MagaVision I

USER MANUAL

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What is Magavision?

Magavision is a system for evaluating seminal quality, specifically designed for processing swine ejaculates in boar studs.

It allows to carry out, easily and in less than 2 minutes, an objective and standardized analysis of the semen quality parameters necessary for the production of commercial seminal doses. It simplifies the daily work of stud technicians by automating the analysis of ejaculates.

Combined with Gesipor 3.0, Magavision calculates the potential number of doses that can be produced from each ejaculate considering the values of concentration and volume predefined by the user in the control parameters.

Necessary Materials

- Micropipette of variable volume from 100 to 1000 µl or from 1 to 10 ml for the 1:10 dilution to evaluate the ejaculate.
- Micropipette of variable volume of 0.50-10 µl to fill the counting chamber.
- Micro tubes or tubes to make a 1:10 dilution.
- Micropipette tips for both micropipettes.
- Counting chambers of known volume for evaluating the ejaculate.

Included in Magavision

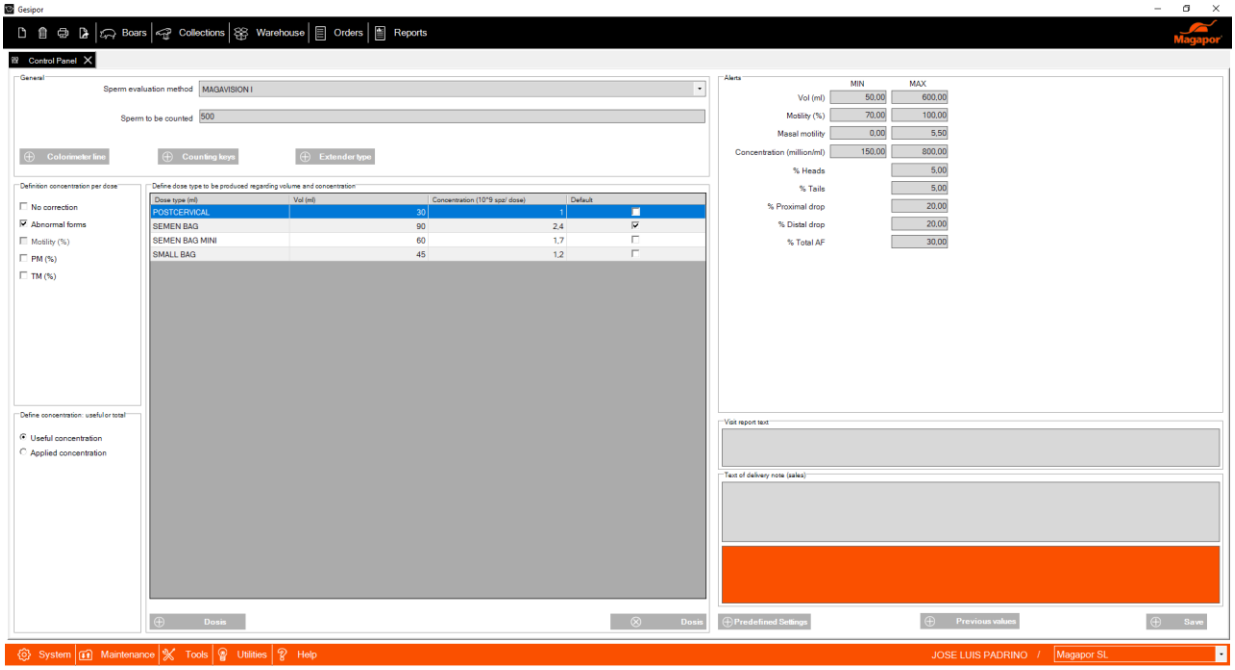
- CPU with software: Gesipor 3.0 and Magavision I
- USB License key Gesipor 3.0 and Magavision I
- Camera and cable USB 3.0
- Microscope
- Objective set
- Green filters
- Set of wrenches
- Thermostat for the heated plate of the microscope
- Micropipette of variable volume 0.5-10 µl
- Bag of 100 micropipette tips

To make the installation simpler and unlike other CASA systems, Magavision is specifically set up to analyze boar semen: camera configuration, motility, sperm limits, type of counting camera…

Once assembled and focused, the equipment does not require any additional software.

