |
| **Number** | 9.000 to 30.000 | 6.000 to 20.000 | 5.500 to 18.800 | 6.000 to 17.500 | 6.000 to 17.000 | 5.000 to 14.500 | 5.000 to 13.500 | 4.000 to 11.000 |

QUALITY ASSURANCE :

For patient samples done in duplicate, the difference between the two counts should not be more than 20 %. For statistical reasons, the precision of the measurement will decrease with the number of counted cells. To decrease the errors for low counts, it may be good to repeat the count, using a lower dilution or counting the cells in more than 4 large squares. (Caution, in these cases, the calculation should be adapted)

It can be good to compare the distribution of the cells in the 4 large squares from the Neubauer chamber. The number of cells, counted in each of the 4 squares should not differ by more than 10 %.

[5] Cf. also addendum 2. References ranges vary in different population and in different laboratories (different techniques). District laboratories should check the above figures for the technique in use with their nearest hematology reference laboratory.

SOURCES OF ERRORS :

- Position of the patient during the blood collection [haemoconcentration].
- Nucleated red cells may cause an erroneously high count of WBC. This can be corrected by determining the proportion of nucleated red cells to white cells on a blood film.
- Prolonged stasis caused by constriction with a tourniquet for more than 1 minute [haemoconcentration].
- Cells counting from a dehydrated patient [heamoconcentration].
- Secondary haemolysis to forcible passage through a fine bore needle.
- Storing the specimen beyond 6-8 hours before performing the test (Disintegration of leukocytes when specimen stands°.
- Blood collection from an arm in which an intravenous infusion is being given [haemodilution].
- Using an inappropriate anticoagulant.
- Sample coagulation.
- When using anticoagulated blood, not mixing the blood sufficiently or not checking the sample for clots.
- Counting chamber or cover glass dirty.
- Incorrect measurement of blood or dilution fluid due to poor technique or using a wet or chipped pipette.
- Dilution fluid contaminated with dust particles.
- Inadequate mixing of blood with diluting fluid.
- Insufficient lyses of RBC may create problems in identification of leucocytes.
- Air bubbles in the counting chamber or in the pipette [volume error].
- Inappropriate covering of the counting chamber [volume error].
- Over-filling a counting chamber or counting cells when sample contains air-bubbles.
- Counting chamber is not sufficiently filled.
- Not allowing sufficient time for the cells to settle in the chamber.
- Use of a too intense light source or not reducing the iris diaphragm to give good contrast.
- Calculation error.
- Administrative error.
- …

# RED CELL COUNT IN BLOOD :

The same kind of technique can be used for the red blood cell count. Unfortunately, the precision is poor and it is not recommended for clinical practice. To calculate the MCV and the MCM indices, an accurate red blood cells count is needed. This needs an electronic cell analyser. Most district laboratories will not therefore be able to calculate these indices. However, examining a well–stained blood film can help to detect macrocytosis or microcytosis.

# RED CELL INDICES

## MCV
### Mean Corpuscular Volume

$$MCV = \frac{\text{PCV (\%)}}{\text{Red blood cell count per µl (in millions)}} \times 10 \times 10^{-15}\ l$$

Reference range : 82 to 92 fl (for adults. Age related variation for children) [a femtolitre (fl) is $10^{-15}$ of a litre]

| FOR ADULTS |
|---|
| microcytic anaemia < 82 normocytic anaemia > 92 macrocytic anaemia |

## MCH
### Mean Corpuscular Haemoglobin

$$MCH = \frac{\text{Haemoglobin (in g/l)}}{\text{Red blood cell count per µl (in millions)}}$$

Reference range: 28 to 32 pg (for adults ; Age related variation for children)

| FOR ADULTS |
|---|
| > 32 “hyperchromic anaemia” |

# ADDENDUM 1 : TECHNIQUES FOR ASSESSING ANAEMIA (PRICE LIST)

## HCS:

- Starter kit containing: 1 Cover-box with 1 dispenser and 200 test-strips, 1 Booklet with Colour scale, 1 instruction manual, 4 refill dispenser with each 200 tests strips
- Price: 21.80 € (Feb. 2004, Copack).
- Refill kit containing:
- 10 dispenser boxes with each 200 test-strips (total 2000 tests)
- Price 32.75 € (Feb. 2004, Copack).

## MICROHAEMATOCRIT CENTRIFUGE:

- Centrifuge DHT 590: 832.25 € (Feb. 2004, DHT)
- Centrifuge Transfer : 1500 € (Feb. 2004, Transfer)
- Capillary tubes heparinised 75 mm (200 tubes): 4.80 € (Feb 2004, DHT)
- Sealing paste for capillary tubes (6): 4€ (Feb 2004, DHT)

## LOVIBOND HAEMOGLOBINOMETER:

- Comparator Lovibond 2000 : 456.22 € (Jan 2004, Transfer)
- Special capillary chamber for Lovibond : 53.33 € (Jan 2004, Transfer)
- Colour standard disc 5/8 A lower haemoglobin : 90.05 € (Jan 2004, Transfer)
- Colour standard disc 5/8 B higher haemoglobin : 161.98 € (Jan 2004, Transfer)

## DRABKIN colorimetric determination:

- Colorimeter WPA C0700D, wavelength range 400-700 nm: 734 € (Feb 2004, DHT)
- Total Haemoglobin kit sigma 525-A 1000 determination, with haemoglobin standard: 80.74 € (Dec 2003) **TRANSPORT AND STORAGE 2-8°C.**
- Sahli pipette: 1.34 € (Feb 2004, DHT)
- Graduated glass pipette 5 ml: 1.93 € (Feb 2004,VWR) or dispenser 4 ml Ceramus 217 € (Feb 2004, VWR).
- Devices for pipetting : 2.92 € (Feb 2004, VWR)
- Optical cuvettes 10 mm light path (100 cuvettes): 4.58 € (Feb 2004,DHT)
- + Bottles
- + Test tubes
- + Test tubes rack

## HEMOCUE:

- Hemocue® B photometer with control cuvette: 555 € (Feb 2004, Hemocue)
- Disposable micro cuvettes Hemocue ® B (50 cuvettes): 60 € (Feb 2004, Hemocue)
- Hemocue® 201 photometer with control cuvette: 327,80 € (July 2006, MSF Supply)
- Disposable micro cuvettes Hemocue ® 201 (200 cuvettes): 84,66 € (March 2007, MSF Supply)
- Hemocue® 301 photometer with control cuvette: 350,00 € (July 2007, Hemocue)
- Disposable micro cuvettes Hemocue ® 301 (200 cuvettes): 75 € (July 2007, Hemocue)
- Batteries 4 (Type N alkaline cells) : 6.35 € (Feb 2004, DHT)

## DHT HAEMOGLOBIN METER:

- Haemoglobin meter DHT HB 523: 543 € (Feb 2004, DHT)
- Ammonia 30 % 1 litre: 5.35 € (Feb 2004, VWR) **! Corrosive and irritant !**
- Sahli pipette: 1.34 € (Feb 2004, DHT)
- Graduated glass pipette 2 ml: 1.93 € (Feb 2004,VWR) or dispenser 2 ml Ceramus 217 € (Feb 2004, VWR).
- Devices for pipetting 2.92 € (Feb 2004, VWR)
- Optical cuvettes 10 mm light path (100 cuvettes): 4.58 € (Feb 2004,DHT)
- Batteries 4 (Type N alkaline cells) : 6.35 € (Feb 2004, DHT)
- + Bottles
- + Test tubes
- + Test tubes rack

## SAHLI:

- Sahli kit : 57.35 € (Feb 2004, VWR)
- Hydrochloric acid 37 % : 9.35 € (Feb 2004, VWR) **! Corrosive and irritant !**

## GENERAL :

- Blood lancet
- Disinfectant (Sodium hypochlorite or chlorine-releasing disinfectants)
- Cotton
- Alcohol 70 %
- …

## ADDENDUM 2 : SOME HAEMATOLOGICAL REFERENCE RANGES (GUIDELINE FIGURES)[6]

| Age | Sex | Haemoglobin | RBC | PCV | MCHC | MCV | WBC |
|---|---|---|---|---|---|---|---|
| Units | | g/100 ml | per µl x $10^6$ | % | g/100 ml | fl | per µl x $10^3$ |
| 3 – 12 months | Male | 10,0 – 14,0 | 3,0 – 5,6 | 26 – 41 | 28,4 – 40,0 | 70 - 105 | 5,5 – 17,5 |
| 3 – 12 months | Female | 10,0 – 14,0 | 3,0 – 5,6 | 26 – 41 | 28,4 – 40,0 | 70 – 105 | 5,5 – 17,5 |
| 1 – 12 years | Male | 10,5 – 15,0 | 3,8 – 5,5 | 32 – 42 | 32,0 – 37,0 | 72 - 94 | 5,0 – 14,0 |
| 1 – 12 years | Female | 10,5 – 15,0 | 3,8 – 5,5 | 32 – 42 | 32,0 – 37,0 | 72 – 94 | 5,0 – 14,0 |
| 12 – 100 years | Male (Europe) | 13,2 – 17,3 | 4,3 – 5,7 | 39 – 49 | 32,0 – 36,0 | 81 - 100 | 4,0 – 11,0 |
| 12 – 100 years | Female (Europe) | 11,7 – 15,5 | 3,8 – 5,1 | 35 – 45 | 31,5 – 36,0 | 81 – 100 | 4,0 – 11,0 |

**N.B. : Man and women (Asia) : 4.000 – 10.000 leukocytes /µl**
**Man and women (Africa) : 2.600 – 8.300 leucocytes /µl**

### Differential WBC reference range :

| CELL TYPE | Percentage (absolute number) | |
|---|---|---|
| **Polymorphonuclear** | **EUROPE + ASIA** | **AFRICA** |
| Basophils | 0 – 1 % (0 - 200) | 0 – 1 % (0 - 200) |
| Eosinophils | 0 – 4 % (0 - 400) | 0 – 5 % (0 - 500) |
| Neutrophils (non segmented) | 0 – 5 % (0 - 700) | 0 – 5 % (0 - 700) |
| Neutrophils (segmented) | 50 – 75 % (1.800 - 7.000) | 30 – 40 % (900 - 4.000) |
| **Monomorphonuclear** | | |
| Lymphocytes | 30 – 40 % (1.000 - 4.000) | 40 – 60 % (1.200 - 6.000) |
| Monocytes | 0 – 8 % (0 - 800) | 0 – 8 % (0 - 800) |

### Reticulocyte count :

**Percentage :** Adults and children : 2 - 15 / 1.000 RBC
Infants at birth : 20 - 60 / 1.000 RBC

**Absolute number :** Adults and children : 25.000 - 160.000 / µl
Infants at birth : up to 150.000 /µl

### Platelet count : 150.000 - 400.000 / µl

[6] References ranges vary in different population and in different laboratories (different techniques). District laboratories should check the above figures for the technique in use with their nearest haematology reference laboratory.

## ADDENDUM 3 : MORPHOLOGY OF BLOOD CELLS IN A MAY-GRÜNWALD-GIEMSA STAINED BLOOD FILM

| CELL TYPE | SIZE | NUCLEUS | | | CYTOPLASM | | |
|---|---|---|---|---|---|---|---|
| **GRANULOCYTES** | µm | FORM | COLOR | CHROMATIN STRUCTURE | QUANTITY | COLOR | GRANULES |
| **LEUKOCYTES : POLYMORPHONUCLEAR GRANULOCYTES** | | | | | | | |
| IMMATURE NON SEGMENTED NEUTROPHILS "Band forms" or "S" shaped | 12 – 15 | Horseshoe, Central curvature is maximum a third part of the width of the lobes [7] | Clear blue purple | Strands of fine chromatin | abundant +++ | Dusty rose (=very small granules) | small granules, light purple or violet Not always present |
| SEGMENTED NEUTROPHILS | 12 – 15 | 2 to 5 lobes [8] | Deep blue purple | Rather thick and coarse | +++ | Rose | Small granules, Pink or pink mauve |
| EOSINOPHILS | 12– 15 | Usually a bi-lobed nucleus | Blue purple | Rather thick and coarse | +++ | Rose | Many large, uniform granules, red orange |
| BASOPHILS | 11 – 13 | Hardly visible lobes, not well separated (polymorph) | Blue purple | Rather thick and coarse, covered by granules | +++ | Light rose | Very large, well separated, variable granules Deep purple Small in number |

[7] **Left deviation of the Arneth formula: an increase over 16 % of non segmented neutrophils, yet immature forms, occurring in inflammations, but also in stress conditions,… 2 to 5 segmented neutrophils are the major fraction of the neutrophils in a normal leukocyte type.**

[8] **Right deviation of the Arneth formula: in contrast with the left deviation, where segmented cells are rarely seen, this image shows hyper segmented neutrophils, with 5 or more lobes. A hyper segmentation is characteristic for megaloblastic anaemia. In the early phase, more than one neutrophil with 6 lobes per 100 granulocytes is found.**

| CELL TYPE | SIZE | NUCLEUS | | | CYTOPLASM | | |
|---|---|---|---|---|---|---|---|
| **AGRANULOCYTES** | µm | FORM | COLOR | CHROMATIN STRUCTURE | QUANTITY | COLOR | GRANULES |
| **LEUKOCYTES : MONOMORPHONUCLEAR AGRANULOCYTES** | | | | | | | |
| SMALL LYMPHOCYTES | 7 -10 | Round or oval Or slightly indented | Deep purple | Big clumps of intensely stained chromatin | (-) or + | Sky blue (Often absent) | |
| LARGE LYMPHOCYTES | 10 – 15 | Round or oval Or slightly indented | Red, purple | Clumps of deep stained chromatin and other clumps which are less intensely stained | ++ | Sky blue | Absent or a few granules azurophils (rose violet) |
| MONOCYTES | 15 – 25 | Round, oval, indented or bean form | Blue to slightly violet | Fine, spongy like | +++ Vacuoles often demonstrable. | Grey or blue grey | Very fine granules (dusty like), azurophils (rose violet) |
| **ERYTHROCYTES** | 6,7 – 7,7 | | | | Biconcave discus shape | rose | none |
| **TROMBOCYTES** | 1,5 – 2 (5) | | | | Slightly blue | | Reddish |

**Prins Leopold Instituut voor Tropische Geneeskunde**
**Institut de Medecine Tropicale Prince Leopold**
**Prince Leopold Institute of Tropical Medicine**
**Instituto de Medicina Tropical Principe Leopoldo**

Nationalestraat, 155
B – 2000 Antwerpen

Stichting van Openbaar Nut 0410.057.701

**POSTGRADUATE IN TROPICAL MEDICINE AND INTERNATIONAL HEALTH**
**MODULE 1**

# BLOOD TRANSFUSION

# (In remote areas)

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# Practical notes

Philippe Gillet, Luc Boel, Jan Jacobs

**November 2007**

## Short summary of basics genetics

The genetic information required for cell life is stored in the DNA. Human cells contain a total of 23 pairs of chromosomes (diploid number). A **chromosome** is the concentration form of the chromatin (DNA + histones) which becomes visible during cell division (mitosis or meiosis).

During the development of male and female reproductive cells, a special type of cell division occurs: the meiosis. This reduces the number of chromosomes in the spermatozoid or the ovum to half the number (haploid number) found in normal body cells. So, when the ovum is fertilized by one spermatozoid, the zygote which results contains the full diploid number of chromosomes (46, 23 from the father and 23 from the mother). The chromosomes from a same pair are **homologous**.

Genes are the small units strung along the length of chromosomes. A **gene** is the factor at a particular point or **locus** on the chromosome which represents a hereditary characteristic. Alternative or slightly contrasting forms of the gene are known as **alleles**. Alleles are generally represented by a character, in capital for the dominant allele and in small character for the recessive allele).

When the locus on a pair of homologous chromosomes is occupied by the same allele, the person is homozygous for the particular gene characteristic (**homozygote ZZ or zz**). If however, the alleles are different, the person is said to be heterozygous for the particular gene characteristic (**heterozygote for instance Zz**).

The alleles inherited for any particular characteristic can be dominant, co-dominant or recessive. A **dominant** allele will always show itself if it is present, whereas a **recessive** allele will only show itself if there is no dominant allele. If both alleles are expressed together, they are **co-dominant**. A recessive allele is manifested only in the homozygote.

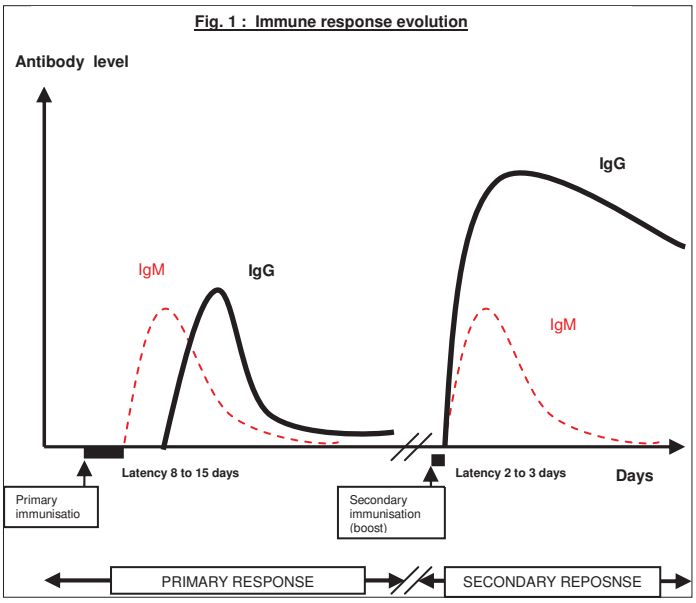
The genetic composition for a particular inherited characteristic is called the **genotype** (gene composition) and its manifestation, or biological effect, is called the **phenotype** (gene expression).

## Short summary of basic immunology

Immune reactions, used or involved, in the blood group determinations, in post transfusion reactions or compatibility tests are mostly humoral immune responses.

**Principal characteristics of the humoral immune response:**

1. Essential contact with an antigen.
2. Immune tolerance. The capacity of the organism to make it unresponsive to "foreign" or "self" antigens.
3. Specific response of the antibody against the antigen.
4. Immunogenecity: The capacity to induce the formation of antibodies is different for different antigens (Antigenic power).
5. A step in the antibody response. Production of different antibody classes.
6. Memory, difference in primary and secondary response.



# BLOOD GROUPING

A blood group is a group of individuals who has a (allotypical) character in common which distinguishes them from other groups. This characteristic, which is carried by the blood elements, has an antigenic activity. However, by language restriction, the expression « blood groups » is only used for the red blood cell groups, but there exist also blood groups for platelets, for polynuclear WBC, lymphocytes and proteins.

The antigens of the blood groups are located on the membrane of the red blood cells (either exclusively, either also on other types of cells). They can be proteins, but most of the time they consists of glucides (sugar) complexes: Glycoproteins, glycolipids etc. They are essentially known by their antigenic capacity; their biological functions are often hardly known: Transporters and membranous channels (proteins assuring the molecule transport through the membrane), of enzymes, of structural proteins of the membrane (« skeleton » of the cell), of adhesion molecules, or of membranous receptors (proteins capable to link with a signal or informative molecule, …). The immunology of blood groups is essentially circulating (immunity with antibody and complement interaction). Their study must thus consider the corresponding antigens and antibodies.

The classification is made on two levels:

1. The first level consists of all the **antigens** of blood groups. Up to now over 650 groups are described. (Examples: Ag A, Ag B, Ag D, Ag $Fy^a$, …).

2. All these antigens are grouped in a second level in **systems**. A blood group system is the total of antigens developed by the alleles of the same genetic mono factorial unit. A system is composed of the total of antigenic variants of membranous components.

Antigens are thus immunologically defined, while the systems have a genetic definition.

29 blood systems are described at this moment. [International Society of Blood transfusion]. (Examples ABO, Rhesus, Duffy, Kell, Lewis, P, Diego, Lutheran, Chido/Rodgers, …)

In the **current minimal transfusion practice**, only the two most important systems are taken into account:

- The **ABO system** that represents the major obstacle in all transfusions by the presence of natural antibodies (it is also a system of tissular histocompatibility antigens HLA).

- And the **Rhesus system**, since the D antigen is the most immunogenic of all the blood group antigens.

The determination of blood groups is following the "**4 x 2 rule**[9]**"**:

- **Two technicians.**

- **Two series of different reagents from different producers, using different techniques (in tubes, on slides, in gel, …).**

- **Two different techniques. (test and counter test, or forward and reverse blood grouping)**

- **Two samples taken at different moments.**

This rule of "4 x 2" permits to give a definite card of blood group.

