



Validation Report – Sperm Vision® Automorph

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Sperm Vision® is widely used in semen production labs for the precise and objective evaluation of motility and sperm concentration.

Sperm Vision® Automorph allows to complete a full semen analysis from sample preparation to the storage of data in the PC in less than 1 minute, including the analysis of motility, sperm concentration and the occurrence of proximal and distal droplets as well as highly bent tails.

The aim of this study was to compare the results of the morphological analysis of boar semen performed by Sperm Vision® Automorph with the results obtained by trained andrology lab personnel.

This validation study was done at the Veterinary University Hannover from March to July 2011 in two parts.

In the first part, the morphological analysis of Sperm Vision® Automorph was scrutinized on a “per cell basis”. On the monitor from 48 semen samples, every sperm cell shown on the screen in 4 analysis fields for each sample was correctly identified and morphologically classified. A total of 12.685 sperm cells were evaluated.

In the second part, the morphological analysis of Sperm Vision® Automorph was compared on a “per sample basis” with the results obtained by a subjective wet mount analysis with phase contrast microscopy in 210 semen samples.

Sperm Vision® Automorph was equipped with a 20x negative phase contrast objective and a 0.63x camera adapter to obtain a total magnification of 12.6x. The semen samples were prepared with a 10µm counting chamber. Four analysis fields were used to analyse the occurrence of morphological defect sperm cells. The morphological analysis was performed on each of the 30 frames per analysis field in order to also capture all the motile sperm cells as potential droplet carriers. Sperm Vision® Automorph analysed 307 +/- 104 spermatozoa per sample of extended semen, totalling a number of 64.377 analysed sperm cells in this trial. In a semen production lab, the dilution rate for the raw semen samples is adjusted to obtain a morphological assessment of the sample based on 600 sperm cells.

The reference system “wet mount” was performed with sperm samples fixed in formol citrate which were evaluated under phase contrast microscopy at 1000x magnification under oil. 200 sperm cells per ejaculate were checked for morphological defects, resulting in a total of 41.800 sperm cells for 210 ejaculates. All assessments were carried out by an experienced lab assistant.

The comparison of the morphological evaluations was focused on the following secondary morphological defects: proximal droplets, distal droplets and highly bent tails.

I: Results of the single cell validation

Definitions for the following table:

- **Sensitivity** shows the percentage of morphologically abnormal sperm cells marked by Sperm Vision® Automorph as proportion of the total number of morphologically abnormal sperm cells.
- **Specificity** shows the percentage of morphologically normal sperm cells marked by Sperm Vision® Automorph as proportion of the total number of morphologically normal sperm cells.
- **The positive predictive value** shows the percentual proportion of sperm cells identified by Sperm Vision® Automorph as morphologically abnormal which are correctly diagnosed.
- **The negative predictive value** shows the percentual proportion of sperm cells which are correctly identified by Sperm Vision® Automorph as morphologically normal.

	SV Automorph: morphologically abnormal cells	SV Automorph: morphologically normal cells	Sum
Expert classification: abnormal cells	2.827	587	3.414
Expert classification: normal cells	366	8.788	9.154
Sum	3.193	9.375	12.568

Sensitivity	82.8 %
Specificity	96.0 %
positive predictive value	88.5 %
negative predictive value	93.7 %

Table 1: Overall conformity between Sperm Vision® Automorph and expert analysis on a single cell basis comparison

Evaluating the Sperm Vision® Automorph results on a per cell basis, the automated analysis correctly identifies 83 % of the morphologically abnormal sperm cells and 96 % of the morphologically normal sperm cells.

II. Results of the sample analysis validation

For the purpose of this validation study semen quality criteria established by the Association of German Pig Producers (ZDS, 2005) were used to decide whether a sample passes or fails quality control. In this study total morphological abnormalities are calculated as an addition of sperm cells with proximal or distal droplets and the sperm cells with bent tails.