Setting up the parameters for semen doses in Gesipor 3.0

In the control panel of Gesipor 3.0, you can define which parameters will be used by Gesipor to estimate the number of doses that can be produced with the ejaculate analyzed by Magavision I.



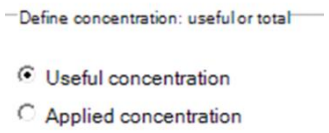
In the mid part, you can define the type of dose according to volume and concentration. For example: SEMEN BAG dose type with a volume of 90 ml and a concentration of 2.5x109spz / dose.

Define dose type to be produced regarding volume and concentration

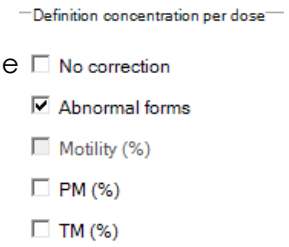
Dose type (ml)	Vol (ml)	Concentration (10^9 spz/ dose)	Default
POSTCERVICAL	30	1	☐
SEMEN BAG	90	2,4	☑
SEMEN BAG MINI	60	1,7	☐
SMALL BAG	45	1,2	☐

You can create as many types of doses as you want and then select them in the analysis screen. Gesipor estimates the number of doses that can be prepared with the ejaculate analyzed and the characteristics selected.

At the left bottom of the screen, you can choose the concentration type (applied/total or useful) of the dose.



On top of the "define concentration, useful or total" box, the section "define concentration by dose" can be used to select the parameters to be used by the software to calculate the concentration of the semen doses produced.



You have the following possibilities and correction combinations:

- Abnormal forms
- TM % (Percentage of total motile)
- PM % (Percentage of progressive motile)
- Abnormal forms and TM%
- Abnormal forms and PM%

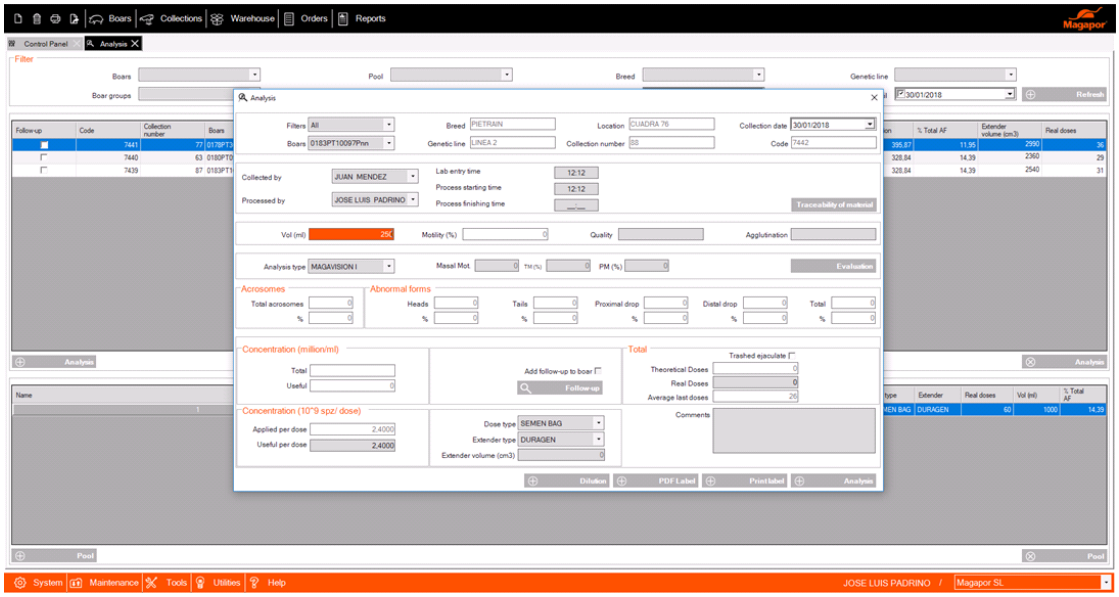
According to the parameters set in the example, the dose type SEMEN BAG, will be a seminal dose of 90 ml and 2.5x109 useful spz (that is, the percentage of abnormal sperm would be added to the 2.5x109 useful spz and that would make the total sperm of the dose).

