

Frozen-thawed/Fresh semen quality analysis (QA)

BY AVARE BIOTECH APP PROTOCOL

Material

Frozen-thawed bovine/equine/ovine semen (3-5 straws from different animals)

Goal

To verify that the app correctly measures semen concentration, total motility, and progressive motility compared to CASA and manual counting.

Tasks

1. Assess three key parameters:	2. Compare results between:
Concentration Total motility Progressive motility	Avare App (MAKSA technology) CASA (e.g., AndroScope, Isperm, etc.) Manual microscope counting

Steps

1 Thawing

- Place a straw in 37 °C water bath for 30–45 sec, dry, gently shake.
- Work quickly: insemination should be performed within ≤ 10 min after thaw.

2 Preparation

- Pre-warm a normal lab or a CASA slide (if you are rich enough) on a heating stage (37–38 °C).

3 Sampling

- Check your cover slip size
- Choose the semen volume based on the table below

Standard Cover-slip Dimensions, mm	Semen Volume, μl
10×10	2
12×12	3
15×15	4.5
18×18	6.5
20×20	8
22×22	10

Pipette only recommended volume μL from the straw and load on the slide (avoid bubbles) using a Microliter Volume Pipettor

4 Testing

- Record 5 videos while moving the slide via microscope according to the instruction of Avare Biotech
- Here is the link to 1 minute video: <https://www.youtube.com/shorts/CdFFHB0CZUg>
- Run the same sample through CASA system on CASA slides (for accuracy if available on site).
- Count sperm manually under microscope.

5 Data Recording

- Enter results for all three methods to a spreadsheet.
- Note agreements or discrepancies.

6 Conclusion

$\pm 10\text{--}15\%$ difference = acceptable validation.

$>15\%$ deviation — attach photo/video evidence and repeat with a new slide.

Example Report

Sample (Bull/Straw)	Parameter s/Method	Avare App (MAKSA)	CASA	Manual Count	Dif. %	Notes
Bull A – Straw 1	Concentration (M/mL)					
Bull A – Straw 1	Total Motility (%)					
Bull A – Straw 1	Progressive Motility (%)					

Practical Note

- Using 10 μL from a 0.25–0.5 mL straw does not prevent insemination if done immediately.
- Best practice in AI stations is to use the same straw taking one drop for Avare analysis and inseminating the rest (90%) right away.