ISAG CONFERENCE 2012, Cairns, Australia

Equine Genetics & Thoroughbred Parentage Testing Standardisation Workshop

Organised by a standing committee: yes

Date and meeting time: 2:30-5:30pm 17th July 2012

Chair: Ann Trezise (ann.trezise@uq.edu.au)

Agenda:

1. Welcome
2. Current ISAG Equine Standing Committee: 2010-2014
3. ISAG Horse Comparison Test 2011-2012
   - 3.1. Report from HCT Duty Laboratory
   - 3.2. Report from HCT Results Analysis Laboratory
     - 3.2.1. HCT Results: Genotyping Accuracy
     - 3.2.2. HCT Results: Parentage Analysis Accuracy
4. Certificates of Participation and Performance in the 2011-2012 ISAG Horse Comparison Test
5. ISAG Horse Comparison Test 2013-2014
   - 5.1. Appointment of Duty Laboratory for 2013-2014 HCT
   - 5.2. Appointment of Results Analysis Laboratory for 2013-2014 ISAG HCT
6. Current ISAG Recommendations for:
   - 6.1. Equine Identification and Parentage Analysis Standards
   - 6.2. Transfer of Equine DNA Profiles (Genotypes) and/or Parentage Analysis Results Between ISAG Member Laboratories
7. Matters for Consideration Regarding the Use of SNP Genotyping for the Parentage Analysis of Horses
8. DNA Testing for Disease and Colour Markers
9. Other Business

Number of participants at meeting: 58

Committee members at commencement of 2012 Workshop

Name	Organisation	Email	Role/Term of Service
Ann Trezise	(AEGRC-UQ, Australia)	ann.trezise@uq.edu.au	Chair: 2010-2014 Member: 2006-2010
Romy Morrin-O'Donnell	(Weatherbys, Ireland)	rmorrin@weatherbys.ie	Member: 2006-2010 & 2010-2014
Sofia Mikko	(SLU, Sweden)	sofia.mikko@hgen.slu.se	Member: 2006-2010 & 2010-2014
Hitoshi Gawahara	(LRC, Japan)	h-gawahara@lrc.or.jp	Member: 2006-2010 & 2010-2012 HCT 2010-12 Duty Lab
Elena Genzini	(LGS Cremona, Italy)	elenagenzini@lgscr.it	Member: 2010-2014
Lee Millon	(VGL-UCD, USA)	lvmillon@ucdavis.edu	HCT Results Lab: 2008, 2010 & 2012.

Summary of the meeting including votes, decisions taken and plans for future conferences

1. Welcome

Ann Trezise welcomed all participants to the 2012 workshop

2. ISAG Horse Comparison Test 2011-2012

2.1. Report from HCT Duty Laboratory - Hitoshi Gawahara, LRC, Japan

2011-12 ISAG HCT Duty Laboratory was the Laboratory of Racing Chemistry, Japan.
- 90 Laboratories requested, and were sent HCT samples

HCT Samples:
20 unknown samples + 1 reference horse genomic DNA sample:
- 11 Thoroughbred
- 6 Westfalen
- 4 Selle Français

HCT Problems for the Duty Lab:
Late requests for HCT samples:
- 68 Labs requested HCT samples before the deadline of 1 July 2011
- 20 additional Labs requested HCT samples before the "extended" deadline of 12 October 2011
- 2 further labs requested samples after this.
- Online registration for the next HCT will deal with this issue

Incomplete, invalid or incorrect courier account information and/or import documentation

- Pre-pay or Valid Courier account number only
- Large, international courier companies only
- Eg. Fedex, DHL, TNT
- ALL import documents need to be correctly completed by the requesting ISAG member Laboratory and provided with original Consignment Request
- This is an on going and unsustainable problem for each Duty Lab.

