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Alternative sample preparation of boar ejaculates for the analysis with AndroVision®

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Correct sample preparation is essential for obtaining accurate and precise concentration measurements and motility analyses. One step of sample preparation is the pre-dilution of the ejaculate, in which a subsample is mixed with extender at a defined ratio.

AndroVision® calculates sperm concentration based on the dilution factor entered in the software. Therefore, it is crucial that this setup accurately reflects the actual dilution, which is affected by any deviation in the pipetted volumes.

Additionally, concentration readings are influenced by the number of cells per field. Too few cells reduce the statistical reliability, while too many cells can cause overlapping, hinder accurate counting, and negatively affect motility assessment. Thus, the recommended sperm count per field is 200 – 500 cells.

This report presents an alternative method for the sample preparation in a boar semen production lab for use with the AndroVision® system with 20 µm counting chambers. The method is based on the use of eFlow sample containers. Compared to the traditional approach, the alternative preparation offers several practical and analytical advantages:

- Higher sample volume
  - Reduced pipetting errors due to higher accuracy and precision (see example)
  - Lower sensitivity to temperature fluctuations during handling
- Container design
  - Optimized geometry ensures more efficient mixing of the sample

Picture 1: Multipette® with Combitip® 50 ml

Picture 2: Electronic mixing pipette

Comparison of sample preparation methods

The following describes the procedures for conventional and alternative methods.

Step-by-step workflow, example with a 1+9 dilution:

	Current preparation	Alternative preparation
Prewarming	Prewarm all materials, extender, and microscope stage to 38 °C	
Ejaculate mixing	Invert 5 times	
Pipetting extender	810 µl with electronic mixing pipette	6300 µl with Multipette®
Pipetting ejaculate	90 µl with electronic mixing pipette	700 µl with electronic mixing pipette
Air bubble	Pull up air bubble	
Tip cleaning	Wipe pipette tip	
Mixing	Prediluted sample mixed in vial with pipette function	Ejaculate and extender mixed in eFlow container with pipette function
Mixing	Mix vial 5 times by inversion (no shaking)	Mix container 5 times by inversion with plug (no shaking)
Load chamber	Pipette ~3 µl into counting chamber	
Measurement	Perform analysis with AndroVision® within 60 seconds	

Dilution

Recommended dilution rates and volumes, to obtain 200 – 500 sperm cells/field:

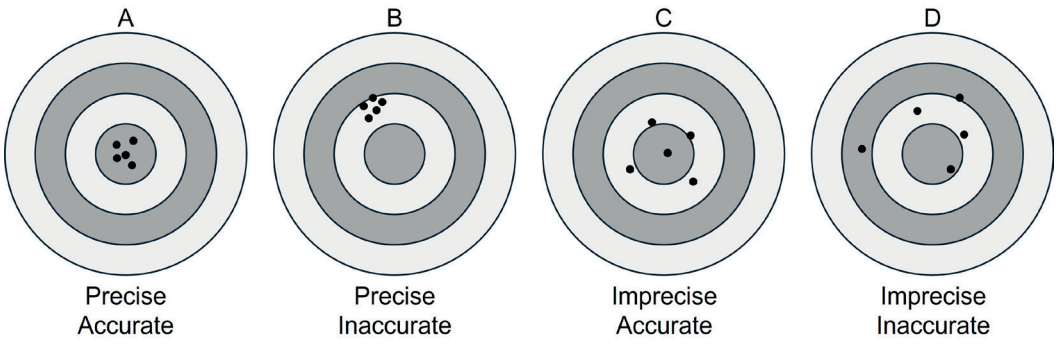
Dilution rate	Raw concentration Min - Max (million/ml)	Volume semen (µl)	Volume extender (µl)
1+4	120 – 300	1500	6000
1+6	165 – 420	1000	6000
1+9	240 – 600	700	6300
1+12	320 – 780	500	6000
1+19	500 – 1200	350	6650

Pipetting accuracy

When preparing samples from raw ejaculates, the accuracy and precision of the pipettes are critical, especially for small sample volumes.

The quality of a pipette is defined by two parameters, the accuracy and the precision. Accuracy describes how close the delivered volume from the pipette is to the true or target volume. A highly accurate pipette delivers liquid very close to the set volume.

Precision describes how consistent the pipette is when you use it repeatedly under the same conditions. A highly precise pipette delivers nearly the same volume each time, even if it is slightly off from the true value.



