

Rules for conducting ISAG Comparison Tests (CT) for animal DNA testing.

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DEFINITIONS

Society = ISAG	Vereniging International Society for Animal Genetics; deed of formation December 21st 2007 (NB Vereniging means association)
Secretary	Elected secretary of the Society Executive
Committee	Executive Committee of the Society
Conferences	Conferences organized on behalf of the Society
Workshops	Meetings about a particular focus related to the aims of the Society
Standing Committee	Committee serving on behalf of the Society with duties that may include conduct of comparison tests.
Chair	Chairperson of one of the Standing Committees.
Institutional members	Institutes or other units that are registered with ISAG to enable participation in Comparison Tests
Reference Sample	A sample chosen by the Duty laboratory, the genotypes of this sample are provided to all participants of the specific Comparison test.
Duty Laboratory	The laboratory responsible for preparing and shipping samples for testing and presenting results of the CT at the corresponding workshop.
Comparison Test	A test conducted to determine concordance for genetic testing and analyses among laboratories.
Genotype Rating System	The mathematical representation of agreement with the agreed genotypes.
Rank	The rank assigned to the CT participants based on absolute genotyping accuracy (including discrepancies and blanks).

COMPARISON TEST (CT)

THE AIM of the Comparison Tests (CT) is to enable laboratories that are genotyping animal DNA samples to maintain high and comparable standards, to have international agreement on nomenclature and rules for kinship testing.

ISAG's involvement is to establish rules for conducting the comparison tests. ISAG does not endorse the performance of laboratories beyond their participation in these comparison tests.

Participating institutions shall agree to the ISAG Policies Governing the Conduct of ISAG Comparison Tests (CT) for Animal DNA Testing.

ISAG Comparison Testing is not an internationally recognized accreditation process and rankings should not be considered or advertised as accreditation.

General Organization *(Also see the separate guidelines for Standing Committees)*

1. The organization of Comparison Tests for each species shall be supervised by a Standing Committee of 3 to 7 ISAG members elected at regular conferences of the Society and consisting of representatives of institutes who actively genotype or study that species. A representative of the Duty Laboratory shall also be a member of the committee.

The duty of the Chair of the Standing Committee is to organize the workshop and prepare and distribute an agenda to participating institutional members prior to the meeting.

 - a. Individuals in the Standing Committee shall serve four-year terms and be eligible for re-election for an additional four-year period.
 - b. A representative of the Duty Laboratory will serve as an ex-officio member of the committee.
 - c. The elected standing committee members will elect a chairperson from amongst themselves. The Chairperson will be the primary contact with the Executive Committee and provide organizational leadership for the endeavors of the standing committee.
 - d. The Standing Committee will prepare the minutes of the workshop and send the minutes in the workshop report format by e-mail within six weeks after the workshop to the ISAG Secretary. The ISAG Secretary will inform all ISAG members that the workshop reports are publicly available on the ISAG website. Requests for revision have to be sent within two weeks by email to the ISAG Secretary. Any requests for revision should include relevant reasons and arguments. Within two weeks, the Standing Committee will – if necessary, at its discretion – adjust the minutes and send the final version of the minutes to the ISAG Secretary. The ISAG secretary will inform all ISAG members that a revised version of a workshop report is publicly available on the ISAG website.
 - e. If any Standing committee member neglects their duties, is involved in any misconduct of their position or misuses the information they receive as a committee member, that member can be suspended from the committee by a majority vote of the other standing committee members and the duties of that member reassigned among the remaining standing committee members. The Executive Committee will be informed of the committee member's suspension.
2. A Duty Laboratory will be responsible for the choice of samples to be analyzed and for dispatching the samples to participating laboratories.
 - a. The selection of a particular laboratory to serve as a Duty Laboratory should depend upon experience in the scientific field with the species of reference and their capacity to provide appropriate samples.
 - b. Import – export restrictions for DNA samples should be reviewed and taken into consideration at the time a Duty Laboratory is being considered.
 - c. The Duty Laboratory and ISAG are not responsible for freight costs, which must be paid by each laboratory for receipt of their set of samples.
 - d. Each participating laboratory is also responsible for obtaining any necessary import permits to receive samples.

