ICAR Proficiency Test
on
Bull Semen (PT BS)

PREFACE

Bull semen analysis is one of the parameters used to assess bull fertility and conception rate in the female served. A robust quality system (QS) to analyse bull semen is strategic for Artificial Insemination (AI) organisations to provide a qualified product to the breeders and farmers. Furthermore, also the instrument manufacturers need QS to evaluate their products and different calibration settings.

To analyse bull semen, computer assisted semen analysis (CASA) and flow cytometry are largely used alongside the microscope method. Nowadays, there is no international standard method for bull semen analyses, consequently the criteria and performance of these tests have not been established. For this reason, it is necessary to collect data on the methods used and to provide tools for their analytical quality control.

ICAR has developed a new service of proficiency testing on bull semen matrix (PT BS) in order to:
- Assess the analytical comparability for different parameters between the different users (7.1.1)
- Provide each participant with feedback on their own objective performance and a robust tool for quality control over time (7.1.2)

SUBSCRIPTION

Please fill in the subscription form at the link below:

https://app.surveymethods.com/EndUser.aspx?A387EBF4A7E0FEF0A1

SAMPLES

SAMPLES ARRIVAL

At arrival, please check the level of liquid nitrogen, and make sure all straws are still frozen.

In case some straws are not frozen please, state it in the results area on the ICAR PT platform. Maintain the samples frozen at -196°C until use.

STRAW PRODUCTION

Semen is collected according to normal procedures and the required ejaculates are fully extended in a phospholipid-based medium to achieve a final concentration of resp. around 10, 15 and 20 x10^6 sperm cells per mini straw.

A clear media was used.

Semen straws will be filled, printed, and sealed before minimum cooling time. After an equilibration time at 4°C for at least 2 hours, the straws will be gradually cooled from 4°C to -140°C in a programmable automatic freezer for 7 minutes and then submerged and stored in liquid nitrogen at -196°C until use.
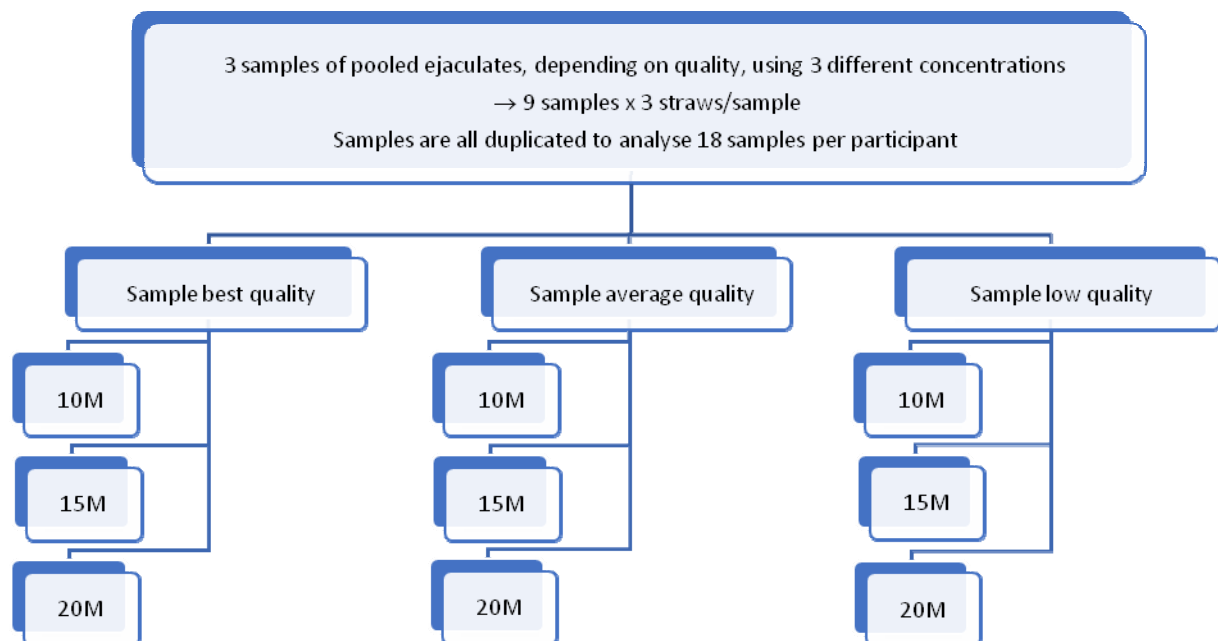
IDENTIFICATION

Each sample will be numbered from 1/18 to 18n/18.
Each sample will include 3 straws, all with the same ID.

