DIY instructions for use

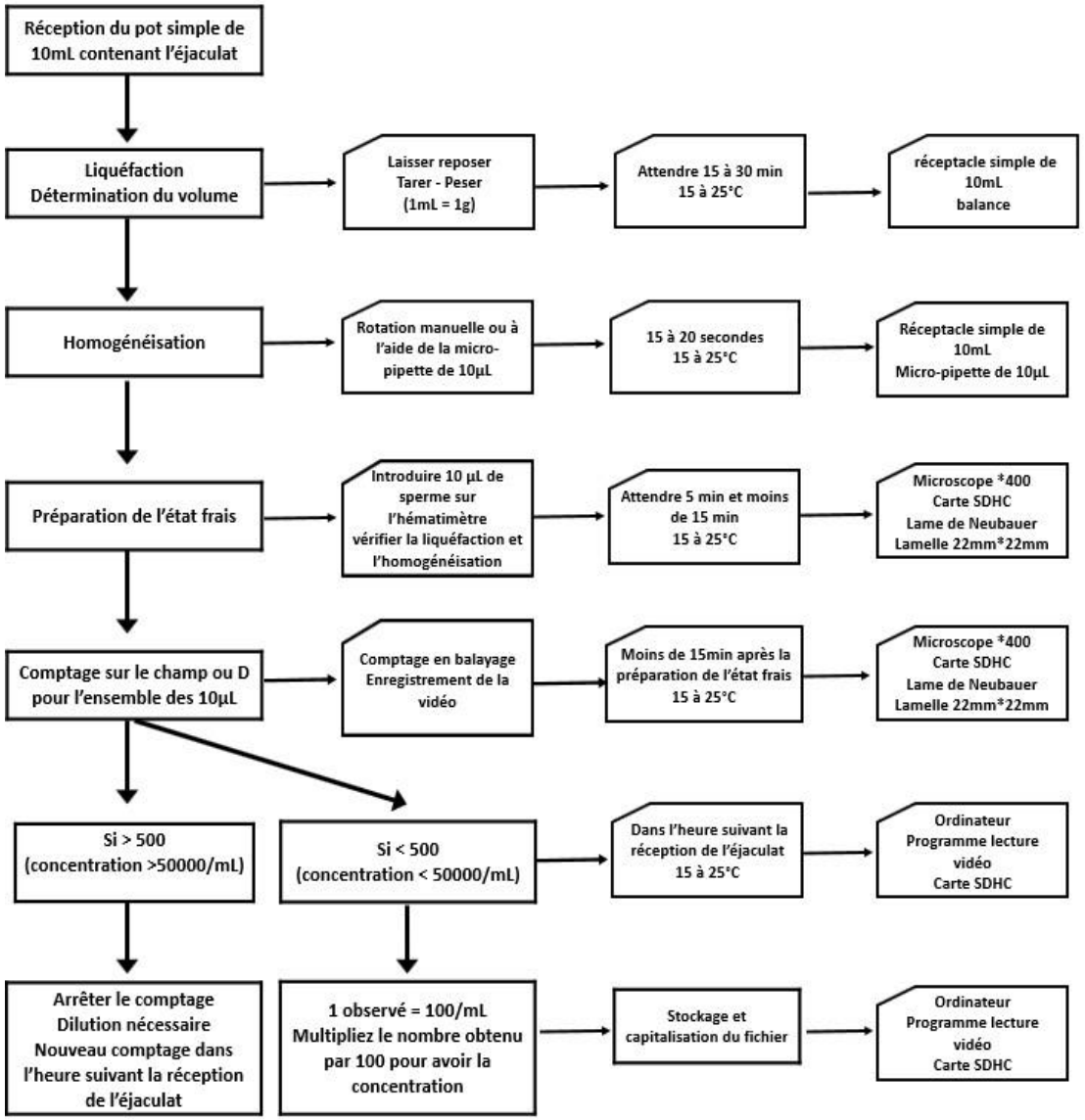
SPERMOGRAM & MALE THERMAL CONTRACEPTION

Presentation	This is a medical examination to analyse various characteristics of sperm. It consists of observing a drop of semen with a microscope to count the sperm and note their characteristics. **This non-invasive, painless** test is performed regularly to check that your sperm concentration is well below the **contraceptive threshold**: sperm concentration **< 1 million/ml.**
Indications in the context of CMT	• The first test assesses the quality of your sperm. If your sperm quality does not meet the standards set by the WHO[1] , your doctor will refer you to other contraceptive methods. • The next 2 tests are done 2-3 months and 3 weeks apart after starting to wear the device. If your concentration is < 1 million/ml, you are contracepted. If not, repeat the test the following month. • For the first 6 months, the review is monthly, then quarterly. • **Caution: If you forget to take the pill or if it is irregular, continue the protocol and take another contraceptive for one month and then do the test.** • When you stop using this contraception, use another method of contraception and have a check-up after 3 months to confirm that your fertility is returning to normal according to WHO standards **with your doctor.**
How do you prepare?	**Abstinence period of 3 days in general.** Drink a litre of water the day before and a large glass of water the day of.

This guide is for information purposes only.

[1] WORLD HEALTH ORGANISATION. WHO laboratory manual for the examination and processing of human semen, Fifth Edition, Geneva, WHO, 2010

Aide mémoire : examen microscopique de l'éjaculat pour une concentration inférieure à 50000/mL



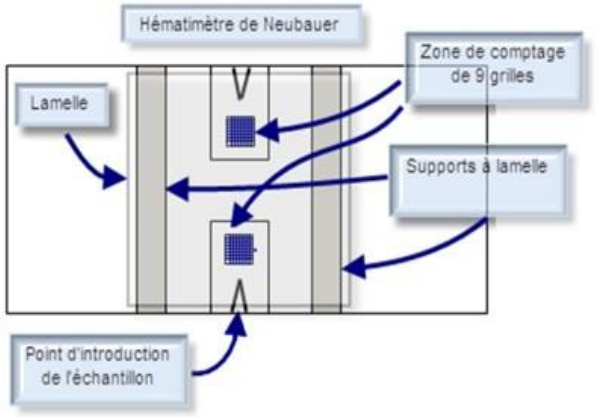


Figure 5: Hématimètre de Neubauer

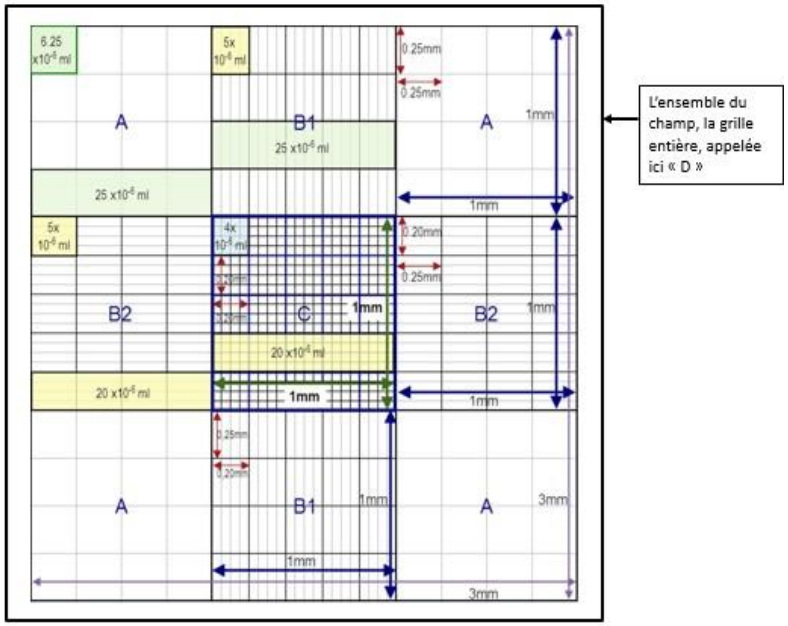
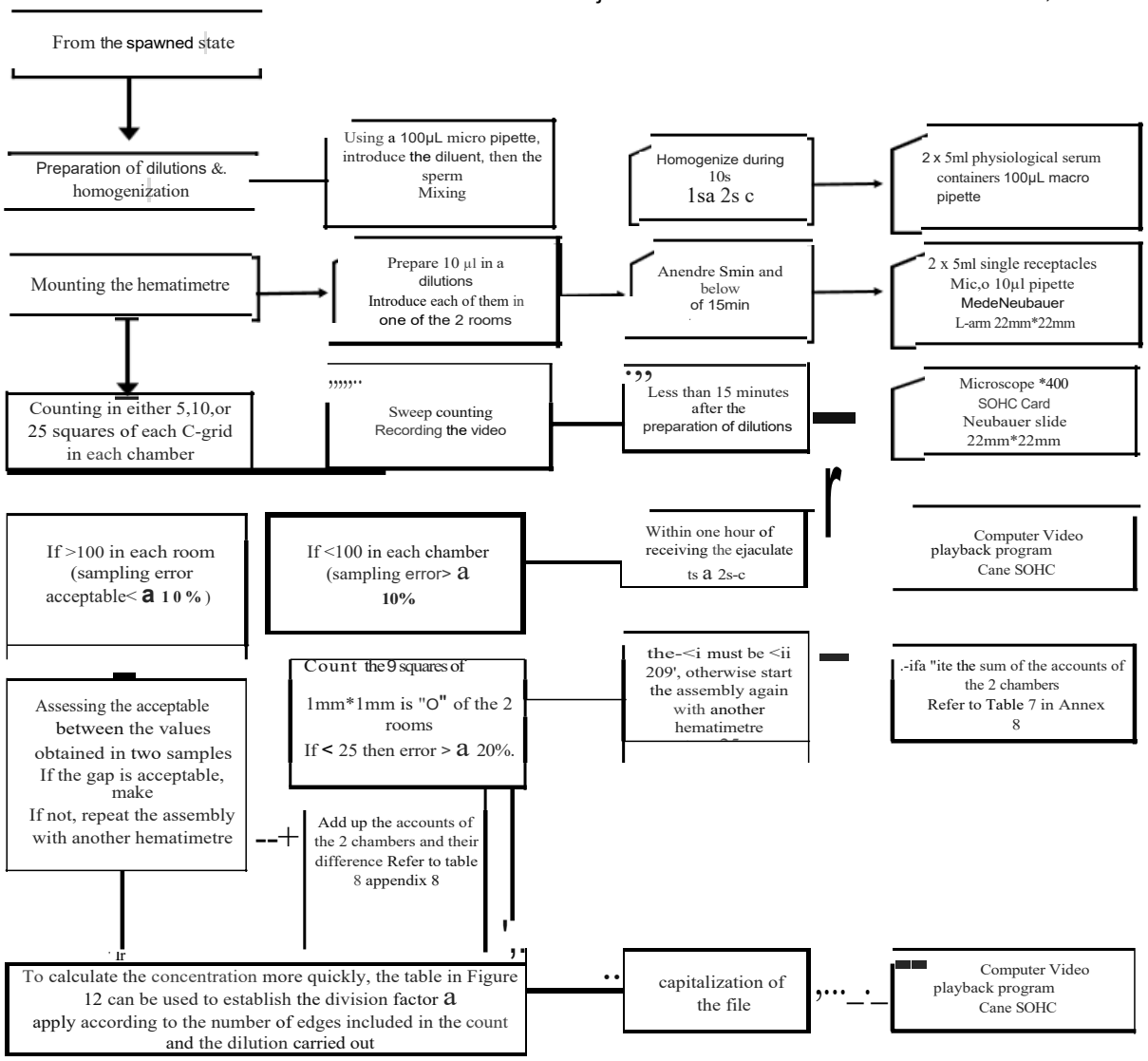


