ISAG
INTERNATIONAL SOCIETY FOR ANIMAL GENETICS

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

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 participants of the Comparison test.**
Duty Laboratory	**The laboratory responsible for preparing and shipping samples for testing**
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.**

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.

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 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 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 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.
e. 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 will be discussed during the workshop meetings. Decisions will be made by a majority vote of those representatives of institutional members present.
4. The Standing Committee will prepare timelines for future Comparison Tests, which should be coordinated with general timelines as indicated in the paragraph "Timelines". The timelines for each CT will be posted on the ISAG website.
5. A standing committee can make changes to those rules with advice of the participants of the workshop. The Standing Committee should inform the ISAG Secretary about this within 3 month after the workshop.

Announcement and Participants

6. Announcements of the Comparison Tests will be distributed by the Secretary of ISAG.
7. 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, including marker name, type of marker (STR/SNP/other) and chromosomal location. For STR markers also repeat sequence information and primer sequences should be made available. For SNP markers also flanking sequences (50 bp downstream and 50 bp upstream of SNP) should be made available.
8. 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. 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.
    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 Aplication 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 the defined timelines. The freight cost for a second set of samples must be paid by the receiving laboratory.
10. The collection and distribution of samples for comparison tests is a tedious task. All laboratories requesting participation are strongly encouraged to report results. If a laboratory fails to report results for two consecutive comparison tests

they can be prohibited from participation in the next CT.

Duty Laboratory Responsibilities

11. Sample Selection
    a) The Duty laboratory selects the samples.
    b) The number of animals to be selected is 21, consisting of a minimum of 1 reference sample and 20 samples that have to be reported.
    c) DNA samples should represent several genotypes per marker.
    d) Two parentage questions are included to test the participant's ability to perform parentage verification.
12. 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 aliquote afterwards, to avoid differences in the quality of the DNA shipped to different laboratories.
13. 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.
14. 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.
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 FASS, the secretary of ISAG and the chair of the Standing Committee.
16. The Duty Laboratory will provide the contact information from FASS to the participants for the submission of results.
17. The Duty Laboratory provides the Secretary of the Society and the Chairperson of the Standing Committee a written report with information about problems in communication and shipping with regard to the Comparison Test.

Rules for Reporting the Results

18. FASS will provide an excel file in the proper format with the reference genotypes and information for reporting data.
19. Results should be reported following the instructions provided by FASS.
20. Only results reported in the correct format will be taken into consideration.
21. 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

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.

FASS Responsibilities

22. If results are received after the deadline, FASS is not obliged to incorporate those into the final compilation.
23. 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.
24. 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.
25. 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)
26. FASS will send the file with compiled results no later than 3 weeks prior to ISAG conference to all participants that reported results.

27. FASS will ask all participants to check if their own results have been copied correctly into the compilation. Requests for revision due to copying errors have to be sent within two weeks by email to FASS. If indeed a copying error occurred FASS will correct the genotype(s) in the compilation.
28. 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 next ISAG conference by email to the chair of the Standing Committee. Any request for correction has to include relevant reasons and arguments.
29. The chair of the Standing Committee will discuss the request(s) for correction within the committee prior to the workshop at the next ISAG conference.
30. The request(s) for correction will be discussed during the workshop at the next 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!
31. 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".
32. 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.
33. 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

34. 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.
35. 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.
36. The arbiter makes a decision within two weeks. The decision of the arbiter is binding.
37. 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

38. 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

39. On the official document of participation it will be reported if the parentage questions have been reported correctly.
40. 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.
41. Compiled results of any Comparison Test are confidential and shall be made available only to those Institutional members that participated in the particular Comparison Tests and submitted results.
42. Participants will be identified in the compilation with their numerical ISAG lab code.
43. The final compilation will be distributed in .pdf or Excel format to all participants.

Timelines

44. 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: no later than 8 weeks after ISAG conference
xi. Provide arbiter with all relevant information: no later than 9 weeks after ISAG conference
xii. Decision of arbiter: 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