**In the practice of a small laboratory these "4 x 2" rule is not applicable. Without giving a blood group card, it is possible to realise transfusions relatively sure, based upon one determination, by one person on one sample. But then the compatibility test or at least the « rapid cross match » must be absolutely performed. (Verification of the ABO compatibility)**

## A The ABO blood group system

[9] **République Française, Ministère de la santé, de la famille et des personnes handicapées : CIRCULAIRE DGS/DHOS/AFSSAPS N° 03/ 582 of 15 december 2003** relative à la réalisation de l'acte transfusionnel et **CIRCULAIRE DGS/SQ 3 N° 99/14 du 12 janvier 1999** relative au respect de la réglementation en vigueur pour la détermination des groups sanguins ABO.

The ABO blood groups system was the first to be discovered. This was attributed to Karl Landsteiner In 1901. For this observation, which he described as the ABO (0) blood group system, Landsteiner was awarded the Nobel Prize for medicine (1930).
The ABO system presents an important characteristic that is at the same time at the origin of techniques of blood grouping and that explains also its crucial role in blood transfusion : the constant presence of antibodies anti-A and anti-B corresponding with the absent antigens on red blood cells of the subject.
The ABO antigens are terminal **sugars** of complex membrane macromolecules. The addition of these sugars is coded by a gene. This gene bears 3 alleles:

1. O : inactive enzyme (no added sugar).
2. A : N-Acetyl galactosamine transferase enzyme.
3. B : Galactose transferase enzyme.

The transmission of the ABO groups follows the laws of Mendel :
A and B are **dominant** over O
A and B are **co dominant**
O is relatively **recessive** to A and B

The ABO Antibodies are principally natural antibodies, complete, cold (optimally at 4°C, but they still agglutinate at 37°C), of **IgM** type. They appear spontaneously during young childhood by cross antigenic stimulation with surface antigens of saprophytic bacteria of the intestinal flora. They appear usually between the 3rd and the 6th month of life and their concentration reaches a maximum at the age of 10 years. They are present on every individual who does not possess the corresponding antigen on his own red blood cells.

**Summary ABO blood group system**

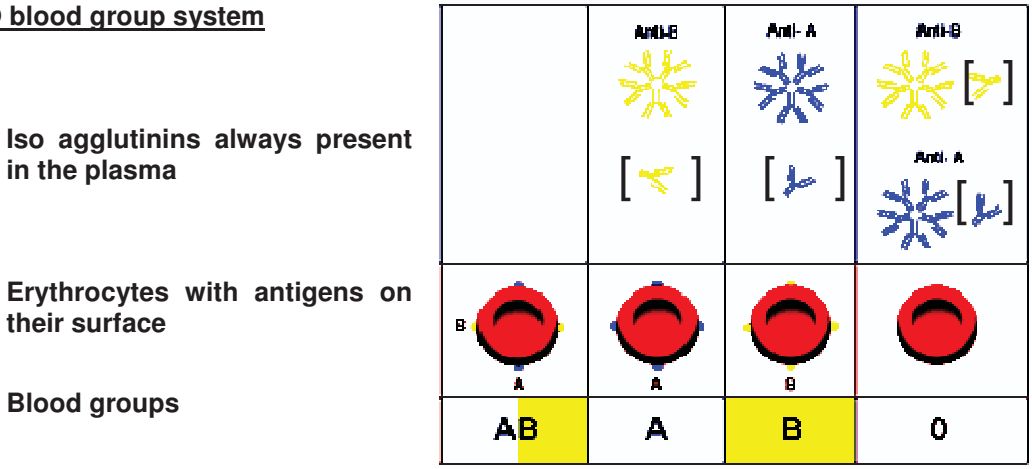



**Table 1 : Estimation of the frequency and function of the skin colour:**

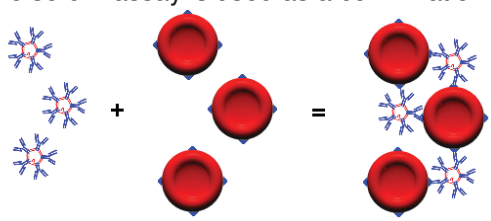
| ABO blood group | AB | A | B | O |
|---|---|---|---|---|
| **Frequency of occurrence of ABO groups (related to skin colour)** [10] | | | | |
| **White** | 4 % | 44 % | 9 % | 43 % |
| **Yellow** | 13 % | 28 % | 23 % | 36 % |
| **Black** | 4 % | 27 % | 21 % | 48 % |

N.B. 1 : **Subgroups $A_1$ and $A_2$** : Antigen A exists as strongly reacting antigen $A_1$, and a weakly reacting antigen $A_2$. Most people who are group A or AB posses $A_1$ antigen, but up to 20 % belong to the subgroup $A_2$ or $A_2B$
Very soon a first complexity level has been reported concerning the phenotype A : The phenotype $A_1$, which is found in 80 % of subjects A and the phenotype $A_2$ in 20 %. The phenotype $A_1$ is characterised by the presence of the $A_1$ antigen while the phenotype $A_2$ does not possess the antigen $A_1$ The number of antigenic sites in phenotype $A_1$ subjects is much higher (1.000.000) than in subjects of phenotype $A_2$ (about 200.000 per red blood cell). This is resulting in weaker intensity reactions for phenotypes $A_2$ than for phenotypes $A_1$ (this explains the importance of the quality of the used reagents in the blood grouping). The practical distinction between these two phenotypes is of no importance in the transfusion context. One can although observe sometimes anti-$A_1$ or irregular natural anti-H, but this concerns most often of cold antibodies of a low titter, without any consequences in the transfusion aspect. Other rare phenotypes have also been described (phenotypes cis-AB, phenotype B(A) and A(B), acquired phenotype A, …). Other variants of group A ($A_3$, $A_{erd}$, $A_{bantu}$, $A_x$, etc.) and more exceptionally of group B weakly reacting have been reported.
N.B. 2 : Occasionally, **IgG** hyper immune anti-A or Anti-B can be found in the serum of group O persons in response to stimulation by A and/or B like substances present in the environment, following pregnancy immunization or following the injection of some vaccines (immune irregular antibodies). Serious haemolytic reactions can occur when Group O whole blood containing anti-A and/or anti-B haemolysins is used to transfuse non group O persons (cf. dangerous O page 6). As IgG anti-A and/or anti-B can cross the placenta, there are also involved in some haemolytic disease of newborn.

**Technique for ABO grouping on card**
**(Beth-Vincent method or forward ABO grouping)**

[10] Approximate average percentages. Marked differences can occur between ethnic groups.

The determination of the ABO group reposes on demonstration of red blood cell surface antigens. Therefore known sera, directed against these antigens, are used. These serums agglutinate the red blood cells possessing antigens against which they are directed. There also exists the inverse determination (reverse blood grouping), which demonstrates antibodies that are present in the serum: known erythrocytes are then used. The serum assay is used as a confirmation of the red blood cell assay.



**Example**: Serum **Anti-A** + Red blood cells **A** = Agglutination

**Samples**:

Receptor blood obtained by capillary sampling (or venous blood on EDTA).

**Reagents**:

Anti-A, anti-B, [anti-AB] humans Diamed (Diaclon) for slide method.
Blood group cart (or on a glass slide or on an opaline plate), timer.

**Technique**: **The manufacture's instructions must be followed**[11].

1. Put on a card 2 [or 3] drops of receptor blood.
2. Near to each blood drop, depose a drop of each anti serum (anti-A, anti-B, [anti-AB]).
3. Mix the blood with the anti serum with the bottom of a tube.
4. Tilt the slide during 1 minute.
5. Read and note the result of the agglutinations immediately.

| Anti-A | Anti-B | Anti-AB | Group ABO |
|---|---|---|---|
| +++ | - | +++ | A |
| - | +++ | +++ | B |
| - | - | - | O |
| +++ | +++ | +++ | AB |

+++ = agglutination (mostly very strong), - = absence of agglutination. Agglutination indicates the presence of the corresponding surface antigen on the red blood cells.

**Possible problems**:

**False negative reactions:**

- Immature antigens A and B (newborns).
- Genetic variants: Weak groups A or B, ...
- Antiserum of bad quality (antibody titre too weak).
- …

**False positive reactions:**

- Coagulation of the blood to be determined
- Presence of cold agglutinins in the tested blood.
- Bacterial contamination of the test reagents
- Chronical infection (rouleaux formation by increased plasmatic proteins).
- Infection of trypanosomiasis (presence of auto agglutinins and rouleaux formation)
- Antigenic modifications during malign pathologies
- ....

[11] This technique is only applicable for the human Diacon antisera (Diamed). The technique may vary in function of the used reagents. Always follow the particular instructions indicated in the note of the company. Verify if the antisera may be used for the determination on slide.

## B The Rhesus system

The discovery of the Rhesus system, as well as numerous other systems of red blood cell groups has been the result of the exploration of transfusion incidents and of haemolytic diseases of neonates.

The attribution of the name « Rhesus » to this system has its origin in a historical confusion with another system: In fact in 1939, Levine and Stetson concluded that the serum of a woman who delivered a baby attacked by a neo-natal haemolytic disease, agglutinating not only the erythrocytes of the child and the father, but also these of 85 % of the tested subjects. Landsteiner and Wiener in 1940 found that the diluted serum of a rabbit immunised with erythrocytes of *Macacus rhesus* agglutinated the erythrocytes in the same subjects. In fact, taking into account that the non diluted serum of the rabbit recognised 100% des subjects, it seemed that these hetero antibodies recognised a different antigen than the D antigen, being present on the majority of human erythrocytes, but from which the antigenic density is more important in subjects bearing the D antigen than in subjects which are deprived of it. The involved antigen in this confusion was named LW (Landsteiner and Wiener) and the term Rhesus has been maintained to design the initially concerned system.

The Rhesus antigens are **membrane proteins**. The addition of these proteins is coded by two genes.
One gene coded for the D, one gene coding for the Cc and Ee. It is a very polymorph system: Nowadays 52 antigens are described (Within these, the DCcEe antigens are the most immunogenic). Its nomenclature, somewhat careless, reposes on 3 genetic hypotheses, the base of 3 theories, of which each is used according the circumstances: It is described as DCE (conception of Fisher and Race), it is named « Rh » (concept of Wiener), and it is exposed in figures (concept of Rosenfield).

For the **current minimal transfusion practice,** only the D antigen is important. It is own to human and to red blood cells. It is the most immunogenic of the blood group systems.

**A subject possessing the D antigen on the surface of his red blood cells is called Rhesus positive (D + or Rh +)**

**A subject who does not possess the D antigen is Rhesus negative (D - or Rh – or d)**

**This system does not possess natural antibodies:** So a « normal » person does not possess any anti Rhesus antibodies in his plasma. They appear by immunisation as a consequence of blood transfusions or by pregnancy. The Rhesus antibodies are immune antibodies, warm, of the **IgG** type, incomplete (non agglutinating in saline solution).

**Table 2 Geographical distribution of the D antigen :**

| Percentages of Rhesus positive persons [12] | |
|---|---|
| South-East Asia and the Pacific | 98 to 100 % |
| Equator and Chilli | 91 to 97 % |
| Brazil and Argentina | 82 to 94 % |
| Africa (Bantus, Ethiopians) | 94 to 97 % |
| Africa (others) | 82 to 94 % |
| Western Europe and North America | 80 to 85 % |

[12] Approximate average percentages. Marked differences can occur between ethnic groups.

## Technique for the Rhesus grouping on slide (restricted to antigen D)

The determination of the Rhesus group rests on the detection of surface antigens of red blood cells. Therefore known serum is used, directed against these D antigens. This antiserum agglutinates red blood cells which possess antigen D. **For the slide technique the D antiserum must be of the IgM type.**

**Samples**:

Receptor blood obtained by capillary sampling (or venous blood on EDTA).

**Reagents**:

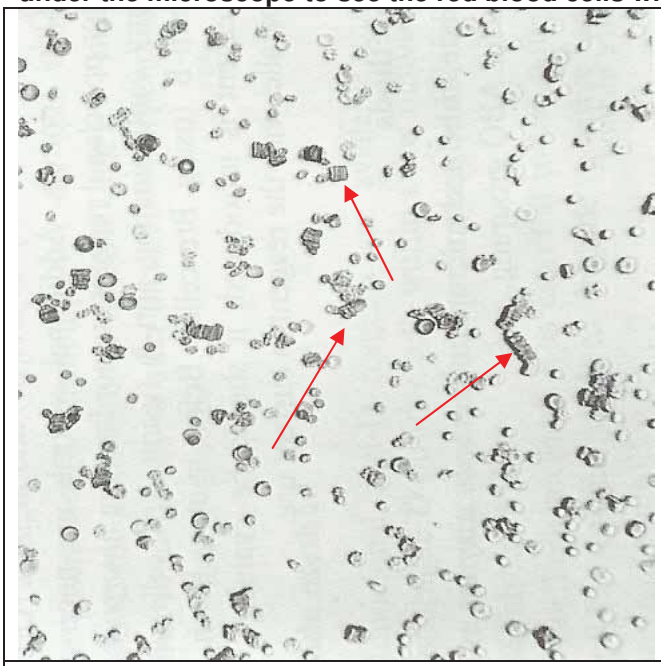
Monoclonal Anti-D Diaclon of Diamed (for slide method).
Blood group cart (or on a glass slide or on an opaline plate), timer, (spirit lamp).

**Technique**[13]**:**

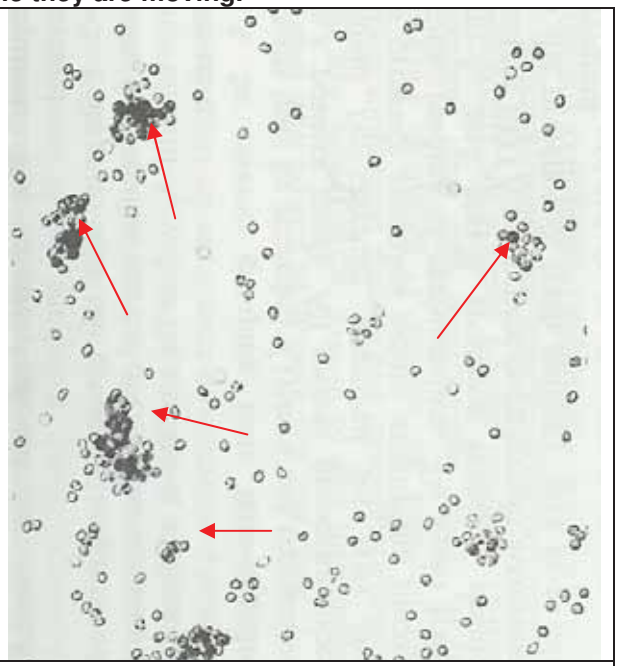
1. [Preheat a glass slide to 37°C.] The quality of the actual antisera is such that heating is only necessary if the test is negative.
2. Put on the glass slide (or on an opaline plate) 1 drop of blood to be tested.
3. Put a drop of anti-D near to the drop of blood.
4. Mix the blood and the anti-serum with the bottom of a tube.
5. Tilt the slide during 1 minute.
6. Read and note the result of the agglutinations immediately.

A positive reaction (presence of agglutination) means Rhesus positive. Absence of agglutination indicates a Rhesus negative result.

**In case of doubt, observe the slide under the microscope (magnification 100x) to distinguish better the agglutinations. In order to make lecture easier, incline slightly the slide before lecture under the microscope to see the red blood cells while they are moving.**



Microphotography of a suspension of red blood cells in serum, presenting a weak proportion of **rouleaux** (red blood cells on a pile of plates). Magnification 100x. [***N.B.** : Rouleaux formation is related to plasmatic proteins concentration].*



Microphotography of a suspension of red blood cells in serum showing a **weak agglutination** (red blood cells in small clusters). Magnification 100x.

[13] This technique is only applicable for monoclonal Diacon (Diamed anti-D antiserum). The technique may vary in function of the used reagent. The manufacture's instructions must always be followed. Check if the antiserum may be used for a reaction on slide and if it contains IgM.

**Possible problems in the Rhesus grouping:**

**False negative reactions**:

- Weak D (weak expression of the antigen)
- Partial D ($D^u$).
- Quality of anti-D. (antibody titre to low, or IgG and not IgM
- …

**False positive reactions**

- Coagulation of the blood to be determined
- Presence of cold agglutinins in the blood to be determined.
- Bacterial contamination of the test reagents
- Chronical infection (rouleaux phenomena by increased plasmatic proteins).
- Antigenic modifications during malign pathology
- Infection of trypanosomiasis (presence of auto agglutinins and rouleaux formation)
- …

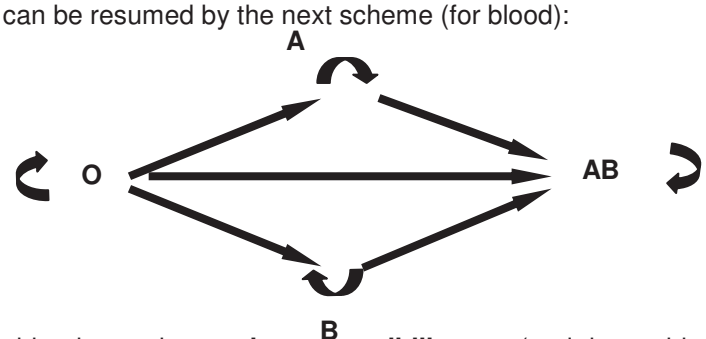
# TRANSFUSIONAL RULES

**1. Avoid Antigen – Antibody conflicts**

Two types of antigen - antibody conflicts can be distinguished: The major and the minor conflicts. The important difference between these two is based upon the quantity and concentration of the involved antibodies.

**Major conflict:**

The blood of the receptor may not possess antibodies directed to the antigens of the red blood cells of the donor (Principle defined by Ottemberg in 1911). This compatibility in the ABO system is an essential condition for transfusions. Based on this principle, the blood compatibility can be resumed by the next scheme (for blood):




Moreover the blood grouping, **major compatibility** test (and the rapid cross match) permits to detect the presence of this type of antibodies.

**Minor conflict:**

It is also important to avoid to transfuse blood (especially when it is complete blood) containing antibodies directed against red blood cells of the receptor. This problem is greatly avoided by transfusing in iso-groups. To determine the presence of other antibodies, a test **of minor compatibility** can also be executed.

**2. Avoid as much as possible the production of antibodies**

Another important remark in blood transfusion is to avoid introduction of an antigen (especially when it is very immunogenic) which the receptor does not possess (Principe *non nocere*). . In fact, this introduction will bring along the production of antibodies which may have dramatical consequences for future transfusions (or for future pregnancies).

## Summary for transfusions on district hospital level (For whole blood, with ABO and Rhesus grouping)

**→ Execute as much as possible transfusions in iso groups ABO and if impossible, follow table 3 on next page.**

**→ A Rhesus negative person must be transfused with Rhesus negative blood.**

**Table 3 Order of choice in the selection of a blood donor (for whole blood, based upon the ABO group and upon the determination of the D antigen of the Rhesus system):**

| Blood group of the receptor | Preferred group of the donor | Order of choice in case that no iso group nor iso-Rhesus blood can be found |
|---|---|---|
| **A Rh +** | A Rh + | A Rhesus –, O Rhesus +, O Rhesus – |
| **A Rh –** | A Rh – | O Rhesus – |
| **B Rhesus +** | B Rhesus + | B Rhesus –, O Rhesus +, O Rhesus – |
| **B Rhesus –** | B Rhesus – | O Rhesus – |
| **AB Rhesus +** | AB Rhesus + | AB Rhesus –, A Rhesus +, A Rhesus –, B Rhesus +, B Rhesus –, O Rhesus +, O Rhesus – |
| **AB Rhesus –** | AB Rhesus – | A Rhesus –, B Rhesus –, O Rhesus – |
| **O Rhesus +** | O Rhesus + | O Rhesus – |
| **O Rhesus –** | O Rhesus – | / |

N.B. In **transfusion situations of complete blood not in iso-groups**, the present IgM anti-A or anti B cause mostly only **small problems** because they are in insufficient quantity for provoking important haemolysis during a standard transfusion. (In all cases of complete blood transfusion, it is as well recommended not to mix the blood pocket to reduce the quantity of transfused plasma by sedimentation). Nevertheless a situation of haemolytic accident risk exists when blood is transfused from a donor presenting IgG anti-A or, more rarely anti-B. This type of rare haemolysin occurs mainly in donors of group O and appears by commutation from IgM anti-A or anti-B (cf. N.B.2 of ABO group). The haemolytic potential of an IgG is much more important than these of IgM which explains that these accidents happen with very low quantities of transfused haemolysin during a standard transfusion (this is even true for concentred cells !).
**Dangerous universal donors** are individuals of group O presenting a haemolysin of the ABO system. Their blood must be reserved for iso-groups transfusions (thus for an O receptor). Since a small laboratory doesn't have the possibility to detect the dangerous O donors, it is very important to privilege as much as possible transfusions in iso-groups. A basic technique permitting to detect **a part** of dangerous O is although described on page 23. This difficult operational technique is rarely used on district level, since dangerous O donors are rare and the preferred iso-group policy.