Access through Gesipor 3.0

Once configured the form of calculating the doses and the parameters to be used by Gesipor to estimate the number of doses to be produced, you can start analyzing the ejaculates with Magavision.

Access the collections tab in the analysis section and choose the boar which ejaculate is going to be analyzed. You can also select the technicians that performed the collection and processed the ejaculate. The rest of the fields are automatically filled in with the information previously saved in the system.

Once entered the ejaculate volume, double click on "Evaluation" to open the program.



With Magavision open, proceed with the evaluation of motility, concentration and percentage of abnormal forms.

First, set the 10xPHN objective and the 10X position of the condenser.

** All the materials used to perform the analysis of the ejaculate should be tempered at 37ºC: counting chambers, heatable stage and micropipette tips.*

Before starting the analysis, make a 1:10 dilution with the ejaculate and extender tempered at 37ºC. For example, take 100 µl of pure semen (previously homogenized) and 900 µl of extender. You can use any volume to make de dilution as long as the ratio ejaculate / extender is 1:10.

After that, homogenize the sample properly, take 3.5 µl. and deposit them on the chamber of known volume by capillarity (placing the tip of the micropipette between the end of the slide and the cover).

Motility

In the field "Motility analysis," you can analyze the percentage of total and progressive motile sperm, that is, the percentage of total sperm that move in the sample, as well as those that move progressively. To do this, the system identifies sperm according to their type of movement and gives a result based on the values predefined in the Magavision I software.

With the counting chamber on the microscope platform, focus the image and click the button “Motility analysis”.

Once you click this button, the following messages appear:

- Recording
- Analyzing

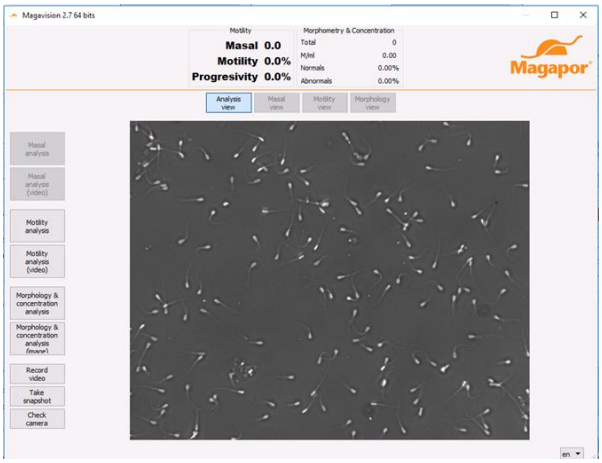
Do not scroll around the counting chamber while these messages are onscreen, so the sample is correctly evaluated.

We recommend to take 3 captures of 3 different fields of the counting chamber to make sure the assessment of ejaculate motility is significant and unbiased.

To perform the motility assessment, you can press the letter "v" on the keyboard instead of the "Mobility Analysis" button.

As you capture fields, the values of Motility and Progression show the average value of the different fields taken.