- e. If additional documentation is required to accompany samples for entry into the institutional member's country, it is the responsibility of the participating lab to ensure all such required documentation is completely filled out and supplied with the application for participation. Failure to do so will result in samples not being sent.
 - f. The Executive Committee may reimburse a Duty Laboratory for costs up to € 5000 based on a description of those costs and the number of samples provided by the Duty Laboratory. More information about reimbursement can be found in appendix 1.
 - g. At the workshop the reimbursement forms will be available and require a signature of the representatives of the Duty Laboratory and the Chair of the Standing Committee. These forms have to be presented at the earliest convenience to the Secretary and Treasurer of ISAG.
3. Proposals for the selection of the Duty Laboratories should be discussed during the workshop meetings with decisions made prior to the completion of the meeting, if at all possible. Decisions will be made by a majority vote of those representatives of institutional members present if done at the meeting. If no duty lab is identified at the meeting, then the final decision will be made by the ISAG secretary or the executive committee.

Announcement and Participants

- 4. Announcements of the Comparison Tests will be distributed by posting on the ISAG website and sending out e-mail to Institutional members
- 5. A list of each set of ISAG recommended (core + additional set) STR or SNP or DNA variant markers should be made available to participants on the ISAG website via a reporting template (or similar), including marker name, type of marker (STR/SNP/other) chromosomal location and how genotypes should be reported. For STR markers also repeat sequence information and primer sequences should be made available. For SNP markers, genome build, also flanking sequences (at least 50 bp downstream and 50 bp upstream of SNP) should be made available.
- 6. Laboratories will indicate their interest in participation in a comparison test for each species by completion of an ONLINE Application form. Only Institutional members whose dues are up to date will be allowed to submit an application online. Institutional Members must maintain their membership through both years of the comparison test process. ISAG will then furnish the Duty Lab with the approved applications and corresponding shipping documents. The online submission will only be available until the application deadline. Only in exceptional cases will applications be accepted after the closing date and these will have to be made to and approved by the Standing Committee in consultation with the Duty Laboratory. Additional fees may apply to late applications.
 - a. Some countries can only apply for an import permit to receive samples at the time samples are distributed. This information must be indicated on the Application form.
 - b. Participating labs should identify themselves with their numerical Institutional Membership Number on the Application form.
- 7. **Participating laboratories are encouraged to first check DNA integrity upon receipt of samples.** Laboratories may request a second set of DNA samples if problems with shipment arise and any compromise of the integrity of the samples may be possible. **Documentation of integrity by the participating lab is**

highly recommended. A request for a second set of samples should occur within the defined timelines. The freight cost for a second set of samples must be paid by the receiving laboratory.

8. The collection and distribution of samples for comparison tests is a tedious task. All laboratories requesting participation are strongly encouraged to report their results. Labs that do not submit results can be prohibited from participation in the next CT.

Duty Laboratory Responsibilities

9. Sample Selection
 - a) The Duty laboratory selects the samples.
 - b) The number of animals to be tested is **20 test samples** and **two reference samples**. To assist with correct genotyping. One of these reference samples should be a sample from the previous year's CT if at all possible .
 - c) DNA test samples should be from a wide variety of breeds or a data set with sufficient genetic diversity to represent a larger number of genotypes per marker (STRs) and minor alleles (SNPs)
 - d) Two parentage questions are included to test the participant's ability to perform parentage verification.
10. DNA can be extracted from different sample types, including blood, tissue, hair, semen and buccal swabs. The Duty laboratories extracts the DNA from each individual samples all at once (one batch) or alternatively, pool batches from the same animal and aliquot afterwards, to avoid differences in the quality of the DNA shipped to different laboratories.
11. Information regarding the DNA samples is provided, including:
 - a. the sample type and DNA extraction method used;
 - b. the DNA concentration of the samples, ranging from ~10 – 100 ng/μl;
 - c. the volume of DNA to be distributed for each sample, with a minimum of 30 μl.
 - d. Integrity analysis (for example agarose gel electrophoresis) performed and accompany documentation provided for each sample prior to shipment.
 - e. This information should be provided to all participants as early as possible and again in the letter sent to participating labs ahead of shipment and again with the shipment of samples.
12. The Duty Laboratory shall provide to the participants the genotypes for the ISAG recommended markers (core + additional panel) of the reference sample(s). The genotypes will be provided in the format consistent with the requested reporting format when dispatching the samples to participating laboratories.
13. The Duty Laboratory provides a report during the workshop that includes a summary of the problems in communication and shipping with regard to the Comparison Test samples and these should be documented in the workshop minutes.