2.2. Report from HCT Results Analysis Laboratory: VGL at UC Davis

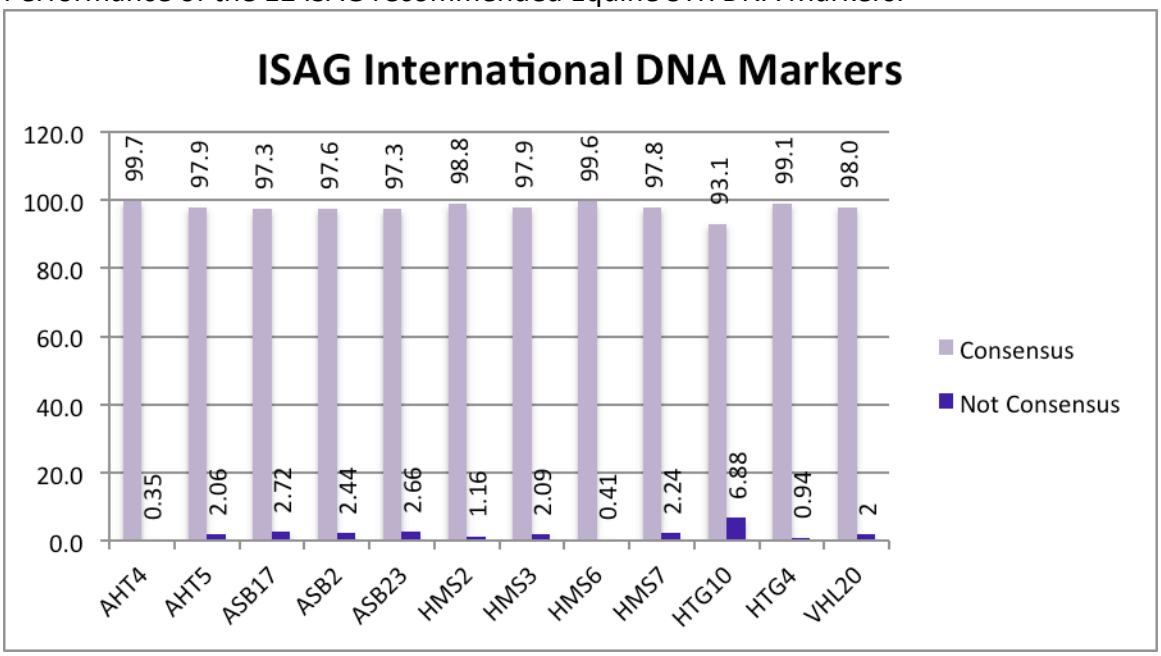
2011-12 ISAG HCT Results were submitted to, compiled and analysed by Lee V. Millon (VGL, UC Davis) and were confirmed and presented by Ann Trezise

85 Labs Reported Results
Overall good performance by Labs and the 12 ISAG recommended Equine STR DNA Markers: AHT4, AHT5, ASB17, ASB2, ASB23, HMS2, HMS3, HMS6, HMS7, HTG10, HTG4, VHL20

The #1 problem for the HCT Results Analysis Laboratory was incorrectly formatted results.
This increases workload, and
Increases manual correction

2.2.1. HCT Results: Genotyping Accuracy

Performance of the 12 ISAG recommended Equine STR DNA Markers:



The most common source of genotyping errors were:

Failure to recognise both alleles in the “LM” genotype in HTG10
Failure to recognise the “M” allele in HMS3
Failure to recognise the “U” allele in ASB23
Failure to recognise the “B” allele in ASB2

The following graph shows the performance of Reporting Laboratories in the 2011-12 HCT when scored for Absolute Genotyping Accuracy in genotyping the twelve ISAG recommended equine STR DNA Markers. The Workshop membership discussed the consequences of sub-optimal performance in the HCT for ISAG member laboratories and the circumstances that are outside the laboratory’s control that could impact on laboratory performance in the HCT. The Workshop membership discussed a range of measures that could be employed to address this issue and to ensure that all participating laboratories had an equal opportunity of achieving 100% genotyping accuracy in the HCT. After considerable discussion the following motion was put to the Workshop:

Motion: When circumstances arise with the HCT process, laboratories may be invited by the Chair of the Standing Committee to review and resubmit their data.

The motion was put to the Workshop by Romy Morrin-O’Donnell and was seconded by Cecilia Penedo. Voting rights, as one vote per ISAG Institutional Member, were confirmed by the President of ISAG, Ernie Bailey.

One representative from each ISAG Institutional Member organisation present at the workshop voted on the motion by a “show of hands”.