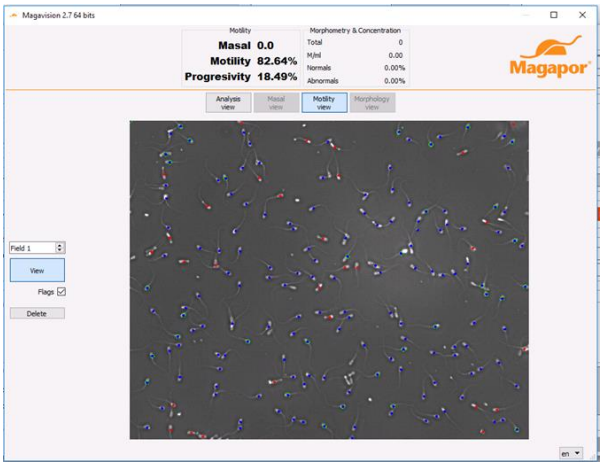
- Motility: It refers to the percentage of mobile sperm present in the fields analyzed.
- Progresivity: Refers to the percentage of sperm with progressive forward movement within the percentage of motile sperm.



**It is recommended to perform the analysis in the center part of each analysis line.*

**Avoid areas with a high level agglutination or dirt.*

Once motility is captured, you can review the videos recorded by Magavision. To do this, click on the "View Motility" button that is in the top center of the Magavision screen.



This section shows how the software has classified each spermatozoon. Sperm are marked in 3 colors:

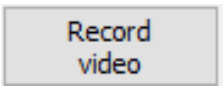
Motile spermatozoa

Motile spermatozoa and with progressive movement

Static Spermatozoa

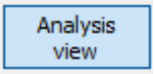
You can remove and set the marks of each type of spermatozoa by removing the tick next to the marks, navigate through the different fields recorded by clicking on the arrows and delete any field. When a field is deleted, the program recalculates the values of motility and progressivity using the remaining fields.

Magavision offers the possibility of recording motility videos. To record, press the button located left of the screen of Magavision which reads "Record video." The program will record a video that can be stored in any folder.



To play a video recorded by Magavision, click on the "Motility analysis (video)" button.

If you want to return to the main screen, click on the "Analysis view" button.

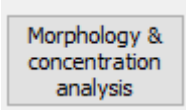


Concentration and abnormal forms

The module of concentration and abnormal shapes is based on the knowledge of the area and depth of the field that appears on the screen. From there, the software receives the morphometric conditions of a spermatozoa so it is thus able to distinguish sperm from the possible artifacts found in the sample (dirt in the ejaculate, foreign bodies, agglutination, bacteria, desquamation cells...). Once the system differentiates sperm from artifacts, the expected morphological pattern has to be entered, so it can discern three cell types: pattern (normal), cytoplasm drops (proximal and distal) and bent tails.

Calculating concentration consists of determining the number of sperm per volume unit. Magavision estimates concentration taking into account the 1:10 dilution made with the extender and the sperm counted automatically as well as the area occupied by the sample on the counting chamber.

To continue with the analysis, click on "Analysis of Morphometry and Concentration" button.