Total morphological abnormalities	≤ 25 %
Sperm cells with head abnormalities	≤ 5 %
Sperm cells with acrosome abnormalities	≤ 10 %
Sperm cells with plasma droplets	≤ 15 %
Sperm cells with bent tails	≤ 15 %
Other morphological abnormalities	≤ 15 %

Table 2: Minimum morphological requirements for boar semen (ZDS, 2005)

A sample was correctly diagnosed for the occurrence of droplets and bent tails, when both the Sperm Vision® Automorph and the wet mount analysis resulted in either more than 20 % abnormalities (total number of abnormalities), or less than 15 % for each of the defects analysed. The difference between the 25 % minimum requirement for total morphological abnormalities and the 20 % threshold used in this validation study served as a safety buffer in case other morphological abnormalities than those evaluated by Sperm Vision® Automorph would be found.

Definitions for the following table:

- **Sensitivity** shows the number of morphologically not acceptable semen samples classified by Sperm Vision® Automorph as a percentual proportion of the total amount of morphologically not acceptable semen samples.
- **Specificity** shows the number of morphologically acceptable semen samples classified by Sperm Vision® Automorph as a percentual proportion of the total amount of morphologically acceptable semen samples.
- **The positive predictive value** shows the percentual proportion of semen samples classified by Sperm Vision® Automorph as morphologically abnormal which were correctly diagnosed.
- **The negative predictive value** shows the percentual proportion of semen samples classified by Sperm Vision® Automorph as morphologically acceptable which were correctly diagnosed.

	SV Automorph: sample with > 20% morphologically abnormal cells	SV Automorph: sample with < 20% morphologically abnormal cells	Sum
Wet mount: abnormal cells > 20%	99	20	119
Wet mount: abnormal cells < 20%	11	80	91
Sum	110	100	210

Sensitivity	83.2 %
Specificity	87.9 %
positive predictive value	90.0 %
negative predictive value	80.0 %

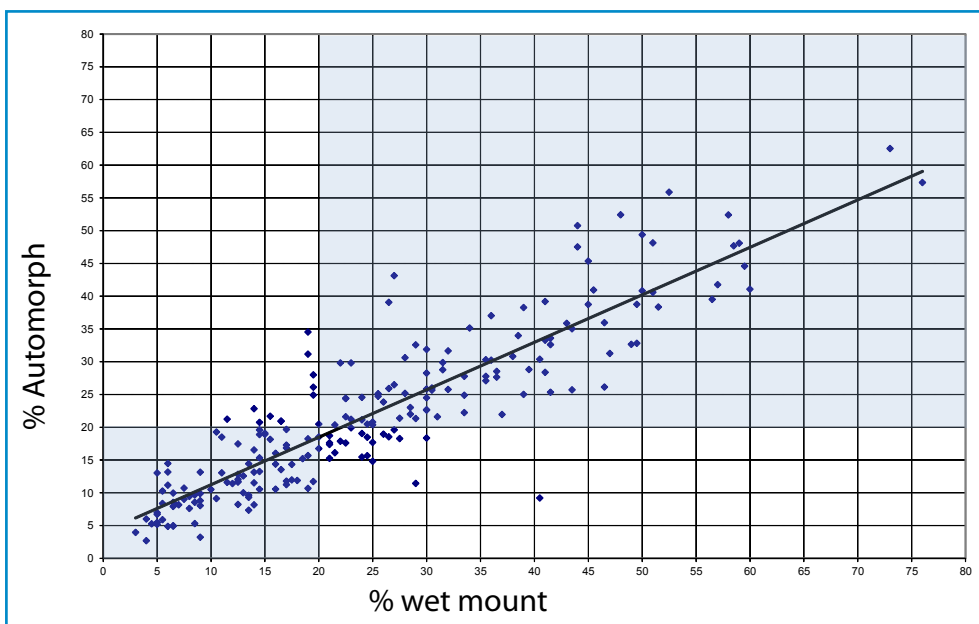
Table 3: Overall conformity between SV Automorph and expert analysis on a sample basis comparison

When applying a threshold of 20% total morphological abnormal sperm cells per sample as maximum acceptable level, Sperm Vision® Automorph correctly detected 83% of the samples which had more than 20% abnormal cells according to the wet mount analysis.