Figure 10 & 11: Zone de comptage de l'hématimètre de Neubauer

Aide memoire: microscopic examination of ejaculate for a concentration above 50,000/ml



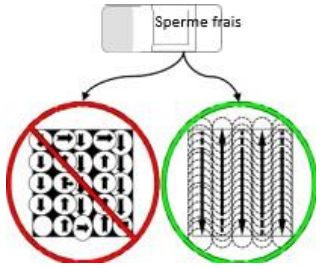


Figure 3: Field of view (scan) to examine the entire surface of the slide covering a known volume of semen.

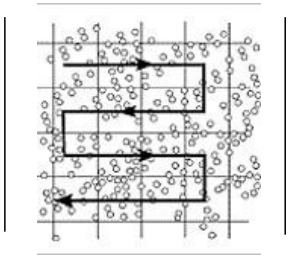


Figure 14 Counting with *elevee* cell concentration.

No. of sperm per field, magnification factor 400	Dilution	Semen (µl)	Oiluant (µl)
Rare	1:2	100	100
Less than 15	1:5	100	400
De15a40	1: 10	50	450
De41a 200	1: 20	50	950
More than 200	1: 50	50	2450

Figure 8: Appropriate dilutions a1-evaluation of the concentration on the Neubauer hematimetre

Dilution	Nb,.of cartCs in the grid C (from ehaqlle room) comoris in the comotae:c			the whole field, the whole grid called • D>
	5	'10	25	
	Di, ision factor			
I : 2	20	40	100	900
1 : 5	8	16	40	360
I: 10	4	8	20	180
I: 20	2	4	10	90
1: 50	08	16	4	36

Figure 12: Division factors to be applied to the total number of sperm counted in the two chambers to calculate fa concentration (in x 10-6/ml)

Table of contents

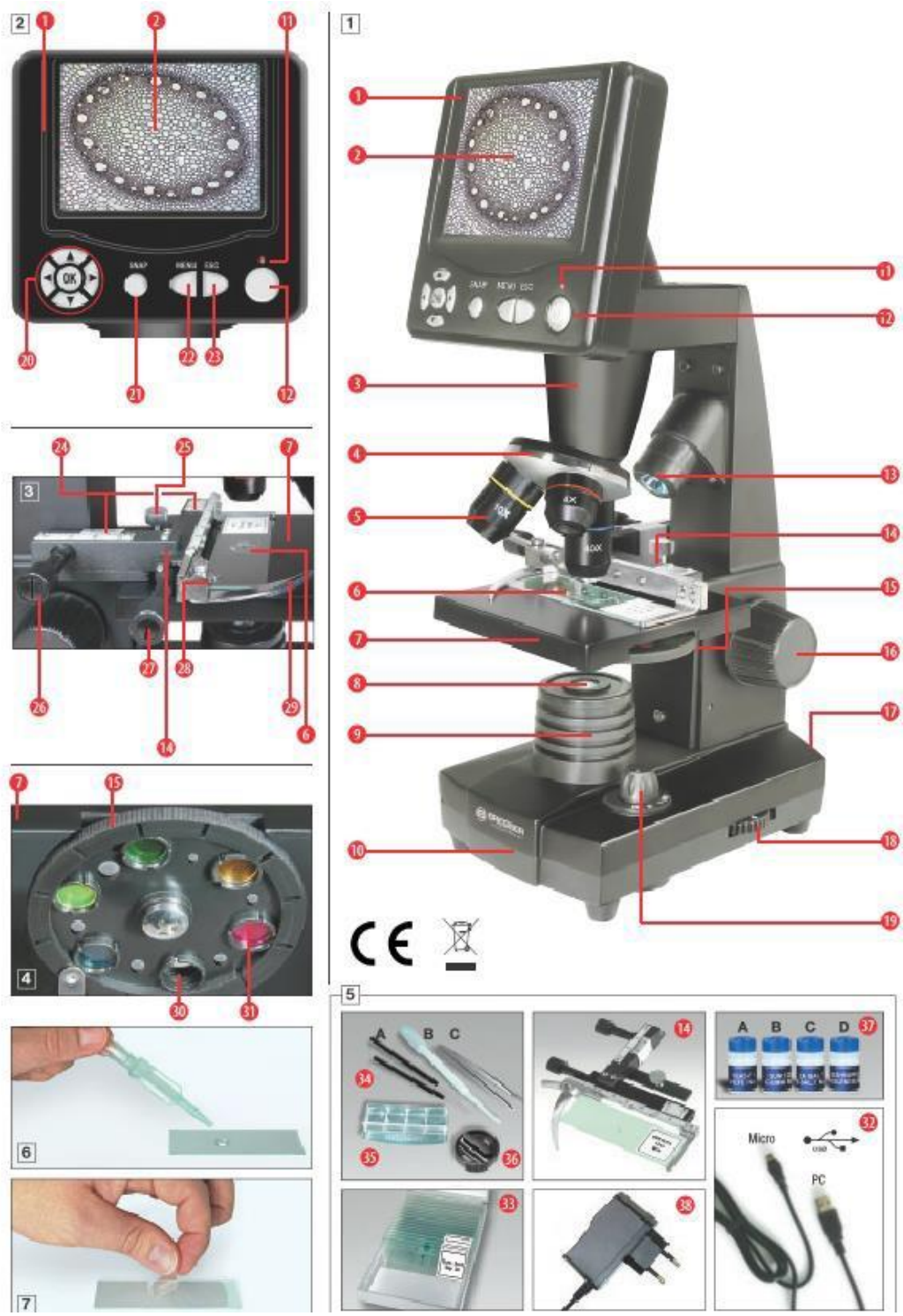
Material

Type	Quantity	Use	Cost total	Hygiene
Antiseptic wipe	1 box of 100	Single Use Material	4 €	Not sterile. Store in a clean, sheltered place of light
Water bottle sterile physiological	1 box of 30 single-dose		8 €	
Sterile pads	1 box of 20 bags		4€	
Examination gloves no powdered	1 box of 100		4€	
Lens papers	1 box of 30		8€	
Sterile tips disposable	1 box	Reusable material	10€	
70° alcohol bottle modified	1 bottle of 200 ml		3€	
Hydroalcoholic solution	1 bottle of 75mL		3€	
Single receptacle for the collection of everything the ejaculate	1 plastic box of 10 mL with cover		5€	Sterilise before use and between each new sampling
Digital scale with gram accuracy	1 new		20€	
Simple receptacles to make the 2 diluted samples and the saline jar thinner	3 x 5 mL plastic boxes		5€	
Laboratory micro-pipette that can hold 10 µl	1 second-hand		35€	
Laboratory micro-pipette that can contain 100 µl	1 second-hand		35€	
Blades Depth of field 0.1 mm Plus surface small squares 0.0025 mm2	2 Neubauer counting cells with 100 coverslips		22€	
SDHC card	1 of 8 GB		7€	
Computer	1 used with SDHC card reader and VLC program video playback		50€	
Microscope	1 BRUSH 8.9cm (3.5") LCD teaching microscope, 50-500x, 2000 (digital)		189€	
		Total	416€	

The cost of performing a spermogram is not negligible. It is advisable to pool the collection of materials to amortise the financial cost. The collection of all the material can serve several users over several years.

