

Guidelines for performance of Comparison Tests (CT) - that is animal DNA testing

The aim of the Comparison Tests is to enable laboratories that are genotyping animal DNA samples to maintain high and comparable standards, to have international agreement on nomenclature and typing procedures, and to encourage research opportunities.

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

1. The organization of Comparison Tests for a species shall be supervised by a Standing Committee of 3 to 5 members elected at regular conferences of the Society by ISAG members affiliated with ISAG institutional members who actively genotype or study that species. All members of Standing Committees for applied genetics/comparison tests must be current individual members of ISAG and affiliated with Institutional members of ISAG.
It is the duty of the Chair of the Standing Committee 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 and the Computing (Data Analysis) Laboratory shall also be elected to 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 a draft will be sent by e-mail within four weeks after the workshop to representatives of the institutional members participating in the workshop. Approval of or comments to the draft version have to be sent within two weeks by e-mail to the sender of the minutes. Any requests for revision has to include relevant reasons and arguments. Within two weeks the Standing Committee will – if necessary at its discretion – adjust the draft version of the minutes and send the final version of the minutes to the participants of the workshop and to the Secretary. The minutes will be made publicly available on the ISAG web.
 - e. A system for correction of clerical errors in reporting and handling of the test results should be instituted by the standing committee.

2. A Duty Laboratory will be responsible for the choice of samples to be analysed 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 the ISAG are not responsible for freight costs, which must be paid by each laboratory for receipt of their set of samples. Each participating laboratory is also responsible for obtaining any necessary import permits to receive samples.
 - d. The Executive Committee may reimburse a Duty Laboratory for other costs up to € 5000 based on a description of those costs and the number of samples provided by the Duty Laboratory. Re-imbusement for the Duty Laboratory is currently (2014) the following:

No. of participants	Duty laboratory	Computing Laboratory
< 25	€ 1.000,00	€ 750,00
26-50	€ 2.000,00	€ 1.500,00
51-75	€ 2.500,00	€ 2.500,00
Above 75	payment to be negotiated	€ 2.500,00

- e. At the workshop the reimbursement forms will be available and have to be signed by the representatives of the Duty and Computing Laboratory and the Chair of the Standing Committee. These forms have to be presented at the earliest convenience to Secretary and Treasurer of ISAG
3. The Computing Laboratory (data analysis laboratory) for each comparison test will be chosen by the representatives of each Institutional member who actively genotype or study that species.
 - a. The Executive Committee may award financial compensation as shown in the table above, for processing Comparison Test data.
 - b. The Duty and Computing Laboratories should be independent laboratories or research groups; however, exceptions can be made at the discretion of the standing committee.
4. Proposals for the selection of the Duty and Computing Laboratories will be discussed during the workshop meetings. Decisions will be made by a majority vote of those representatives of institutional members present.
5. The Standing Committee will prepare timelines for future Comparison Tests, which should be coordinated with general timelines established by the Society and other committees.

Announcement and Participants

6. Announcements of the Comparison Tests will be distributed by the Secretary of ISAG, in accordance with the timelines determined by the individual Standing Committees, in coordination with the Society and other species' Comparison Tests.
 - a. Suggested timelines:
 - i. Announcement of Comparison Tests: 18 months prior to the next ISAG conference
 - ii. Last Date to Receive Consignment forms requesting participation:
 - iii. Distribution of samples: 6 – 9 months before next ISAG conference
 - iv. Second deadline to receive samples: within 3 months of first distribution
 - v. Deadline to report results to Computing Laboratory: 2 months prior to ISAG conference
 - vi. Preliminary report to participating laboratories: 1 week prior to ISAG conference
 - vii. Final report to ISAG secretary: 2 weeks after ISAG conference
 - viii. Distribution of certificates: 1 - 2 months after ISAG conference
7. Participants in Comparison Tests organized on behalf of ISAG must be institutional members of the Society and must abide by these test guidelines.

All participants must declare that their institution agrees to the following terms and conditions:

- b. Any liability of ISAG for participation in the Comparison Test, the execution of the test and its results is excluded.
 - c. The participating institution and its employees will not and cannot claim damages arising out or in connection to the Comparison Test and its results from ISAG.
 - d. These conditions are also stipulated on behalf of the Duty Lab, Computing Lab, members of the Standing Committee and other assisting organizations and as well on behalf of the board, employees and other co-operators of these organizations.
8. Laboratories will indicate their interest in participation in a comparison test for each species by completion of an ONLINE consignment form. Only Institutional members whose dues are up to date will be allowed to submit an application on line. ISAG will then furnish the Duty Lab with the approved applications. 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.
 - e. Some countries can only apply for an import permit to receive samples at the time samples are distributed. Please indicate this information on the Consignment form.
 - f. Participating labs should identify themselves with their numerical Institutional Membership Number on the consignment form.

9. 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. A request for a second set of samples should occur within 3 months of the first distribution. The freight cost for a second set of samples must be paid by the receiving laboratory.