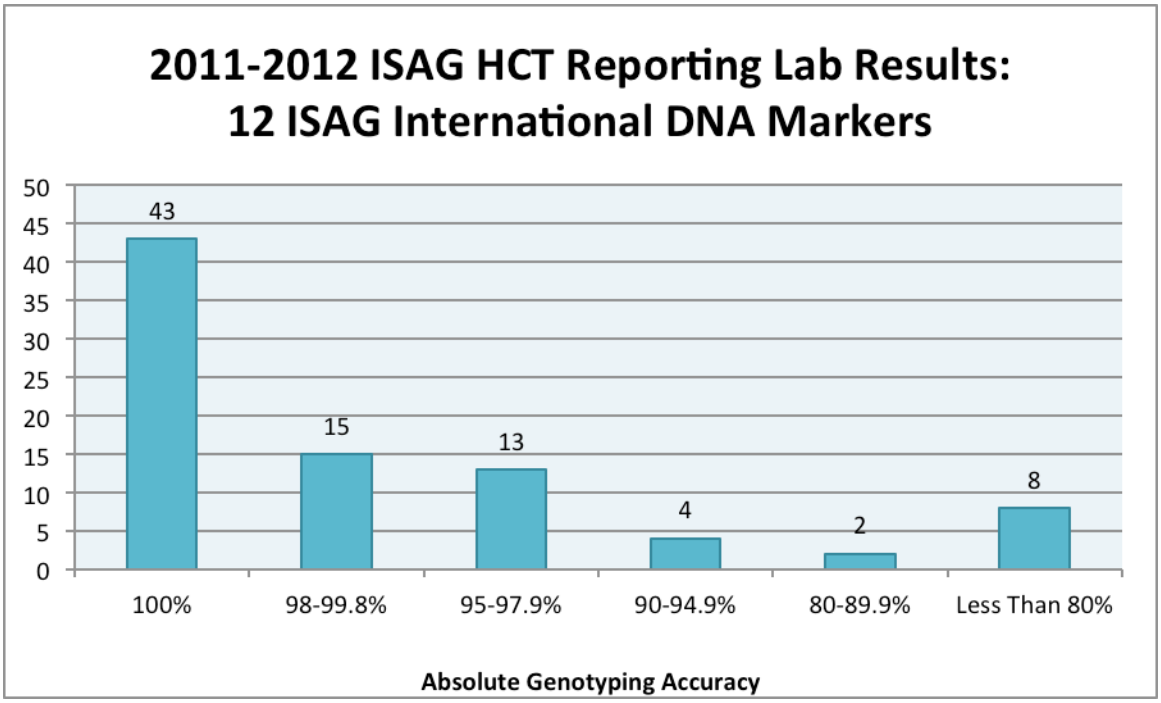
The result of the vote count was:

	TALLY
IN FAVOUR of the Motion	28
OPPOSED to the Motion	0
ABSTAIN	0

The Motion was CARRIED unanimously.

Recommendation: The Workshop also recommended that Two (2) Reference Samples be included, along with the Twenty (20) Unknown Samples in future Horse Comparison Tests.

No objection was made to the recommendation therefore adoption of this recommendation was the formal decision of the workshop.



2.2.2. HCT Results: Parentage Analysis Accuracy

The workshop discussed the importance of the correct application of parentage analysis principles, as distinct from the accuracy of genotyping the Equine STR DNA Markers Many Reporting Laboratories excluded parentage on the basis of two STR markers only: ASB23 and LEX3.

The Workshop recommended that parentage exclusions should not be made on the basis of X chromosome linked STR DNA markers such as LEX3.

In cases such as this, it is recommended that the ISAG recommended secondary Panel of 12 of the 15 TKY STR DNA Markers are genotyped in order to determine parentage.

3. Certificates of Participation and Performance in the 2011-2012 ISAG Horse Comparison Test

Certificates of Participation will be issued for the 2011-12 Horse Comparison Test and these will include the individual Laboratory's Rating calculated on percentage absolute genotyping accuracy. Absolute Genotyping Accuracy counts both "incorrect alleles" and "blanks" or "missing alleles" as genotyping errors when calculating a laboratory's genotyping accuracy and rating that will be reported on the 2011-12 Horse Comparison Test Participation Certificate. The following Table shows the percentage absolute genotyping accuracy associated with each Rating, which are the same as for the 2009-10 Horse Comparison Test. .