Getting started with the microscope

The components of the microscope & First handling of the microscope



Liste complète des divers éléments (graph. 1-5) :

1. Module d'écran
2. Écran LCD
3. Tube
4. Revolver porte-objectifs
5. Objectif
6. Lamelle porte-objet (ici : échantillon préparé)
7. Platine de microscope
8. Lentille collectrice
9. Éclairage LED (éclairage diascopique)
10. Pied du microscope
11. Témoin lumineux
12. Interrupteur marche-arrêt pour module d'écran
13. Éclairage LED pour éclairage épiscopique (utiliser uniquement avec l'objectif 4x)
14. Table à mouvements croisés
15. Disque à filtre chromatique
16. Molette de mise au point
17. Raccordement électrique
18. Variateur
19. Sélecteur d'éclairage
20. Touches d'entrée
21. Touche de prise de vues
22. Touche de menu
23. commutateur
24. Échelles verniers
25. Vis de serrage
26. Molette avant/arrière de la table à mouvements croisés
27. Molette droite/gauche de la table à mouvements croisés
28. Levier pour dispositif de fixation par serrage
29. Dispositif de fixation par serrage
30. Diaphragme libre (sans filtre chromatique)
31. Filtre chromatique
32. Câble USB
33. Boîte de 10 lamelles porte-objet, 10 lamelles couvre-objet et 5 préparations permanentes
34. A) Instruments de microscopie; B) Pipette; C) Pincette
35. Écloserie de crevettes
36. Microtome
37. Préparations: A) Levure; B) «Gum-media»; C) Sel de mer; D) Œufs de crevettes

Be careful with your microscope by holding it carefully. Be careful not to put your fingers on the eyepiece, which is very sensitive. The same applies to the objective and the preparations you will be observing to prevent them from getting dirty and interfering with the observation.

Avoid sudden movements and instead carefully pick up the microscope by the stem, the handle or by lifting the base.

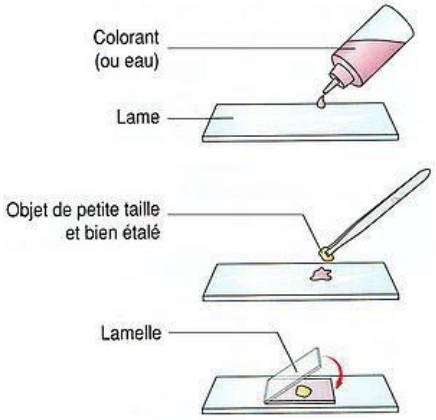
Now switch on the lighting and set it to maximum first.

Next, open the diaphragm completely to adjust the intensity of the light. Of course, these settings can be readjusted according to the observation to be made.

The otic tube can also be lowered using the macrometer screws. Caution:

Always use the smallest lens first.

Microscope preparation, focusing, observation, storage of the microscope



The preparation consists of an object slide, an object cover slip and the small object to be observed.

Place the preparation on the stage and proceed to observation.

The object to be observed is in the centre of the stage, then raise the stage as close as possible to the objectives. Be careful that the blade does not break.

To focus at low magnification, while looking through the eyepiece, carefully lower the stage with the macrometer screw until the object to be observed is sharp. To improve the focus, make an adjustment with the micrometer screw. You can then adjust the illumination by closing or opening the diaphragm little by little.

Fix the preparation with the jacks.

Use screws to move back and forth and right and left.

You can then look at your object through the eyepiece and change the lens if you need to. By
For example, if the magnification is not sufficient, choose the higher magnification objective. Then refocus with the small screw.

To calculate the magnification, multiply the eyepiece magnification by the objective magnification.

Put the microscope back on the smallest objective and reopen the diaphragm to the maximum. Clean the microscope thoroughly, especially the stage, and remove any dust that may have settled on the lenses. To do this,

use the lens paper previously soaked in alcohol. Finally, lower the optical tube and carefully place the microscope in its cover or case.

To find out more, go to:

https://fr.wikihow.com/utiliser-un-microscope

https://www.bresser.de/fr/Microscopie/BRESSER-Microscope-d-enseignement-LCD-8-9cm-3-5.htm

The use of the microscope requires time to adapt in order to make quality observations.

Hygiene and prevention of risks of exposure to biological fluid

The equipment you use to collect semen must be sterilized or maintained as indicated:

- 1 Single receptacle
- 1 x 3 ml micro-pipette
- 1 Neubauer counting cell with 1 coverslip

Boiling water sterilisation

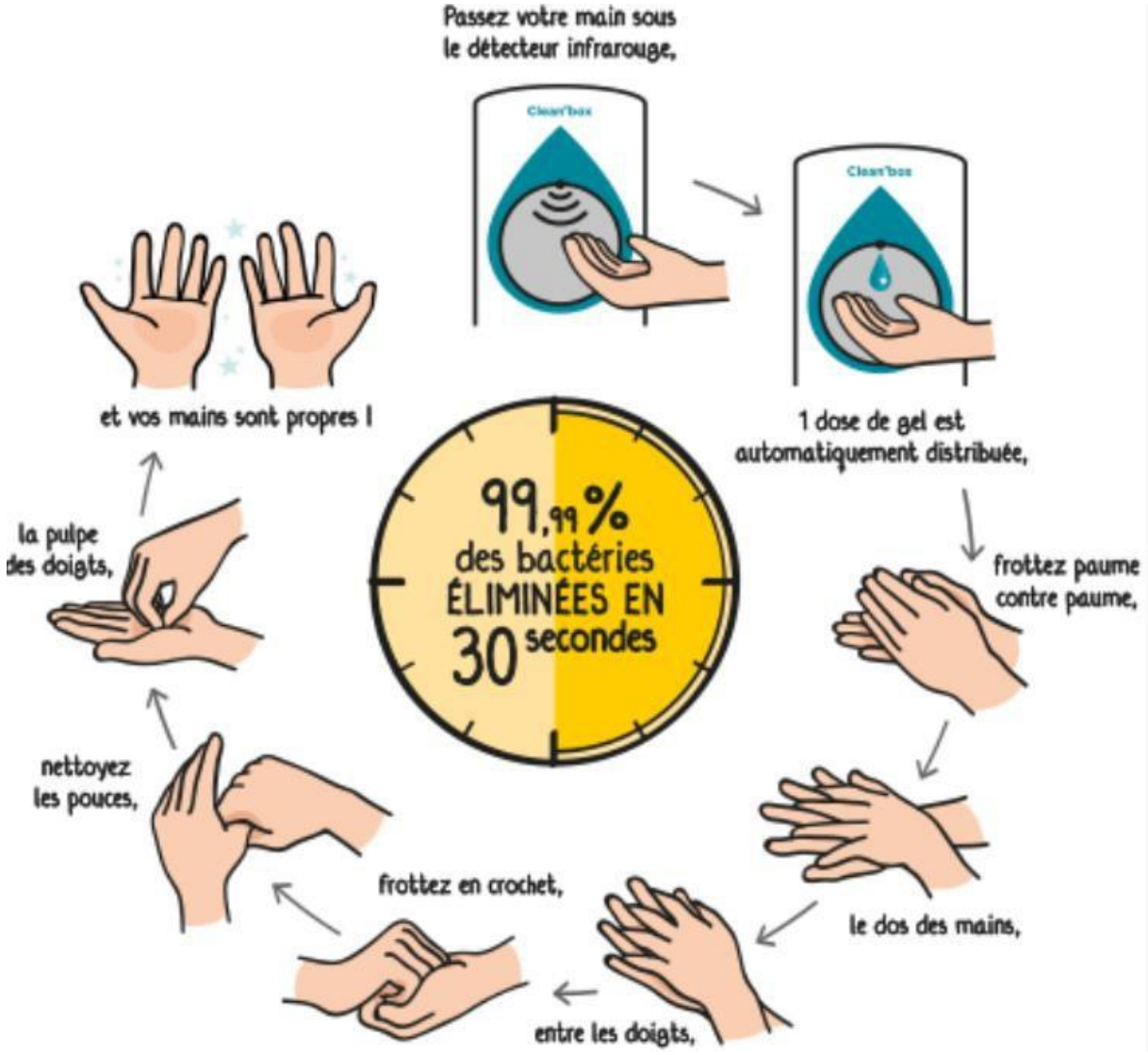
This is the oldest and simplest system:

- Take a pressure cooker or a large saucepan.
- Fill it three-quarters full of water and boil it for at least a quarter of an hour, or fill a container with 1/3 tap water and 2/3 with water previously heated in an electric kettle.
- Immerse the material in it for 5 minutes (it may become deformed after that).
- Remove it from the water with pliers.
- Leave to dry on sterile compresses.

Maintenance

- Wipe gently with lens paper to remove any sperm or debris.
- Disinfect with 70° alcohol to limit the risk of contamination.
- Rinse with water to remove the disinfectant.

Hand washing with hydroalcoholic solution:



N.B. If you need to perform a spermogram on someone other than yourself, be careful not to come into skin contact. Wear gloves when working with someone else's sperm.

Course of action

Step 1: Preparation of the material

Wash your hands once the equipment has been set up and sterilised, Step 2: Ejaculate collection

Urinate before collecting.
Wash hands with hydroalcoholic solution.
Disinfect the penis with an antiseptic wipe.
Rinse the penis with the bottle of sterile physiological water to remove any traces of disinfectant. Dry the penis with a sterile compress.

Wash your hands with the hydroalcoholic solution.
Open the container
Perform semen collection by masturbation in the sterile receptacle provided.
N.B. It is very important to collect all the ejaculate, especially the first few jets, as these contain a greater quantity of sperm than the end of the ejaculate. If some of the ejaculate is not collected, it is best to start again with a 3-day abstinence period.
Seal the receptacle well
Wash hands with soap and water or hydroalcoholic solution. Step 3: Sampling

& microscopic observations & video recording

Put on your gloves for the micropipette sampling, placing a drop on the slide of
Neubauer, placing the coverslip, reading under the microscope and recording on the SDHC card. Step 4:

Cleaning the equipment

Keep your gloves on when cleaning non-sterile equipment and sterilising equipment that has been in contact with semen.

Step 5: Counting & data capitalisation

Wash your hands. There is no longer any risk of exposure to the biological fluid (semen). Take the SDHC card and insert it into the computer. Take a first reading with vlc, then start counting. Once this is done, save the file on the computer or other, naming it as follows: year_month_day_name or nickname_D number of days since D0_number of spermatozoids_M_ml, adding the following information if necessary: pathology in the 3 previous months, febrile episodes, current treatment, age.

Follow the protocol and repeat it, it is by practicing that you will acquire the gestures and the technique. Beyond the risk of exposure to the biological liquid, impeccable hygiene is necessary to avoid contaminating the sample and to have a reading of the best quality.

