

## SECTION 4.2 - ICAR RULES AND GUIDELINES FOR LABORATORY ACCREDITATION OF PARENTAGE TESTING IN CATTLE

Considering the need for high quality standards in bovine parentage testing and identity verification due to the impact incorrect parentage assignment or identity may have in the estimation of genetic indexes and in national and international genetic evaluations, and based on two years of work by the genetic analysis task force, ICAR has decided to define the minimum requirements for laboratories performing DNA parentage testing and identity verification. Guidelines for accreditation are provided for microsatellite- and SNP-based analyses in cattle. Minimum requirements for additional species and DNA tests will be defined in the future.

Laboratories requesting microsatellite- and/or SNP-based accreditation will have to apply by downloading and filling out the appropriate forms (Annex II for microsatellites; Annex V for SNPs) on the ICAR website. The forms must be filled out accurately and completely, providing necessary documentation as required. The application will be evaluated by a Committee of Experts appointed by ICAR that will either approve it, request additional information, or reject it. In case of rejection, the applicant may submit a new form at least one year after her/his failed application. Accreditation will be given for a two-year period, at the end of which a new application is to be completed and submitted after participation in additional ISAG comparison tests.

Section 4.2.1 and 4.2.2 contain the rules and guidelines for laboratory accreditation of DNA paternity testing in cattle using microsatellites and the rules and guidelines for SNP-based parentage testing in cattle

Annex I and Annex II contain the application form for laboratory accreditation of DNA paternity testing in cattle using microsatellites and the list of recommended ISAG microsatellite markers, respectively.

Annex III, and Annex IV contain the application form for laboratory accreditation for SNP-based testing in cattle and the list of recommended SNP markers, respectively.

~~Laboratories requesting accreditation will have to apply for it downloading and filling a questionnaire (Annex I) that will be made available on the ICAR website. The form is to be filled accurately, providing necessary documentation, when required. Accreditation will be released for a two year period, at the end of which a new application is to be completed and submitted. The application will be evaluated by a **Committee of Experts** appointed by ICAR that will approve it, require additional information or reject it. In case of rejection the applicant may submit a new form at least one year after her/his failed application.~~

### **4.2.1 ICAR rules and guidelines for accreditation of DNA paternity testing in cattle**

The present Rules contain **Minimum requirements** for accreditation of DNA paternity testing in cattle.

Annex I contains the questionnaire to be filled by applicants and e-mailed to the ICAR secretariat (dna@icar.org). Annex II contains the list of microsatellite markers recommended by ISAG and the method for calculating 1 parent and 2 parent exclusion probabilities.

## **1. Laboratory identification**

The applicant must be clearly identified by providing the following:

- Name of the laboratory and Institution if relevant,
- Institution if relevant
- Address and Country
- Contact person at the lab, as well as all information necessary for getting in touch with her/him quickly.

## **2. Education and training of lab supervisor and operators**

The minimum requirements for education and training of the laboratory supervisor and senior operator are:

- Bachelor degree, or higher, in a scientific discipline for laboratory head or supervisor, and
- At least five years of experience in molecular diagnostics for the laboratory senior operator.

Experience is considered a key factor in data production and in the interpretation of results.

## **3. Equipment**

- Equipment used to run and score microsatellite must be described.
- The year of purchase and last revision must be provided - this allows ICAR to evaluate the appropriateness of the technology being used and ensure each lab is following a proper maintenance program that should enable it to generate high quality data.
- Yearly revision is considered a minimum requirement.
- A personal opinion on the performance of the laboratory set up available is asked to foresee the need for improvements in quality standards.

## **4. Certification**

- International ISO17025 or ISO9001 is a minimum requirement for ICAR accreditation.