# COMPATIBLITY TESTS

Moreover the ABO antibodies (mostly natural and regular), one can find other antibodies directed against **non ABO** erythrocyte antigens. Generally they are irregular immune antibodies which take particular techniques to be demonstrated. Their presence in the blood of an individual is mostly due to an immunisation against one or more antigens during a **preceding blood transfusion** or in women during **pregnancy**. The risks depend on the immunogenecity of antigens: by range of importance in the Rhesus system D, E, c,e,C ; the K of the Kell system; the $Fy^a$ of the Duffy system; the $Jk^a$ of the Kidd system,… To do well, all these antigens must be taken into account (or at least the most immunogenic antigens) before executing a transfusion. This is obviously impossible, even for a well equipped laboratory. In isolated situations, where only the ABO groups and the D of the Rhesus antigen are determined, the approach of the haemolysin (compatibility tests) is therefore most important.

The aim of the compatibility tests is to prevent an immunological transfusion reaction by demonstrating the incompatibility between donor and receptor. It permits thus to assure to the receptor the benefit of a transfusion with reduced immunological risk. In case of a positive compatibility test (presence of haemolysin), the search for compatible blood will not be easy : Without knowing neither the antigen or the concerning antigens, neither the principal donor's blood group, finding compatible blood will just depend on the perseverance in the search of a donor and in a great part of luck.

In this notes only the « rapid cross match» and the major compatibility in saline medium, associated with the indirect Coombs in LISS albumin medium, will be practiced. The majority of the considered techniques in a district laboratory are resumed in table 5 (page 19) and 6 (page 20). In this table, the most important advantages, but also the most important inconveniences are considered for each technique.

## Rapid test (rapid cross match)

To avoid confusion errors of the patient, it is recommended to do an ultimate bed side control of the ill person in order to detect the ABO errors. In basic laboratory conditions, this minimal compatibility test can be performed in the laboratory as a "compatibility test". Nevertheless it cannot replace the major compatibility test (detection of IgM and IgG). **It is only useful for transfusions in iso-groups (presence of antibodies anti-A and anti-B in the blood of a person of group O). This test has thus only a restricted capacity, since it will only detect, almost exclusively, an ABO grouping error (restricted detection of IgM) in the context of an iso-group transfusion.**

**Reagents**:

Alcohol 70°.

**Material**:

Lancets, slides, needles.

**Technique:**

1. Verify the identity of the patient to be transfused. If he is able to, confirm this information by the patient, loudly spoken.
2. Verify if the blood group of the patient matches the blood group of the pocket.
3. Prepare an object slide.
4. Disinfect with alcohol 70° the tubing of the blood pocket and the arm of the patient.
5. Prick by means of a sterile lancet the finger of the receptor
6. Put a drop of blood of the receptor on the slide.
7. Prick by means of a needle the tubing of the blood pocked (!!! In order to avoid contamination of the blood pocket, there must be pricked between two knots or between two weldings).
8. Put a blood drop of the tubing on the slide.
9. Mix both blood drops with the corner of another slide.
10. Move and tilt during at least one minute.
11. Watch if any agglutination appears.

**If agglutination is observed: ABO incompatibility or presence of antibodies directed against red blood cells of IgM type. DO NOT TRANSFUSE THE BLOOD BAG. Check the blood group of the donor and receptor.**

**If absence of agglutination: The blood bag can be transfused.**

**In case of doubt, observe the slide under the microscope (magnification 100x) to distinguish better the agglutinations. In order to make lecture easier, incline slightly the slide before lecture under the microscope to see the red blood cells while they are moving. (cf. microphotography page 8 for interpretation).**

**<u>Possible problems in the « rapid cross match »</u>**

**False negative reactions:**

- Too weak red blood cell concentration, resulting in a difficult lecture (p.e. a very anaemic patient, with not much red blood cells).
- Too short reaction time.
- …

**False positive reactions:**

- Coagulation of the blood to be determined.
- Presence of cold agglutinins in the blood to be determined.
- Chronical infection in the donor or the receptor (rouleaux phenomena by increased plasmatic proteins).
- Infection of trypanosomiasis (presence of auto agglutinins and rouleaux formation).
- …

# More complete tests

Complete Major compatibility test in saline medium
followed by an indirect Coombs test[14] in LISS-Albumin medium.

Antibody examination of the receptor versus erythrocytes of the donor. (IgM in Saline medium and IgG in LISS-Albumin).

**Sample**:

Serum of the receptor.
Red blood cells of the donor (blood taken on anticoagulant).

**Reagents**:

- Polyvalent Coombs Serum directed against human IgG and the fractions C3 of the complement. Diaclon Coombs serum (Diamed).
- LISS Diamed medium (DiaLISS - albumin). [LISS = Low Ionic Strength Solution]
- Physiological water 0.9 % (p/v) in NaCl (= saline or saline solution).

| | |
|---|---|
| Sodium chlorite (NaCl)..... | 9 g |
| Distilled water..... | 1000 ml |

**CONSERVATION :** a few months.
**CONDITIONS :** Brown **or** white flask of 1000 ml.
Label : physiological water (or saline solution) and note the date of preparation.

**Material**:

Haemolysis tubes in plastic, 10mm x 75mm,
Plastic Pasteur pipettes,
Bulb pipettes for physiological water,
Haematological centrifuge,
Vacuum pump,
Water bath 37°C,
Microscope mirror [slides, microscope].

**Technique** [15] :

1. Take a sample of red blood cells of the donor in a haemolysis tube.
2. Wash the red blood cells 3 times with physiological water.
3. Dilute the red blood cells to 5 % in physiological water (50 µl of the pellet of the washed red blood cells + 950 µl physiological water). Homogenise the tube well.
4. Take 2 haemolysis tubes:

[14] The principle of the reaction is explained on page 6.
[15] This technique is only applicable for Diaclon Coombs antiserum (Diamed), associated with DiaLISS-albumin medium (Diamed). The technique may vary in function of the used reagents. Always follow the particular instructions which are indicated in the user manual of the producer.

## TUBE 1

## Saline medium

2 drops of red blood cells of the donor 3 times washed and diluted at 5 % in physiological water.
2 drops of serum of the receptor.

Incubate 5 minutes at room T° (22°C).

Centrifuge 1 minute at 1.000 RPM (100*g*).

Read and evaluate the result.

**[a test is positive if there is presence of agglutination or haemolysis (total or partial) of red blood cells]**

**Demonstration of antibodies (complete of IgM type) :**

ABO Error
Cold allo antibodies
Cold auto antibodies

**ACTIONS** (CF. MORE DETAILED EXPLANATIONS ON PAGE 6)

**EXCLUDE THE ABO ERROR BEFORE TRANSFUSION:**

Verify ABO group of donor
Verify ABO group of receptor
Verify if false positive
(Perform an auto-test with the receptor)

**TUBE 2**

**Coombs indirect LISS albumin Medium**

2 drops of red blood cells of the donor 3 times washed and diluted at 5 % in physiological water
2 drops of serum of the receptor.
4 drops of LISS (DiaLISS-Albumin).

Incubate 5 minutes at 37°C. **(sensibilisation step)**

Wash three times in physiological water; pour off well the supernatant after the last wash.

Add 2 drops of Coombs polyvalent serum. **(demonstration step)**

Centrifuge 1 minute at 1.000 RPM (100*g*).

Read and evaluate the result.

**[a test is positive if there is presence of agglutination or haemolysis (total or partial) of red blood cells]**

**Demonstration of antibodies (incomplete of IgG type) :**

Warm allo antibodies
Warm auto antibodies
False positives

**ACTIONS**

Verify if false positive
This always concerns dangerous antibodies

**DO NOT TRANSFUSE, FIND ANOTHER DONOR**

## PROBLEMS AND INTERPRETATION OF THE MAJOR COMPATIBILITY TESTS

**False negative reactions (in saline medium and/or in LISS albumin medium) :**

- Incorrect cell concentration involving a difficult lecture.
- Incorrect wash of the red blood cells resulting in an inhibition of the Coombs serum.
- Unclean material bringing along an inhibition of the Coombs serum.
- Quality of the Coombs serum (expired, specificity, activity, titre, …).
- Incorrect concentration of the reagents (Coombs or LISS-albumin).
- Temperature and/or incubation time incorrect.
- …

***POSITIVE REACTION IN SALINE MEDIUM :***

- **False positives**
  - Presence of fibrin or bacterial contamination of the serum
  - …

    1 **Haemolysis** :

    - Concentration of NaCl incorrect of physiological water
    - Centrifugation too fast,
    - …

    2 **Rouleaux><agglutination :** check agglutination under a microscope (see microphotograpy page 8 for interpretation):

    - Chronical infections (rouleaux formation caused by plasmatic proteins increase).
    - Trypanosomiasis infection (presence of auto agglutinins and rouleaux formation).
    - …
- **ABO error** : Do not transfuse, verify the blood group of the donor and receptor and find a compatible donor

Execute an auto test (see page 17) [Not so useful in a district laboratory].

Exclusion of ABO error and exclusion of false positives, with positive auto-test : presence **of cold agglutinins** (cold agglutinins are cold auto antibodies which are active between 4 and 22°C). Practically, blood may be transfused at 37°C (except if the indirect Coombs is positive).

Exclusion of ABO error and exclusion of false positives, with negative auto-test : Presence of **cold allo antibodies** (these cold allo antibodies are not dangerous from the point of view of transfusion on condition that they are not active at 37°C. Practically, blood may be transfused at 37°C (except if the indirect Coombs in LISS-albumin medium is positive).

**POSITIVE REACTION IN INDIRECT COOMBS IN LISS ALBUMIN MEDIUM :**

- **False positives** :
  - Presence of fibrin or bacterial contamination of the serum
  - Quality of the Coombs serum (adsorption of antibodies against human red blood cells).
  - Insufficient washed red blood cells or contaminated solution by quartz

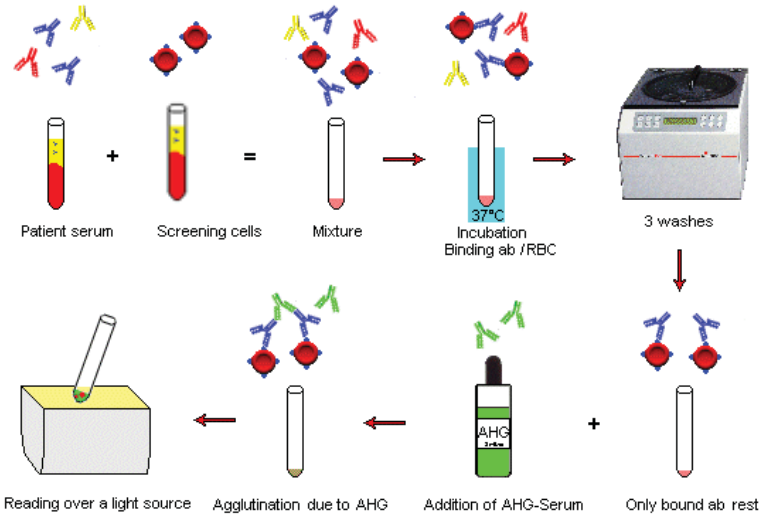
  1. **Haemolysis :**

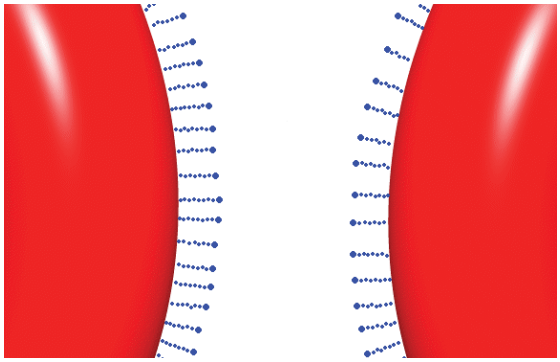
     - Concentration of NaCl incorrect of physiological water
     - Centrifugation too fast,
     - …
  2. **Rouleaux><agglutination :** check agglutination under a microscope (see microphotograpy page 8 for interpretation):

     - Chronical infections (rouleaux formation caused by plasmatic proteins increase).
     - Trypanosomiasis infection (presence of auto agglutinins and rouleaux formation).
     - **…**

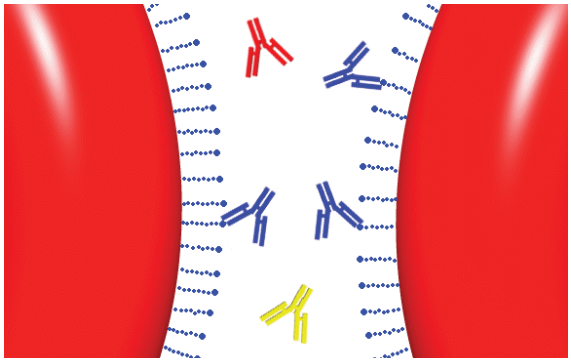
  If a false positive can be excluded, it always concerns a dangerous antibody from the point of view of transfusion. **DO NOT TRANSFUSE, FIND ANOTHER DONOR**.

# PRINCIPLE OF THE MAJOR COMPATIBILITY, INDIRECT COOMBS TEST

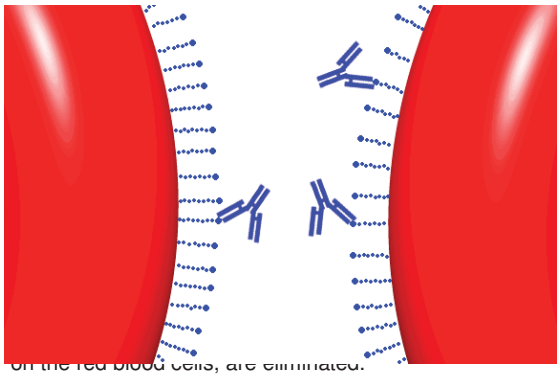


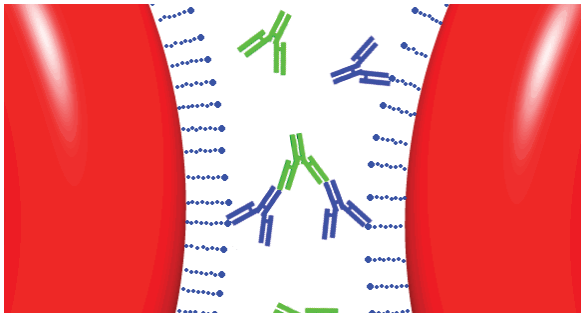
1. Antigens present on the surface of the red blood cells of the donor. (in blue for instance the antigens Fya).



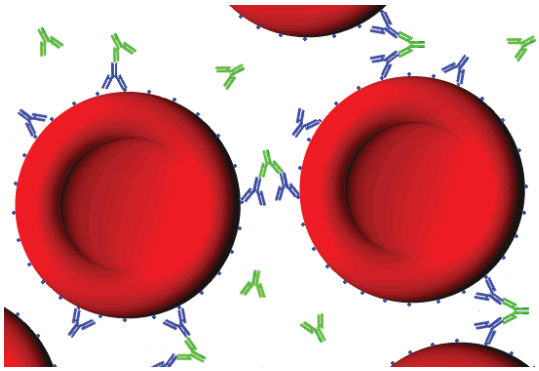
2. The serum of the receptor is added. The antibodies anti Fya, present in the serum will bind on the corresponding antigen. All the other present antibodies stay free in the serum**. Sensibilisation step.**



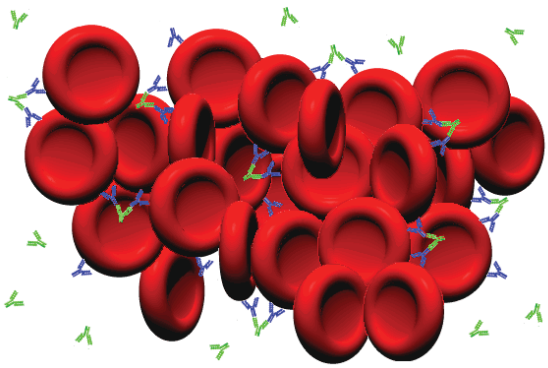
on the red blood cells, are eliminated.



4. After adding Coombs serum, the anti- human antibodies are binding with the anti-Fya antibody. **Revelation step.**



5. The fixation of the anti-human antibodies on two different anti-Fya antibodies are forming a “bridge” between the red blood cells resulting in an agglutination



6. This agglutination is macroscopically visible in the tube as a clot. Auto-test on the receptor

## Auto test on the receptor
## (In case of positive compatibility tests in saline medium)

The autotest can be executed in case the of a positive compatibility test in saline medium, in order to make the distinction between cold agglutinins and cold allo antibodies. As these two types of antibodies are not very dangerous in the context of transfusion and in practice one may transfuse the blood in both situations (**but at 37°C)**, this test is not very useful on district laboratory level.

**Sample**:

Serum of the receptor.
Red blood cells of receptor (blood taken on EDTA anticoagulant).

**Reagents**:

Physiological water [or saline solution] (cf. page 6).

**Material**:

Plastic haemolysis tubes of 10mm x 75mm, plastic Pasteur pipettes, bulb pipette for physiological water, haematological centrifuge, vacuum pump, fridge, microscope mirror [slides, cover slips 22mm x 22mm, microscope].

**Technique**:

1. Take a blood tube of the receptor (on EDTA).
2. Wash the red blood cells 3 times with physiological water.
3. Dilute the red blood cells at 5 % in physiological water.
4. Take 1 haemolysis tube.
5. Bring 2 drops of red blood cells of the receptor, washed 3 times and diluted to 5 %, in physiological water (point 3).
6. Add 2 drops of serum of the receptor.
7. Incubate 5 minutes at 22°C, centrifuge 1 minute at 1.000 RPM, read and evaluate the results.
8. Incubate 20 minutes at 4°C, centrifuge 1 minute at 1.000 RPM, read and evaluate the results.

## A test is positive if there are agglutinations.

**Table 4 Interpretations of an auto-test.**

| 4°C | 22°C | Interpretations |
|---|---|---|
| + | + | Presence of cold agglutinins (if major compatibility is also positive in saline medium). |
| + | - | Presence of cold agglutinins (if major compatibility is also positive in saline medium). |
| - | - | Presence of cold allo antibodies (if major compatibility is also positive in saline medium). |
| - | + | Incorrect test |

+ = agglutination, - = absence of agglutination

## Test of minor compatibility

Examination of antibodies of the donor versus red blood cells of the receptor. **This test makes only sense for transfusions of blood in iso-group** (presence of antibodies anti-A and anti-B in the serum of a person of group O).

This test is only rarely executed in routine in a small laboratory. Taken into account that the minor test detects the antibodies of the donor and that these are much diluted in the circulation of the receptor, this test is of restricted interest.

**Of course, in case of plasma transfusion, the minor compatibility test is the most important.**

The executing of the test is similar to the major compatibility, but by inversing donor and receptor. Washed red blood cells of the receptor are brought in contact with the serum of the donor.