Theoretical data on the spermogram

Spermogram

Data to be recovered

Volume: Amount of semen released, it varies from 1.5 ml to 5 ml. Appearance (colour, viscosity) and Ph are laboratory data. Using a gram scale, pre-weigh the bottle before introducing the collected semen. 1 ml of semen corresponds to 1 gram, so it is easy to determine the volume collected.
Counting: This involves counting the various elements present in the semen:

- Spermatozoa (million/mL), the sperm concentration,
- Sperm in the entire ejaculate (millions), i.e. the sperm count,
- Round cells (spermatids),
- Leukocytes,
- Red blood cells,

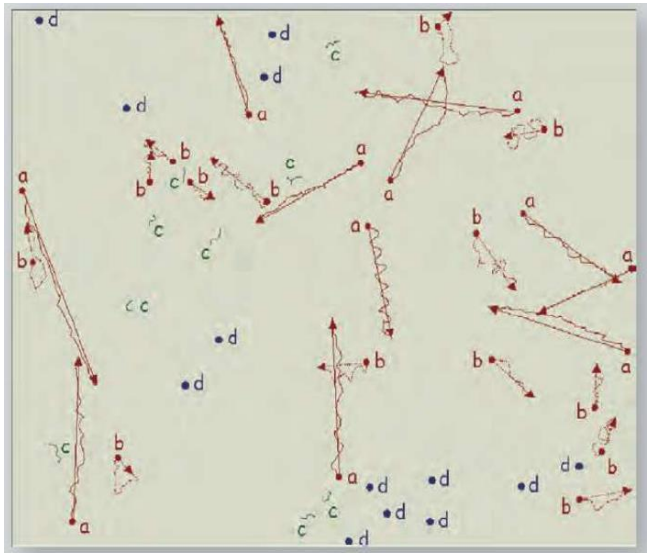
The sperm concentration is obtained by dividing the count by the volume of the ejaculate.

At present, the only indicator of a man's contraceptive status is the sperm count (million/mL). The count is carried out with the help of the Neubauer slide reading grid by counting all the spermatozoa present, regardless of their mobility. Once the count is complete, a simple calculation will be explained below to give you an estimate of your daily count.
It is important to perform at least 2 Neubauer slide counts from 2 different drops extracted with the micro-pipette, to check the acceptability of the measurement differences between the different counts.

For information:

Mobility: It includes 4 categories according to the type of travel observed:

- Type A: progressive, fast, straight - Progressive
- Type B: slow progressive or zigzag - Progressive
- Type C: On-site mobility - Non-progressive
- Type D: immobile



Depending on the number of sperm counted, it is
It is interesting to evaluate the percentage of those that are progressive (A+B). This is not essential, but remember that the progressives have a high chance of being able to fertilise the egg.
Vitality: This is the percentage of live sperm 1 hour after emission. This examination is carried out with the help of a contrast medium.
Normal sperm morphology: There are different categories of abnormalities:

- Head abnormality (elongated, thinned, micro or macrocephalic, multiple, irregular, abnormal or absent acrosome)
- Cytoplasmic abnormalities (cytoplasmic remnant, small or thickened intermediate piece, angulation)
- Flagellum abnormality (absent, shortened, irregularly sized, coiled, multiple, isolated)

There are many more parameters. Remember that only the concentration (million/mL) is necessary. Certainly, the assessment of mobility and vitality can be elements interesting to calculate, but they are not currently essential in the evaluation of your practice of WCL.

Reference values according to the WHO 2010 guide

CHARACTERISTICS OF SEMEN	*STANDARD VALUES*	*STANDARD VALUES UNDER MALE CONTRACEPTION THERMAL (after 3 months of wear)*
VOLUME	> 1.5 ml	> 1.5 ml
CONCENTRATION	>15 million/ml (infertility threshold)	**< 1 million/ml (contraceptive threshold)**
PROGRESSIVE MOBILES (A+B)	> 32%	< 10%
VITALITY (MOBILITY ONE HOUR AFTER EJACULATION)	> 58%	< 40%
NORMAL SPERM MORPHOLOGY	> 4%	< 4%

Variability in concentration within the same subject

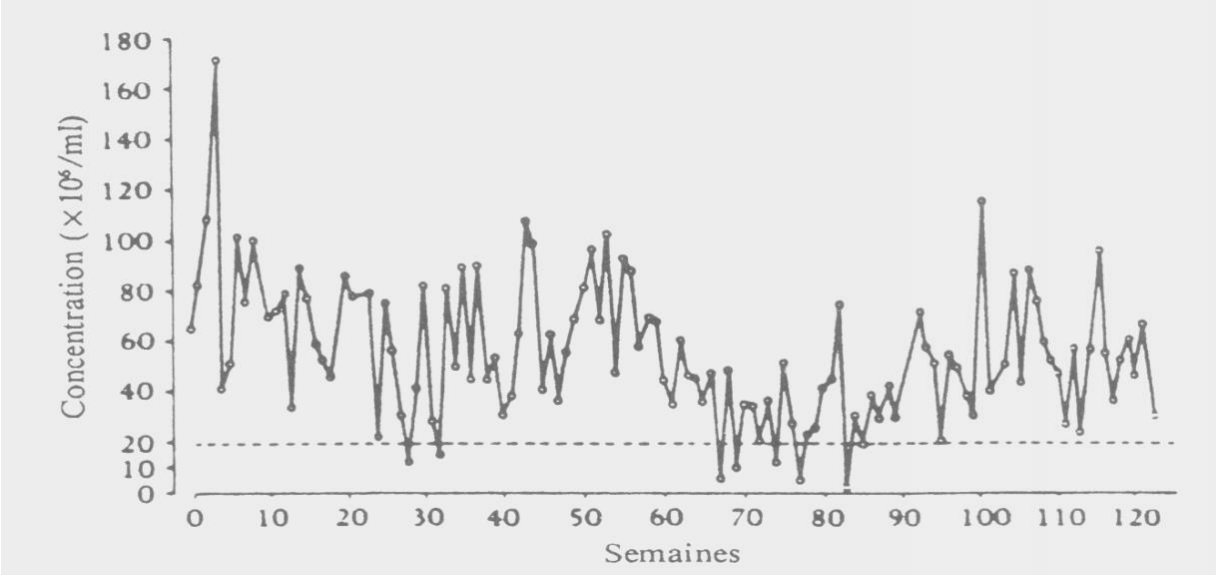


Figure 1 Bi-weekly sperm concentration in the same subject for a period of 120 weeks. During this period the subject received no treatment and no febrile episodes were noted (after Paulsen, WHO, 1992, unpublished data).

Keep in mind that your concentration can vary greatly over a few months. Parameters such as age (especially over 50), a medical condition that occurred in the previous 3 months, a febrile episode, treatment or drug use can reduce the quantity and quality of spermatozoa. In all the examinations carried out on men practising CMT, there was no evidence of

such variations. Namely, that a man below 1 million/mL has never been above
This threshold is reached if the WCL protocol is followed. It is likely that resting spermatogenesis by CMT also leads to a reduction in the amount of variability in concentration.

Variability in sperm characteristics according to the time of abstinence

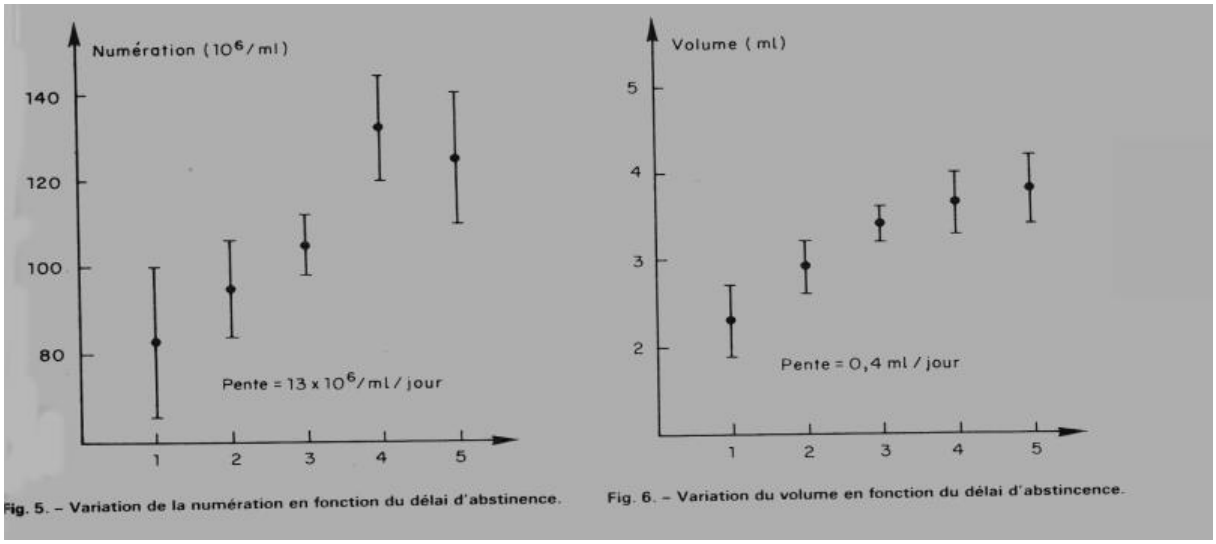


Fig. 5. – Variation de la numération en fonction du délai d'abstinence.

