

RACING SERVICES TASMANIA

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EXPLANATORY NOTES FOR SEMEN EVALUATION BY VETERINARIAN/AUTHORISED PERSON

COLLECTION

A bitch in season may or may not be available; to facilitate collection of the ejaculate but their presence often helps considerably. Sires approved for AI are less likely to require such motivation.

Collection should be made into a chemically clean, dry container, warmed to body temperature (38-39°C). Protect the fresh semen from excess light, heat and cold.

Semen evaluation should be commenced as soon as possible following the collection.

MATERIAL REQUIRED

- Chemically clean dry container.
- Warming facility for collection container (eg. Microbiology incubator; container of warm water; hot-air hair dryer; heat lamp).
- Microscope glass slides and cover slips – also warmed to body temperature for motility estimation.
- Pasteur pipettes, or AI pipettes for transfer of semen from container to slides. **Note** that the silicon lubricated plastic hypodermic syringes used for injections may be spermicidal and reduce the number and motility of live sperm, leading to an inaccurate evaluation.
- UNOPETTE for Red Blood Cell Estimation No 5851 – this is used for direct sperm count procedures.
- Standard Neubauer haemocytometer with coverslip.
- Microscope – to 400xhigh power.

EVALUATIONS

LIBIDO	-	Rate from one (poor) to three (good).
COLOUR OF SEMEN	-	Indicate findings of visual examination of fresh ejaculation.
MOTILITY	-	Using a warmed Pasteur pipette or AI pipette, transfer a drop of fresh semen to a warmed glass slide. Apply a cover slip and examine under the microscope using 400xhigh power. Record quality from 0-5 using the following guidelines.
0	=	Dead
1	=	Slow side to side – no forward progression
2	=	Slow side to side – forward progression in spurts
3	=	Rapid side to side – forward progression in spurts
4	=	Slow forward progression
5	=	Fast forward progression

Now estimate percentage live to dead sperm and record.

SPERM COUNT PER ML – USING UNOPETTE NO.5851 – (AS USED FOR RED BLOOD CELL ESTIMATION).

Open by inverting top and marking hole in container of fluid by downward push of cap. Fill capillary pipette with fresh semen by touching capillary pipette to surface of ejaculate. Remove excess semen from exterior of capillary pipette by wiping swiftly with a tissue. Squeeze Unopette container to remove some air, insert capillary pipette firmly, and release pressure on container so that the vacuum created draws the semen from the pipette into the container. Wash the solution up and down the pipette 3-4 times by gently squeezing and releasing the sides of the container. Remove the cap and squeeze it to empty the pipette into the container. Invert cap and push firmly into place to seal the container.

Rotate Unopette for 45-60 seconds to ensure even mixing of contents – **Do Not Shake.**

Invert and squeeze Unopette to remove 5-6 drops of fluid then proceed immediately to fill the clean dry haemocytometer chamber. If chamber overfills, repeat the process. Count the sperm in the four (4) corner squares – then add four zeros (0000) to the total number of sperm counted in the five (5) squares to give the total number of sperm per cubic millilitre.

ALTERNATIVELY – USING UNOPETTE NO.5855 – (AS USED FOR WHITE BLOOD CELL ESTIMATIONS).

Count the sperm in the central area of small squares on both sides of the Neubauer Hemocytometer, and divide the total by 2 to obtain the mean (average) of the two areas.

Multiply this figure by 1000 to obtain the number of sperm per cubic millilitre.

TOTAL SPERM COUNT

- equals sperm count per ml multiplied by volume of ejaculate in mls.

VOLUME OF EJACULATE

- as the motility assessment has already been completed, the volume measurement may be undertaken using a measuring pipette, a graduated test tube, or a 5ml standard hypodermic syringe. Record ejaculate volume to nearest 0.5ml.

GROSS AND DETAILED MORPHOLOGY

- return to the slide used for motility estimate, or, prepare a fresh similar slide. Count at least 200 sperm cells using 400x high power. Record normals and abnormal for each 100 cells counted. Record also the R.B.C. and W.B.C. for each of 4 separate fields at 400x magnification and record the average per field for each.

Record cellular debris seen over at least 4 separate fields as per R.B.C. and W.B.C.

VETERINARY/AUTHORISED PERSON ASSESSMENT

- a reasonable fertility rating should take into account a desirable requirement of 10 million sperm per pup; so that for a litter of 10 pups one should look to 100 million live normal sperm per ejaculate.

If the evaluation is unsatisfactory in any aspect, the procedure should be repeated in 48-96 hours and/or appropriate therapy be instituted to correct any underlying clinical problem.

REFERENCE: *Laboratory Procedures – Using The Unopette Brand System.*; Benton & Dickson & Co.