Rating	% Correct Genotypes
1	98-100%
2	95-98%
3	90-95%
4	80-90%
5	less than 80%

2011-12 Horse Comparison Test Participation Certificates will be emailed to HCT email contact addresses provided on the HCT consignment form.

4. ISAG Horse Comparison Test 2013-2014

ISAG members were reminded that they must have a valid ISAG Institutional Membership for 2013 and 2014 in order to participate in and receive a Participation Certificate for the 2013-2014 Horse Comparison Test.

4.1. Appointment of Duty Laboratory for 2013-2014 HCT

Institutional Members of ISAG were given the opportunity to nominate from the floor for the role of "Duty Laboratory", for preparation and distribution of purified Horse DNA samples (2 reference samples plus 20 unknown samples) for the 2013-2014 ISAG Horse Comparison Test.

Both Cecilia Penedo (VGL, UC Davis) and Sofia Mikko (SLU, Sweden) offered to undertake the role of Duty Laboratory. Following some discussion the workshop agreed to accept the offer from Dr Sofia Mikko to undertake the role of Duty Laboratory.

Dr Sofia Mikko, representing the Animal Genetics Laboratory at the Swedish University of Agricultural Sciences was appointed as the Duty Laboratory for the 2013-2014 ISAG Horse Comparison Test.

The participants of the workshop expressed their sincere thanks to Dr Sofia Mikko, on behalf of the Animal Genetics Laboratory (SLU, Sweden), for undertaking this significant and very important role.

4.2. Appointment of Results Analysis Laboratory for 2013-2014 ISAG HCT

Lee Millon from the Veterinary Genetics Laboratory, University of California at Davis (VGL-UC Davis), USA, volunteered to undertake the role of "Results Analysis Laboratory" for the collation and analysis of results reported by participating laboratories for the 2013-2014 ISAG Horse Comparison Test.

Other Institutional Members of ISAG were given the opportunity to nominate from the floor for the role of Results Analysis Laboratory for the 2013-2014 ISAG HCT. No other nominations were made.

Lee Millon, on behalf of VGL-UC Davis (USA), was appointed as the Results Analysis Laboratory for the 2013-2014 ISAG Horse Comparison Test.

The participants of the workshop expressed their sincere thanks to Lee Millon, on behalf of VGL-UC Davis (USA), for undertaking this increasingly complex and very important role.

5. Current ISAG Recommendations for:

5.1. Equine Identification and Parentage Analysis Standards

The current ISAG recommended standards for Equine DNA Profiling and Parentage Analysis are:

1. Organisations must be Institutional Members of the International Society of Animal Genetics.
2. Organisations must participate in each, biennial ISAG Horse Comparison Test.
3. Organisations must achieve Rating 1 (98-100% accuracy) in the ISAG Horse Comparison Test.
4. From 1 January 2011, the twelve ISAG recommended equine DNA Markers (ISAG Primary Equine DNA Panel) are: AHT4, AHT5, ASB17, ASB2, ASB23, HMS2, HMS3, HMS6, HMS7, HTG10, HTG4, VHL20.
5. For Equine parentage analysis: in the case of a single-system exclusion occurring in the twelve ISAG recommended equine DNA Markers (Primary Equine DNA Panel), at least twelve additional DNA Markers must be tested in the sire, dam and foal.
6. The twelve additional DNA Markers must be twelve, of the available fifteen, TKY DNA Markers (ISAG Secondary Equine DNA Panel). Ref: Tozaki et al., *J Vet Med Sci.* **63**(11):1191-7
7. If, after testing both the Primary and Secondary Equine DNA Panels, only a single-system exclusion remains, then the foal cannot be excluded (qualifies) as the offspring of the nominated sire and dam.

8. Equine identification can be accurately determined by use of nine of the twelve ISAG recommended equine DNA Markers (Primary Equine DNA Panel).
9. Horse International DNA Certificates must include the following minimum information:
    a. Complete DNA Profile information for the ISAG recommended DNA Markers (9 DNA Markers prior to 2011, and 12 DNA Markers after 1-1-2011),
    b. The Date the DNA Profile was determined,
    c. The Organisations' ISAG Institutional Membership Number.
10. Blood Typing is no longer recommended by ISAG for either equine parentage or identification analysis

The 2010 workshop recommended that ASB17, ASB23 and HMS2 Equine STR DNA Markers be included in the ISAG International Panel of Equine STR DNA Markers for all new Equine DNA Profiles produced after 1st January 2011.