Duty Laboratory Responsibilities

10. A list of each set of microsatellite or SNP or DNA variant markers should be made available to participants on the ISAG website, including primers, flanking DNA sequence and other technical information. Additional questions for genotyping can be directed to the Duty Laboratory.
11. Sample Selection
 - a. DNA samples should be selected to represent the broadest diversity of microsatellites and SNP markers possible. DNA samples should be selected to represent breeds of the broadest interest, but regional populations should also be considered.
 - b. Selection of animals from which samples are to be taken should be limited largely to animals whose genotypes for the more important genetic systems are reasonably well established. However, this should not preclude the possibility of the Duty Laboratories including some doubtful and unknown samples in the test.
 - c. The number of animals to be sampled should normally be 20 including or in addition to one reference sample, which may be decided by the standing committee.
 - d. A small family may also be included that would test the participants ability to establish parentage.
12. DNA can be extracted from different sample types, including blood, tissue, hair and buccal swabs. The Duty laboratories should extract the DNA from each sample all at once (one batch) or alternatively, mix several batches from the same animal and splitting afterwards, to avoid differences in the quality of the DNA shipped to different laboratories.
13. Information regarding the DNA samples should be provided, including:
 - a. the sample type and DNA extraction methods used;
 - b. the DNA concentration of the samples, ranging from ~10 – 100 ng/ul;
 - c. the volume of DNA to be distributed for each sample (should be 50ul).
14. The Duty Laboratory shall provide to the participants a copy of the microsatellite or SNP or DNA variant genotypes of the reference sample(s). The genotypes will be provided in the format consistent with the requested reporting of results. This information should be included when dispatching the samples to participating laboratories, both by e-mail and included in the package with the samples.
15. At the time of sample distribution, the duty laboratory will send a list of participants and their contacts with the corresponding ISAG numerical code to both the computing laboratory and to the secretary of ISAG.
16. The Duty Laboratory will provide the contact of the Computing Laboratory to the

participants for the submission of results.

Rules for Reporting the Results

17. The Computing Laboratory will provide an excel file with the reference genotypes and the proper formats and examples for reporting data.
18. Results should be reported following the instructions provided by the Computing Laboratory.
19. Only results following the officially adopted nomenclature will be taken into consideration by the Computing Laboratory, excepting that laboratory nomenclature may be used for new microsatellites/SNP or other DNA variant markers.

Computing Laboratory Responsibilities

20. If results are received after the deadline, the Computer lab is not obliged to incorporate those into the final compilation.
21. The most frequently reported genotype for each sample and marker will be considered as the “concordant” genotype and will be shown in a different font to “discordant” genotypes. Concordance of genotypes that may not be correct will be discussed at the workshop.
22. The Genotype Rating System will be employed only for the ISAG recommended markers and not for other markers.

The 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)

- Number of Samples (reference sample not included) x Number of ISAG recommended Markers

Relative number of Genotypes (Ngr)

= Nga - number of genotypes not reported

Absolute genotyping Accuracy (Aga) for ISAG recommended Markers

= (Nga - Gea) / Nga (As Percentage)

Relative genotyping Accuracy (Rga) for ISAG recommended Markers

= (Ngr - Ger) / Ngr (As Percentage)

23. If for a certain genotype no consensus exists or if a lab does not agree with the “concordant” genotype, the results of this genotype will be discussed during the workshop at the next iSAG conference. If necessary a voting can take place during the workshop to decide if this genotype needs to be excluded from the rating system. Therefore, the final rating scores can only be calculated after the workshop!
24. The Computing Laboratory or the Chair of the Standing Committee should provide all participants with a copy of a short summary of the results with

comments and suggestions. This short summary should be discussed at the relevant workshop session during the next regular conference of the Society. Based on the conclusions of this session, an overall summary should be established by the Standing Committee and reported to the Society.

25. The Duty Laboratory should provide the Secretary of the Society and the Chairperson of the Standing Committee with a written report on problems in communication, shipping and expenses and on other general information with regard to the Comparison Test.
26. Each participating institutional member will receive an official proof of participation from ISAG. The document may include the Genotyping Accuracy values and an anonymous overview of the results (as exemplified below) provided by the standing committee.

Example Reporting Format:

Absolute genotyping Accuracy Total # labs: 60		Relative genotyping Accuracy Total # labs: 60	
Rate	% Labs	Rate	% Labs
1: 100 – 98%	70	1: 100 – 98%	77
2: 97,9 – 95%	10	2: 97,9 – 95%	8
3: 94,9 – 90%	7	3: 94,9 – 90%	6
4: 89,9 – 80%	8	4: 89,9 – 80%	7
5: 80%	5	5: 80%	2

27. 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.
28. Compilation results of any Comparison Tests are confidential and shall be made available only to those Institutional members that participated in the particular Comparison Tests and submitted results.
29. Participants will be identified in the compilation with their numerical ISAG lab code. A list of data from participants and contacts ordered by ISAG numerical code will be provided along with the compiled results.
30. The final compilation will be distributed in .pdf or Excel format to all participants.
31. 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.
32. The collection and distribution of samples for comparison tests is a tedious task. All laboratories requesting participation are strongly encouraged to report results. Failure to report results for two consecutive comparison tests may prohibit the laboratory from future participation.