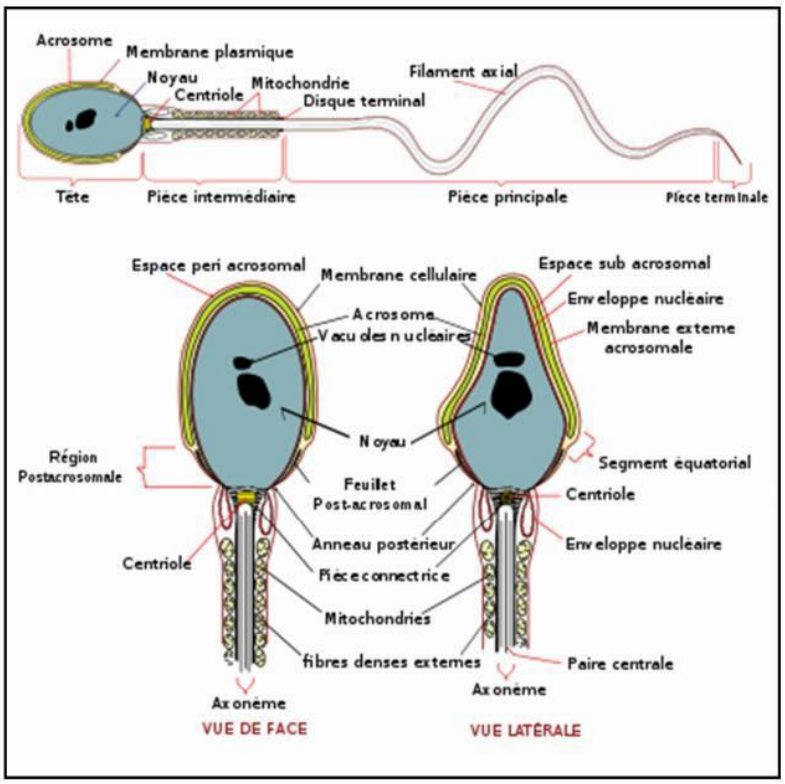
Fig. 6. – Variation du volume en fonction du délai d'abstincence.

Figure 2 Variability in sperm characteristics as a function of abstinence time, Paulsen, unpublished data.

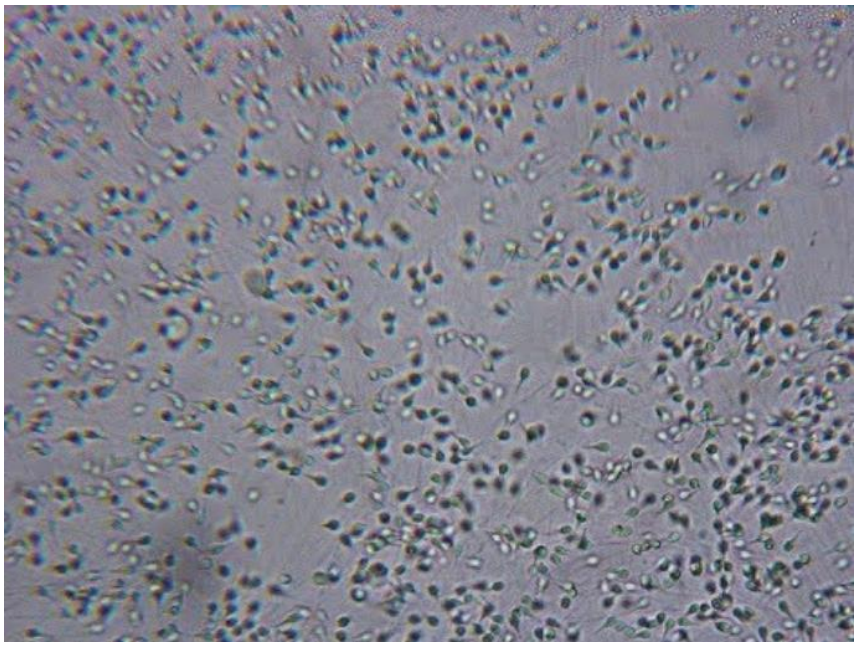
The period of abstinence has an impact on the concentration and volume emitted. The 3-day period is therefore important to respect.

In addition, certain molecules, treatments, pathologies, fever and age (especially over 50) can reduce the quantity and quality of spermatozoa. It is important to take note of this information when taking the sample and to respect the 3-day abstinence period.

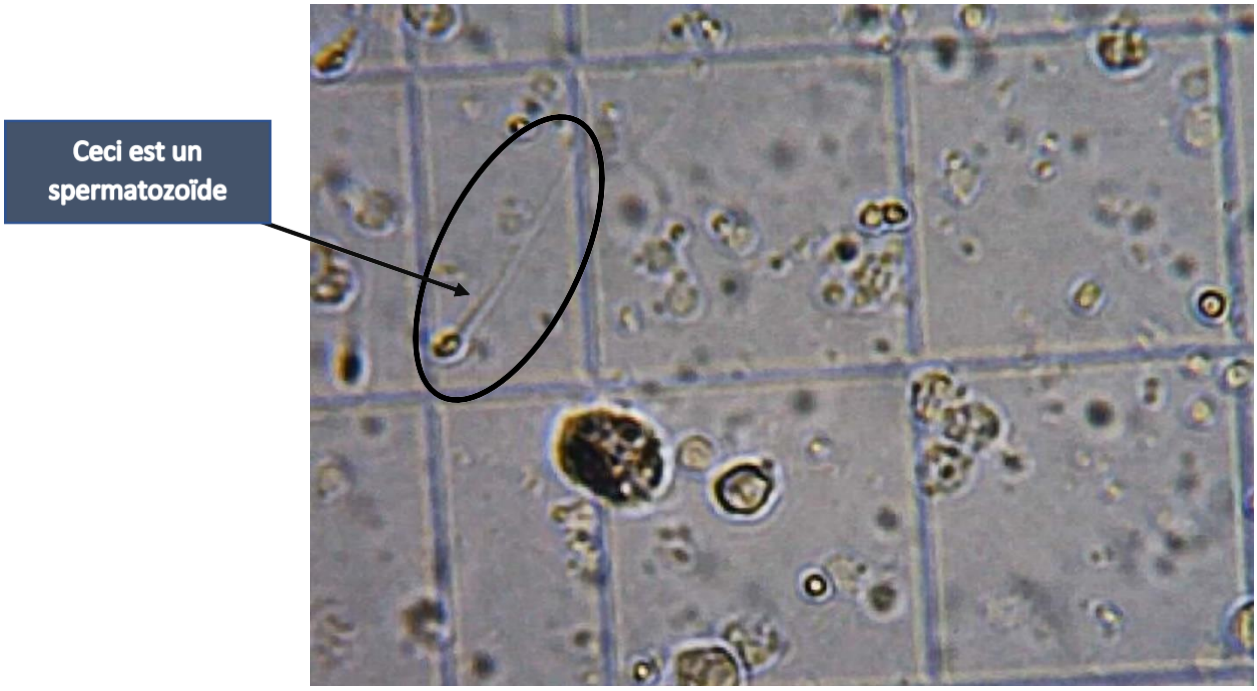
Morphology of the spermatozoon



What you will see under the microscope without the counting grid:



What you will see on the 8.9cm LCD BRESSER microscope at *400 magnification with part of a Neubauer C-counting grid square:



Sperm identification is fairly straightforward once you are aware of their characteristic shape.

Semen handling and preparatory techniques

Work for this part with the laboratory micropipette that can hold 10 µl.

Ideally, semen should be kept at 15°C & 25°C, which may be room temperature.

Standard set-up of the hematimeter or Neubauer counting cell :

- Place the cover slip on the Neubauer haematizer, and place it in a horizontal position on the table, where it is easy to handle the micro-pipette.
- Insert a disposable tip into the end of the propette.
- Adjust the micropipette (which holds 10 µl) to draw up 10 µl of liquid. Usually this adjustment is made by guiding the plunger knob to select the desired volume.
- Insert the tip of the propette into the sample.
- Press the plunger gently until it reaches the end of its travel.

- Remove the tip of the propette from the sample and keep it in a vertical position to take it to the Neubauer haematimeter.
- Place the tip of the pipette on the edge of the cover slip at the end of the Neubauer haematizer. The idea is to let the liquid penetrate between the plate and the cover from the side by capillary action:
- Release the document plunger while you check that the liquid is entering the hematimeter correctly and evenly.
- If bubbles appear, or the cover has moved, or any other anomaly, repeat the operation.

The Neubauer haematizer is then mounted and ready for microscopic reading.

This procedure is valid each time you mount the Neubauer haematimeter of a sample.

Liquefaction of the ejaculate

15 to 30 minutes after ejaculation, ideally wait 30 minutes but can take up to 1 hour. As long as If the ejaculate is not liquefied, sperm movement, sample homogeneity and the ability to make good microscopic observations are limited. A simple method to validate liquefaction is to use a pipette. If the semen collected in the pipette flows dropwise, liquefaction is complete, but if the flow is stringy, viscous, liquefaction is incomplete.

Homogenisation of the ejaculate

Either by rotating the container: Rotate the container in circles with the wrist for 15 to 20 seconds.

Or with a micro-pipette: Aspirate and gently expel the sample (about ten times) with the pipette in taking care to avoid the formation of bubbles.

Preparation of the fresh state

Introduction of the 10 µl sample into the Neubauer plate

Under the microscope, the fresh ejaculate should be examined quickly and systematically (scanning motion) to prevent the preparation from drying out.

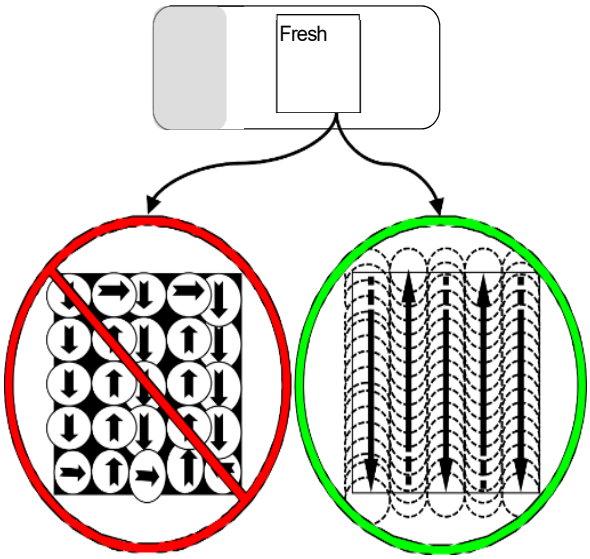


Figure 3 Shifting the field of view (scanning) to examine the entire surface of the slide covering a known volume of sperm.