Rules for Reporting the Results

14. FASS will provide an excel file in the proper format with the reference genotypes and information for reporting data based on what is provided by the duty lab.
15. Results should be reported following the instructions provided by FASS.

16. Only results reported in the correct format will be taken into consideration.
17. Results from the two parentage questions are to be reported according to the rules for parentage verification.

STR:

- 0 mismatches in core panel: Parentage qualifies
- 1 mismatch in core panel: Parentage doubtful. Type additional panel, if 1 mismatch remains, qualify parentage and assume that a mutation occurred
- 2 or more mismatches in core (+ additional) panel: Parentage excluded

SNP:

*Case with offspring and one parent tested (considering 100 SNP panel)**

Minimum number of common SNPs in verification offspring: 90

- 0---1 mismatches: parentage accepted
- 2---3 mismatches: parentage doubtful, type additional panel, if 2---3 mismatches remain, qualify parentage and assume that mutation(s)/error(s) occurred
- > 3 mismatches: parentage excluded.

Case with offspring and both parents tested

Minimum number of common SNPs in verification offspring: 85

- 0---2 mismatches: parentage accepted
- 3---4 mismatches: parentage doubtful, type additional panel, if 3---4 mismatches remain, qualify parentage and assume that mutation(s)/error(s) occurred
- > 4 mismatches: parentage excluded.

* These rules for SNP parentage were developed many years ago based on the original cattle SNP panel of 100 markers. Panels are now larger and there are species specific information. It is necessary to scale up based on the number of ISAG approved SNPs however, please refer to the species-specific information on the ISAG website or check with the committee chairman for any species-specific recommendations on SNP parentage exclusions.

FASS Responsibilities

18. If results are received after the deadline, FASS is not obliged to incorporate those into the final compilation.
19. The most frequently reported genotype for each sample and marker will be considered as the “concordant” genotype.
NB: “Concordant” does not mean correct. If a participant can prove that the “concordant” genotype is not the correct genotype two options arise, the correct genotype will be used in the rating system or the genotype will be excluded from the rating system the decision on which falls to the committee.
20. FASS will compile the results from all participants into an excel file. “Concordant” genotypes” will be shown in a different font (highlight) to “discordant” genotypes.
21. The Genotype Rating System will be employed only for the ISAG recommended markers in the core panel and not for other markers.

The Genotype Rating System:

- Absolute genotype Error at locus for Sample (Gea):
One or both alleles incorrectly reported or not reported
 - Relative genotype Error at locus for Sample (Ger):
One or both alleles incorrectly reported
 - Absolute number of Genotypes (Nga)
= 20 x Number of ISAG recommended Markers (core panel)
 - Relative number of Genotypes (Ngr)
= Nga --- number of genotypes not reported
 - Absolute genotyping Accuracy (Aga) for ISAG recommended Markers in core panel
= (Nga --- Gea) / Nga (As Percentage)
 - Relative genotyping Accuracy (Rga) for ISAG recommended Markers in core panel
= (Ngr --- Ger) / Ngr (As Percentage)
22. FASS will send the file with compiled results no later than 3 weeks prior to ISAG conference to all participants that reported results.
23. FASS will ask all participants to verify that their own results have been correctly included in the compilation. Requests for revision must be submitted to FASS by email within two weeks of receiving the draft results and will only be considered when an error has been introduced during the **FASS compilation process**. If FASS confirms such an error, the affected genotype(s) will be corrected in the compilation.
24. FASS will ask all participants to check that the “Concordant” genotype is the correct genotype. If a participant does not agree that the “Concordant” genotype is the correct genotype and can prove this, the participant has to send a request for correction of that genotype no later than one week before the corresponding ISAG conference by email to the chair of the Standing Committee. Any request for correction has to include relevant reasons, documents, and arguments.
25. The chair of the Standing Committee will discuss the request(s) for correction within the committee prior to the workshop at the next corresponding conference.
26. The request(s) for correction will be discussed during the workshop at the corresponding ISAG conference. If necessary, a vote can take place during the workshop to decide if a “concordant” genotype is the correct genotype or if a genotype needs to be excluded from the rating system. Therefore, the final rating scores can only be calculated after the workshop!
27. The chair of the Standing Committee informs the participant(s) that sent a request for correction about the decision to accept or reject their request within one month after the ISAG conference. If the participant does not agree with the decision of the Standing Committee, he/she can appeal by sending an email to the ISAG Secretary within six weeks after the conference. Any appeal has to include relevant reasons and arguments why the participant does not agree with the decision. For details about the appeal process see paragraph “Appeal process”.
28. Within one month after the ISAG conference, the Chair of the Standing Committee will report to FASS the decisions from the workshop about corrections of “concordant” genotypes or exclusions of genotypes from the rating system.