NUMBER OF SAMPLES AND LEVELS OF CONCENTRATION

Three levels of concentration are required and combined with 3 levels of quality, and are duplicated. Each participant will receive 54 straws (18 samples IDs x 3 straws).

ANCHOR SAMPLE

These samples will serve to compare the laboratory performance over time.

In the different PT rounds, the same characterized samples will be added to the new sample set. The sample analysed is the same over time. This allows to calculate characterized ZS score fix where the assigned value is the characterized value, and the SD is defined (SR of the method or of the PT). If there is a variation in the instrument setting, it is possible to visualize it thanks to the anchor samples.

HOMOGENEITY

The homogeneity check (variance between straws of the same samples) will be calculated according to ISO 13528. The acceptance limits will be established when a clearer picture on the precision of the methods used will be provided by the proficiency tests. The homogeneity will be calculated on the total number of sperm and on the motility.

SAMPLE PREPARATION

Three straws per sample (e.g 1/18-1/18-1/18) will be thawed at 37°C for 30 s and emptied in an Eppendorf cup, using a piston.

For each sample a range of sperm concentration will be indicated. According to their protocol, participants will decide if it is necessary to apply procedure 1 or 2.

Procedure 1 - sample dilution
A subsample of 200µl is taken and diluted in prewarmed (37°C) media (indicate used media at the questionnaire). Dilution rate depends on required number of samples per field, depending on the system used (also indicate the dilution rate used at the questionnaire). Samples will be incubated for 5 minutes at 37°C.

Procedure 2 – undiluted sample
If you do not need to dilute the samples, incubate the samples for 5 minutes at 37°C.

When using a CASA system with standardized counting chambers, a sample of 3 µL of diluted semen is loaded into a pre-warmed analysis chamber (indicate in the questionnaire the type of counting chamber used) and analysis is performed. Next to the analysis results, the number of sperm cells analysed is recorded. If possible, the videos of the CASA analyses should be stored.

When using a microscope, a drop of the pooled non diluted sample will be placed on a pre-warmed (37°C) glass slide and covered with a cover slip. The evaluation will be performed at magnification X 200 by a phase contrast microscope and a thermal plate (if a different method is used, please indicate it in the questionnaire). The percentage of motile and progressive motile sperm is estimated in four fields. The laboratory technician will give a percentage of (progressive) motile cells.

When using a flow cytometry, please describe system and protocol used.

This procedure will be repeated for each of the 18 samples.

SAMPLE ANALYSIS AND UPLOADING OF RESULTS

Each of the 18 samples have to be analyzed in two replicates with your analytical system.

If you wish to use an additional analytical system, you can use the same samples and to upload two or more sets of results on the ICAR PT platform.

PARAMETERS AND UNIT OF MEASUREMENT

The parameters considered are:

Parameter	Unit of measurement	Note
Number of cells analysed	n	Record number of cells analysed
Total concentration	number of cells / ml	Value including using dilution factor
Total motility	%	
Progressive sperm motility	%	Indicate settings in questionnaire
Optional additional parameters		
VAP	µm/s	Velocity Average Path
VSL	µm/s	Velocity Straight Line
VCL	µm/s	Velocity Curvilinear

ALH	µm	Amplitude of Lateral Head
BCF	Hz	Beat Cross Frequency
If flow cytometry is used		
Acrosome integrity	%	Percentage of cells with an intact acrosome
Membrane integrity	%	Percentage of cells with an intact membrane

The participant can determine one or more parameters according to the analytical system used.

STATISTICAL ANALYSIS

The following criteria will be calculated in the final report:

QUANTITATIVE PARAMETERS

REPEATABILITY

(r)

The absolute difference between two independent single test results (r), obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, should in no more than 5 % of the cases be greater than the values established for a certain method.

Repeatability conditions are:

- a) The same measurement procedure or test procedure;
- b) The same operator;
- c) The same measuring or test equipment used under the same conditions;
- d) The same location;
- e) Repetition over a short period of time.

Instrument repeatability It can be calculated considering the two replicates that each laboratory executes with one method on sample (e.g 1/18 as the results of 3 straws commingled) each straw under the repeatability conditions indicated above.

Method repeatability it can be calculated considering the 4 replicates that each laboratory executes with one method on one sample in double blind (e.g 1/18 and 12/18 is the same sample and two results of each have to be reported)

INTRA-LABORATORY REPRODUCIBILITY R $_{INTRA}$

The absolute difference between two independent single test results (Rintra), obtained using the same method on identical test material in the same laboratory by possibly different

operators using different instruments at different times (within at most a few hours), should in no more than 5 % of cases be greater than the values established for a certain method.