***Table 5 :*** *Comparison of different (major) compatibility tests useful on district laboratory level.*

| Type of compatibility test | Principle | Advantages | Inconveniences |
|---|---|---|---|
| « Rapid Cross match » | Mix on a slide a drop of **whole blood** of the donor and a drop of **whole blood** of the receptor. Observe eventual agglutination of the red blood cells. | ▪ Detection of **ABO errors** (and irregular antibodies of IgM type).<br>▪ Fast (+/- 2 minutes).<br>▪ Easy.<br>▪ Feasible on the bed side of the patient.<br>▪ Neither equipment nor electricity needed.<br>▪ Low costs.<br>▪ … | ▪ Only applicable for **iso-group** transfusions.<br>▪ Detects only IgM, so almost exclusively ABO errors.<br>▪ … |
| « Improved Cross match » [16] | Mix on a slide or in a tube, a drop of **whole blood** of the donor + a drop of **serum** of receptor. Observe eventual agglutination of red blood cells. | ▪ Detection of **ABO errors** (and irregular antibodies of IgM type).<br>▪ Also applicable for transfusions of **non iso-groups**.<br>▪ Moderate fast (+/10 minutes).<br>▪ Moderate easy.<br>▪ … | ▪ Detects only IgM, so almost exclusively ABO errors.<br>▪ Necessitates an **electric centrifuge.**<br>▪ Necessitates a big enough quantity of receptor blood (serum) → Small anaemic children?<br>▪ … |
| Major Compatibility (in saline medium) | Mix in a tube 2 drops of washed red blood cells of donor + 2 drops of serum of the receptor. Incubate 5 minutes at room T° (22°C), next centrifuge 1 minute at 1.000 rpm (100 *g*). Observe eventual agglutination of the red blood cells. | ▪ Detection of **ABO errors** (and of irregular antibodies of IgM type).<br>▪ Also applicable for transfusions of **non iso-groups**.<br>▪ … | ▪ Detects only IgM, so almost exclusively ABO errors.<br>▪ Necessitates a big enough quantity of receptor blood (serum) → Small anaemic children?<br>▪ Necessitates an **electric centrifuge.**<br>▪ Slow (+/30 minutes).<br>▪ Rather complex.<br>▪ … |
| Polybrene Method on slide[17] (cf. article in annex 4 page 6) | On a slide, 1 drop of washed and diluted at 20 % blood of the donor + 2 drops of serum of the receptor + 3 drops of a medium with weak ionic strength. Mix for 1 minute next add 1 drop of Polybrene, mix and observe eventual agglutination of red blood cells. | ▪ Detection of **ABO errors** (and irregular antibodies of IgM type and of certain **IgG**).<br>▪ Also applicable for transfusions of **non iso-groups**.<br>▪ Low costs<br>▪ Rather fast (+/- 30 minutes).<br>▪ … | ▪ Bad detection of certain irregular antibodies of IgG type (antibody anti-Kell for instance, so feasible in Asia, but not often in Africa or in Europe)<br>▪ Necessitates an **electric centrifuge.**<br>▪ Necessitates a big enough quantity of receptor blood (serum) → Small anaemic children?<br>▪ Reagents rather difficult to prepare locally.<br>▪ Rather complex.<br>▪ … |
| Major compatibility in saline medium + Coombs indirect in albumin medium) | Rather complex, see protocol compatibility in LISS- albumin medium page 6, but when using albumin instead of LISS-albumin, the incubation time has to be increased. | ▪ Detection of **ABO errors** and irregular antibodies of IgM and **IgG** type.<br>▪ Also applicable for transfusions of **non iso-groups**.<br>▪ … | ▪ Sensitivity and specificity not as good as in LISS-albumin medium.<br>▪ Necessitates a big enough quantity of receptor blood (serum) → Small anaemic children?<br>▪ Necessitates a **centrifuge** and a water bath (**electricity**).<br>▪ Moderate expensive.<br>▪ Rather complex.<br>▪ Slow (+/- 60 minutes).<br>▪ … |
| Major compatibility in saline medium + indirect Coombs in LISS-albumin medium) | Rather complex, see protocol page 6 . | ▪ Detection of **ABO errors**, of irregular antibodies of IgM and **IgG** type.<br>▪ Also applicable for transfusions of **non iso-groups**.<br>▪ Rather fast (+/- 30 minutes).<br>▪ High sensitivity.<br>▪ High specificity.<br>▪ … | ▪ Necessitates a big enough quantity of receptor blood (serum) → Small anaemic children?<br>▪ Necessitates a **centrifuge** and a water bath (**electricity**).<br>▪ Rather expensive<br>▪ Rather complex.<br>▪ … |

[16] Une autre amélioration supplémentaire est d'utiliser des globules rouges lavés du donor, ceci complique et rallonge le test pour un résultat équivalent.
[17] Marie Lin. Compatibility testing without a centrifuge : the slide polybrene method. Transfusion 2004; 44: 410-413.

***Table 6 :*** *Use of compatibility tests on district laboratory level described in the notes.*

| Type of test | Use | Demonstrated antibodies | Questions / Actions | Necessary Equipment(s) | | |
|---|---|---|---|---|---|---|
| | | | | Electricity | Centrifuge | Water bath |
| **Rapid Cross Match.** | Transfusion of whole blood or of concentrated red blood cells:<br><br>In Iso-group. | IgM in the receptor against the red blood cells of the donor :<br><br>ABO Error<br>Cold Auto antibodies<br>Cold Allo antibodies | ¿ Error of **ABO** grouping?<br><br>**Do not transfuse if +** | | | |
| **Major** compatibility test in **saline** medium**.** | Transfusion of whole blood or of concentrated red blood cells:<br><br>In iso-group.<br>In non iso-group. | IgM in the receptor against the red blood cells of the donor :<br><br>ABO Error<br>Cold Auto antibodies<br>Cold Allo antibodies | ¿ Error of **ABO** grouping?<br><br>¿ Does the receptor have antibodies (**IgM**) against red blood cells of donor?<br><br>If + and ABO error excluded**, transfuse at 37°C** | **X** | **X** | |
| M**ajor** compatibility test in **indirect Coombs** in **LISS-albumin** medium**.** | Transfusion of whole blood or of concentrated red blood cells:<br><br>In iso-group.<br>In non iso-group. | IgG in the receptor against the red blood cells of the donor :<br><br>Warm Allo antibodies | ¿ Does the receptor have antibodies (**IgG**) against red blood cells of donor?<br><br>**Do not transfuse if +** | **X** | **X** | **X** |
| **Minor** compatibility test in **saline** medium**.** | Transfusion of whole blood or of concentrated red blood cells:<br><br>In iso-group.<br><br>Transfusion of plasma :<br><br>In iso-group.<br>In non iso-group. | IgM in the donor against the red blood cells of the receptor :<br><br>ABO Error<br>Cold Auto antibodies<br>Cold Allo antibodies | ¿ Does the receptor have antibodies (**IgM**) against red blood cells of donor?<br><br>Whole blood or concentrated red blood cells :<br><br>If + and ABO error excluded**, transfuse at 37°C**<br><br>Plasma or concentrated red blood cells :<br><br>**Do not transfuse if +** | **X** | **X** | |
| **Minor** compatibility test in **indirect Coombs** in **LISS-albumin** medium**.** | Transfusion of whole blood or of concentrated red blood cells:<br><br>In iso-group.<br><br>Transfusion of plasma :<br><br>In iso-group.<br>In non iso-group. | IgG in the donor against the red blood cells of the receptor :<br><br>Warm Allo antibodies | ¿ Does the receptor have antibodies (**IgG**) against red blood cells of donor?<br><br>Whole blood or concentrated red blood cells :<br><br>**Do not transfuse if +**<br>Plasma :<br><br>**Do not transfuse if +** | **X** | **X** | **X** |

***Table 7 :*** *Blood transfusion on district hospital level: from minimum until extra possibilities.*

| Activities | The minimum | More…<br>(in function of possibilities, listed for each activity in order of importance) |
|---|---|---|
| **Blood donor** | • None remunerated familial blood donors. | • Living blood bank.<br>• Mini blood bank for emergencies (1 to 2 blood bags).<br>• Blood bank based on none remunerated and regular voluntary blood donors.<br>• … |
| **Selection of donors** | • Questionnaire and clinical selection. | • Delay of reflexion between the first screening test and the first blood donation (for the voluntary donors).<br>• … |
| **Type of serological screening** | • Rapid tests **after**/**during** the taking of the blood unit. | • Rapid tests **before** the blood taking.<br>• … |
| **Serological screening on the donors** | • HIV (antibodies).<br>• HBsAg (antigens).<br>• Syphilis (non treponemal test - RPR). | • HIV (antigens).<br>• HCV (antibodies).<br>• Syphilis (Test treponemal - TPPA type).<br><br><u>And in function of the region a screening for :</u><br>o Chagas Disease ?<br>o African Trypanosomiasis ?<br>o Leishmaniasis ?<br>o Microfilaria ?<br>• … |
| **Type de transfusion** | • « Warm » transfusion of whole blood<br><br>o Iso-group (general rule).<br>o Non iso-group (exception). | • «Cold» transfusion of whole blood (blood bank).<br>• Transfusion of packed cells (blood bank).<br>• [Transfusion of plasma (blood bank)].<br>• … |
| **Blood grouping** | • ABO grouping: Forward blood grouping<br>• Rhesus grouping: Limited to antigen D on slide. | • ABO grouping: Reverse blood grouping.<br>• ABO grouping in tube.<br>• Rhesus grouping in tube.<br>• … |
| **Compatibility test** | • Rapid Cross Match. | • Major compatibility test in saline medium associated with an indirect Coombs in LISS-albumin medium.<br>• [Minor compatibility test].<br>• [Auto-tests].<br>• … |

# *ANNEX 1*

## Wash of the red blood cells

The purpose of the wash of the red blood cells is to eliminate all the plasmatic antibodies which are free or non specifically fixed on the surface of the red blood cells.

**Sample**:

Blood taken on anticoagulant (EDTA).

**Reagents**:

Physiological water (or saline solution cf. preparation page 6).

**Material**:

Plastic haemolysis tubes 10mm x 75mm,
Plastic pipettes Pasteur,
Bulb pipette for physiological water,
Haematological centrifuge,
[Vacuum pump].

**Technique** :

1. Centrifuge sample 5 minutes at 3000 RPM.
2. Aspire the plasma with a vacuum pump or take off with a Pasteur pipette.
3. Bring 1 volume of the cell clot in the haemolysis tube.
4. Add at least 10 volumes of physiological water to the red blood cells clot with a bulb pipette (use the pressure of the pipette to bring the red blood cells in suspension).
5. Centrifuge the tube 5 minutes at 3000 RPM.
6. Aspire the plasma with a vacuum pump or take off with a Pasteur pipette.
7. Repeat two times steps 4 to 6.

# *ANNEX 2*

## Screening of dangerous O donors

It can happen, in case of emergency, that it is necessary to transfuse O blood to a receptor A, B or AB. The plasma of certain O persons may contain an important quantity of antibodies anti A or more rarely anti B which may cause a reaction with the red blood cells A, B or AB of the receptor. These persons, dangerous O donors, must be detected and may not be considered as "universal donors". Blood of « dangerous O donors » may only be transfused to receptors of group O. The screening of dangerous O donors is only possible in the context of a blood bank and should be systematically performed at the moment of the arrival of the pocket of the O blood in the fridge (duration of the test). Nevertheless, for reason of rareness of dangerous O donors and the maximal use of iso-group transfusion, this test is little useful.

The fresh serum of an O donor is incubated with small quantities of red blood cells $A_1$ and B. If there are too many antibodies anti A or anti B these red blood cells will be haemolysed and the serum will be pink stained.

**Sample**:

Serum of the donor.
Known red blood cells $A_1$ and B, diluted at 5 % in physiological water.

**Reagents**:

Physiological water (or saline solution, cf. preparation page 6).

**Material**:

Plastic haemolysis tubes of 10mm x 75mm
plastic Pasteur pipettes
bulb pipette for physiological water
haematological centrifuge
vacuum pump
fridge
microscope mirror [slides, cover slips 22mm x 22mm, microscope].

**Technique** :

1. Take the serum of the donor O to be tested. The serum must be used within 6 hours.
2. Wash the known red blood cells $A_1$ and B 3 times with physiological water.
3. Dilute these red blood cells at 5 % in physiological water.
4. Take 2 haemolysis tubes.
5. Bring in a tube 1 drop of red blood cells $A_1$ diluted to 5 % and in the other 1 drop of red blood cells B diluted to 5 %.
6. Add to each tube 9 drops of serum of the donor O to be tested.
7. Incubate 2 hours at 37°C.
8. Bring the red blood cells back in suspension by slightly tapping the haemolysis tubes.
9. Centrifuge 1 minute at 1.000 RPM.
10. Control the colour of the supernatant.

If the serum is **yellow**, with sediment of red blood cells, this donor O can be considered as **universal**.

If the serum is **pink**, with limited sediment of red blood cells, this donor O must be considered as « dangerous donor ». His blood may only be transfused to a receptor of group O.

# *ANNEX 3*

## Screening for infectious diseases

Blood transfusion is known to be an efficient way for transmitting infectious diseases. It is therefore important to screen blood before its potential use in order to discard any blood unit capable of infecting a recipient.

**Viral and related diseases :**

- Hepatitis B, C, (A)? D,E G, (VHA), VHD, VHE, VHG/VGB-C
- HIV 1 / 2 (Human Immunodeficiency Virus).
- HTLV 1 / 2 (Human T-Lymphocytotrophic Virus).
- CMV (Cytomegalovirus).
- EBV (Epstein Barr virus).
- TTV (TT virus)
- HHV-6, HHV-8 (human herpes virus type 6 and 8)
- SEN-V (SEN virus
- HPB19 (human parvovirus)
- Creutzfeld-Jacob disease (and other prions).
- [Hemorrhagic fevers].
- …

**Parasitical disease :**

- Malaria.
- Leishmaniasis.
- Toxoplasmosis.
- Chagas disease.
- African trypanosomiasis.
- Babesiosis.
- (Microfilaria).
- …

**Bacterial diseases :**

- Syphilis
- Borreliosis.
- Brucellosis.
- …

**Bacterial contamination of blood products**. This is another often observed risk disorder directly associated with blood transfusion. Most commonly associated with contamination during blood collection or during handling of blood products, and on occasion, associated with bacterial infection of the donor, it is sometimes recognizable by obvious changes in the appearance of the blood product. When grossly contaminated, blood appears haemolysed and dark in colour.

Sometimes, infectious agents can be detected directly in blood (for example HBs antigen detection reflects directly an HBV infection). More often blood will be analyzed in order to detect specific antibodies. For some infectious diseases, the presence of antibodies may reflect a past infection and does not mean that the blood is infectious (hepatitis for example); in other cases, on contrary, antibodies may reflect a current transmissible infection (for example anti-HIV antibodies).

The latency, characterizing some infections, has also to be taken into account for two main reasons : first, the latent phase is often infectious, secondly, tests detecting viral antigens before antibodies become detectable are not always available (for example HCV).

In many developing countries, the prevalence of infectious diseases in the general population is high. For that reason, high rates of infected blood donors can be expected, and proportionally, a high rate of co-infections. Laboratory testing of blood donors for infectious diseases is therefore an essential phase in assessing blood safety. On the other hand, this high prevalence will also increase the risk of missing some detection because of the latency (windows period and/or sensitivity limitation)… This problem can be partially solved by a good donor's selection.

## ***LEVELS OF SCREENING STRATEGY***

Laboratory testing should be considered at least at three levels:

**1.** Screening tests applied to blood units: To be useful in the improvement of blood safety, screening tests have to be applied systematically on all blood units in order to identify any potentially dangerous blood. In this context, a positive test result is by itself a sufficient reason to discard the blood unit from therapeutic use. **Therefore, for the purpose of blood safety, the most sensitive test should be recommended for the screening of blood units.**

2. Laboratory testing may acquire a role of diagnosis when blood donors ask for the results obtained by analyzing their blood for infectious agents. In this context, the results of screening tests have to be confirmed by confirmatory methods with high specificity.

**3.** The results performed in a blood bank can also be used as indicators of effectiveness of the selection criteria applied to blood donors. Indeed, the rate of sample found to be "reactive" with the screening test will give information about the prevalence in the selected population and **may help to revise and / or reorient the criteria used in order to recruit and select "safer" blood donors.**

## ***PARAMETERS INFLUENCING THE SCREENING STRATEGY***

Before dealing with technical considerations, one should keep in mind that environmental parameters as well as some intrinsic characteristics of the infectious agents themselves are likely to have an effect on the prevention of transmissible diseases in developing countries.

- Ideally, any blood for transfusion purposes should be tested for the presence of all those agents which are prevalent in a given population, and if transmitted, can cause serious disease for the recipient.
- Epidemiological data (if available) in the local population have to be taken into account.
- In endemic areas, the probability for an adult recipient, being infected prior to transfusion, and to have achieved immunity, depends on the prevalence of the disease in the population…This is not true for young children…
- Some infectious agents are only present in cells and are not transmitted by cell free blood components such as plasma (malaria for example) [this aspect is to be considered only if separation of blood components is feasible]. Other agents are present and infectious in cells and cell free components.
- Some infectious agents are killed or at least their virulence is weakened after blood storage for 72 hours at 4 to 7 °C (syphilis, trypanosomes). This could be kept in mind if storage is feasible and safe.
- Information given to blood donors in order to teach them about at-risk behaviour and to encourage them to "self-deferral" is less expensive, less dangerous and probably as useful as testing to discard dangerous blood units (more relevant for sexually transmitted diseases).

When financial support is limited, local priority should be given to various screening tests according to the prevalence of the carrier state in the general population, the consequences of infection for the recipient's health and the age of recipient.

***WHICH TESTS CAN BE USED IN A REMOTE AREA ?***

***TECHNICAL CONSIDERATION***

- Number of blood transfusions ?

  Blood bank type ?
  Cold blood ?
  Test before /during / after blood unit's collection ?

- Test availability ?
- Test complexity ?
- Time ?
- Human resources ?
- …

**HIV** : In 2005 the WHO estimated that 5 % of HIV infections in Africa might be caused by transfusion. Therefore HIV screening is mandatory. A single positive screening test result is sufficient to decide to discard the blood unit. If the donor is to be informed, all precautions should be taken: the positive result should lead to performance of alternative tests according the adapted strategy of the prevalence. Sensitive, specific and rather cheep rapid tests are available (detection of antibodies and/or antibodies and antigens).

**HBV** : Hepatitis B is an important transfusion hazard since it is established that blood infected with hepatitis B virus is infectious in almost 100 % of cases. In developing countries, the rate of people infected with HBV is most of the time very high and may reach 90 % in adults. It is essential that every blood unit should be screened for HBsAg for the following reasons: a great number of transfusion indications refers to paediatric patients who have not been immunized; besides, the consequences of transfusing blood infected with hepatitis B, to immunized individuals are not known.

**HCV** : Few epidemiological data are available for developing countries. Screening for HCV antibodies is 2 times more expensive than for HIV. HCV is responsible for more than 90 % of post transfusion hepatitis, if HBV has been excluded (European data). Estimates are that 80 % of the persons receiving a transfusion with blood infected with HCV will seroconvert, and probably more than 50 % of the persons who seroconvert will develop chronic liver disease with possible serious complications 10 to 20 years after infection (liver cirrhosis, hepatic cellular carcinoma).

**HBA** : Hepatitis A has rarely been associated with transfusion, and the infection is clinically mild; screening whole blood donors is not anticipated.

**CMV :** The prevalence of the CMV antibody ranges from 50% to 80% of the population. Blood contaminated with CMV can cause problems in neonates or immune compromised patients. Potential problems in selected patient populations can be prevented by transfusing CMV negative blood. Donor blood is not routine tested for CMV. Tests are expensive and complex.

**HTLV 1 / 2 :** No systematic screening recommended except in areas where the disease is frequent (epidemiological data are incomplete, but there are three known high prevalence areas : Central and South America and the Caribbean, southern Japan and sub-Saharan Africa). Risk of transmission in the United States at this time is said to be 1 in 641,000. The risk of developing HTLV-1 disease, adult T-cell leukaemia/lymphoma or tropical spastic paraparesis, is estimated to 1 or 2 per 1.000 HTLV-1 positive cases per year after an incubation period averaging 20 years. The actual estimates are that about 60 % of the persons receiving blood containing HTLV-1 will seroconvert. The test (EIA and particle agglutination assay) gives many false positive results. Screening and confirmation tests are expensive and complex.

**Malaria :** The best method for the diagnosis of malaria is to examine a thick blood film for parasites. However, since this method requires microscopic examination of each sample, it is not suitable on a large scale. Even in endemic areas, the absence of parasites in a thick blood film, will not say that the blood is not infected (sensitivity limitation). Antibody detection is not applicable in endemic countries. In endemic areas, a medical history, seeking evidence of recent fever and illness is essential. The use of therapeutic (or prophylactic) anti-malarial drug for transfusion recipients has to be considered.