For clarity, the workshop confirmed that there is no expectation that existing Equine DNA Profiles, produced prior to the 1st January 2011, be re-tested to include the three new DNA STR Markers (ASB17, ASB23 and HMS2). Therefore, parentage analysis cases involving Sire and Dam DNA Profiles determined prior to 2011 may be analysed across 9 common Equine STR DNA Markers.

No dissent was made and these recommendations were again endorsed by the Workshop.

5.2. Transfer of Equine DNA Profiles (Genotypes) and/or Parentage Analysis Results Between ISAG Member Laboratories

ISAG member laboratories have previously agreed a set of principles in respect to sharing Horse DNA Profiles between laboratories. These principles are:

1. The client, i.e. breed association or horse owner, owns the DNA Profile/genotype information and the results of any analysis (e.g. parentage).
2. DNA Profiles, genotypes and analysis results should never be given to any 3rd party (eg. another laboratory, individual or breed society/registry), without prior written approval or instructions from the client – the owner or breed society/registry that submitted the original sample for DNA analysis.
3. ISAG member laboratories will send the DNA profile/genotype of a horse to another ISAG member laboratory at the specific written request of the client, the horse owner or breed society/registry, usually for use in a specific parentage analysis or identification case.
4. If an anomaly (possible exclusion) arises in a case involving a DNA profile provided by separate laboratory, then all DNA profiles are confirmed with the laboratory/ies that

originally determined/provided each DNA profile before reporting the parentage anomaly (possible exclusion) result.

These principles were again endorsed by the Workshop.

6. Matters for Consideration Regarding the Use of SNP Genotyping for the Parentage Analysis of Horses

The Workshop again discussed the matter of using single nucleotide polymorphisms (SNPs) for equine parentage analysis as an alternative to the currently used Equine STR DNA Markers. Many of the issues that were discussed at the 2010 Workshop were again considered (see 2010 Report for details). In addition the workshop discussion was informed by:

1. The requirement for 100% accuracy in genetics testing and in identification and parentage analysis for the international horse racing and breeding industries, and the recreational and sport/eventing horse industries. The industry expectation of 100% accuracy is a reflection of the exceptionally high value of individual horses (particularly Thoroughbred horses), and very high average value of Thoroughbred horses, compared to other economically important species.
2. The experience of industry groups that have trialled, or are using, SNP-based parentage and identification analysis for other economically important species. The cattle industry is moving to SNP-based parentage and identification analysis. Research results presented at the ISAG Conference on the imputation of STR DNA Profiles from high-density SNP genotypes (over 700,000 SNPs) can reduce transition costs associated with genotyping animals using both SNP and STR methods. These research findings indicate 98% accuracy in imputation of STR DNA Profiles from high-density SNP genotypes in four dairy cattle breeds. To date, the imputation of STR DNA Profiles from high-density SNP genotypes has not been successful in beef cattle breeds. Developments in this area will continue and will be monitored for their relevance and potential application to equine identification and parentage analysis.
3. The experience and decision taken by the international human forensic DNA Profiling community to continue to use 13 internationally agreed, core human STR DNA Markers for human parentage and identification analysis for use in legal cases (Gill *et al.,* 2004 *Science and Justice* 44: 51-53).
4. Consideration of necessary transition arrangements, such as: the need for equine SNP Panel trialling/selection/agreement/an equine SNP comparison test, new capital equipment and database requirements, validation of SNP-based equine parentage analysis across many horse breeds.
5. Consideration of potential benefits of SNP-based equine parentage analysis, such as combining equine parentage and identification analysis with genetic screening tests for inherited diseases, coat colours and other characteristics.

Overall, the workshop decided that STR-based parentage and identification analysis continues to best serve the requirements of the international horse racing and breeding industries as an independent, highly accurate method of verifying horse pedigrees. The workshop also decided to continue to monitor the development and application of SNP-based parentage and identification analysis in other economically important species.

7. DNA Testing for Disease and Colour Markers

The question of whether there is a need for an international standardised nomenclature for reporting the results of genetic screening/diagnostic tests for inherited disease, coat colour and other characteristics in horses.