Intra-laboratory conditions:

Conditions where test results are obtained with the same method, on identical test or measurement items in the same test or measurement facility, under some different operating conditions, e.g. time, calibration, operator, equipment, sample batch, sample preparation and others.

It can be calculated considering the 4 replicates that each laboratory executes on each sample (e.g sample 1/18 and sample 12/18) using different instruments.

Note: These criteria will be calculated if enough results obtained in the intra-laboratory conditions will be provided e.g 5 different method/instrument for each laboratory

REPRODUCIBILITY

(R)

The absolute difference between two independent single test results (R), obtained using the same method on identical test material in different laboratories within a short interval of time, should in no more than 5 % of cases be greater than the values established for a certain method.

Reproducibility conditions:

Conditions where test results are obtained with the same method, on identical test or measurement items in different laboratories, under some different operating conditions, e.g. time, laboratory, calibration, operator, equipment.

It can be calculated considering all the double replicates that all the laboratories execute on each sample using the different instruments. If there are enough data from each method, different statistical elaborations can be provided e.g., one report for Microscope / CASA (containing the results of the motility parameters) and one report for Flow cytometry (containing the results of the other parameters).

LABORATORY

BIAS FROM THE ASSIGN VALUE

Difference between the laboratory mean of each sample and the PT assign value (robust mean) for each sample.

RANKING

It is the laboratory ranking based on the calculation of the Euclidian distance (D)

$$D = \sqrt{d^2 + sd^2}$$

where:

d=mean of the differences

sd=standard deviation of differences

The best result is to obtain a small D, it means a performance closer to the centre of the distribution.

ZETA SCORE

The Zeta score calculation will be provided for each sample and for each laboratory.

The result reported by a participating laboratory for a measurand is compared to the assigned value by calculating the Zeta score value.

The zscore value is calculated as follows:

$$ZScore = \frac{(mlab - AV)}{SDpt}$$

where:

- mlab is the mean value reported by the participating laboratory.

- AV is the PT assign value.

- SDpt is the proficiency test standard deviation after outlier deletion.

The result is considered successful, if the result of the zscore is $-1 \leq Zscore \leq 1$. In this case the participating laboratory agrees with the reference value within the stated uncertainty of the two.

If a laboratory obtains a Zscore < -1 or En > 1, it is expected that the laboratory activates some alert to improve the performance.

If a laboratory obtains an unsuccessful Zscore < -2 or Zscore > 2, it is expected that the laboratory investigates the reason for the disagreement and implements corrective action.

The laboratory that will send two sets of data obtained with two different instruments will receive two evaluations of its performance, one for each instrument.

QUALITATIVE PARAMETERS

How the laboratory classify the sample (low-medium-best)

o   All participants

o   Participants using the same system

ICAR PT REPORTS

Each participant can download a short individual report with their own performance, and a general report where all the received data, anonymized, will be reported and represented with tables and graphics.

ANNEX 1 - PARTICIPATION FORM – QUESTIONNAIRE

PARTICIPATION FORM

Please fill in the participation form at the link below:

https://app.surveymethods.com/EndUser.aspx?A387EBF4A7E0FEF0A1

QUESTIONNAIRE

xxxxxxxxxxxxxxxxxxxx

ANNEX 2 PRACTICAL REMARKS

DISTRIBUTION

- Logistics – each participant pays for individual shipment of the samples.

In order to optimize the costs and customs procedures, for the USA laboratories a distribution center will be identified. From Europe, multiple parcels will be delivered to a distribution center in USA. From the distribution center, the parcel will be delivered to the laboratory with express couriers.

RESULTS

You will receive detailed instructions by email on how to upload your results online and the deadline.

If you are not registered yet, please follow the ICAR PT instructions.

For any further clarifications, please do not hesitate to contact the ICAR secretariat:

Mrs. Silvia Orlandini, Technical Manager, at silvia@icar.org.

TIMETABLE (PROVISIONAL)

- May 1st announcement,
  - July ICAR webinar
  - September ICAR webinar

- September   final registration before 15 September
- October   ICAR Subcontractor prepares the straws and shipment for the participants.
- October   Samples shipment
- November   Participants receive straws and perform measurements
- November   Participants upload results on the ICAR PT platform.
- December   ICAR processes results and creates reports.
- January   ICAR delivers reports and results to the participants.