If the number of sperm per field varies considerably, the sample is not homogeneous. The preparation must be discarded and a new one made, paying particular attention to homogenisation. Examination of fresh semen is to begin as soon as the cellular elements of the sperm have stopped drifting between the slide and the coverslip.

Microscopically, the liquefaction of the fresh semen preparation is also validated. The presence of viscous currents indicates that liquefaction is incomplete and may compromise the count. The observation of viscous currents, as shown in the photograph, is delicate. Use the drip technique to check for liquefaction.

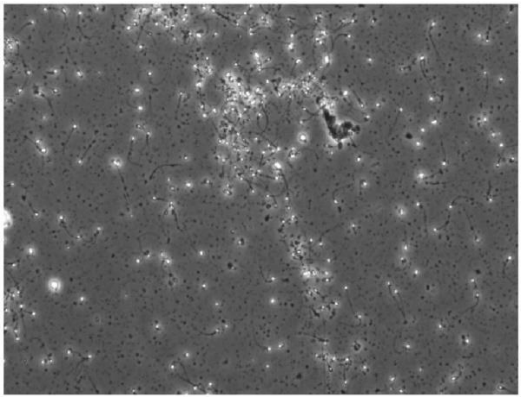


Figure 4 Viscous currents visible under the microscope showing incomplete liquefaction of semen

Neubauer haematometer

The Neubauer haematimeter remains the reference counting chamber for manually assessing the This is the only method of sperm concentration that will be described in this guide.

The Neubauer haematimeter consists of two separate counting chambers, each comprising a 3 mm x 3 mm counting area subdivided into 9 squares of 1 mm x 1 mm, each consisting of squares of known size (see Figures 5 and 10). It is used with a coverslip placed on

a built-in support to obtain a spread of constant thickness of 0.1 mm. By multiplying the area of a square by the thickness of the spread, the volume of sperm in that square can be obtained and the concentration calculated by dividing the number of sperm counted in a known volume (concentration = number/volume).

Note: 1 mm3 = 0.001 ml = 1 µl.

Sperm should be counted on different sections of the counting area, depending on the dilution performed and the number of sperm to be counted per sample

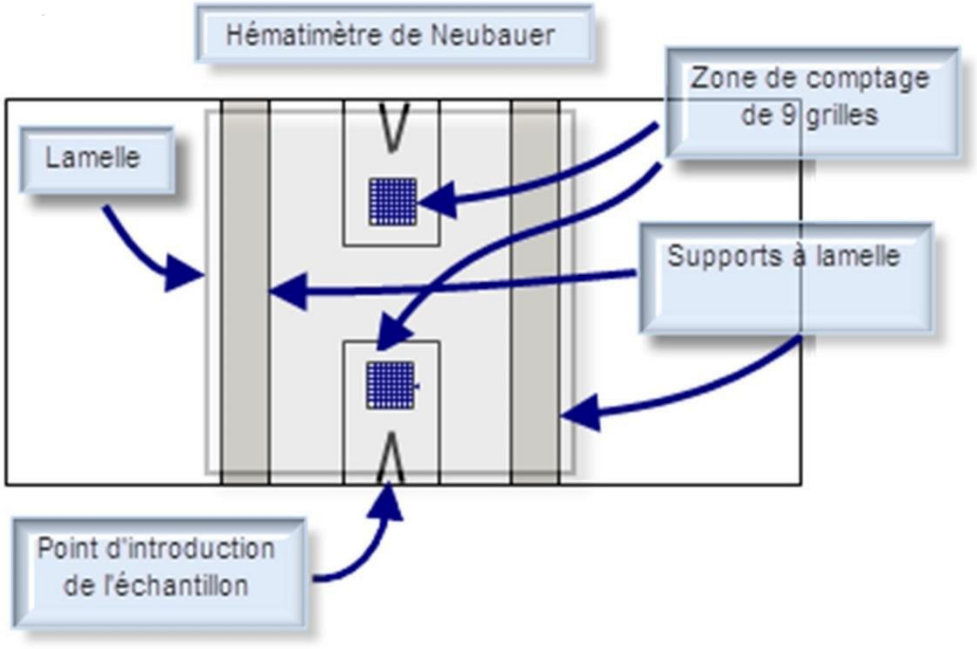


Figure 5 Neubauer haematometer, top view

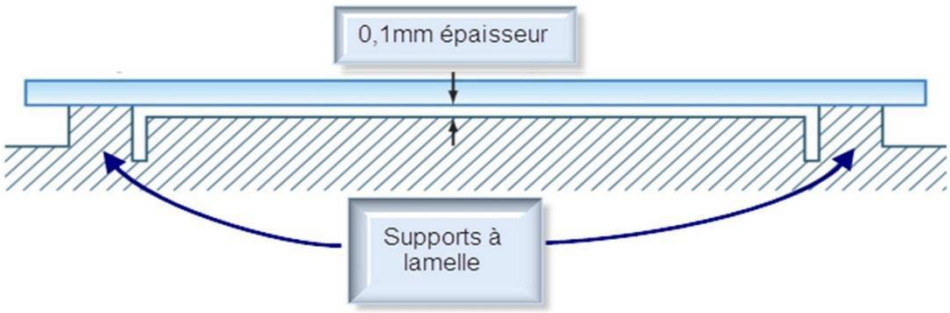


Figure 7 Neubauer haematometer, side view.

Selection of the appropriate dilution for the evaluation of the concentration on the Neubauer

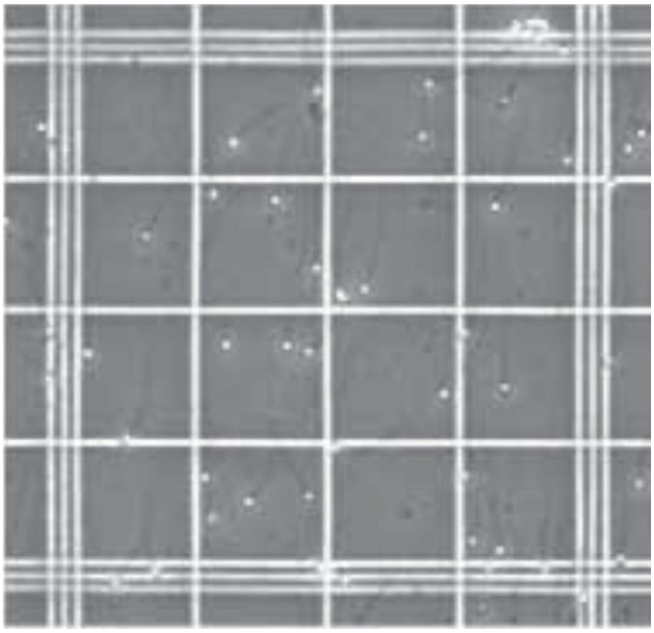


Figure 8 One of the 25 squares in the central counting area of grid C, subdivided into 16 other squares and bordered by 3 parallel lines.

Semen examined on the Neubauer haematizer must be diluted for the following reasons:

- Immobilise the spermatozoa,
- Promote spreading by capillary action under the coverslip by making the semen less viscous,
- Facilitate counting when sperm are very numerous and overlapping,
- Facilitate enumeration when the sample contains a lot of debris.

The number of sperm per field, assessed in 10 µl of fresh semen spread under a 22 mm x 22 mm coverslip, guides the choice of the appropriate dilution for the Neubauer haematizer set-up (table in Figure 9) with the aim of counting at least 100 sperm per sample. The dilution is made with saline.

Nbre de spermatozoïdes par champ, facteur de grossissement de 400	Dilution	Sperme (µl)	Diluant (µl)
Rare	1 : 2	100	100
Moins de 15	1 : 5	100	400
De 15 à 40	1 : 10	50	450
De 41 à 200	1 : 20	50	950
Plus de 200	1 : 50	50	2450

Figure 9 Dilutions suitable for concentration assessment on the Neubauer haematizer.

Assessment of sperm concentration

Work for this part with the laboratory micropipette that can hold 100 µl.

Concentration refers to the number of sperm, expressed in millions, in one millilitre (n^{bre} x 10^6 /ml) of ejaculate.

It should be noted that if the sperm concentration is less than 1.0 x 10^6 /ml, the relevance of the assessment of vitality and morphology may be questioned. An accuracy of 20% is considered acceptable for limits below 1.0 x 10^6 /ml. Errors between 20% and 30% are common with this counting method due to the handling of the micropipette, statistical errors for having an unrepresentative sample, errors in the volume of the sample actually introduced on the plate, etc.

Homogenisation and dilution of the ejaculate

Due to the viscous nature of semen and the relatively small volume of diluted samples, it is essential that the semen is thoroughly homogenised before the desired volume is taken for dilution.
The two counting chambers of the Neubauer haematizer allow the counting of two separate samples from the ejaculate. To reduce the risk of error, the two samples are diluted separately, in two different tubes (i.e. the two counting chambers are not filled with samples from the same dilution, nor is counting performed twice in the same chamber, otherwise sampling, homogenisation or dilution errors would not be detected).

Dilutions can be made in clean 5 ml tubes (glass or plastic). First place the diluent and then the semen in the dilution tube. Before transferring the liquid or semen into the dilution tube, wipe the outside of the propette, taking care not to touch the opening of the tip so as not to absorb any liquid with the paper. To collect all the semen, rinse the tip of the propette in the diluent (saline) by aspirating and expelling a few times.

If there is a delay between the preparation of the dilution and the assembly of the haematizer, precautions must be taken to avoid evaporation.