The Chair suggested that the Standing Committee should discuss this question and provide feedback to the ISAG member community. No dissent was made so this was the formal decision of the workshop.

8. Other Business: Committee Membership

The term of the majority of current committee members is ongoing (see Table below).

Hitoshi Gawahara (LRC, Japan) is retiring from the ISAG Equine Genetics & Thoroughbred Parentage Testing Standardisation Standing Committee. The Chair thanked Dr Gawahara and the Laboratory of Racing Chemistry – Japan, on behalf of the members of the Workshop and the wider ISAG community, for his service on the Standing Committee since 2006, and the essential role they fulfilled as Duty Laboratory for the 2011-2012 Horse Comparison Test.

The Chair called for nominations from the floor for membership for one Four year Term of the ISAG Equine Genetics & Thoroughbred Parentage Testing Standardisation Standing Committee.

Two nominations were made:

1. Lucie Genestout from Labogena, France.
2. Paula Hawthorne from AEGRC-UQ, Australia.

The Chair asked the Workshop membership if there were any objections to both nominees joining the Committee.

No objections were raised during the workshop and both nominees were accepted as members of the ISAG Equine Genetics & Thoroughbred Parentage Testing Standardisation Standing Committee.

Committee members at Conclusion of 2012 Workshop

Name	Organisation	Email	Role/Term of Service
Ann Trezise	AEGRC-UQ, Australia	ann.trezise@uq.edu.au	Chair: 2010-2014 Member: 2006-2010
Romy Morrin-O'Donnell	Weatherbys, Ireland	rmorrin@weatherbys.ie	Member: 2006-2010 & 2010-2014
Sofia Mikko	SLU, Sweden	sofia.mikko@hgen.slu.se	Member: 2006-2010 & 2010-2014 HCT Duty Lab: 2013-2014
Elena Genzini	LGS Cremona, Italy	elenagenzini@lgscr.it	Member: 2010-2014
Lee Millon	VGL-UCD, USA	lvmillon@ucdavis.edu	HCT Results Lab: 2008, 2010, 2012 & 2014.
Lucie Genestout	Labogena, France	lucie.genestout@jouy.inra.fr	Member: 2012-2016
Paula Hawthorne	AEGRC-UQ, Australia	p.hawthorne@uq.edu.au	Member: 2012-2016

9. Any Other Business:

The Chair asked if the members present at the Workshop if there was any other business. No other matters were raised.

There being no other business the meeting of the ISAG Equine Genetics & Thoroughbred Parentage Testing Standardisation Workshop was closed.

10. Workshop Meeting Closed.

2011-2012 Horse Comparison Test

If yes:

Number of enquiries – requests for consignment forms:	Unknown
Number of participants receiving samples:	90
Number of samples:	20 Unknown plus 1 Reference
Number of participants reporting results:	85

Duty laboratory:

Hitoshi Gawahara
Laboratory of Racing Chemistry – Japan
h-gawahara@lrc.or.jp

Comments (see main report)

1. Incomplete, invalid or incorrect courier account information and/or import documentation.
2. 25% of Participating Labs registered late for the Horse Comparison Test

Computing Laboratory:

Lee Millon
Veterinary Genetics Laboratory, University of California, Davis, USA
lvmillon@ucdavis.edu

Comments: The #1 problem for the HCT Results Analysis Laboratory was incorrectly formatted results.

List of recommended markers:

12 ISAG recommended Equine STR DNA Markers: AHT4, AHT5, ASB17, ASB2, ASB23, HMS2, HMS3, HMS6, HMS7, HTG10, HTG4, VHL20

2013-2014 Horse Comparison Test

If yes:

Number of enquiries – requests for consignment forms:	
Number of participants receiving samples:	
Number of samples:	20 Unknown plus 2 Reference
Number of participants reporting results:	

Duty laboratory:

Sofia Mikko
Animal Genetics Laboratory, Swedish University of Agricultural Sciences, Sweden
sofia.mikko@hgen.slu.se

Computing Laboratory:

Lee Millon
Veterinary Genetics Laboratory, University of California, Davis, USA
lvmillon@ucdavis.edu

List of recommended markers:

12 ISAG recommended Equine STR DNA Markers: AHT4, AHT5, ASB17, ASB2, ASB23, HMS2, HMS3, HMS6, HMS7, HTG10, HTG4, VHL20