



Minitube photometers

Precision in ejaculate concentration measurement

Rudolf Grossfeld, PhD

Minitube offers different devices to measure the sperm concentration in a given ejaculate from several species in a semen laboratory. Among these measurement devices are the SDM 1 and SDM 6. The measuring principle of both photometers is based on analysing the turbidity of an ejaculate sample. The turbidity of an ejaculate increases with increasing semen concentration.

Working principle of photometers

The measurement unit of Minitube's SDM 1 and SDM 6 consists of a light source, an optical filter, a holder for the sample and a light detector. This set up is typical for a photometer.

The light source emits light which is reduced to a certain wave length in the optical filter and is then sent through the semen sample. The light detector measures the amount of light that reaches the detector after passing through the sample.

When the light passes through the sample the amount of light is reduced due to the turbidity of the sample. This fact is based on the Beer-Lambert law. That means a higher concentrated semen sample takes more light away than a lower concentrated semen sample does. Both samples have different turbidity and they reduce the amount of light differently. This reduction of light is called extinction. The precision of the reduction of light in dependency of the turbidity of a sample is based on the sample characteristics and the wave length of the light. For semen concentration measurement a wave length of 546nm has been proven to result in the most precise measurement.

If the amount of light that reaches the optical sensor without any turbidity (the zero value) is known then a photometer can calculate the loss of light, when comparing the zero value and the value for the sample. The turbidity is correlated to the extinction value. If the turbidity and the extinction value of a semen sample with a certain concentration is known, it is possible to calculate the semen concentration of the sample based on the turbidity of a sample.

Fortunately we can teach the photometer these values.

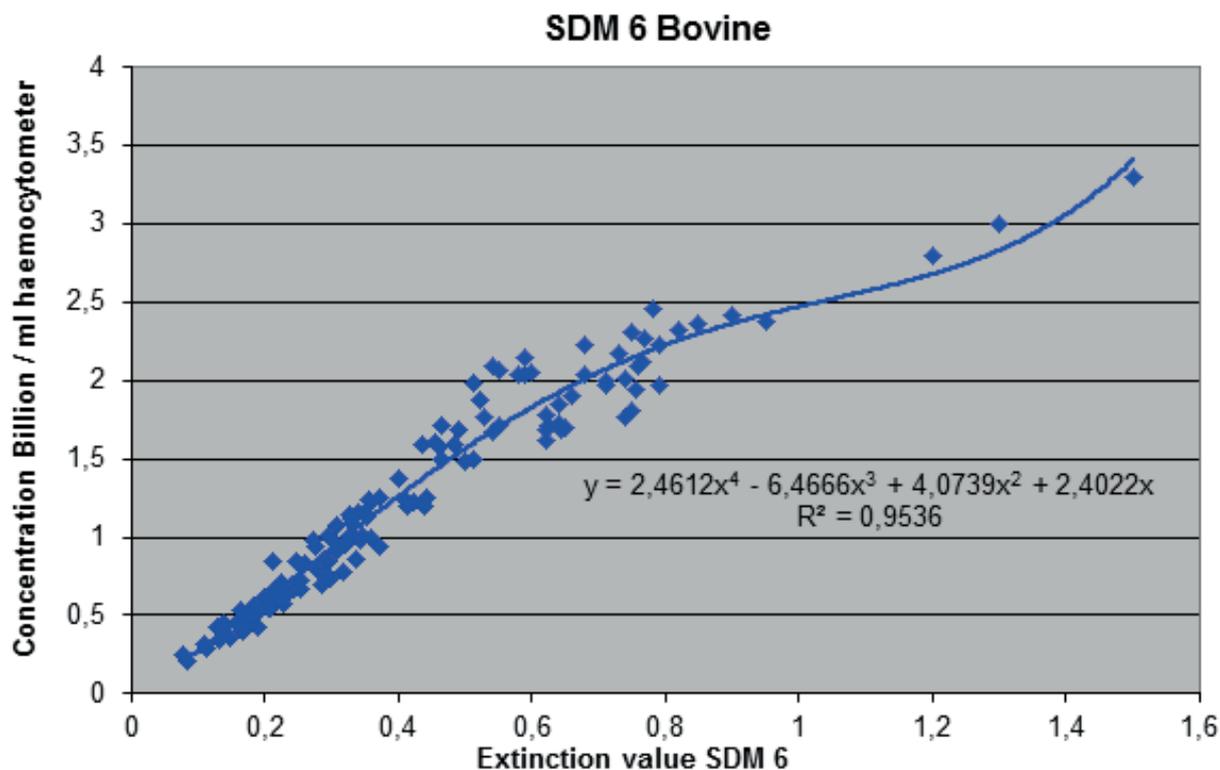
Calibrating the underlying curve for photometers

For this purpose the sperm concentration of several ejaculates from an individual species are measured for sperm concentration i.e. with a haemocytometer. The same semen sample is measured with the photometer SDM 1 and/or SDM 6 and the extinction value is recorded. This calibration procedure is repeated many times with different ejaculates until enough examples are available in all concentration ranges for this species. According to these values a correlation is calculated. An example graph and correlation curve is shown in Graph 1.



These tests require significant work. Minitube assigns them to independent, specialized institutes of Universities or other research facilities. For each species individual curves need to be calibrated.

Based on the correlation, a mathematical equation can be determined that is programmed into the SDM 6. From this equation the SDM 6 will calculate the semen concentration value from the measured extinction reading. The procedure for the SDM 1 is exactly the same.



Graph 1: Example Calibration SDM 6

Exactness of the correlation

After the initial calibration of the SDM 6 or SDM 1, the device can calculate the sperm concentration solely based on the extinction of a semen sample by using the equation. The correlation of extinction and corresponding semen concentration is not linear. That means a two-fold turbidity (=extinction) in the semen sample does not indicate a two-fold semen concentration. Therefore the calculation of the SDM 6 is based on a fourth degree equation. This equation is shown as formula and curve in Graph 1 and represents the correlation of extinction and corresponding semen concentration much better than a linear correlation.

A statistical value, the so called coefficient of determination (R^2) indicates how well data values fit into a line or curve. A 100% fit would result in a value of $R^2 = 1$. The R^2 of the SDM 6 curve for bovine for example is 0,9536 (Graph 1).

Important technical terms

Turbidity: Cloudiness or haziness of a fluid caused by the characteristics of the fluid.

Extinction: The loss of light (absorbance), when a light beam passes through a sample in a photometer. Caused by turbidity of a fluid.

Correlation: Statistical dependence of two variables. i.e. the turbidity and the semen concentration of an ejaculate sample are highly correlated.

R^2 : Statistical value describing how well data points fit a line or curve. Value is used to describe the quality of a correlation. The closer to 1 the better the correlation of a calculated curve with the data points.

Conclusion

Minitube's SDM 1 and SDM 6 photometer are calibrated by independent specialized institutes. The ejaculate samples taken to calculate the basis for semen concentration measurement cover the whole range of semen concentrations of an individual species. Higher equations are the basis for semen concentration measurements in order to get the highest possible correlation between extinction and semen concentration measurement. All this results in a very high precision of the semen concentration results measured with the SDM 1 and SDM 6.