90% of the samples detected by Sperm Vision® Automorph as morphologically not acceptable were confirmed by the wet mount analysis.

When applying a threshold of 20% total morphological abnormal sperm cells per sample as maximum acceptable level, Sperm Vision® Automorph correctly detected 88% of the samples as morphologically acceptable.

80% of the samples detected by Sperm Vision® Automorph as morphologically acceptable semen samples were confirmed by the wet mount analysis.

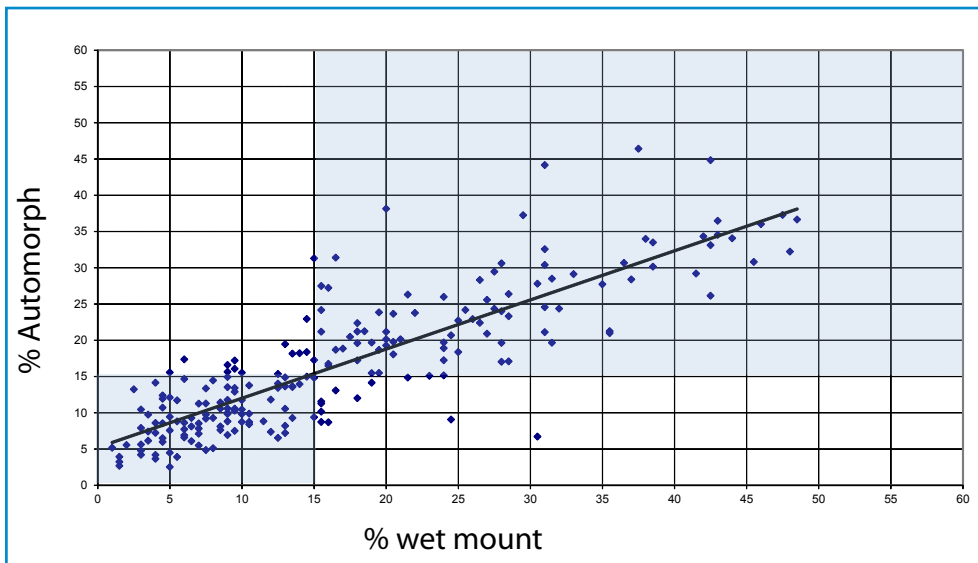


Graph 1: Sample classification according to total morphological abnormalities (proximal droplets, distal droplets and bent tails)

During this validation study, 105 out of 110 ejaculates classified as “bad” by Sperm Vision® Automorph, were confirmed by the wet mount analysis with a minimum of 19 % morphologically abnormal sperm cells. Only 5 ejaculates with good semen morphology would have been discarded by Sperm Vision® Automorph.