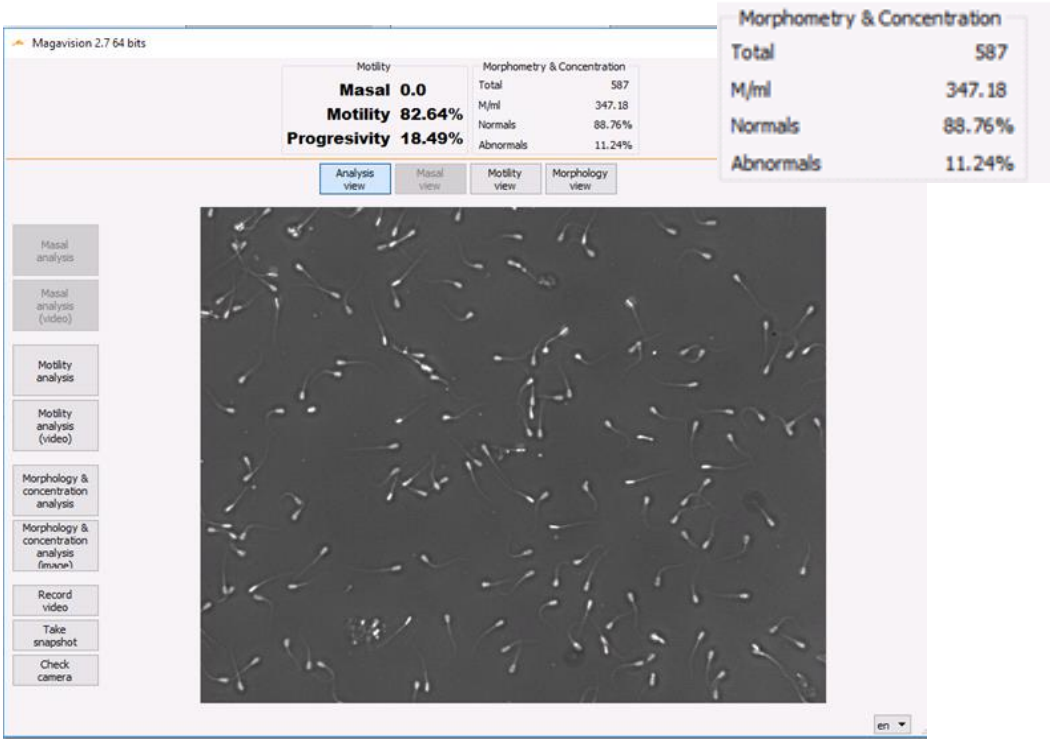


Once we click on that button, the message "Analyzing" appears on the screen. Do not move the counting chamber while the message is onscreen.

We recommend taking 5 snapshots from 5 different fields with at least 500 sperm. In the case of not reaching 500 sperm in the counting, it is recommended to take additional captures till such value is obtained so the assessment of concentration and morphoanomalies of the ejaculate is significant and unbiased.

To perform the evaluation, you can also press the letter "m" on the keyboard, instead of the "Morphometry and concentration analysis" button.

As you take pictures, the total number of sperm counted in the concentration and morphometry section will increase.



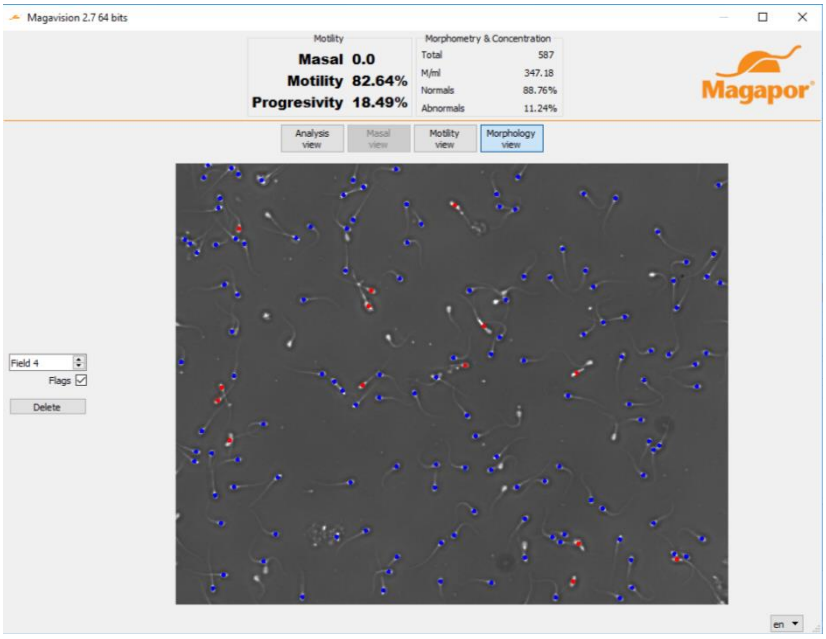
** It is recommended to perform the analysis in the central part of each analysis field*

** Avoid areas with a high level of agglutination or dirt*

On the section of Morphomety and concentration, you see 4 values:

- Total: Nº of spermatozoa counted
- M/ml: Total concentration of spermatozoa in million per ml
- Normal: percentage of normal spermatozoa in the sample
- Abnormal: percentage of abnormal spermatozoa in the sample (proximal droplets, distal droplets and bent tail).