## **5. Participation and performance in ring test**

- The participation and performance in ISAG and national ring (comparison) tests must be disclosed, and certificates provided, when available. Applicants must also sign a release allowing ISAG to directly disclose their ring test results to the ICAR Genetic Analysis Working Group.
- The participation in at least two ISAG ring tests is a minimum requirement.
- Beginning with the 2009-10 ISAG ring test, lab typing performance for the official set of 12 ISAG microsatellites (see Annex II) must be disclosed (previous ISAG ring test reporting can be limited to 9 microsatellites).

- The Committee of Experts will decide performance thresholds for each ring test with due consideration for the structure of the ring test and the average performance of laboratories in the ring test that year.

## **6. Microsatellite markers**

- The names of all microsatellites typed on all animals (marker set I) and of the additional ones assayed in the case of unresolved parentage (marker set II) must be declared, as well as the number of animals typed in at least the last two years.
- The minimum requirement for international exchange is the complete set of 12 official ISAG microsatellite markers.
- To ensure sufficient experience within the lab, analysis of 500 animals per year is set as minimum requirement for certification.
- Exclusion probability (PE; 2 parents and 1 parent) of each marker and of the complete marker sets must be calculated and provided in the application. The type of population and number of animals (minimum 150) used for computations are to be described. ICAR recommends using Holstein as a reference group when possible. The ICAR Committee of Experts will evaluate that an appropriate PE is reached for accreditation, on the basis of the population analyzed.

## **7. Marker nomenclature**

- Nomenclature of markers must be described.
- ISAG nomenclature is required for the official ISAG 12 marker set.

### **4.2.2 ICAR rules and guidelines for SNP-based parentage testing in cattle**

The present Rules contain **Minimum requirements** for accreditation of SNP-based DNA parentage testing in cattle.

Annex III contain the application form for laboratory accreditation for SNP-based testing in cattle that has to be emailed to the ICAR secretariat (dna@icar.org). Annex IV contains the list of recommended SNP markers.

#### **1. Laboratory identification**

The applicant must be clearly identified by providing the following:

- Name of the laboratory.
- Institution if relevant.
- Address and Country.
- Contact person at the lab, as well as all information necessary for getting in touch with her/him quickly.

#### **2. Education and training of lab supervisor and operators**

The minimum requirements for education and training of the laboratory supervisor and senior operator are:

- Bachelor degree, or higher, in a scientific discipline for laboratory head or supervisor.

- At least five years experience in molecular diagnostics for the laboratory senior operator.

Experience is considered a key factor in data production and in the interpretation of results.

### **3. Equipment**

- Equipment used to run and score SNPs must be described, as well as the methods used.
- The year of purchase and last revision must be provided – this allows ICAR to evaluate the appropriateness of the technology being used and ensure each lab is following a proper maintenance program that should enable it to generate high quality data.
- Yearly revision is considered a minimum requirement.

### **4. Certification**

- No certification is presently required. In the future, ISO17025 and/or ISO9001 will be a minimum requirement.

### **5. Participation and performance in ring test**

- The participation and performance in ISAG ring (comparison) tests must be disclosed and certificates provided, when available. Applicants must also sign a release allowing ISAG to directly disclose their ring test results to the ICAR Genetic Analysis Working Group.
- Participation in at least one ISAG ring tests is considered a minimum requirement at this time.
- A Committee of Experts will decide performance thresholds for each ring test with due consideration for the structure of the ring test and the average performance of laboratories in the ring test that year.

### **6. SNP markers**

- The name of all SNPs typed on all animals (marker set I) and of the additional markers assayed in the case of unresolved parentage (marker set II) must be declared, as well as the number of animals typed in at least the last two years.
- It is a minimum requirement to use at least 95 SNPs from the set recommended by ISAG (see Annex VI) on all animals typed.
- To ensure sufficient experience within the lab, genotyping 500 animals per year is set as a minimum requirement for accreditation.
- Exclusion probability (PE; 2 parents and 1 parent) of the complete marker sets used must be calculated and declared. The type of population and the number of animals (minimum 150) used for computations are to be described. ICAR recommends using Holstein as a reference group when possible. The ICAR Committee of Experts will evaluate that an appropriate PE is reached for accreditation, on the basis of the population analyzed.