Assembly of the haematimeter and sedimentation of spermatozoa

Mix the first dilution thoroughly by shaking for about 10 seconds or with a propette. Immediately take 10 µl of the dilution and bring the tip of the propette to the point of introduction of the haematiometer without touching the slide. Be careful not to overload or lift the coverslip, otherwise the thickness of the spread will change.

R e p e a t with the second dilution in the second chamber. Handle gently
the hematimeter to avoid moving the slide and leave it horizontal.

Allow the sperm to settle at room temperature for at least 4 minutes, but not more than 15 minutes, otherwise the preparation will dry out and the sperm concentration will be higher.

Assessment of concentration

Remember that at least 2 counts should be made on 2 chambers from two different drops of the sample.

Absence of sperm in fresh semen

If no sperm is observed after examination of the complete slide.

Less than 500 sperm in fresh semen

If less than 500 sperm are counted on examination of the whole slide or on the 9 C-squares, record the number of sperm observed in 10 µl of fresh (undiluted) semen.

If fresh semen is spread on the slide according to the instructions and the
If the slide is complete, the concentration per ml can be estimated as follows:

One observed sperm = 100 sperm/ml = 0.0001 x 10-6/ml

Example: 12 sperm in 10 µl of semen equals 1200 sperm per ml (0.0012×10^6/ml).

The threshold of 500 spermatozoa was established as follows: as the presence of 1 spermatozoon on a 10 µl slide is equivalent to approximately 100 spermatozoa per ml, the presence of 500 spermatozoa on the same slide corresponds to a concentration of 50,000 spermatozoa per ml. The haematology method presented has a detection limit of 56,000 sperm per ml. The threshold of 500 sperm in 10 µl therefore allows the concentration to be assessed, even below the detection limit of the haematometer.

At least 500 sperm in fresh semen

As soon as 500 sperm are reached on the 10 µl slide, the sperm count should be stopped. Do not report the number of sperm on this slide and proceed immediately to the hematimetric count. Only the result of the haematology count will be reported.

Sperm count in each of the prepared samples

Proceed with the count without delay or interruption as soon as the 4-minute time limit has passed and not beyond 15 minutes of waiting.

It is accepted that the assessment of 100 sperm per sample and therefore an error of 7.1% are sufficient to ensure the quality of the analysis.

Counting is done under total magnification of 200 or 400.

The decision to count a sperm cell or not depends on the position of its head, regardless of the orientation of the flagellum. The boundary of a square is the centre line of the three parallel lines along its sides. The sperm is counted if most of its head lies between the two inner lines of the three-line border. It is not counted if most of its head lies between the two outer lines of the three-line border.

If most of the sperm head is on the centre line, only sperm touching the edges of two sides of the square should be counted, by convention the left and bottom sides (Figure 9), to avoid counting the same sperm twice in adjacent squares.

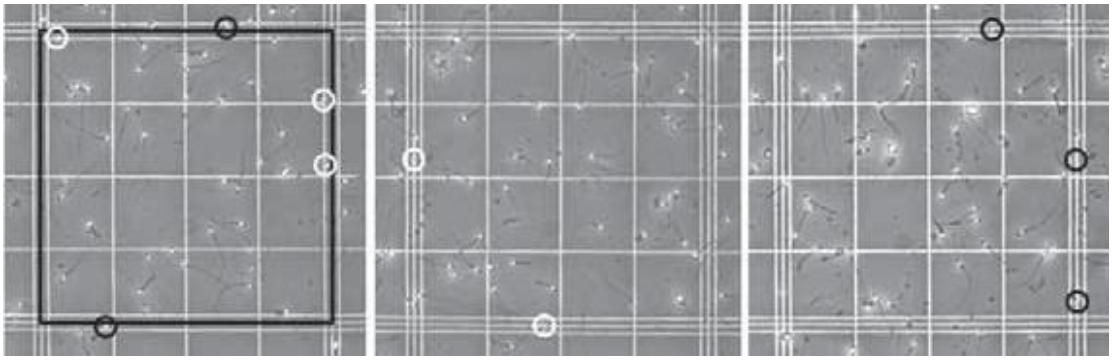


Figure 10 Sperm to be counted in a square in grid C (bounded by a 3-line border).

To count sperm touching the edges of the grid, see explanations above. Sperm circled in black are not counted, while sperm circled in white are counted.

Count the sperm in 5, 10 or 25 grid squares C (see Figure 10 and 11) until at least 100 sperm are counted (maximum of 25 squares, whether 100 sperm are reached or not). Move to the second counting chamber of the haematizer and count the sperm in the same number of grid squares C (i.e. the same volume) as for the first sample, even if the final number of sperm counted is less than 100. Each grid square C has a volume of 4 x 10-6 ml.

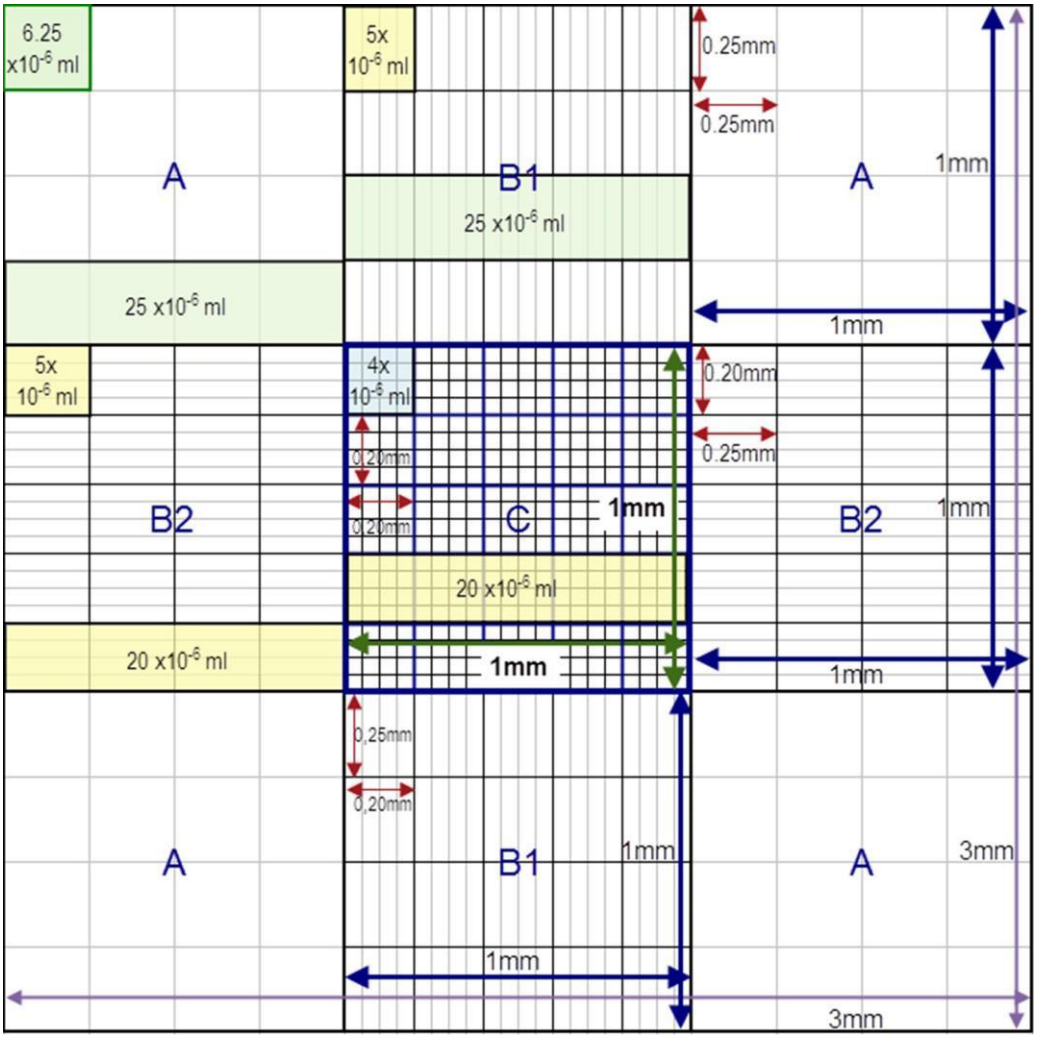


Figure 11 Counting area of the Neubauer haematometer chamber.

If the cell concentration is very high, it is easy to get lost in the counting, a zigzag counting sequence is used as described in Fig. 7 and 12.

- Write down on a sheet the results of the number of cells counted in the first frame.

- Repeat the process for the other frames you wish to count, noting the result for each. The more frames you count, the more accurate the measurement will be.