**Chagas disease :** As far as blood transmission of Chagas disease by blood is concerned, the problem is most serious in South America. However, migration of people from endemic to non endemic areas has resulted in the presence of infectious individuals in previously non endemic areas (America). No systematic screening is recommended except in areas where the disease is frequent. Laboratory testing in the early phase of infection is by examination of tick blood film in order to detect the protozoa. In the acute phase, the parasite can be cultured from blood samples. None of these two methods is applicable to the screening of blood donors. Several serological tests are available for the detection of antibodies that are produced in 50 % of acute phase patients, and in 95 % with chronic infection, but their sensitivity and specificity remain questionable. In some *T. cruzi* endemic areas, gentian violet is added to donor blood (125 mg / 500 ml blood) followed by storage at 2-8 °C for 24 hours to kill the parasite. Guidelines regarding the most appropriate test to use in a particular area should be obtained from the nearest Chagas disease reference laboratory.

**African trypanosomiasis** : African trypanosomiasis can be transmitted when donor blood contains *T.b. gambiense* (or less probably *T. b. rhodesiense*). It can occur in areas of high prevalence but very few instances have been reported (Quality of the data ?). The CATT test may be useful in endemic areas. If donor is to be informed, all precautions should be taken: the positive result should lead to perform of confirmation tests.

**Leishmaniasis** : Cases of transfusion-associated leishmaniasis are growing each year world wide. This is increasingly associated with patients who are positive for HIV. Transfusion-associated leishmaniasis requires that the parasites be present in the peripheral blood of the donor, survive processing and storage in the blood bank, and infect the recipient. In endemic areas for visceral leishmaniasis, where the population of potentially infected individuals may be much higher, a serological screening process should be used. These tests are expensive, they take a long time and are a little bit complex.

**Microfilaria** can be transmitted in blood and may cause allergic reactions but the larvae are unable to develop further in the recipient and therefore filariasis cannot occur. Wet blood examination may be used to detect infected blood.

**Syphilis** : Testing for syphilis (RPR or VDRL) is recommended. However, a positive result does not always mean that the blood unit is infectious. Besides, retention of blood for 3 days at 4°C inactivates the infecting agent. Although the risk of post-transfusion is quite low, the screening of infected donors may be used as a marker of individual risk of STD infections (HIV, …) on account of their sexual behaviour. If the donor is to be informed, all precautions should be taken : the positive result should lead to performance of a confirmation test.

**Borreliosis** : In areas with endemic recurrent fever, a good donor selection is the best manner to exclude the risk. The best method for diagnosis of recurrent fever is to examine a thick blood film for bacteria. However, since this method requires microscopic examination of each sample, it is not suitable for a large scale. Even in endemic areas, the absence of bacteria in a thick blood film doesn't mean that the blood is not infectious (sensitivity limitation).

**…**

**Distribution of some infectious markers (example):**

| | RWANDA 1991 (n = 500) | | CONGO 2005 (n= 2500) | |
|---|---|---|---|---|
| **Marker** | **POSitives** | **%** | **POSITIVES** | **%** |
| HIV | 18 | 3,5 | 199 | 8 |
| HBsAg | 30 | 6 | 175 | 7 |
| Syphilis | 15 | 3,0 | 50 | 2 |
| HCV Ab | 15 | 3,0 | (113) ? | 4,5 (on 250) [18] |
| total | 78 | | 537 | |

**Co-infections :**

**Rwanda** 18. As much as 12 % of blood units collected must be discarded and not transfused.
**Congo** 134. As much as 16 % of blood units collected must be discarded and not transfused.

[18] N = 250

# ANNEX 4

# Compatibility test on slide, Polybrene method



## Compatibility testing without a centrifuge: the slide Polybrene method

*Marie Lin*

BACKGROUND: A simple and rapid slide Polybrene method (SP) for pretransfusion compatibility testing is described. SP is particularly suitable for use in developing countries where, due to limited resources, centrifuges and biologic reagents may not be readily available.
STUDY DESIGN AND METHODS: The original manual Polybrene method (MP) was modified for use on glass microscope slides, eliminating the need for test tubes and centrifugation. The sensitivity of SP for detecting allo-antibodies to RBC antigens was compared with that of MP and the IAT.
RESULTS: Both SP and MP were more sensitive than the IAT for detecting anti-E. SP detected 21 of 23 examples of anti-M and 7 of 8 examples of anti-E. Kidd and Diego system antibodies were also readily detectable by SP, although the reactions were weaker than those observed with both MP and IAT. However, both SP and MP failed to detect some examples of antibodies to Kell system antigens.
CONCLUSIONS: SP is an acceptable method for compatibility testing in developing countries, particularly in populations where the frequency of K is low (e.g., southeast Asia). The reagents are inexpensive and can be prepared in-house. SP is simple to use, does not require a centrifuge, and can be performed by personnel with minimal training.

Most developed countries have introduced standardized methods for pretransfusion testing that require the use of specific reagents and equipment (e.g., antiglobulin serum, centrifuges, etc.). However, for underdeveloped countries such items may be unavailable or too expensive to purchase. At a recent "Manual Polybrene" Workshop in Laos (December 2000), Lao Red Cross Blood Center, Vientiane[1] and an "Immunohematology in Taiwan" Workshop in Vietnam (December 2002, Viet-Duc University Hospital, Hanoi), it was discovered that very few centrifuges were available for use in the transfusion services of these countries. Therefore, the development of a compatibility testing procedure that did not require the use of a centrifuge or expensive reagents was of particular importance.

Past experience in developing a national blood program for Taiwan in the 1980s[2] demonstrated the importance of having a simple, rapid, and inexpensive method for compatibility testing to standardize pretransfusion testing procedures. Before 1969, transfusion medicine was an area of low priority in Taiwan and only a few teaching hospital blood banks had adequate equipment, finance, and staff to incorporate the more expensive and time-consuming standard Western procedures. However, in 1993 the manual Polybrene (MP)[3] method, which is a simple and rapid procedure for the detection of RBC alloantibodies, was introduced at Mackay Memorial Hospital. Within a few years, MP was incorporated successfully into routine pretransfusion testing procedures throughout the whole of Taiwan (including both large and small institutions).[4]

In this report, the slide Polybrene (SP) method, which is even simpler to perform than MP, is described. The reagents required for the SP method are inexpensive and can be prepared in-house. In addition, blood-bank personnel require only minimal training to perform the test satisfactorily, and the use of a centrifuge is not required. SP also demonstrates good sensitivity for the detection of all antibodies that are of clinical significance in southeast Asia.

ABBREVIATIONS: MP = manual Polybrene; SP = slide Polybrene.

From the Immunohematology Reference Laboratory, Mackay Memorial Hospital, Taipei, Taiwan.
*Address reprint requests to:* Marie Lin, MD, FCAP, Immunohematology Reference Laboratory, Mackay Memorial Hospital, 92, Sec 2, Chung Shan N Road, Taipei, Taiwan; e-mail: marielin@ms2.mmh.org.tw.

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## MATERIALS AND METHODS

### Reagents

Low-ionic-strength medium was prepared as for the MP method described by Lalezari and Jiang.[3]

A 10-percent stock solution of Polybrene (hexadimethrine bromide, Sigma Chemical, St. Louis, MO) in normal saline was also prepared as described by Lalezari and Jiang.[3] A 0.1-percent working Polybrene solution was prepared by appropriate dilution of the 10-percent stock solution in saline.

Resuspending solution for reversing the nonspecific Polybrene induced aggregation was prepared by mixing 60 mL 0.4 mol/L trisodium citrate solution and 40 mL 5-percent dextrose.

(Note: the working Polybrene solution and trisodium citrate concentrations used in SP were double the concentrations that were used in MP.)

### SP method

Sensitization phase. An ellipse, 3 × 1.5 cm in size, is drawn on a glass microscope slide with a wax pencil to prevent overflowing of reagents. Three drops of low-ionic-strength medium are added to the slide followed by 2 drops of test serum (or plasma) and 1 drop (50 µL) of 20-percent RBCs in saline (or 10 µL packed RBCs). The reagents are mixed thoroughly with an applicator stick and incubated at room temperature (~22°C) for 1 minute.

Polybrene aggregation phase. One drop of the 0.1-percent Polybrene working solution is then added to the reagents on the slide, mixed with an applicator stick, and incubated at room temperature for 1 minute. RBC aggregation usually begins to appear about 30 seconds after the addition of the 0.1-percent Polybrene solution and is complete within 1 minute.

Resuspension phase. One drop of resuspending solution is added, and the slide is gently rocked by hand for about 10 seconds until any nonspecific Polybrene-induced aggregation has dispersed. True antibody-induced agglutination does not dissociate and can be readily observed macroscopically. For weaker reactions, the agglutinates can be read using a magnifier. Results should be evaluated as soon as possible, and certainly no later than 3 minutes after the resuspending solution has been added.

Controls. Daily quality controls should include a weakly reacting anti-E or anti-D as a positive control and fresh AB serum as a negative control.

### MP method

MP was performed as described by Lalezari and Jiang[3] except that no supplementary antiglobulin phase was performed. Tests were examined microscopically.

### IAT

A standard saline IAT was performed by incubating at 37°C for 30 minutes, followed by washing and the addition of antihuman IgG (Gamma Biologicals, Houston, TX). Tests were examined microscopically.

## RESULTS

A comparison of the sensitivities of SP, MP, and IAT for the detection of various alloantibodies are shown in Tables 1 and 2.

From Table 1, it can be seen that SP and MP are more sensitive than IAT for the detection of anti-E. However, IAT is more sensitive than both SP and MP for the detection of anti-$M^{[6]}$, -$K_t$, -$Jk^a$, and -$Jk^b$.[7]

From Table 2, it can be seen that SP detected 21 of 23 examples of anti-$M^{[6]}$ and 7 of 8 examples of anti-E. More significantly, SP readily detected all ABO incompatibilities (Table 2). SP also readily detected other important alloantibodies of clinical significance, including antibodies against antigens of the Kidd and Diego blood group systems, although reactions were weaker than those observed with MP and IAT.

TABLE 1. Comparison of alloantibody titers by SP, MP, and IAT

| Methods* | Anti-E | Anti-E | Anti-E | Anti-E | Anti-E | Anti-E | Anti-$D_1$ | Anti-$D_1$ | Anti-$M^a$ | Anti-$M^a$ | Anti-$M^a$ | Anti-$M^a$ | Anti-$M^a$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SP | neat | 4 | 8 | 16 | 128 | 512 | 200 | 1600 | neat | 2 | 2 | 4 | 8 |
| MP | neat | 8 | 128 | 64 | 1024 | 2048 | 1600 | 6400 | 2 | 2 | 4 | 16 | 8 |
| IAT | neat | 8 | 2 | neat | 512 | 256 | 200 | 3200 | neat | 4 | 16 | 64 | 32 |

| Methods | Anti-$K_t$ | Anti-$K_t$ | Anti-$Jk^a$ | Anti-$Jk^a$ | Anti-$Jk^a$ | Anti-$Di^a$ | Anti-$Fy^a$ | Anti-$Fy^a$ | Anti-$Fy^a$ | Anti-$Fy^a$ | Anti-$Fy^a$ | Anti-$Fy^a$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SP | 2 | 16 | 2 | 4 | 16 | 2 | 4 | 4 | 4 | neat | 4 | 4 | neat |
| MP | neat | 16 | neat | 4 | 16 | 16 | 64 | 16 | 16 | 64 | 16 | 16 | 16 |
| IAT | 64 | 512 | 4 | 8 | 32 | 4 | 128 | 8 | 4 | 128 | 8 | 4 | 4 |

* Diluents for antibody titration were 3-percent albumin (BSA, Sigma Chemical) in saline. Except for the two Mabs initially diluted 1:100 and followed by 1:2 step dilutions, the rest of the antibodies were titrated in 1:2 dilution steps.
† FDA-approved commercial Mabs.
‡ FDA-approved commercial antisera.





TABLE 2. Numbers of patients in whom antibodies were detected and methods of detection

| Anti- | Number of patients | SP | MP | IAT |
|---|---|---|---|---|
| "M" | 23 | 21 | 23 | 23 |
| E | 8 | 7 | 8 | 7 |
| D | 5 | 5 | 5 | 5 |
| C | 1 | 1 | 1 | 1 |
| $Jk^a$, $Jk^b$, $Jk3$ | 8 | 5 | 8 | 8 |
| $Kp^b$ | 2 | 0 | 0 | 2 |
| $Di^a$, Di | 6 | 3 | 6 | 6 |
| S | 1 | 1 | 1 | 1 |
| M | 4 | 4 | 4 | 2 |
| $P1$ | 2 | 2 | 2 | 0 |
| $Le^a$, $Le^b$ | 2 | 2 | 2 | 1 |
| A | 10 | 10 | 10 | 10 |
| B | 10 | 10 | 10 | 10 |
| $H^a$ | 1 | 1 | 1 | 1 |
| I | 2 | 2 | 2 | 0 |
| Total | 85 | 74 | 83 | 77 |

* Bombay phenotype.

Among 85 antibodies from patients that were tested, 74 antibodies were detectable by SP, including 10 anti-A and 10 anti-B. Anti-$Kp^b$ could only be detected by IAT (Table 2) and higher anti-K titers were obtained by IAT than by both SP and MP (Table 1). Therefore, the main disadvantage of the two Polybrene methods is that a small number of antibodies of the Kell blood group system will not be detected. However, because the frequency of K in oriental populations is very low, this is not clinically significant.

## DISCUSSION

Slide methods have generally been considered inferior to tube methods with regards the detection of clinically significant alloantibodies. This is mainly due to the fact that in tube methods, RBCs are forced close together by centrifugal force, which thus enhances hemagglutination. However, in the SP method RBCs are brought close together by the action of the positively charged 0.1-percent Polybrene reagent, resulting in nonspecific RBC aggregation. Heparin interferes with the test, and 2 to 3 times of Polybrene should be added if heparinized samples are used (i.e., 2 or 3 drops of Polybrene). The Polybrene-induced aggregation can be quickly reversed by adding 1 drop of 0.4 mol/L citrate resuspending solution leaving any specific antibody-induced agglutination intact.

In this study, the sensitivity and efficacy of SP in detecting alloantibodies were compared simultaneously with MP and IAT by testing antibodies that were encountered during antibody screening and cross-matching in the Blood Bank, Mackay Memorial Hospital; antibodies obtained from the "Serum, Cells and Rare Fluids International Immunohematology Exchange Group" (SCARF), which included antibodies rarely found in Taiwan; two highly diluted commercial MoAb (anti-D and anti-E), which were routinely used as daily positive controls for SP and MP; and also several commerical antisera as shown in Table 1. The results show that SP detected most alloantibodies of clinical significance, especially anti-E and anti-$M^{[6]}$. These two antibodies are the most common alloantibodies of clinical significance in Taiwan[2] and most likely also in the rest of southeast Asia. Anti-$M^{[6]}$ is used in Taiwan to describe antibodies that react with RBCs of the MiIII phenotype.[5] Other alloantibodies such as anti-D, -K, -$Jk^a$, -$Jk^b$, -$Py^a$, and -$Py^b$ were also detected by SP with a sensitivity similar to that of MP. Although SP (and MP) are not as sensitive as IAT for the detection of anti-K, because most patients and donors in southeast Asia are K negative,[7,8] then the incidence of anti-K would be expected to be very rare. This is indeed the case, and during the past 20 years in Taiwan, only one antibody against an antigen of the Kell blood group system has been found (anti-Ku in a $K_{null}$ person).[3] Therefore, the implementation of a sensitive procedure for the detection of antibodies to antigens of the Kell blood group system would appear to be of low priority in routine compatibility procedures for southeast Asia.

SP is extremely rapid (about 5 min), cost effective (reagents for the test can be prepared simply and in-house), and is easy to perform. Personnel require only 1 day's training to perform the test with confidence. Therefore, the introduction of SP in countries with limited resources, and especially in countries where pretransfusion testing is limited to ABO grouping, will help significantly to improve transfusion safety. In such countries, many antibodies of clinical significance, which until now have been undetected, can now be detected.

Transfusion services in developing countries, not only lack centrifuges but also lack finances for purchasing the reagents and training new staff. In such situations, SP is the method of choice for routine pretransfusion testing so as to improve patient safety.

### ACKNOWLEDGMENTS

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# *ANNEX 5*

## Indicative price list (Diamed, blood grouping) *http://www.diamed.ch/*

| Items | Pkg size | Reference (Diamed) | Price (€) 09/2005 | Number of tests[19] |
|---|---|---|---|---|
| Coombs-serum, polyvalent anti-IgG (rabbit), anti-C3d (monoclonal),Diaclon, green | 10 ml | 107140 | 29,0 | 100 |
| LISS modified for red cell suspension Dialiss (Liss-albumin) | 10 ml | 106510 | 11,2 | 50 |
| Anti-A or anti-B, blood grouping monoclonal IgM Diaclon for slide and tube test | 10 ml | 100810 | 8,5 | 200 |
| Anti-AB, blood grouping monoclonal IgM Diaclon for slide and tube test | 10 ml | 100910 | 8,5 | 200 |
| Anti-D, blood grouping monoclonal IgG and IgM antibodies, D(VI-) Diaclon for slide and tube test | 10 ml | 101070 | 17,7 | 200 |

| Items | Package | Price (€) 08/2006 | Number of feasible tests |
|---|---|---|---|
| Hematological centrifuge for blood bank : Immufuge II (Baxter©) | 1 | 675 | S.O |

**Indicative price list (serological screening):**

| Items | Package | Price (€) 03/2006 | Number of feasible tests [20] |
|---|---|---|---|
| Determine HIV 1 / 2 | 100 | 80 | 100 |
| Determine HBsAg | 100 | 90 | 100 |
| HCV SPOT | 100 | 325 | 100 |
| CATT | 250 | 150 | 250 |
| RPR card antigen suspension Becton Dickinson | 500 | 150 | 500 |

[19] Without taking into account the controls and the losses.
[20] Without taking into account the controls and the losses.

# ANNEX 6

## HIV rapid test example

# Determine™

## HIV-1/2

This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are deviations from the instructions in this package insert.

NAME AND INTENDED USE
The Abbott Determine™ HIV-1/2 is an *in Vitro*, visually read, qualitative immunoassay for the detection of antibodies to HIV-1 and HIV-2 in human serum, plasma or whole blood. The test is intended as an aid to detect antibodies to HIV-1/HIV-2 from infected individuals.

SUMMARY AND EXPLANATION OF THE TEST
AIDS (Acquired Immunodeficiency Syndrome) is characterized by changes in the population of T-cell lymphocytes, in an infected individual, the virus causes depletion of helper T-cells, which leaves the person susceptible to opportunistic infections and some malignancies. The virus that causes AIDS exists as two related types known as HIV-1 and HIV-2. The presence of the AIDS virus elicits the production of specific antibodies to either HIV-1 or HIV-2.[1,11]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE
Determine HIV-1/2 is an immunochromatographic test for the qualitative detection of antibodies to HIV-1 and HIV-2.

Sample is added to the sample pad. As the sample migrates through the conjugate pad, it reconstitutes and mixes with the selenium colloid-antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient window site.

If antibodies to HIV-1 and/or HIV-2 are present in the sample, the antibodies bind to the antigen-selenium colloid and to the antigen at the patient window, forming a red line at the patient window site.

If antibodies to HIV-1 and/or HIV-2 are absent, the antigen-selenium colloid flows past the patient window, and no red line is formed at the patient window site.

To insure assay validity, a procedural control bar is incorporated in the assay device.

CONTENTS
Abbott Determine HIV-1/2 Serum/Plasma Assay (List No. 7D23-12), 20 Tests
- Determine HIV-1/2 Test Card, 2 cards (10 tests/card), HIV-1/2 recombinant antigen and synthetic peptide coated.

Abbott Determine HIV-1/2 Whole Blood Assay (List No. 7D23-32), 20 Tests
- Determine HIV-1/2 Test Card, 2 cards (10 tests/card), HIV-1/2 recombinant antigen and synthetic peptide coated.
- 1 Bottle (2.5 mL) Chase Buffer (List No. 7D22-11) prepared in phosphate buffer. Preservatives: Antimicrobial Agents.

ACCESSORIES (required but not provided)

| Serum/Plasma or Whole Blood (venipuncture assay) | | Whole Blood (fingerstick assay) | |
|---|---|---|---|
| Pipette | No. 7D22-51 | Lancets* | No. 7D22-31 |
| Pipette Tips | No. 7D22-61 | EDTA Capillary Tubes | No. 7D22-21 |

*Not available in European Union countries.