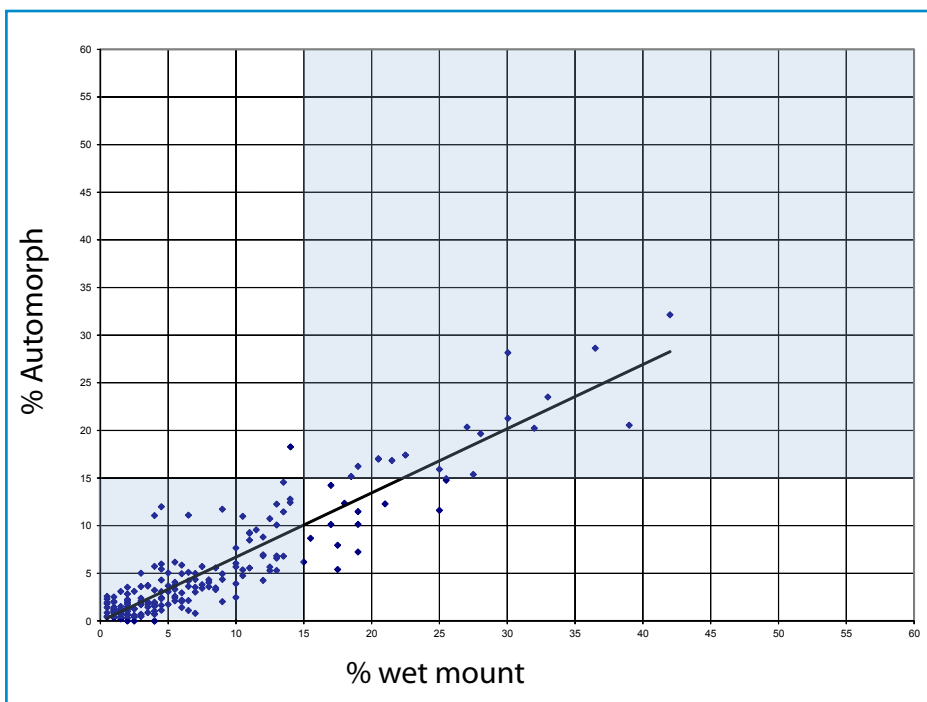
On the other hand, 98 out of 100 ejaculates classified as “good” by Sperm Vision® Automorph either were confirmed by the wet mount analysis of being within the maximum of 20 % morphologically abnormal sperm cells (80 ejaculates) or were within a tolerable range from 20 % to 29 % morphologically abnormal sperm cells. Only 2 ejaculates out of 100 with clearly unacceptable semen quality would have passed into production.

The validation study also included a detailed investigation into the accuracy Sperm Vision® Automorph achieved within the 15 % threshold for each of the individual morphological abnormalities.



Graph 2: Sample classification according to proximal and distal droplets only

When applying the threshold of 15 % sperm cells per sample with a proximal or distal droplet as maximum acceptable level, 86 % of the samples analysed by Sperm Vision® Automorph as not acceptable for this parameter were confirmed by the wet mount analysis.



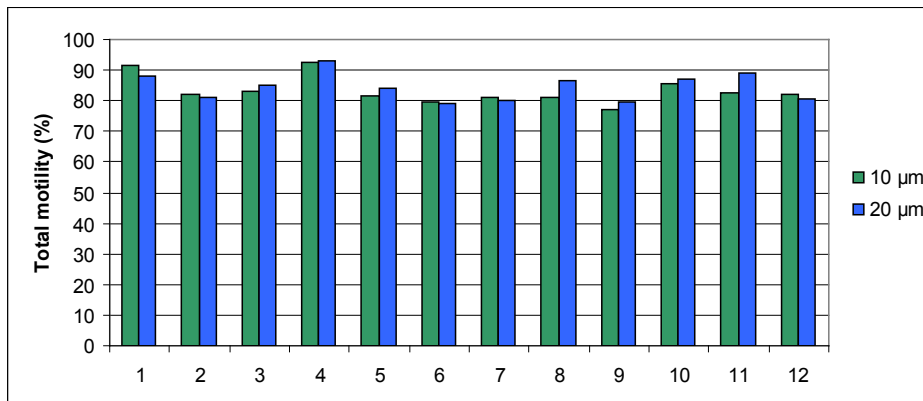
Graph 3: Sample classification according to bent tails only

When applying the threshold of 15 % sperm cells per sample with a bent tail, 95 % of the samples analysed by Sperm Vision® Automorph as not acceptable for this parameter were confirmed by the wet mount analysis.