Once the images have been taken, you can check the images used by Magavision to estimate concentration and morphologies. To do so, click the "Morphology view" button located on the upper part of the Magavision screen.



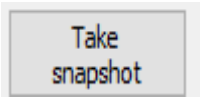
In this section, the software marks each spermatozoa with 2 colours

Normal spermatozoa

Abnormal spermatozoa

You can remove and set the marks for each type of sperm by removing the "tick" next to the marks, navigate through the different fields captured by clicking on the arrows and delete any of the fields. When you delete a field, the program recalculates the values of concentration and abnormal forms using the remaining fields.

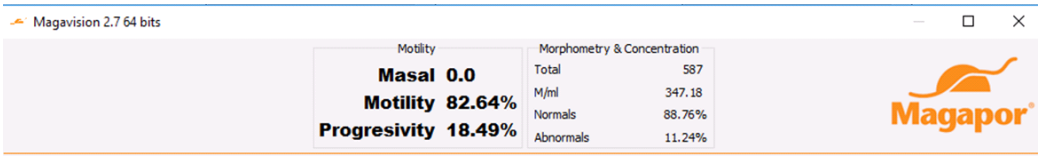
Magavision offers the possibility of taking photos. For this, use the button in the lower left part of the Magavision screen named "take snapshot." The program takes a photo that can be saved in any folder.



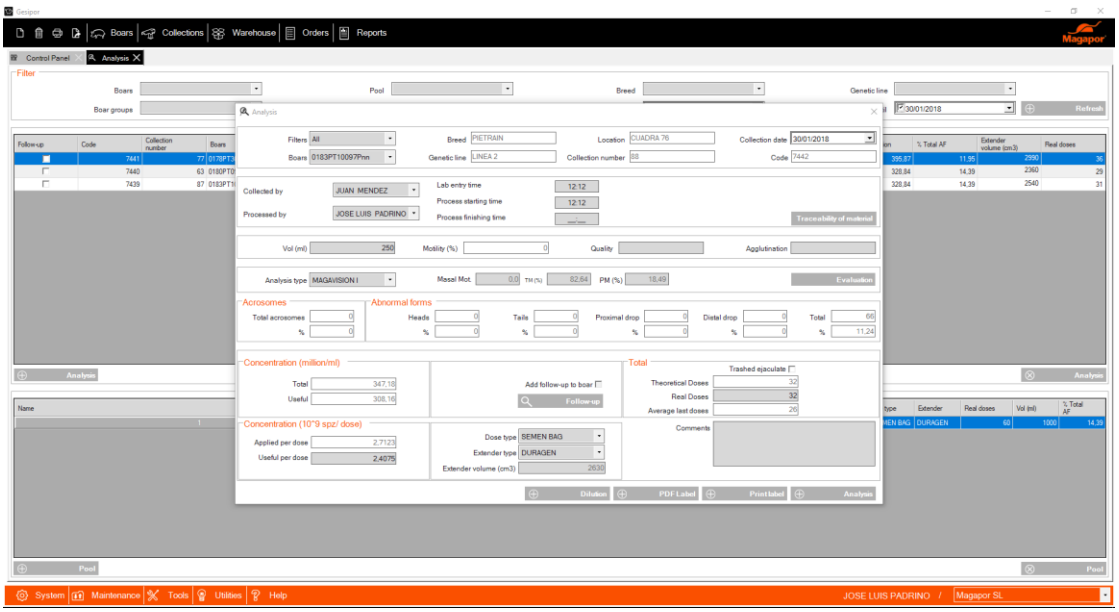
You can load the pictures taken by clicking on the "Analysis of Morphometry and Concentration (image) button.

To return to the main screen, click on "Analysis View".

Once the analysis is finished, close the Magavision screen using the X on the right upper part of the screen.



The collection detail fields will be automatically filled and show the number of doses that can be produced from the ejaculate considering the parameters previously introduced in the GESIPOR 3.0 control panel.