WARNINGS AND PRECAUTIONS
For *In Vitro* Diagnostic Use.
CAUTION:
Appropriate biosafety practices[11] should be used when handling specimens and reagents. These precautions include, but are not limited to the following:
- Wear gloves.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
- Clean and disinfect all spills of specimens or reagents using a suitable disinfectant, such as 0.5% sodium hypochlorite.[11]
- Decontaminate and dispose of all specimens, reagents, and other potentially contaminated materials in accordance with local regulations.[11]

STORAGE
The Abbott Determine HIV-1/2 Test Cards and Chase Buffer must be stored at 2-30°C until expiration date.

Kit components are stable until expiration date when handled and stored as directed. Do not use kit components beyond expiration date.

SPECIMEN COLLECTION
Serum, Plasma, and Whole Blood Collection by Venipuncture
Human serum, plasma, and whole blood collected by venipuncture should be collected aseptically in such a way as to avoid hemolysis.

NOTE: For whole blood and plasma specimens, EDTA collection tubes must be used.
Whole Blood Collection by Fingerstick™
Before collecting a fingerstick specimen, place an EDTA capillary tube on a clean dry surface.

1. Choose the fingertip of the middle, ring, or index finger (whichever is the least callused) for adults and children older than one year. Warm the hand as needed with a warm, moist towel or warm water to increase blood flow.

2. Clean fingertip with alcohol; allow to air dry. Position the hand palm-side up.

3. Use a new lancet for each person. Place the lancet off-center on the fingertip. Firmly press the lancet against the finger and puncture the skin. Dispose of the lancet in an appropriate biohazard sharps container.

4. Wipe away the first drop of blood with a sterile gauze pad.
5. Hold the finger lower than the elbow and apply gentle, intermittent pressure to the base of the punctured finger several times. Touch the tip of the EDTA Capillary Tube to the drop of blood". Avoid air bubbles.

*If EDTA Capillary Tubes (No. 7D22-21) will be used, fill the tube with blood between the 2 marked lines.

SPECIMEN STORAGE
- Serum and plasma specimens should be stored at 2-8°C if the test is to be run within 7 days of collection. If testing is delayed more than 7 days, the specimen should be frozen (-20°C or colder).
- Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 7 days of collection. Do not freeze whole blood specimens.
- Whole blood collected by fingerstick should be tested immediately.

TEST PROCEDURE
The desired number of test units from the 10-test card can be removed by bending and tearing at the perforation.
NOTE: Removal of the test units should start from the right side of the test card to preserve the lot number which appears on the left side of the test card.
1. Remove the protective foil cover from each test.
2. For serum or plasma samples:
   a. Apply 50µL of sample (precision pipette) to the sample pad (marked by the arrow symbol).
   b. Wait a minimum of 15 minutes (up to 60 minutes) and read result.
3. For whole blood (venipuncture) samples:
   a. Apply 50 µL of sample (precision pipette) to the sample pad (marked by the arrow symbol).
   b. Wait one minute, then apply one drop of Chase Buffer to the sample pad.
   c. Wait a minimum of 15 minutes (up to 60 minutes) and read result.
4. For whole blood (fingerstick) samples:
   a. Apply 50 µL of sample (by EDTA capillary tube) to the sample pad (marked by the arrow symbol).
   b. Wait until blood is absorbed into the sample pad, then apply one drop of Chase Buffer to the sample pad.
   c. Wait a minimum of 15 minutes (up to 60 minutes) and read result.

QUALITY CONTROL
To insure assay validity, a procedural control is incorporated in the device and is labeled "Control". If the control bar does not turn red by assay completion, the test result is invalid and the sample should be retested.

INTERPRETATION OF RESULTS
POSITIVE (Two Bars)
Red bars appear in both the control window (labeled "Control") and the patient window (labeled "Patient") of the strip. Any visible red color in the patient window should be interpreted as positive.

NEGATIVE (One Bar)
One red bar appears in the control window of the strip (labeled "Control"), and no red bar appears in the patient window of the strip (labeled "Patient").

INVALID (No Bar)
If there is no red bar in the control window of the strip, and even if a red bar appears in the patient window of the strip, the result is invalid and should be repeated. If the problem persists, contact your local Abbott Customer Service and Support Center.

NOTES:
- The test result is positive even if the patient bar appears lighter or darker than the control bar.
- If an invalid test result occurs repeatedly, or for technical assistance, contact your local Abbott Customer Service and Support Center.

LIMITATIONS OF THE PROCEDURE
- The Abbott Determine HIV - 1/2 test is designed to detect antibodies to HIV - 1 and HIV - 2 in human serum, plasma, and whole blood. Other body fluids or pooled specimens may not give accurate results.
- The intensity of the patient bar does not necessarily correlate to the titer of antibody in the specimen.
- A negative result with Determine HIV does not exclude the possibility of infection with HIV. A false negative result can occur in the following circumstances:
  - low levels of antibody (e.g., early seroconversion specimens) are below the detection limit of the test
  - infection with a variant of the virus that is less detectable by the Determine HIV assay configuration
  - HIV antibodies in the patient that do not react with specific antigens utilized in the assay configuration
  - specimen handling conditions which result in loss of HIV antibody multivalency

For these reasons care should be taken in interpreting negative results. Other clinical data (e.g., symptoms or risk factors) should be used in conjunction with the test results.
- Positive specimens should be retested using another method and the results should be evaluated in light of the overall clinical evaluation before a diagnosis is made.
- Whole blood or plasma specimens containing anticoagulants other than EDTA may give incorrect results.

PERFORMANCE CHARACTERISTICS
SPECIFICITY
A total of 1,594 serum and plasma specimens from Asia, West Africa, and North America were tested by Abbott Determine HIV-1/2 and a commercially available test (Table I).

Table I
Specificity of Abbott Determine HIV-1/2

| Population | Number of Specimens Tested | Negative by Abbott Determine HIV-1/2 | Negative by a Commercially Available Test |
|---|---|---|---|
| Seronegative Serum | 908 | 907/908 (99.89%) | 908/908 (100.00%) |
| Plasma | 403 | 403/403 (100.00%) | 403/403 (100.00%) |
| Pregnant Females | 58* | 57/57 (100.00%) | 57/57 (100.00%) |
| West Africans | 49 | 48/49 (97.96%) | 48/49 (97.96%) |
| Disease States Other than HIV and Potentially Interfering Substances | 176* | 173/175 (98.86%) | 174/175 (99.45%) |
| Total | 1,594 | 1,588/1,592 (99.75%) | 1,590/1,592 (99.87%) |

*One specimen from a pregnant female and an HCV positive patient were positive by both Abbott Determine and the commercially available test. Both specimens confirmed positive by HIV-1 Western Blot.

A total of 368 seronegative whole blood specimens from Thailand were tested with paired serum and plasma by Abbott Determine HIV-1/2. Thirty-nine of the whole blood specimens were collected by both venipuncture and fingerstick (Table II).

Table II
A Comparison of Abbott Determine HIV-1/2 Specificity in Seronegative Whole Blood and Paired Serum and Plasma Specimens

| Specimen Type | Number of Specimens Tested | Negative by Abbott Determine HIV-1/2 |
|---|---|---|
| Serum | 368 | 368/368 (100.00%) |
| Plasma | 368 | 368/368 (100.00%) |
| Whole Blood (venipuncture) | 368 | 368/368 (100.00%) |
| Whole Blood (fingerstick) | 39 | 39/39 (100.00%) |

SENSITIVITY
A total of 869 HIV-1 and HIV-2 antibody positive serum and plasma specimens from Asia, Africa, North and South America were tested by Abbott Determine HIV-1/2 and a commercially available test (Table III).

Table III
Sensitivity of Abbott Determine HIV-1/2

| Population | Number of Specimens Tested | Positive by Abbott Determine HIV-1/2 | Positive by a Commercially Available Test |
|---|---|---|---|
| HIV-1 Positive | 521 | 521/521 (100.00%) | 521/521 (100.00%) |
| HIV-2 Positive | 114 | 114/114 (100.00%) | 114/114 (100.00%) |
| HIV-1 Subtypes A-G | 222 | 222/222 (100.00%) | Not Tested Not Tested |
| HIV-1 Group O | 12 | 12/12 (100.00%) | Not Tested Not Tested |
| Total | 869 | 869/869 (100.00%) | 635/635 (100.00%) |

A total of 102 seropositive whole blood specimens from Thailand were tested with paired serum and plasma by Abbott Determine HIV-1/2. Thirty-two of the whole blood specimens were collected by both venipuncture and fingerstick (Table IV).

Table IV
A Comparison of Abbott Determine HIV-1/2 Sensitivity in Seropositive Whole Blood and Paired Serum and Plasma Specimens

| Specimen Type | Number of Specimens Tested | Positive by Abbott Determine HIV-1/2 |
|---|---|---|
| Serum | 102 | 102/102 (100.00%) |
| Plasma | 102 | 102/102 (100.00%) |
| Whole Blood (venipuncture) | 102 | 102/102 (100.00%) |
| Whole Blood (fingerstick) | 32 | 32/32 (100.00%) |

# ANNEX 7

## HBsAg rapid test example

# Determine™

## AgHBs

Lire attentivement cette notice avant l'utilisation du dosage. Les instructions d'utilisation doivent être suivies en conséquence. La fiabilité des résultats du dosage ne peut pas être garantie si ces instructions ne sont pas strictement respectées.

**DEFINITION ET DOMAINE D'APPLICATION**

Abbott Determine AgHBs est un dosage immunologique qualitatif *in vitro* à lecture visuelle pour la détection de l'antigène de surface de l'hépatite B (AgHBs) dans le sérum, le plasma ou le sang total humain. Ce test constitue une aide pour la détection de l'AgHBs chez les sujets infectés.

**RESUME ET EXPLICATION DU TEST**

Les dosages de l'AgHBs sont utilisés pour le dépistage de l'AgHBs dans le sang et les produits dérivés, afin d'éviter la transmission du virus de l'hépatite B (VHB) aux receveurs de ces produits. Les dosages de l'AgHBs sont également utilisés de façon routinière pour le diagnostic d'une infection par le VHB suspectée et le suivi de l'état des patients infectés, à savoir si l'infection du patient a été guérie ou si le patient est devenu un porteur chronique du virus.[1]

**PRINCIPES BIOLOGIQUES DE LA METHODE**

Determine AgHBs est un test immunochromatographique pour la détection qualitative de l'antigène de surface de l'hépatite B (AgHBs).

L'échantillon est déposé sur la zone de dépôt de l'échantillon. Comme l'échantillon migre jusqu'à la zone de dépôt du conjugué, il se reconstitue et se mélange avec le conjugué colloïde de sélénium-anticorps. Ce mélange continue à migrer sur la phase solide jusqu'aux anticorps immobilisés au niveau de la fenêtre-patient.

Si l'AgHBs est présent dans l'échantillon, l'antigène se lie à l'anticorps du conjugué anticorps-colloïde de sélénium et à l'anticorps de la fenêtre-patient en formant une ligne rouge.

Si l'AgHBs est absent, le conjugué anticorps-colloïde de sélénium traverse la fenêtre-patient sans former de ligne rouge.

Une barre de contrôle de la procédure est incluse dans ce système de dosage afin d'assurer la validité du test.

**COMPOSITION**

Dosage Abbott Determine AgHBs Sérum/Plasma (Réf. 7D25-12), 100 tests

- Test Determine AgHBs, 10 cartons (10 tests par carton) recouverts d'anticorps anti-HBs (souris, monoclonaux).

Dosage Abbott Determine AgHBs Sang total (Réf. 7D25-32), 100 tests

- Test Determine AgHBs, 10 cartons (10 tests par carton) recouverts d'anticorps anti-HBs (souris, monoclonaux).
- 1 flacon (2,5 ml) de tampon de fixation (Réf. 7D22-11) préparé dans du tampon phosphate. Conservateurs : Agents antimicrobiens.

**ACCESSOIRES (nécessaires mais non fournis)**

| Sérums/Plasma ou Sang total (ponction veineuse) | | Sang total (bout du doigt) | |
|---|---|---|---|
| Pipette | Réf. 7D22-51 | Lancettes* | Réf. 7D22-31 |
| Embouts pour pipette | Réf. 7D22-61 | Tubes capillaires avec de l'EDTA | Réf. 7D22-21 |

* Non disponibles dans les pays de l'Union Européenne

**PRECAUTIONS ET RESTRICTIONS D'EMPLOI**

**Pour diagnostic *in vitro*.**

**ATTENTION :**

**Les échantillons et réactifs doivent être manipulés conformément aux règles biologiques en vigueur.[2,3] Ces précautions comprennent, entre autres, les mesures suivantes :**

- **Porter des gants.**
- **Ne pas effectuer de pipetages à la bouche.**
- **Ne pas manger, boire, fumer, ni manipuler des produits cosmétiques ou des lentilles de contact dans les locaux où sont manipulés ces matériaux.**
- **Nettoyer et désinfecter toutes les éclaboussures d'échantillons et de réactifs à l'aide d'un désinfectant antimicrobien tel qu'une solution d'hypochlorite de sodium à 0,5%.[4,5]**
- **Décontaminer et éliminer tous les échantillons, réactifs et autres substances susceptibles d'avoir été contaminées conformément à la réglementation en vigueur.[4,7]**

**CONSERVATION**

Les tests Abbott Determine AgHBs et le tampon de fixation doivent être conservés entre 2 et 30°C jusqu'à la date de péremption.

Les composants du kit sont stables jusqu'à la date de péremption s'ils sont conservés et manipulés selon les indications du fabricant. Ne pas utiliser les composants du kit au-delà de la date de péremption.

**PRELEVEMENT DES ECHANTILLONS**

**Prélèvement de sérum, plasma et sang total par ponction veineuse**

Le sérum, le plasma et le sang total humains prélevés par ponction veineuse doivent être recueillis dans des conditions d'asepsie, de manière à éviter l'hémolyse.

**REMARQUE : Pour les échantillons de sang total, il faut utiliser des tubes de prélèvement avec de l'EDTA.**

**Prélèvement de sang total sur le bout du doigt***

Avant de prélever un échantillon sur le bout du doigt, placer un tube capillaire avec de l'EDTA sur une surface propre et sèche.

1. Pour les adultes et les enfants de plus d'un an, choisir le bout du majeur, de l'annulaire ou de l'index (choisir le moins calleux). Chauffer la main avec une serviette chaude et humide ou bien avec de l'eau chaude afin d'augmenter le flux sanguin.
2. Nettoyer le bout du doigt avec de l'alcool ; laisser sécher à l'air. Placer la main paume vers le haut.

3. Utiliser une lancette différente pour chaque personne. Placer la lancette sur un côté du bout du doigt. Appliquer une ferme pression sur la lancette placée sur le doigt et piquer la peau. Jeter la lancette dans un récipient pour déchets biologiques pointus.

4. Essuyer la première goutte de sang avec une gaze stérile.

5. Maintenir le doigt un peu plus bas que le coude et appliquer par intermittence de faibles pressions à la base du doigt piqué. Effleurer la goutte de sang avec l'extrémité du tube capillaire contenant de l'EDTA*. Eviter la formation de bulles d'air.

*Si l'on utilise les tubes capillaires contenant de l'EDTA (Réf. 7D22-21), remplir le tube de sang jusqu'à un niveau situé entre les 2 traits.

**CONSERVATION DES ECHANTILLONS**

- Si le test est effectué dans les 7 jours qui suivent le prélèvement, les échantillons de sérum et de plasma doivent être conservés entre 2 et 8°C. S'ils sont analysés plus de 7 jours après le prélèvement, ils doivent être congelés (à une température inférieure ou égale à -20°C).
- Si le test est effectué dans les 7 jours qui suivent le prélèvement, le sang total prélevé par ponction veineuse doit être conservé entre 2 et 8°C. Ne pas congeler les échantillons de sang total.
- Le sang total prélevé sur le bout du doigt doit être analysé immédiatement.

**PROCEDURE D'ANALYSE**

Le nombre souhaité de tests peut être détaché du carton de 10 tests en pliant et déchirant au niveau de la perforation.

**REMARQUE : Détacher les tests en commençant par la droite du carton de tests afin de préserver le numéro de lo apparaissant sur la gauche de ce carton.**

1. Enlever la protection plastique de chaque test.
2. Pour les échantillons de sérum ou de plasma :
   a. Distribuer 50 µl d'échantillon (à l'aide d'une pipette de précision) sur la zone de dépôt de l'échantillon (symbole : flè
   b. Attendre au moins 15 minutes (maximum : 24 heures) et lire le résultat.
3. Pour les échantillons de sang total (ponction veineuse) :
   a. Distribuer 50 µl d'échantillon (à l'aide d'une pipette de précision) sur la zone de dépôt de l'échantillon (symbole : flèche).
   b. Attendre une minute, puis distribuer une goutte de tampon de fixation sur la zone de dépôt de l'échantillon.
   c. Attendre au moins 15 minutes (maximum : 24 heures) et lire le résultat.
4. Pour les échantillons de sang total (bout du doigt) :
   a. Distribuer 50 µl d'échantillon (avec un tube capillaire contenant de l'EDTA) sur la zone de dépôt de l'échantillon (symbole : flèche).
   b. Attendre que le sang soit absorbé par la zone de dépôt, puis distribuer une goutte de tampon de fixation sur la zon dépôt de l'échantillon.
   c. Attendre au moins 15 minutes (maximum : 24 heures) et lire le résultat.

**CONTROLE DE QUALITE**

Un contrôle de la procédure annoté "Control" est inclus dans ce système afin d'assurer la validité du test. Si la barre de con ne vire pas au rouge à la fin du dosage, le résultat du test n'est pas valide et l'échantillon doit être réanalysé.

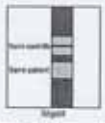
**INTERPRETATION DES RESULTATS**

**POSITIF (deux barres)**

Les barres rouges apparaissent dans la fenêtre-contrôle (annotée "Control") et la fenêtre-patient (annotée "Patient") sur la bandelette. Toute couleur rouge visible dans la fenêtre-patient doit être interprétée comme un résultat positif.

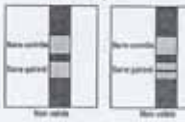
**NEGATIF (une barre)**

Une barre rouge apparaît dans la fenêtre-contrôle (annotée "Control"). la barre rouge de la fenêtre-patient (annotée "Patient") n'apparaissant pas sur la bandelette.



**NON VALIDE (pas de barre)**

Si la barre rouge n'apparaît pas dans la fenêtre-contrôle de la bandelette et même si une barre rouge apparaît dans la fenêtre-patient de la bandelette, le résultat n'est pas valide et le test doit être recommencé. Si le problème persiste, contacter votre Service Clients Abbott.



**REMARQUES :**

- Le résultat du test est positif même si la barre-patient est plus claire ou plus foncée que la barre-contrôle.
- Si un résultat non valide venait à se répéter ou en cas de questions, contacter votre Service Clients Abbott.

**LIMITES DE LA METHODE**

- Le test Abbott Determine AgHBs est destiné à détecter l'antigène de surface de l'hépatite B (AgHBs) dans le sérum, plasma et le sang total humains. D'autres fluides physiologiques ou pools d'échantillons peuvent donner des résultat imprécis.
- L'intensité de la barre-patient n'est pas nécessairement proportionnelle au titre d'antigène de l'échantillon.
- Aucun test ne peut garantir de façon absolue qu'un échantillon ne contient pas de faibles concentrations en AgHBs, con celles présentes à un stade très précoce de l'infection. C'est pourquoi un résultat négatif n'exclut pas la possibilité d'u exposition à l'AgHBs ou d'une infection par celui-ci.
- Les échantillons de sang total contenant des anticoagulants autres que l'EDTA peuvent donner des résultats incorrect
- Afin de pouvoir diagnostiquer et distinguer une infection aiguë par le VHB d'une infection chronique, la détection de l'AgHBs doit être associée aux symptômes que présente le patient et aux autres marqueurs sérologiques viraux de l'hép B.

**CARACTERISTIQUES SPECIFIQUES**

**SPECIFICITE**

Un total de 1908 échantillons de sérum, de plasma et de sang total provenant d'Asie, d'Afrique de l'Ouest et d'Amérique du N ont été analysés par les dosages Abbott Determine AgHBs et Abbott Dainascreen AgHBs (tableau I).