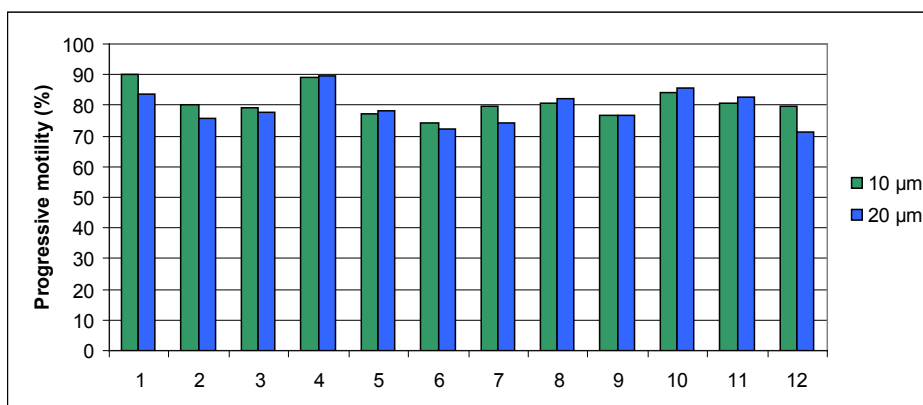
III. Motility and semen concentration

Sperm Vision® Automorph is designed to perform morphological analysis of highly motile semen samples with up to 200 sperm cells per frame. Good focus quality and a sample depth of 10µm are recommended to obtain good results. Therefore a 10µm counting chamber was used for this validation study. Since all other Sperm Vision systems for motility and semen concentration measurements typically use a 20µm counting chamber, the effect of a 10µm sample depth on motility parameters and cell distribution in the counting chamber was investigated. Six analysis fields were used for the analysis of concentration. For comparison of motility measurements between different counting chambers ten analysis fields were used. The reference system for the semen concentration measurement was a Thoma "neu" counting chamber.

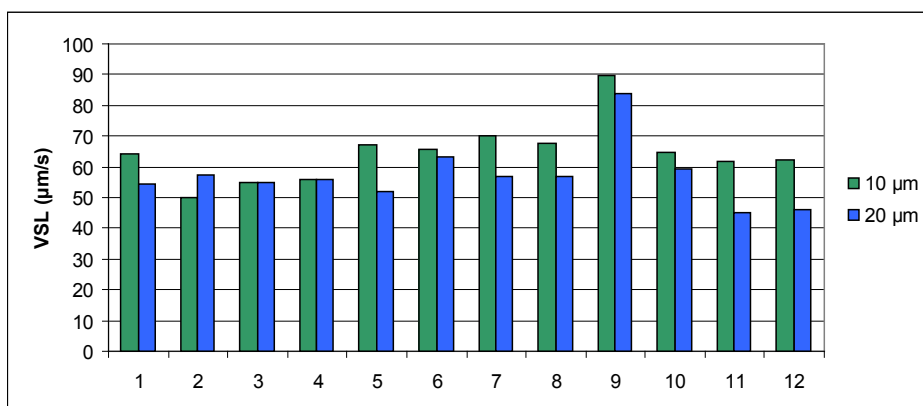
12 ejaculates were analysed for total motility, progressive motility and VSL (Velocity straight line, µm/sec). No significant differences for total and progressive motility could be observed ($p > 0.05$). The average VSL of spermatozoa tended to be higher in chambers of 10µm depth ($p < 0.05$).



Graph 4: Total motility of extended boar semen analysed in a 10µm and a 20µm counting chamber (n=12).