Picture 3: Illustration of precision and accuracy in pipetting. (A) Precise and accurate measurements are tightly clustered around the true target. (B) Precise but inaccurate measurements are consistent but systematically offset. (C) Imprecise but accurate measurements vary widely but average near the true value. (D) Imprecise and inaccurate measurements are scattered and far from the target.

Using the electronic mixing pipette and the Multipette® as an example, the manufacturer's specifications of accuracy and precision vary depending on the target volume.

Electronic mixing pipette, 1 ml	Accuracy (%)	Precision (%)
90 µl	> ±3 %	> ±0.6 %
700 µl	±0.8 %	±0.18 %
810 µl	±0.8 %	±0.17 %

Electronic mixing pipette, 2 ml	Accuracy (%)	Precision (%)
90 µl	> ±3 %	> ±0.6 %
700 µl	±1.6 %	±0.35 %
810 µl	±1.3 %	±0.3 %

Multipette ® with 50 ml tip	Accuracy (%)	Precision (%)
6000 µl	±0.3 %	±0.5 %

Multipette ® with 10 ml tip	Accuracy (%)	Precision (%)
6000 µl	±0.4 %	±0.25 %

This demonstrates that pipetting smaller volumes introduces a higher relative error, which should be considered when designing sample preparation protocols. Using larger volumes for initial dilution reduces pipetting errors, improving measurement reliability with AndroVision®.

Example calculation of the effect of pipetting errors

An ejaculate with a raw concentration of 421 million/ml corresponds to 350 sperm per field, assuming ideal pipetting accuracy in a 1+9 dilution.

The potential impact on the final concentration measurement can be demonstrated by assuming that all pipettes operate at their maximum error in terms of accuracy. To introduce the maximal possible error, the two pipettes deviate from the target volume in opposite directions, i.e., the pipette for the raw ejaculate pipettes more, the pipette for the extender less.

In conventional preparation, the target volumes are 90 µl of ejaculate and 810 µl of extender. With maximal pipetting errors of +3% and -0.8%, respectively, this corresponds to 92.7 µl of ejaculate and 803.52 µl of extender, leading to an overestimation of the concentration by +3.71%.

When larger pipetting volumes are used, as suggested in the alternative preparation using eFlow sample containers, this overestimation is reduced. For example, instead of 700 µl of raw ejaculate the pipetted volume may be 705.6 µl, and instead of 6300 µl of extender the actual volume may be 6281.1 µl. In this case, the concentration is overestimated by only +1.1%, which represents the maximal possible error due to pipette accuracy.

Sample preparation	90 + 810	700 + 6300
Max. error raw ejaculate	90 µl + 3% = 92.7 µl	700 µl + 0.8% = 705.6 µl
Max. error extender	810 µl – 0.8% = 803.52 µl	6300 µl – 0.3% = 6281.1 µl
Instead of 350 sperm/field	363 sperm/field	354 sperm/field
Instead of 420.84 million/ml	436.47 million/ml	425.65 million/ml
Overestimation of concentration	+ 3.71 %	+ 1.10 %

If the maximal possible errors happen in the other direction (pipette for the raw ejaculate pipettes less, the pipette for the extender more), the measured concentration is underestimated by 3.86% for the conventional sample preparation and by 1.16% for the alternative method.

Conclusion

The alternative sample preparation method using eFlow containers demonstrates clear advantages over the conventional approach. By employing larger pipetting volumes and improved container geometry, this method significantly reduces pipetting errors and ensures more reliable concentration measurements with AndroVision®, which makes it the recommended method for porcine AI centers.

Required materials for the alternative method

- 12510/0200 - Stand for 5x eFlow sample container for warming plate
- 12510/0100 - Sample container for eFlow
- 12510/0101 - Plug for sample container (reusable)
- 12427/5065 - Multipette® E3
- Combitip® for Multipette®, 2 options:
  - 12427/5067 - Combitip® for Multipette®, 10 ml
    OR
  - 12427/5066 - Combitip® for Multipette ®, 50 ml
- Electronic mixing pipette, 2 options:
  - 12050/0516 - Electronic mixing pipette, 0.1 – 1 ml
  - 12050/0512 - Pipette tip 0.1-1 ml, 1000/bag
  - 12050/0513 - Pipette tip 0.1-1 ml, 96/rack
    OR
  - 12050/0517 - Electronic mixing pipette, 0.2 – 2 ml
  - 12050/0554 - Pipette tip 0.2-2 ml, 1000/bag
  - 12050/0555 - Pipette tip 0.2-2 ml, 60/rack

Picture 4: Sample container for eFlow