**Tableau I**
**Spécificité du test Abbott Determine AgHBs**

| Population | Nombre d'échantillons analysés | Négatifs par Abbott Determine AgHBs | | Négatifs par Abbott Dainascreen AgHBs | |
|---|---|---|---|---|---|
| Séronégatifs | | | | | |
| Sérum | 682 | 681/682 | (99,85%) | 681/682 | (99,85%) |
| Plasma* | 498 | 498/498 | (100,00%) | 498/498 | (100,00%) |
| Sang total | 458 | 458/458 | (100,00%) | 458/458 | (100,00%) |
| Femmes enceintes | 56** | 57/57 | (100,00%) | 57/57 | (100,00%) |
| Africains de l'Ouest | 50 | 50/50 | (100,00%) | 50/50 | (100,00%) |
| Maladies autres que l'infection par le VHB et substances potentiellement interférentes | 152** | 159/159 | (100,00%) | 159/159 | (100,00%) |
| Total | 1908 | 1903/1904 | (99,95%) | 1903/1904 | (99,95%) |

*Pour 92% (458/498) des échantillons pour lesquels le plasma et le sang total étaient disponibles, les résultats étaient concorda

**Un échantillon provenant d'une femme enceinte et 3 échantillons positifs pour le VIH-1 étaient positifs par les tests Determ et Dainascreen. Ces 4 échantillons ont été confirmés positifs par le dosage Abbott AxSYM AgHBs.

**SENSIBILITE**

Un total de 434 échantillons de sérum et de sang total séropositifs pour l'AgHBs, provenant d'Asie et d'Amérique du Nord ont analysés par les dosages Abbott Determine AgHBs et Abbott Dainascreen AgHBs (tableau II).

**Tableau II**
**Sensibilité du test Abbott Determine AgHBs**

| Population | Nombre d'échantillons analysés | Positifs par Abbott Determine AgHBs | | Positifs par Abbott Dainascreen AgHBs | |
|---|---|---|---|---|---|
| Positif pour l'AgHBs | | | | | |
| Sérum | 373 | 353/373 | (94,64%) | 336/373 | (90,08%) |
| Sang Total | 61 | 60/61 | (98,36%) | N/A | N/A |
| Total | 434 | 413/434 | (95,16%) | 336/373 | (90,08%) |

# ANNEX 8

## HCV rapid test example

ONE STEP Anti-HCV Test

**SD BIO LINE HCV**

### 1. Explanation of the test

Hepatitis C virus (HCV) now is recognized as a major agent of chronic hepatitis, transfusion-acquired non-A, non-B hepatitis and liver disease throughout the world. HCV is an enveloped positive-sense, single-stranded RNA virus. Clinical diagnostic issues related to HCV is the detection of HCV antibodies in human serum, plasma or whole blood by immunoassay. We have constructed HCV genes for the expression of recombinant antigens in bacterium systems such as *E. coli* and focused on structural and non-structural regions of HCV-encoded polyprotein, which are definitely immunogenic. The major immunoreactive antigens of these proteins have been reported as core, NS3, NS4 and NS5 regions of HCV genome, which are known to be highly immunodominant regions. For diagnosis of HCV infection, these recombinant proteins were used as capture materials of a immunochromatographic (rapid) test. Compared to the first generation HCV test using single recombinant antigens, multiple antigens using recombinant proteins have been added in new serologic tests to avoid non-specific cross-activity and to increase the sensitivity of the HCV antibody test.
The SD BIOLINE HCV test is a immunochromatographic (rapid) test for the qualitative detection of antibodies specific to HCV, in human serum, plasma or whole blood.
The SD BIOLINE HCV test contains a membrane strip, which is pre-coated with recombinant HCV capture antigen (core, NS3, NS4 and NS5) on test band region. The protein A - colloid gold conjugate and serum sample moves along the membrane chromatographically to the test region (T) and forms a visible line as the antigen-antibody-protein A gold particle complex forms with high degree of sensitivity and specificity. This test device has a letter of T and C as "Test Line" and "Control Line" on the surface of the case. Both the Test Line and Control Line in result window are not visible before applying any samples. The Control Line is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control line are working.

### 2. Materials provided

1) SD BIOLINE HCV test device
2) Assay Diluent
3) Instructions for use

### 3. Precautions

The SD BIOLINE HCV test devices should be stored at room temperature. The test device is sensitive to humidity and as well as to heat. Perform the test immediately after removing the test device from the foil pouch. Do not use it beyond the expiration.

### 4. Specimen collection and storage

1) [whole blood] Collect the whole blood using the suitable anti-coagulant.
2) [serum or plasma] Centrifuge whole blood to get plasma or serum specimen.
3) If specimens are not immediately tested they should be refrigerated at 2~8 degrees C. For storage periods greater than three days, freezing is recommended. They should be brought to room temperature prior to use.
4) Specimens containing precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.
5) The whole blood may be used for testing immediately or may be stored at 2~8 degrees C up to three days.

### 5. Warnings

1) For in vitro diagnostic use only.
2) Do not eat or smoke while handling specimens.
3) Wear protective gloves while handling specimens. Wash hands thoroughly afterwards.
4) Avoid splashing or aerosol formation.
5) Clean up spills thoroughly using an appropriate disinfectant.
6) Decontaminate and dispose of all specimens, reaction kits and potentially contaminated materials, as if they were infectious waste, in a biohazard container.
7) Do not use the test kit if the pouch is damaged or the seal is broken.

### 6. Procedure of the test

1) Remove the test device from the foil pouch, and place it on a flat, dry surface.
2) Add 10 μL of serum, plasma or whole blood to the sample well, and then add 3 drops of assay diluent (Figure 1).
3) As the test begins to work, you will see purple color move across the results window in the center of the test device.
4) Interpret test results at 5~20 minutes. Do not interpret test result after 20 minutes.

Figure 1

**Caution** : The above interpreting time is based on reading the test results at room temperature of 15 to 30 degrees C. If your room temperature is significantly lower than 10 degrees C, then the interpreting time should be properly increased.

### 7. Interpretation of the test

1) A color band will appear in the left section of the result window to show that the test is working properly. This band is the Control Band.
2) The right section of the result window indicates the test results. If another color band appears in the right section of the result window, this band is the Test Band.

**Negative results** : The presence of only one band within the result window indicates a negative result (Figure 2).

Figure 2

**Positive results** : The presence of two color bands ("T" band and "C" band) within the result window, no matter which band appears first, indicates a positive result (Figure 3).

Figure 3

**Invalid results** : If the purple color band is not visible within the result window after performing the test, the result is considered invalid (Figure 4). Some causes of invalid results are: not following the directions correctly or the test may have deteriorated beyond the expiration date. It is recommended that the specimen be re-tested using a new test kit.

Figure 4

**Note** : A positive result will not change once it has been established at 20 minutes.
However, in order to prevent any incorrect results, the test result should not be interpreted after 20 minutes.

### 8. Limitations of the test

A negative result does not preclude the possibility of infection with HCV. Other clinically available tests are required if questionable results are obtained. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

### 9. Performance Characteristics

1) Sensitivity and Specificity
The SD BIOLINE HCV have tested with positive and negative clinical samples tested by a leading commercial anti-HCV ELISA test. The result shows that the SD BIOLINE HCV is very accurate to other commercial ELISA kit.

| Reference | | SD BIOLINE HCV | | Total Results |
|---|---|---|---|---|
| Method | Result | Positive | Negative | |
| Commercial ELISA | Positive | 206 | 2 | 208 |
| | Negative | 3 | 497 | 500 |
| Total Results | | 209 | 499 | 708 |

In a comparison of the SD BIOLINE HCV versus a leading commercial anti-HCV ELISA test, results gave sensitivity of 99.0% (206/208), a specificity of 99.4% (497/500), and a total agreement of 99.3% (703/708).
2) Precision
(1) Within run precision was determined by using 10 replicates of four different specimens containing different concentrations of antibody. The negative and positive values were correctly identified 100% of the time.
(2) Between run precision was determined by using the four different specimens containing different concentrations of antibody in 3 different replicates with 3 different lots of test devices. Again negative and positive results were observed 100% of the time.

### 10. Bibliography of suggested reading

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SD STANDARD DIAGNOSTICS, INC.
575-34 Pajang-dong, Jangan-ku, Suwon-si, Kyonggi-do, Korea

# *ANNEX 9*

## *RPR test example*

# *BD Macro-Vue. RPR Card Tests* ver: 26/09/2005

### *18 mm Circle Qualitative and Quantitative*

***Brewer Diagnostic Kit for the Serologic Detection of Syphilis***

***INTENDED USE***

The ***Macro-Vue.*** RPR (Rapid Plasma Reagin) 18 mm Circle Card Test is a nontreponemal testing procedure for the serologic detection of syphilis.1,2

***SUMMARY AND EXPLANATION***

The ***Macro-Vue*** RPR Teardrop Card Test (using finger puncture blood) was the original Card Test and was developed for field use where testing could be performed without laboratory equipment.3,4 By incorporating machine rotation, ringed test surfaces, and certain other technical changes, the RPR Circle Card Test was developed for use in large scale testing in public health and clinical laboratories. The RPR 18 mm Circle Card Test is recommended when venous blood collection is employed and a large volume of serum is available, such as generally prevails in public health and clinical laboratories.5-12 When a specimen contains antibody, flocculation occurs with a coagglutination of the carbon particles of the RPR Card antigen, which appear as black clumps against the white background of the plastic-coated card. By contrast, nonreactive specimens appear to have an even light-gray color. In special situations when nontreponemal test results are needed rapidly and the specimen is collected as EDTA plasma, the RPR 18 mm Circle Card Test can be used if the test is performed within 24 h.13,14

***PRINCIPLES OF THE PROCEDURE***

RPR Card antigen suspension is a carbon particle cardiolipin antigen1 which detects “reagin", an antibody-like substance present in serum or plasma from syphilitic persons, and occasionally in serum or plasma of persons with other acute or chronic conditions. The reagin binds to the test antigen, which consists of cardiolipin-lecithin-coated cholesterol particles, causing macroscopic flocculation.

***REAGENT***

The ingredients* of the RPR Card antigen suspension are1: 0.003% cardiolipin, 0.020-0.022% lecithin, 0.09% cholesterol, 0.0125 M EDTA, 0.01 M Na2HPO4, 0.01 M KH2PO4, 0.1% thimerosal (preservative), 0.02% charcoal (specially prepared, BD), 10% choline chloride, w/v, and deionized/distilled water. *Adjusted and/or supplemented as required to meet performance criteria.

***Warnings and Precautions:***

For *in vitro* Diagnostic Use. Pathogenic microorganisms, including hepatitus viruses and Human Immunodeficiency Virus, may be present in clinical specimens. Standard Precautions.15-18 and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids.

***Antigen:*** Refrigeration is recommended for the RPR Card antigen suspension only. Storage in bright sunlight or temperatures above 30°C should be avoided; such conditions may cause a rough appearance of the antigen when used with nonreactive sera. If the ampule of antigen is frozen during shipment, it can be reconstituted once by warming to room temperature; avoid repeated freezing and thawing. Immediate use of a refrigerated antigen may result in decreased sensitivity of the test. Therefore, upon
removal from the refrigerator, allow the antigen to warm to room temperature (23 to 29°C) before use. Do not use antigen beyond the expiration date.

***Diagnostic Test Cards:*** Specially prepared, plastic-coated cards designed for use with the RPR Card antigen. In handling, take care not to fingermark the card test areas, as this may result in an oily deposit and improper test results. When spreading specimen within confines of test areas, avoid scratching the card with the ***Dispenstirs “*** device or stirrer” If the specimen does not spread to the outer perimeter of test area, use another test area of card.

***Dispenstirs. and Capillaries:*** In performing the Card Tests, a ***Dispenstirs*** device (18 mm Circle qualitative test only) or capillary may be used to transfer the specimen to the card surface. A new ***Dispenstirs*** device or capillary must be used for each test specimen. When transferring from the collecting tube, the specimen must not be drawn up into the rubber bulb attached to the capillary, as this will cause incorrect readings on subsequent tests.

***Needles:*** To maintain clear passage for accurate drop delivery, upon completion of the tests, remove the needle from the dispensing bottle and rinse the needle with deionized/distilled water. Do not wipe the needle since this will remove the silicone coating and may affect the accuracy of the drop of antigen being dispensed.

***Reading of Card Test Results:*** Read immediately following rotation in the “wet” state under a high intensity incandescent lamp or strong daylight.

***Rotation:*** The recommended speed for mechanical rotation is 100 ± 2 rpm. The rotator should circumscribe a circle approximately two centimeters in diameter in the horizontal plane. A moistened humidifying cover should be used to prevent drying of test specimens during rotation.

***Storage of Antigen:*** Refrigerate at 2 to 8°C. All other components of the kit should be stored in a dry place at room temperature in the original packaging. See “Warnings and Precautions” for additional information. Once placed in the *dispensing bottle* (provided in each kit) and refrigerated (2 to 8°C), the antigen reactivity remains satisfactory for approximately three months, or until the expiration date, if it occurs sooner. Label the dispensing bottle with the antigen lot number, expiration date, and date antigen was placed in the bottle.

***SPECIMEN COLLECTION AND PREPARATION***

No special preparation of the patient is required prior to specimen collection.

***To Test Unheated Serum:*** Collect blood by venipuncture into a clean, dry tube without anticoagulant and allow to clot. Centrifuge the specimen at a force sufficient to sediment cellular elements. Keep the serum in the original collecting tube or transfer the serum into a clean, dry test tube if testing is to be delayed. Serum, removed from the clot, may be refrigerated at 2 to 8°C, for up to 5 days or frozen at -20°C or below in a Pyrex (or equivalent) vial or capped test tube.1 Avoid repeated freeze.thawing of specimens.

***To Test Heated Serum:*** After collection and centrifugation, as for unheated serum, transfer to a clean dry tube and place in 56℃ water bath, or a heat block for 30 min.
***To Test Unheated Plasma:*** Collect blood by venipuncture into a tube containing anticoagulant such as EDTA, heparin, potassium oxalate, potassium sequestrene or sodium fluoride. EDTA and heparin have the advantage of not being critical with respect to concentration; as little as 1 mL of blood in a tube normally used to collect 7 mL of blood produces satisfactory results. With the other anticoagulants, it is advisable to collect no less than one half a tube of blood. Centrifuge as above. Keep plasma in the original collecting tube, and if stored, store the specimen at 2 to 8℃. Test specimen within 24 h of blood collection.

***PROCEDURES AND RESULTS***
***Materials Provided:*** Various RPR Card Test kits are available (see .Availability.) which contain sufficient card antigen suspension to perform the specified number of daily control card and card tests, and the required dispensing bottle, dispensing needle, cards and either capillaries, stirrers, or ***Dispenstirs*** devices.
***Materials Required But Not Provided:***
1. Controls with established patterns of graded reactivity should be included in each day's testing to confirm optimal reactivity of the antigen. See "Availability" for ***Macro-Vue*** RPR 18 mm Circle Card Test Control Cards.
2. A rotator, 100 ± 2 rpm, circumscribing a circle 2 cm in diameter, with automatic timer, friction drive, and a cover containing a moistened sponge or blotter.
3. Saline (0.9%) for use in quantitative testing. Prepare by adding 900 mg dry sodium chloride, ACS to 100 mL deionized/distilled water.
4. Serum Nonreactive to syphilis in 0.9% saline; required for diluting test specimens giving a Reactive result at the 1:16 dilution. Also required is the necessary equipment and labware used in preparation, storage and handling of serologic specimens.

***Preliminary Preparations:*** Review "Warnings and Precautions" and "Specimen Collection and Preparation" prior to performance of card tests. When tests are to be performed, the antigen suspension should be checked with controls of graded reactivity using the particular test procedure. Only those antigens which give the prescribed reactions should be used. Controls, RPR Card antigen suspension and test specimens should be at room temperature when used. Before use, vigorously shake the ampule for 10 to 15 s to resuspend the antigen and disperse any carbon particles lodged in the neck of the ampule. If any carbon should remain in the neck of the ampule after this shaking, no additional effort should be made to dislodge it as this will only tend to produce a coarse antigen. Check delivery of the needle by placing the needle firmly on a 1 mL pipet or syringe; fill the pipet or syringe with antigen suspension, and holding the pipet or syringe in a vertical position, count the number of drops delivered in 0.5 mL. The correct number of drops is given in the table opposite: Attach the needle to the tapered fitting on the dispensing bottle. Be sure the antigen is below the breakline; snap the ampule neck and withdraw all of the antigen into the dispensing bottle by collapsing the bottle and using it as a suction device. Label the dispensing bottle with the antigen lot number, expiration date, and date antigen was placed in the bottle. Shake the antigen dispensing bottle gently before each series of antigen droppings. *The needle and dispensing bottle should be discarded when the kit is used up. It is imperative techniques as described herein be followed in detail.*

| Test Method | Color of Needle Hub | Number of Drops in 0.5 mL |
|---|---|---|
| 18 mm Circle | Yellow, 20 G | 30 ± 1 drop |

***18 mm Qualitative Card Test Using Dispenstirs. Devices :***
1. Hold a ***Dispenstirs*** device between thumb and forefinger near the stirring or sealed end. Squeeze and do not release pressure until open end is below surface of specimen, holding the specimen tube vertically to minimize stirring up of cellular elements when using original blood tube. Release finger pressure to draw up the sample.
2. Holding in a vertical position directly over the card test area to which the specimen is to be delivered (not touching card surface), squeeze ***Dispenstirs*** device allowing one drop to fall onto card (approx. 0.05 mL; *each* ***Dispenstirs*** *device is designed to expel slightly in excess of 0.05 mL to compensate for small amount of specimen retained by stirring end*).
3. Invert ***Dispenstirs*** device and with sealed stirring end, spread the specimen filling entire surface of circle. (If desired, sample remaining may be discharged into specimen tube from which it was drawn.) Discard ***Dispenstirs*** device. Repeat procedure for number of specimens to be tested.
4. Gently shake antigen dispensing bottle before use. Holding in a vertical position, dispense several drops in dispensing bottle cap to make sure the needle passage is clear. Place one "free-falling" drop (20 G, yellow hub needle) onto each test area. *Do not restir; mixing of antigen and specimen is accomplished during rotation*. Pick up the pre-dropped antigen from bottle cap.
5. Rotate for 8 min (± 30 s) under humidifying cover, on mechanical rotator at 100 ± 2 rpm. Following rotation, to help differentiate Nonreactive from Minimally Reactive results, a brief rotating and tilting of the card by hand (3 or 4 to-and-fro motions) must be made. Immediately read macroscopically in the "wet" state under a high intensity incandescent lamp or strong daylight. Report as: Reactive : Showing characteristic clumping ranging from slight but definite (minimal-to-moderate) to marked and intense. Nonreactive : Showing no clumping. See the Reading Guide.
***Note:*** *There are only two possible final reports with the Card Test : "Reactive or Nonreactive" regardless of the degree of reactivity. Reactivity minimal-to-moderate (showing slight, but definite clumping) is always reported as Reactive. Slightly granular or "rough" reactions should be repeated using an alternative procedure. For donor screening, these tests may bereported as "indeterminant" pending further evaluation. See "Limitations of the Procedure".* All reactive syphilis tests should be repeated using an alternative procedure.

***18 mm Qualitative Card Test Using Capillaries:***
1. Using a new capillary, attach rubber bulb to capillary and remove 0.05 mL of specimen from blood collecting tube by allowing specimen to rise to measuring line on capillary, taking care not to transfer cellular elements. (If desired, a serologic pipette may be used, but do not pipette by mouth.)
2. Place measured specimen onto circle of diagnostic test card, by compressing rubber bulb, while holding one finger over the hole in the bulb.
3. Using a new stirrer (broad end) for each specimen, spread to fill entire circle. Discard stirrer. Repeat procedure for number of specimens to be tested.
4. Gently shake antigen dispensing bottle before use. Holding in vertical position, dispense several drops in dispensing bottle cap to make sure the needle passage is clear. Place one "free-falling" drop (20 G, yellow hub needle) onto each test area. *Do not restir; mixing of antigen and specimen is accomplished during rotation*. Pick up the pre-dropped antigen from bottle cap.
5. Rotate for 8 min (± 30 s) under humidifying cover, on mechanical rotator at 100 ± 2 rpm. Following rotation, to help differentiate Nonreactive from Minimally Reactive results, a brief rotation and tilting of the card by hand (3 or 4 to-and-fro motions) must be made. Immediately read macroscopically in the "wet" state under a high intensity

incandescent lamp or strong daylight. Report as: Reactive : Showing characteristic clumping ranging from slight but definite (minimum-to-moderate) to marked and intense. Nonreactive : Showing no clumping. See the Reading Guide.
*Note: There are only two possible final reports with the Card Test : Reactive or Nonreactive, regardless of the degree of reactivity.Reactive minimal-to-moderate (showing slight, but definite clumping) is always reported as Reactive. Slightly granular or "rough" reactions should be repeated using an alternative procedure. For donor screening, these tests may be reported as "indeterminant" pending further evaluation. See Limitations of the Procedure.* All reactive syphilis tests should be repeated using an alternative procedure.