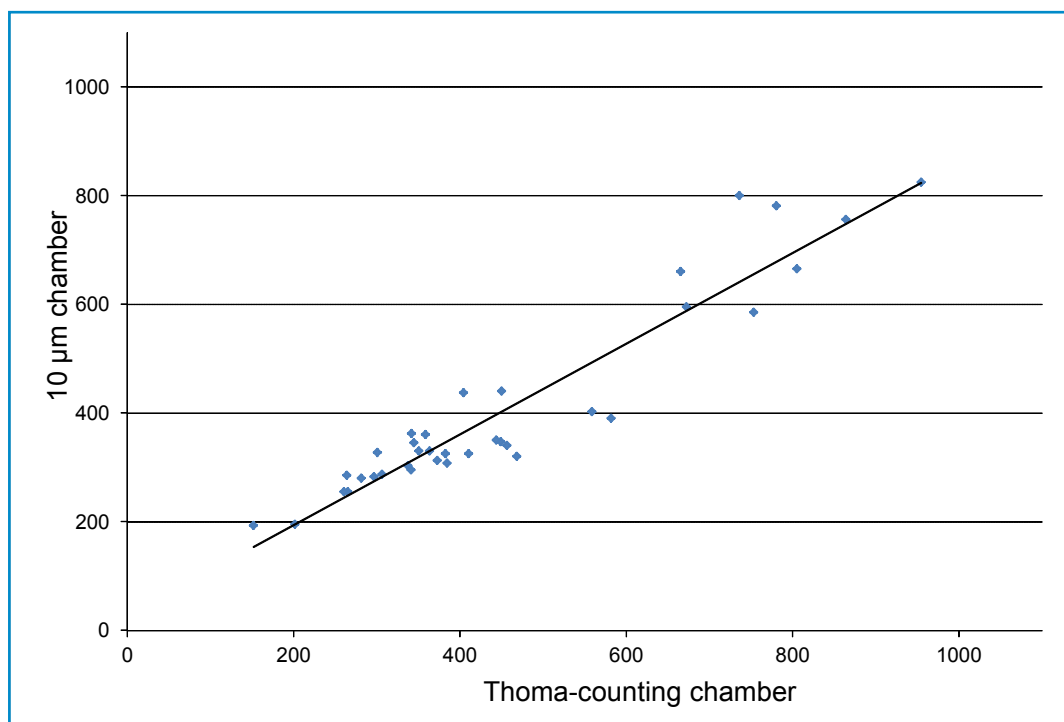


Graph 5: Progressive motility of extended boar semen analysed in a 10µm and a 20µm counting chamber (n=12).



Graph 6: VSL (velocity straight line) of extended boar semen analysed in a 10µm and a 20µm counting chamber (n=12).

The semen concentration measurements comparing the Thoma counting chamber with a 10µm counting chamber show very high correlations.



Graph 7: Semen concentration measurements in a Thoma counting chamber and a 10µm counting chamber in 10^6 x sperm cells/ ml (n=24).

Ejaculates with a semen concentration between 150 and 600 million sperm cells/ml raw ejaculate can be measured with the standard dilution rate of 1+9, which was used to prepare the samples for the Sperm Vision® Automorph analysis in this study. Ejaculates with higher semen concentration should be diluted further in order to provide optimal readings with a 10µm counting chamber.

Conclusions:

Sperm Vision® Automorph provides a fast and reliable semen analysis, including motility, semen concentration and morphology with only one sample. It is the first CASA system enabling boar semen production labs to automatically analyse each ejaculate for morphological fitness, before passing it on to the production process.

Sperm Vision® Automorph provides motility as well as semen concentration measurements, which correlate very well with the reference standards.

When applying a threshold of 20 % total morphological abnormal sperm cells per sample as maximum acceptable level, Sperm Vision® Automorph showed a sensitivity of 83,2 % and a specificity of 88 % according to the wet mount analysis of the samples.

During this validation study, 105 out of 110 ejaculates classified as “bad” by Sperm Vision® Automorph were confirmed by the wet mount analysis with a minimum of 19 % morphologically abnormal sperm cells. Only 5 ejaculates with good semen morphology would have been discarded by Sperm Vision® Automorph.

On the other hand, 98 out of 100 ejaculates classified as “good” by Sperm Vision® Automorph were confirmed by the wet mount analysis of being within the maximum of 20 % morphologically abnormal sperm cells (80 ejaculates) or being within a tolerable range from 20 % to 29 % morphologically abnormal sperm cells. Only 2 ejaculates out of 100 with clearly unacceptable semen quality would have passed into production.

Sperm Vision® Automorph provides a very effective and easy-to-use quality control tool for the analysis of boar semen, identifying over 98 % of the ejaculates with critical morphology problems (> 30 % abnormal sperm cells). 100 % morphology screening of all collected ejaculates prior to production and distribution will help to optimize boar management, improve the quality of the produced semen doses and support reproductive performance of the sow farms.