### **7. Marker nomenclature**

- Nomenclature of markers must be described.
- ISAG nomenclature is required for the ISAG SNP marker set.

## **4.3 ICAR Guidelines for the Accreditation of applying the results of genotype analysis for parentage verification**

With the advent of SNP testing, the functions of analysis and verification have the potential to be separated. Consequently, ICAR has a separate accreditation for applying the results of genotype analysis, which may be taken by laboratories, herd books and any other organisation involved in parentage verification. It is concerned with utilising the results that are delivered by the laboratories from DNA sample analysis and so is a data warehouse function. These organisations may act as service providers between laboratory accredited for SNP-based DNA parentage testing and end user.

Service providers could use different laboratories for different breeds/species.

Organisations requesting accreditation for applying the results of genotype analysis for SNP based parentage verification will have to apply by downloading and filling in the appropriate form. (Annex VII). This form must be filled out accurately and completely, providing necessary documentation as required, and submitted to ICAR. The form will be evaluated by a Committee of Experts appointed by ICAR that will either approve it, request further information or reject it. In the case of rejection, the applicant may submit a new form at least one year after the failed application. Accreditation will be given for a four year period, at the end of which a new application is to be completed and submitted.

Annex VII contains the questionnaire to be filled in by participants and e-mailed to the ICAR secretariat.

### **Section 4.3.1 ICAR Rules and Guidelines for the accreditation of applying the results of genotype analysis for parentage verification**

The present Rules contain **Minimum requirements** for accreditation of applying the results of genotype analysis for SNP based parentage verification.

#### **1. Organisation identification**

The applicant must be clearly identified by providing the following:

- Name of the Organisation.
- Address and Country of the Organisation.
- Contact person at the Organisation, as well as all information necessary for getting in touch with her/him quickly (email and telephone number).

#### **2. Education and training of institution**

The minimum requirements for education and training of the responsible person of the Organisation are:

- Bachelor degree, or higher, in a scientific discipline.
- Understanding and experience in molecular biology, data handling and interpretation of results.
- An in-depth knowledge of the principles of parentage verification using data from genotype analysis.

Experience is considered a key factor in the interpretation of results from genotype analysis.

### **3. Accreditation**

- Some organisations currently performing this function will be members of ISAG, or ICAR and some not members of ICAR or ISAG. There are organisations who would wish to become accredited such as herd books who won't necessarily be members of ICAR.
- Organisations must provide evidence of their competence to provide parentage verification service. ISO17025 accreditation is acceptable for this. When not available, initially similar evidence of fitness will be acceptable
- If an organisation acts as a service provider for data handling and interpretation of results obtained from certified laboratory, such organization does not have to have ISO17025.

### **4. Participation and performance in ring test**

As this accreditation is concerned with data handling and interpretation of the SNPs for parentage verification, as part of the accreditation a set of animals SNPs will be sent to the organisation, plus animals of known and unknown parentage and the question posed as to who the parents were:

- Participation in the data handling and interpretation test is mandatory.
- The test must be performed every four years.
- A panel will devise and monitor the test.

### **5. SNP Nomenclature**

- The name of all SNPs genotyped (ISAG200) and of the additional markers assayed in the case of unresolved parentage (other SNP sets such as 500SNP, 800SNP or larger) must be declared, as well as the number of animals typed in at least the last 2 years.
- ISAG200 - ISAG100 core panel plus additional ISAG SNPs
- ISAG200plus – ISAG200 plus additional user defined SNPs

### **6. SNP Markers and interpretation**

- When using ISAG200, follow the guidelines on the ISAG website [www.isag.us/Docs/Guideline-for-cattle-SNP-use-for-parentage-2012.pdf](http://www.isag.us/Docs/Guideline-for-cattle-SNP-use-for-parentage-2012.pdf)
- ISAG200plus can be used for both validation and discovery, if this is done it is a minimum requirement to use at least 100 SNPs from the set recommended by ISAG (see annex VI – ISAG100 panel) on all animals typed.
- A maximum cutoff of 1% SNP misconcordance between parent and child is allowed for parentage verification and discovery.
- If a parent is SNP excluded then 3 options can be used