This screen shows the evaluation data loaded with Magavision I, the number of theoretical and real doses to be produced and the extender volume needed, as well as total and useful concentration according to the values configured in the control panel of Gesipor 3.0.

The fields number of real doses to be produced and concentration x useful dose can be modified manually. These parameters will also change if you select a different configuration in "Type of dose," because the new dose type will be configured with different values of concentration and volume .

Evaluation of seminal doses

Magavision can analyze seminal doses within the quality control established by the boar stud. The parameters to be controlled are motility, concentration and abnormal shapes. To do this, fill the counting chamber with 3.5 µl of the seminal dose and perform the analysis just as in the estimation of semen doses per ejaculate.

Magavision will express the value obtained in spermatozoa x 105/ml.

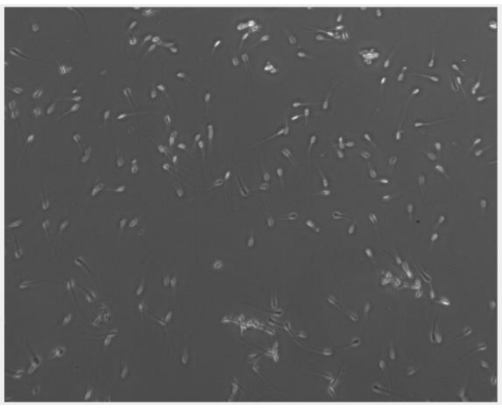
**Before evaluating the sample, temper it at 37 ° for at least 5 minutes.*
(Exact time may vary depending on the extender used).

Troubleshooting:

While working with Magavision, the reading of the image may be affected by some issues.

For example:

Microscope centered incorrectly: Spermatozoa are out of focus and the image is analyzed incorrectly. Center the equipment following the steps in the user manual enclosed with the equipment.

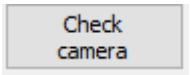


Incorrect counting of sperm: if the systems fails to detect more than 5% of the sperm (whether in motility, concentration or abnormal shapes) because the image is not focused correctly, focus the image again using the macrometric and micrometric knobs.

On the Magavision screen, the area where the sample is visualized blinks: light is not correctly regulated. Check the aperture of the diaphragm, the condenser flange and the light intensity knob are fully open.

On the Magavision screen, the area where the sample is visualized appears white: Magavision has been opened twice. Close one and the image will be restored.

The message "camera not detected" or Camera no Responding appears on the screen: click the "Check Camera" button.



When parts of the demarcation lines of the counting chamber are displayed on the screen, move to another area because the analysis is not being correctly performed. It is recommended to take the images for the analysis in the central part of the counting chamber.

Bad sperm count is higher than 5 % due to contamination in the counting chamber. It is essential to keep the material and microscope in proper cleaning conditions and keep them away from high levels of humidity.

It is essential to keep the micropipettes properly calibrated and pipette the samples correctly to prevent sampling errors.

** Reminder, how to micropipette: Press till the first stop of the micropipette plunger and, holding this position, insert the tip into the liquid to be absorbed (ejaculate, extenders, 1:10 dilution or seminal dose) (if the liquid contains spermatozoa, remember to homogenize it first.), Release the plunger finger of the micropipette slowly to take the correct volume.*

Image freezes during the motility analysis: The image is frozen and the equipment does not respond. Restart the system to resume the work with Magavision. This occurs when the sample moves during the analysis. Do not move the microscope stage while the words "recording" or "analyzing" appear on the screen.

Recommendations for the correct use of the equipment

Connected to a stable power source:

- Microscope
  - Input: AC100 = 240V 50/50 Hz
  - Ouput: LED 3W
  - Fuse: 3.15 A

- Heatable stage thermostat
  - Input: 100-240 Vac, 2.0A 50-60 Hz
  - DC OUTPUT: 19 V - 4.74 A

- CPU:
  - Input: 110-220 V

Avoid exposure to environments with relative humidity over 60-65% and dust.
Clean the equipment periodically with 70% ethanol and paper that does not scratch or leaves fibers.

Protect the equipment with the cover after each work day.

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