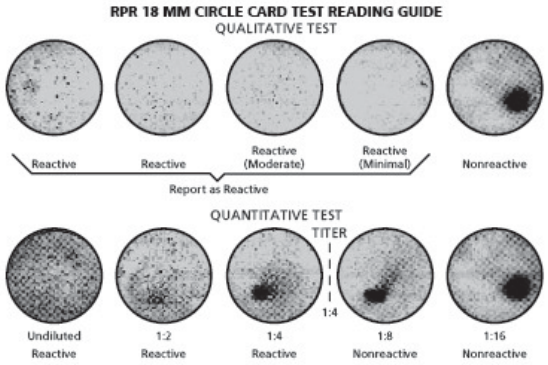
***18 mm Circle Quantitative Card Test:***
1. For each specimen to be tested, place 0.05 mL of 0.9% saline onto circles, numbered 2 to 5. A capillary (red line), or serological pipette, 1 mL or less, may be used. DO NOT SPREAD SALINE!
2. Using a capillary (red line graduated at 0.05 mL, to the tip) with rubber bulb attached, place 0.05 mL of specimen onto circle 1.
3. Refill capillary to red line with test specimen, and holding in a vertical position, prepare serial two-fold dilutions by drawing saline and test specimen mixture up-and-down capillary 5 to 6 times. Avoid formation of bubbles. Transfer 0.05 mL from circle 2, to 3, to 4, to 5, mixing after each transfer. Discard 0.05 mL after mixing contents in circle 5.
4. Using a new stirrer (broad end) for each specimen, start at highest dilution of serum (circle 5) and spread serum, filling the entire surface of circle. Proceed to circles 4, 3, 2 and 1 and accomplish similar spreading.
5. Gently shake antigen dispensing bottle before use. Holding in vertical position, dispense several drops in dispensing bottle cap to make sure needle passage is clear. Place one "free-falling" drop (20 G, yellow hub needle) onto each test area. *Do not restir; mixing of antigen and specimen is accomplished during rotation*. Pick up the pre-dropped antigen from bottle cap.
6. Rotate for 8 min (± 30 s) under humidifying cover, on mechanical rotator at 100 ± 2 rpm. Following rotation, to help differentiate Nonreactive from Reactive minimal-to- moderate (RM) results, a brief rotating and tilting of the card by hand (3 or 4 to-and-fro motions) must be made. Immediately read macroscopically in the wet state under a high intensity incandescent lamp or strong daylight. Report in terms of the highest dilution giving a Reactive including minimal-to-moderate reaction.

*Examples:*
(Prozone reaction : see "Limitations of the Procedure")

R = Reactive
N = Nonreactive
RM = Reactive minimal-to-moderate

| (Und.) 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | Report |
|---|---|---|---|---|---|
| RM | N | N | N | N | Reactive, 1:1 dilution |
| R | R | R | N | N | Reactive, 1:4 dilution |
| R | R | R | R | N | Reactive, 1:8 dilution |

***Unheated or Heated Serum:*** If the highest tested (1:16) is Reactive, proceed as follows.
1. Prepare a 1:50 dilution of Nonreactive serum in 0.9% saline. (This is to be used for making 1:32 and higher dilutions of specimens to be quantitated)
2. Prepare a 1:16 dilution of the test specimen by adding 0.1 mL of serum to 1.5 mL of 0.9% saline. Mix thoroughly.
3. Place 0.05 mL of 1:50 Nonreactive serum in circles 2, 3, 4, and 5.
4. Using capillary, place 0.05 mL of 1:16 dilution of test specimen in circle 1.
5. Refill capillary to red line, make serial two-fold dilutions and complete tests as described under steps 3 to 6. (See "18 mm Circle Quantitative Card Test") Higher dilutions are prepared if necessary in 1:50 Nonreactive serum.
***Plasma:*** If a baseline is to be established from which changes in titer can be determined, the test should be repeated on unheated serum (see section "Unheated Serum").
***Reading and Reporting the Macro-Vue. RPR Card Tests:*** Individual reactions should be evaluated in the "wet" state, under a high intensity incandescent lamp or strong daylight. Immediately following rotation read and record as Reactive or Nonreactive.




***Quality Control***
Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent NCCLS guidance and CLIA regulations for appropriate Quality Control practices.

***LIMITATIONS OF THE PROCEDURE***
The diagnosis of syphilis should not be made on a single reactive result without the support of a positive history or clinical evidence. Therefore, as with any serological testing procedure, Reactive card test specimens should be subjected to further serologic study. Serum specimens which are Reactive in qualitative testing should be quantitated to establish a baseline from which changes in titer can be determined, particularly for evaluating treatment.1 The use of plasma specimens to establish a baseline from which changes in titer can be determined has not been evaluated. False-negative results can occur because of failure to recognize prozone reactions. Prozone reactions occur in 1% to 2% of patients with secondary syphilis. These specimens may exhibit a nonreactive pattern that is slightly granular or .rough. Upon dilution, the reactivity will increase and then decrease as the endpoint titer is approached. All tests with a rough appearance should be further evaluated. False-

negative nontreponemal test results are also seen in incubating primary and late syphilis.1 It is not necessary to perfom the quantitative procedure on reactive donor samples. The RPR Card Tests cannot be used for testing spinal fluids. The ideal specimen for neonatal testing is the infant's serum as obtained by heel stick procedure. However, cord blood may be used for baseline screening when no other specimen is available.1 With cardiolipin type antigens, biological false positive reactions have been reported in diseases such as infectious mononucleosis, leprosy, malaria, lupus erythematosus, vaccines and virus pneumonia. In leprosy, Portnoy3 reported no false positives; Achimastos19 reported 14 of 50 leprosy cases were Reactive and Scotti20 reported 1 out of 208 cases was reactive with RPR Card which were nonreactive with the FTA-ABS and TPI tests. Dorwart 21 studied the incidence of chronic BFP reactions in various connective tissue disorders. Six out of 41 cases of systemic lupus erythematosus were reactive in the Card Test, whereas only 5 were reactive in the VDRL slide test. Only 1 out of 23 cases of rheumatoid arthritis was reactive with both RPR Card and VDRL slide tests. In pregnancy, several reports indicated the occurrence of false positive reactions.11, 22 Narcotic addiction and autoimmune diseases also may give false positive reactions.23 Pinta, yaws, bejel and other treponemal diseases produce positive reactions in this test.1 Lipemia will not interfere with the card tests, however, if the degree of lipemia is so severe as to obscure the state of the antigen particles, the specimen should be considered unsatisfactory for testing. Do not test specimens that are grossly hemolyzed, contaminated or extremely turbid; report as "Specimen unsatisfactory for testing".1

### *EXPECTED VALUES AND PERFORMANCE CHARACTERISTICS*

RPR Card antigen suspension is tested for the established pattern of reactivity against reference antigen suspensions and meets the U.S. Centers for Disease Control and Prevention (CDC) product specifications for performing the RPR 18 mm Circle Card Tests. These performance characteristics were established from a large number of papers which have appeared in the scientific literature, from routine daily test performances in syphilis serology testing laboratories and are in conformity with CDC specifications.

Reported studies show the RPR Card Tests have adequate sensitivity and specificity in relation to clinical diagnosis and a reactivity level similar to that of the VDRL slide test.6,10, 24, 25 Heating of serum specimens at 56°C for 30 min has been shown to have no effect on reactivity.20 A qualitative comparison of 1104 simultaneously collected serum and EDTA plasma specimens was conducted using the ***Macro-Vue*** RPR 18 mm Circle Card Test. There was complete agreement in test results which included 134 reactive and 970 nonreactive pairs. In other studies comparable results were found between plasma and serum pairs (306 specimens) with RPR Card Tests both in qualitative and quantitative procedures.14, 26

### *AVAILABILITY*

***Cat. No. Description***

***Macro-Vue.*** RPR Card Tests:

| | |
|---|---|
| 274449 | Kit No. 104: (300 qualitative tests), contains: two 3 mL amps. antigen, 20 G needle, dispensing bottle, 350 stirrers, 30 cards with ten 18 mm Circle spots ea. and 300-0.05 mL capillaries. |
| 275005 | Kit No. 110: (500 qualitative tests), contains: three 3 mL amps. antigen, 20 G needle, dispensing bottle, 50 cards with ten 18 mm Circle spots ea. and 500-0.05 mL ***Dispenstirs.*** devices. |
| 275239 | Kit No. 112: (150 quantitative tests), contains: five 3 mL amps. antigen, 20 G needle, dispensing bottle, 200 stirrers, 50 cards with fifteen 18 mm Circle spots ea. and 150-0.05 mL capillaries. |
| 275539 | Kit No. 115: (150 qualitative tests), contains: one 3 mL amp. antigen, 20 G needle, dispensing bottle, 15 cards with ten 18 mm Circle spots ea. and 150-0.05 mL ***Dispenstirs.*** devices. |
| 275110 | Bulk Kit No. 510: (5,000 qualitative tests). |
| 275692 | Bulk Kit No. 532: (10,000 qualitative tests). |
| 276709 | ***Macro-Vue.*** RPR Card Test Control Cards containing graded reactivity specimens, (R, RM and N 18 mm circles). Box of 10. |
| 272905 | ***Dispenstirs.*** (single use, plastic pipettes), 0.05 mL, Box of 500. |

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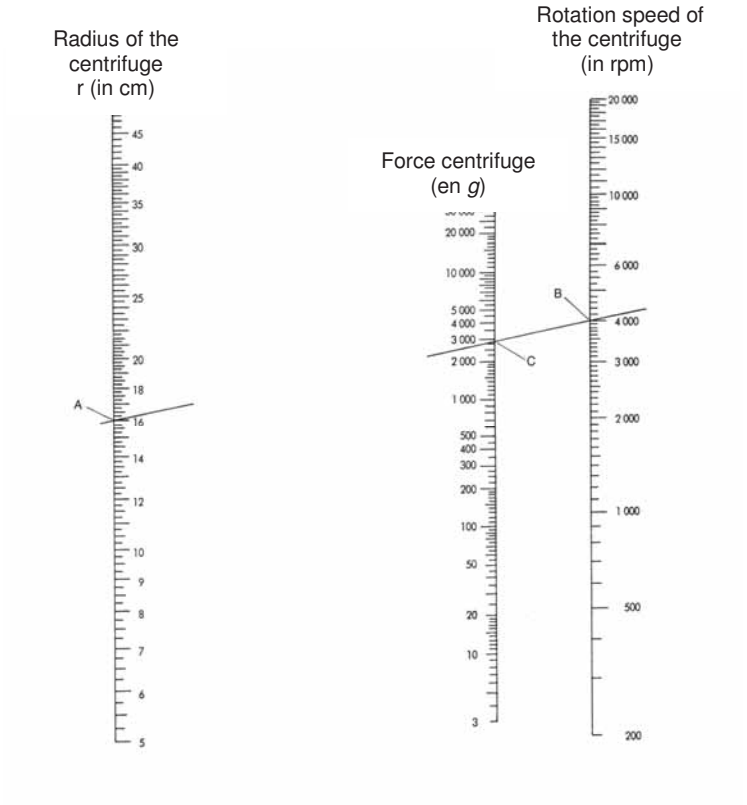
# *ANNEX 10*

## *Relation rotation speed of a centrifuge and the centrifugal power:*




**Relation between centrifugal (*g*) and the number of rotations per minute (rpm) in function of the radius of the rotor of the centrifuge (r**). Combining the two values r and *g* with a line, an intersection is obtained with the line of the rotation speed of the centrifuge, the value in rpm to use for this centrifuge and this rotor. The relation formulae between g and rpm is :
$g = 0.00001118 \times r \times rpm^2$. (N.B. : the radius of the centrifuge is expressed in cm)

# *ANNEX 11*

## Useful internet sites

World Health Organization in French, English and Spanish (documents can be downloaded from blood transfusion):

http://www.who.int/topics/blood_transfusion/fr/

International Society of blood transfusion in French and English

http://www.isbt-web.org/

Public Health Agency (Canada) in French and English :

http://www.phac-aspc.gc.ca/hcai-iamss/tti-it/risks_f.html#tab2

Site of the Canadian Society of blood in French and English :

http://www.medecinetransfusionnelle.ca/

Site de l'hémovigilance en français :

http://www.hemovigilance.org/

Site français de l'Institut National de transfusion sanguine (INTS) en français :

www.ints.fr

Site du service du sang de la croix rouge de Belgique en français :

http://www.transfusion.be/

# GLOSSARY

**Agglutinins** : antibodies which provoke agglutination of the red blood cells. It always concerns IgM.

**Allo antibodies** : (= iso antibodies) antibodies form against « foreign » antigens.

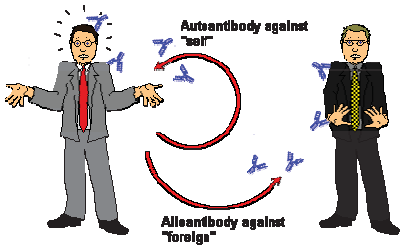
**Antibody** : specialized proteins (immunoglobulins) which bind specifically and react with the antigen. . As a rule, antibodies to an antigen are only formed if the corresponding antigen is missing in the antibody forming organism. One Immunoglobulin will react only with a specific antigen (specificity). There are 5 classes : IgM, IgG, IgA, IgE, IgD.

**Antibodies (warm-)**: (IgG) antibodies with a maximal activity at 37 °C.

**Antibodies (cold-):** (IgM) antibodies with a maximal activity at 4°C.

**Antigen** : A substance which is recognized as foreign by a living organism and as a result induces a specific immune response. Every molecule recognized by the immune system is described as an antigen. This predominantly concerns proteins which occur either in the pure form or combined with other substances.

**Auto antibodies** : Antibody form against « own » antigens.




**Blood donor (familial -)**: family member who is voluntary giving his blood.

**Blood donor (voluntary-)**: altruistic blood donor, who is not compensated for it in any way.

**Blood donor (regular, volontary-)**: regular altruistic blood donor, who is not compensated for it in any way.

**Blood donor (Remunerated-)**: a person who is selling his blood.

**Blood donor (Replacement-)**: familial blood donor in the context of a blood bank. If a blood bank doesn't have enough voluntary blood donors, they can ask the family of a transfused patient to replace one or more transfused blood units. In this case, it is not the "familial" blood that is given to the patient, but a bag of stored blood. This type op donor permits to "reconstitute" the blood stock in the context of a non self sufficient blood bank with voluntary, non remunerated donations.

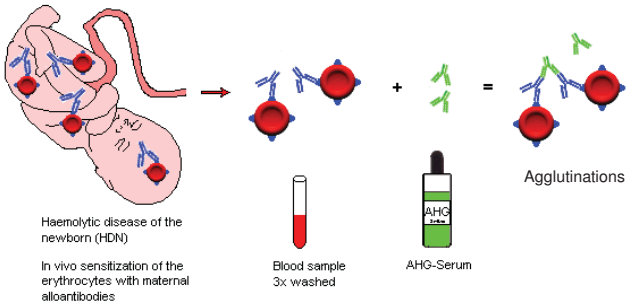
**Centrifuge for blood bank :** Rotor, tubes and speed adapted for wash of red blood cells and for centrifugation for reading of agglutinations. Examples : model Immufuge-II de Baxter©, model Diacent-12 de Diamed©. There also exist automatic red blood cell washers of the type Diacent-CW de Diamed©. These washers permit to make the work easier and to reduce duration compatibility test, at the price of a moderate fragile instrument..

**Cold agglutinins :** antibodies found in some persons. These antibodies agglutinate the own red blood cells of the subject, but only at low temperature (maximal agglutination at 4° C, weaker agglutination at 22°C). These antibodies are not active at 37°C. It always concerns about IgM. They are meanly directed against I and i antigens.

**Complete antibodies** : (also called agglutinins) Some antibodies, of the **IgM** class, can agglutinate erythrocytes directly in a saline medium They are cold antibodies with a maximal activity at 4 °C.


**Coombs serum** : anti-human globulin antibodies. Obtained by immunisation of animals (rabbit or goat). The Coombs serum can be **polyvalent** (directed against all human immunoglobulins : IgM, IgG, IgA,…) or **mono specific** (directed against just one specific human antibody).

**Direct Coombs Test (or : direct anti-globulin test)** : Test using the Coombs serum, permitting to demonstrate non agglutinating antibodies (IgG) *in vivo* fixed on the red blood cells. This test permits for instance determination of maternal allo antibodies fixed on the red blood cells of the newborn or of the fœtus (p.i. in case rhesus incompatibility).




**Globulins**: plasmatic proteins containing the antibodies or the immunoglobulins.

**Haemolysin** : substance that is destroying the red blood cells. Generally it concerns antibodies, but this expression is also used for other substances (p.i. the continence of the venin of certain serpents).

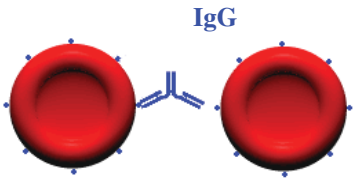
**HLA** : Human leukocyte antigens. The HLA system is a human major histocompatibility system. A part located on the human genome from which the genes code especially for the major histocompatibility antigens which intervene in the control of the immune response and in the phenomena of transplantation rejection.

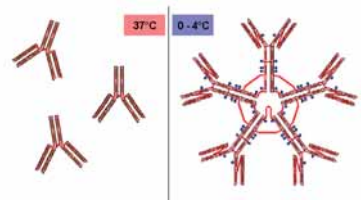
**Humoral immunity**: Mechanism of defence of an organism, involving antibodies.

**Indirect Coombs Test (or : indirect anti-globulin test) :** Test using the Coombs serum, permitting to demonstrate the presence of non agglutinating antibodies in serum (IgG). It is used p.i. for the major compatibility test.

**Incomplete antibodies** (**IgG)** : their fixation on the membrane of the red blood cells is not sufficient to provoke agglutination. These are warm antibodies, with a maximal activity at 37°C. Their detection is based upon the use of artificial techniques which permit to bring red blood cells together.
This is provided for example by:

- bovine albumin
- photolytic enzymes
- Coombs serum.




**Irregular antibodies**: appearing after immunisation, in certain subjects, when the corresponding antigen does not exist on the surface of their red blood cells.

**Iso antibodies**: antibodies developed by an organism, as an answer to an antigen from another individual of the same species. (synonym for Allo antibody)

**LISS albumin medium**: solution with Low Ionic Strength, enhancing the fixation of antibodies on the erythrocytes, associated with macromolecules (albumins) which increase the dielectric constant of the medium (decreasing so the rejection of the red blood cells). This medium increases thus the possibilities of the reactions between red blood cells and antibodies. It is associated with the Coombs test and permits the demonstration of irregular, incomplete antibodies of type IgG.

**Living blood bank**: registered group of people living nearby the hospital, with a known blood group, which accept to donate freely their blood in case of need.

**Monoclonal antibodies** : Very specific antibody products from one single cell or identical precursor of this cell, directed against a specific epitope of an antigen. (As the opposite of polyclonal antibodies)

**Natural antibodies** : Antibodies found in the serum without apparent pre immunisation from the corresponding antigen. They appear apparently spontaneously. If this is not the case, they are called immune antibodies.

**Packed cells**: blood bag, containing mainly red blood cells, obtained after elimination of the plasma (and the white blood cells).

**Regular antibodies** : antibodies present in all subjects in absence of the corresponding antigen. (As the opposite of irregular antibodies). Irregular antibodies occur also naturally, but not in all subjects.

**Saline medium** : physiological medium that contains 9 g natrium chloride (NaCl) per litre of distilled water. This is an isotonic solution that permits to preserve the cell volume.

**Transfusion of full blood**: transfusion of a blood bag, containing red blood cells, white blood cells and plasma. As an opposite of fractioned blood, permitting to transfuse packed cells (erythrocytes), plasma, etc.

**Transfusion (Warm)**: transfusion of a blood bag immediately after the taking, without storing in a refrigerator. This system is meanly applied for familial blood donors.

**Transfusion (Cold)**: Transfusion of a blood bag that has been stored in the fridge. This system is applied with voluntary blood donors and blood bank.