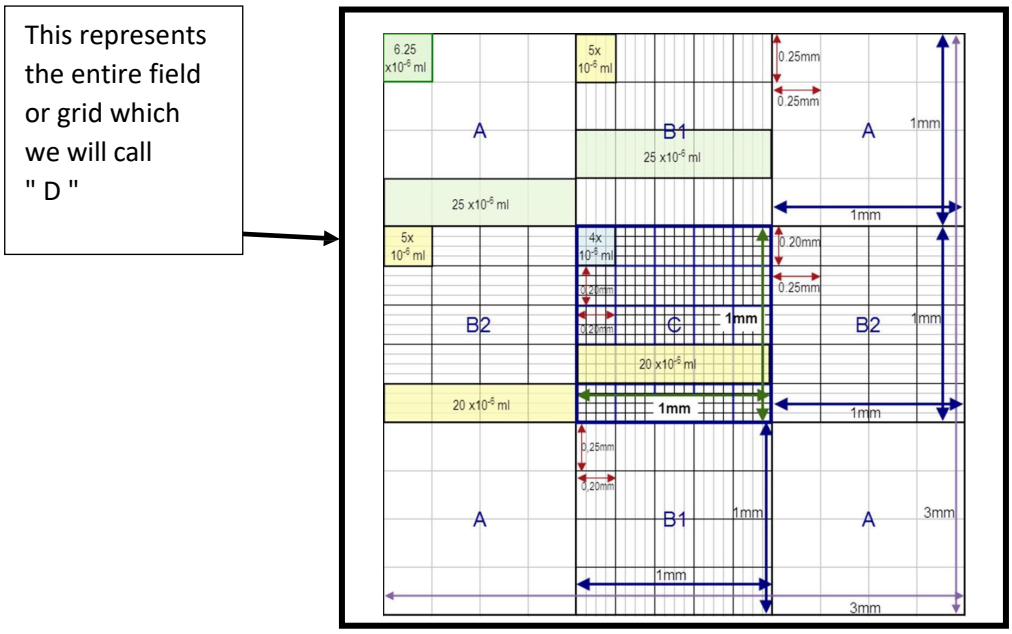


Figure 12 Counting area of the

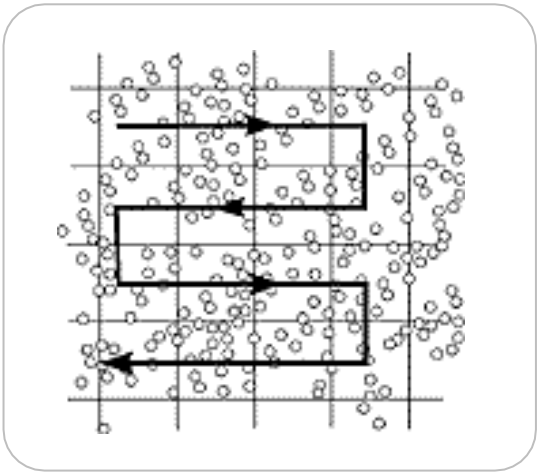


Figure 13 Counting with high cell concentration.

However, if fewer than 100 sperm are counted per 1:2 sample, the sampling error will be greater and the degree of certainty lower. In this case, the 9 1 mm x 1 mm squares of the 2 counting chambers (see Figure 11) should be examined to try to achieve an acceptable sampling error (20%).

Calculate the sum of the two counts (e.g. 1st sample: 75 sperm in the 9 1 mm x 1 mm squares; 2nd sample 70 sperm in 9 1 mm x 1 mm squares of the second chamber, totalling 145).

See Table 1 in the Appendix for information on the percentage of sampling error depending on the number of sperm counted, if this is less than 20%, the sampling error is considered acceptable.

Checking the acceptability of measurement differences between samples

Calculate the sum and difference of the two counts (e.g., 1st sample: 126 sperm in 10 C-grid squares; 2nd sample 134 sperm in 10 C-grid squares of the second chamber, totaling 260. Difference between the two counts: 8).

Establish the acceptability of the difference between the numbers by referring to Table 2 in the Appendix.

Example. For 260 sperm, the acceptable difference is 32; the difference obtained here, 8, being less than 32, is acceptable and we can proceed to the calculation of the concentration.

If the difference is too great to be accepted, it is recommended that both dilutions are repeated, taking care to homogenise the semen at each stage, and that the count is repeated. Repeat this procedure up to two times (three sets of two samples). If the difference is still too great after three rounds (ejaculate is particularly viscous and heterogeneous), average the six numbers obtained.

Calculation of the concentration

The sperm concentration (C) is equal to the number (N) of sperm divided by the volume (V) of semen in which they were counted, multiplied by the dilution factor (D).

Example:

- D = 20 (1:20 dilution).
- N = 260 (1st chamber = 126, 2nd chamber = 134).
- V = 10 squares (x 2 samples) of 4 x 10-6 ml each, totalling 80 x 10-6 ml.
- C = (N/V) x D (260/80 x 10-6) x 20 = 65 x 10-6 per ml.

Dilution	N^{bre} de carrés de la grille C (de chaque chambre) compris dans le comptage			The whole field, the whole grid called " D "
	5	10	25	
	Facteur de division			
1 : 2	20	40	100	900
1 : 5	8	16	40	360
1 : 10	4	8	20	180
1 : 20	2	4	10	90
1 : 50	0,8	1,6	4	36

Figure 14 Division factors to be applied to the total number of sperm counted in both chambers to calculate the concentration (in x 10-6/ml).

To calculate the concentration more quickly, the table in Figure 12 can be consulted to establish the division factor to be applied according to the number of squares included in the count and the dilution made.

Let's go back to the previous example:

- N = 260 (2 rooms)
- Division factor = 4 (intersection of the 10 squares column and the 1:20 dilution row)
- N/Division factor = C
- 260/4 =65
- C = 65 x 106/ml

Detection limit

The proposed method has a detection limit of 56,000 sperm per ml (so that the sampling error does not exceed 20%). Therefore, this method is inaccurate if there are less than 25 sperm per counting chamber (total of 50 sperm)

Annexes

Tables of acceptable differences between samples

Table 1 is used to calculate the sampling error according to the number of sperm counted in the haematometer.

Table 1. Rounding error (%) by total sperm count

Total (number)	Sampling error (%)	Total (number)	Sampling error (%)	Total (number)	Sampling error (%)
1	100	25	20	88	10,8
2	70,7	30	18,3	90	10,5
3	57,7	35	16,9	95	10,3
4	50	40	15,8	100	10
5	44,7	45	14,9	150	8,2
6	40,8	50	14,1	200	7,1
7	37,8	55	13,5	250	6,3
8	35,4	60	12,9	300	5,8
9	33,3	65	12,4	350	5,3
10	31,6	70	12	400	5
15	25,8	75	11,5	450	4,7
20	22,4	80	11,2	500	4,5

[1] WORLD HEALTH ORGANISATION. WHO laboratory manual for the examination and processing of human semen, Fifth Edition, Geneva, WHO, 2010

Example: 100 sperm are counted in each chamber of the haematizer. According to the Table 7, the sampling error is 7.1% per 200 sperm.

Tables of acceptable differences between samples

Table 2 is used to establish the acceptability of the difference in the number of sperm counted in two haematology samples.

Table 2. Acceptable difference between the values obtained in two samples, taking into account the total number of spermatozoa

Total	Difference	Total	Difference	Total	Difference
35 à 40	12	144 à 156	24	329 à 346	36
41 à 47	13	157 à 169	25	347 à 366	37
48 à 54	14	170 à 182	26	367 à 385	38
55 à 62	15	183 à 196	27	386 à 406	39
63 à 70	16	197 à 211	28	407 à 426	40
71 à 79	17	212 à 226	29	427 à 448	41
80 à 89	18	227 à 242	30	449 à 470	42
90 à 98	19	243 à 258	31	471 à 492	43
99 à 109	20	259 à 274	32	493 à 515	44
110 à 120	21	275 à 292	33	516 à 538	45
121 à 131	22	293 à 309	34	539 à 562	46
132 à 143	23	310 à 328	35	563 à 587	47

*Based on a rounded confidence interval of 95%.

[1] WORLD HEALTH ORGANISATION. WHO laboratory manual for the examination and processing of human semen, Fifth Edition, Geneva, WHO, 2010

Example : First sample : 126 spermatozoa; second sample: 134 spermatozoa, totalling 260. Difference of 8.

According to Table 8, for a total of 260 sperm, the difference should be equal to or less than 32. The difference of 8 is therefore acceptable.

Bibliography

WORLD HEALTH ORGANISATION. *WHO laboratory manual for the examination and processing of human semen,* Fifth Edition, Geneva, WHO, 2010.

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION. *ISO15189:2012(E) Laboratories for Medical biology - Requirements for quality and competence,* third edition (corrected version 2014-08-15), Geneva, ISO, 2012.

MINISTÈRE DE LA SANTÉ ET DES SERVICES SOCIAUX. *Compliance of medical laboratories with the CAN/CSA-15189 standard "Laboratoire d'analyses de biologie médicale - Exigences particulières concernant la qualité et la compétence".* 2005-03-21.

PROFESSIONAL ORDER OF MEDICAL TECHNOLOGISTS OF QUEBEC. *Quality in the laboratoires de biologie médicale: Règles de pratique,* second edition, Montréal, OPTMQ, 2009.

CANADIAN STANDARDS ASSOCIATION. *CAN/CSA-Z15190 Medical laboratories - Requirements for safety.* Mississauga: Canadian Standards Association, 2005.

CSA GROUP. *Z316.7-12 Primary Specimen Collection Facilities and Medical Laboratories - Patient Safety and Quality of Care - Requirements for Specimen Collection, Transport and Storage,* Mississauga, CSA Group, Update No. 1 March 2014.

CHROMSRIMEK, Natta, et al. "Effect of Time between Ejaculation and Analysis on Sperm Motility", *Thai Journal of Obstetrics and Gynaecology,* April 2008, Vol. 16, No. 2.

SUCKCHAROEN N., et al. "A comparison of Mackler counting chamber and improved Neubauer hemocytometer in sperm concentration measurement, *J Med Assoc Thai,* September 1994, Vol. 77, No 9.

CHRISTENSEN, P., et al. "Discrepancies in the determination of sperm concentration using Bürker-Türk, Thoma and Makler counting chambers", *Theriogenology,* March 2005, Vol. 63, No 4.

HAMAMAH, S. and BARTHELEMY, C. Spermogram and fertility tests: Interest and limitations. *JTA Chapter V- Fertility and male infertility.*

KRUGER, Thinus F. and FRANKEN, Daniel R. Atlas of Human Sperm Morphology Evaluation, London, *Taylor & Francis*, CRC press, 2004, 86.

Cooper, T.G., et al. "Azoospermia: Virtual Reality or Possible to Quantify?" *Journal of Andrology*, 2006, Vol. 27, No. 4.

KORTHORST, Ruben A., et al. "Clearance after vasectomy with a single semen sample containing < than 100 000 immotile sperm/mL: analysis of 1073 patients", *BJU International*, 2009, Vol. 10.