29. If no appeals are received, FASS will send the final file with compiled results 7 weeks after the ISAG conference to all participants that reported results, the Chair of the committee and to the Secretary. For more information about the appeal process see paragraph “Appeal process”.

Appeal process

30. Within two weeks after the appeal has been received by the ISAG Secretary, the Standing Committee and the appealing participant will select a mutually agreed arbiter from inside or outside ISAG or they will be reviewed by an appointed *ad hoc* CT review committee serving as the arbiter in years when multiple appeals are submitted from different species. This committee will be appointed by the ISAG Secretary and should include the secretary, FASS staff, and at least three other ISAG members.
31. The chair of the Standing Committee and the appealing participant will provide all relevant information concerning the appeal to the arbiter within one week after the appeal committee has been formed.
32. The arbiter makes a decision within two weeks. The decision of the arbiter is binding.
33. FASS will send the final file with compiled results 1 week after the decision of the arbiter to all participants that reported results, the Chair of the committee and to the Secretary.

Final compilation and official document of participation

34. Each participating institutional member will receive an official document of participation from ISAG. The document includes the absolute genotyping accuracy (Aga) rank of the laboratory itself and an anonymous overview of the Aga of all participants combined.

Example anonymous overview of the Aga of all participants combined :

Absolute genotyping Accuracy Total # labs: 60	
Rank	% Labs
1: 100 – 98%	70
2: 97,9 – 95%	10
3: 94,9 – 90%	7
4: 89,9 – 80%	8
5: 80%	5

35. On the official document of participation, it will be reported if the parentage questions have been reported correctly.
36. Labs may use the certificate to demonstrate their competence to their clients. Each lab is free to decide if and with whom they share their own Genotyping Accuracy values. **However, this report should not be communicated or marketed as an official accreditation.**
37. Compiled results of any Comparison Test are confidential and shall be made available only to those Institutional members that participated and submitted results in the particular Comparison Tests.
38. Participants will be identified in the compilation with their numerical ISAG lab code.
39. The final compilation will be distributed in .pdf or Excel format to all participants.

Timelines

40. In accordance with the timelines below, the individual Standing Committees will define the exact time schedule for CT(s) they organize, in coordination with the Society.

Timelines:

- i. Announcement of Comparison Tests: 14--- 16 months prior to the next ISAG conference
- ii. Last Date to receive Application forms requesting participation: 10 --- 12 month before next ISAG conference
- iii. Distribution of samples: 6 – 9 months before next ISAG conference
- iv. Deadline to request second batch of sample: within 2 months of distribution
- v. Deadline to distribute second batch of samples: within 3 months of first distribution
- vi. Deadline to report results to FASS: no later than 6 weeks prior to ISAG conference
- vii. Preliminary report to participating laboratories: no later than 3 weeks prior to ISAG conference
- viii. Request to FASS for revision due to copying error(s): no later than 1 week prior to ISAG conference (see paragraph 27)
- ix. Request to the Chair of the Standing Committee for correction of genotype(s): no later than 1 week prior to ISAG conference (see paragraph 28)
- x. Decision by workshop participants about request for correction of genotype: during the workshop at the ISAG conference
- xi. Chair of the Standing Committee informs the participant that sent a request for correction and FASS about the decision of the workshop: within 1 month after the ISAG conference
- xii. Appeal on decision of the participants of the CT: no later than 6 weeks after ISAG conference

If no appeal received:

- x. Final report on typing results to ISAG Secretary, chair of the committee and participants: no later than 7 weeks after ISAG conference
- xi. Distribution of certificates to participants: no later than 3 months after ISAG conference

If appeal received:

- x. Selection of arbiter/*ad hoc* committee: no later than 8 weeks after ISAG conference
- xi. Provide arbiter/*ad hoc* committee with all relevant information: no later than 9 weeks after ISAG conference
- xii. Decision of arbiter/ *ad hoc* committee: no later than 11 weeks after ISAG conference
- xiii. Final report to ISAG secretary, chair of the committee and participants: no later than 3 month after ISAG conference
- xiv. Distribution of certificates to participants: no later than 4 months after ISAG conference