- If ISAG100 or ISAG200 have been used for validation, ISAG200plus can be used for discovery. At low SNP levels genotyping errors can cause false exclusions that are just over the 1% SNP misconcordance rate
- Run parentage validation using the ISAG200 panel against other possible parents suggested by the farmer or herdbook
- Run parentage discovery using ISAG200plus
- For Parentage discovery at least 500 SNP (ISAG200plus or ISAG200 and 300 additional markers with MAF >0.40) are recommended. Caution is advised when using smaller panels, as multiple sire and dams may be predicted. This will depend upon the population and breed composition.
- If multiple parents are predicted then with the exception of identical twins, a larger SNP set must be used to determine the most likely parent.
- To ensure sufficient experience within the organisation, it is recommended that parentage analysis of 500 animals per year is set as a minimum requirement for accreditation.
- Exclusion probability (PE; 2 parents and 1 parent) of the complete marker sets used must be calculated and declared. The type of population and the number of animals (minimum 150) used for computations are to be described. The panel will evaluate that an appropriate PE is reached for accreditation, on the basis of the population analyzed.

## 7. Recommendations

- Use the same set of SNP for parentage validation and discovery (if possible) instead of using a smaller set for initial validation and a larger set for discovery.
- A standardised format is required for data exchange from laboratory to service provider.

This is an example of Final report from two different laboratories:

1. SNP Name Sample ID Allele1 - Forward Allele2 – Forward Allele1 – Top Allele2 – Top Allele1 – AB Allele2 – AB GC Score X Y
2. SNP Name Sample ID Allele1 - Forward Allele2 – Forward Allele1 – AB Allele2 – AB Allele1 – Top Allele2 – Top GC Score X Y

- It is recommended that sufficient SNPs should be used to reduce the likelihood of assigning incorrect parentage for either validation or discovery. The number of SNPs will be determined by technology, in herd parentage recording and SNP availability.



ICAR Parentage Recording Working Group Questionnaire for Applying the Results of Genotype Analysis for Parentage Verification

SECTION 1. GENERAL INFORMATION

1. ADDRESS DETAILS (fill out)

Country: ..... Organisation name: ..... Contact person: ..... Address: ..... Telephone: ..... E-mail: .....

2. EDUCATION, TRAINING, AND EXPERIENCE OF THE RESPONSIBLE PERSON

- a. Level of education of the responsible person (tick the box and describe) [ ] Ph.D. in ..... [ ] Masters of Science in ..... [ ] Bachelors of Science in ..... [ ] Other ..... [ ] None

3. CERTIFICATION

Organisations must provide evidence of their competence to provide parentage verification service. ISO17025 certification is acceptable for this. When not available, initially similar evidence of fitness will be acceptable

- a. Certification (tick the box, describe, and send a copy of the certification, and English translation as necessary, to the ICAR Secretariat; please note that no certification is required by ICAR at the moment). [ ] International certification ..... [ ] National certification ..... [ ] No certification (certification will be a minimum requirement at some point in the future)
- b. If you have no certification, describe briefly: Procedure for receiving, storing and retrieving information .....



Describe quality management system used

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**4. DATA ANALYSIS**

Describe the marker panels used and how the panel was selected

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Parentage analysis technique (describe)

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Procedure for error and repeatability checking

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**5. PARTICIPATION AND PERFORMANCE IN RING TESTS**

a. Participation and performance in international ring (comparison) tests (tick)

$\geq 1$  international ring tests within 4 years

I have not yet completed a ring test

\_\_\_\_\_year of the most recent international ring test

Please submit a copy of the report of the most recent ring test with this application.