



CONFERENCE 2014, Xi'an, China

Equine Genetics and Thoroughbred Parentage Testing Workshop

STANDING COMMITTEES / WORKSHOPS

Organised by a standing committee **YES**

Date and meeting time: 29th July 2014 2pm-5pm

Chair, name and contact email: Ann Trezise (ann.trezise@uq.edu.au)

Agenda / programme (items 1-8 summarized below)

Number of participants at meeting: 68

Summary of Meeting:

1. Welcome

Ann Trezise welcomed all participants to the 2014 workshop.

2. Current ISAG Standing Committee 2010-2014

Name	Organisation	Email	Role/Term of Service
Ann Trezise (Chair, Retiring)	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: 2010-2013 (retired Dec 2013)
Lee Millon	VGL-UCD, USA	lvmillon@ucdavis.edu	Member since 2006 Results Lab: since 2008
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

3. New Guidelines for Standing Committees and Comparison tests.

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:

- **Any liability of ISAG for participation in the Comparison Test, the execution of the test and its results, is excluded.**
- The participating institution and its employees **will not and cannot claim damages** arising out of or in connection with the Comparison Test and its results from ISAG.
- 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.

Comparison test

Upcoming changes being considered for HCT process include:

- Consignment Form sign-up: via Institutional Membership Logon at ISAG website ONLY
 - Tick Box – I agree to exclude Liability
 - Institutional Members were asked to obtain advice from their organizations regarding the legal ramifications for them with respect to being required to agree to this Liability Exclusion by ISAG in order to participate in Comparison Tests. Institutional Members are asked to provide their feedback to the Standing Committee and the ISAG Executive.
- HCT Result Reporting: **Deadline is End of February.**
- Results to be uploaded via ISAG website ONLY
 - via Institutional Membership Log on
 - Incorrect Format and file will not upload
 - An example HCT results file and instructions for completion and submission of HCT Results via upload to the ISAG website will be placed on the ISAG website
 - FASS will make an initial compilation of the HCT Results
 - FASS and Standing Ctte will review and prepare HCT Report and ranks

The motion for FASS to make the initial compilation of the HCT results and for FASS and the Standing Ctte to review and prepare HCT Report with ranks was put to vote, with 23 in favor, 0 against and 0 abstain.

With respect to laboratory performance in HCT, it was noted that the International Studbook Committee for Thoroughbreds has enacted the following policy. If a Stud Book is using a laboratory that does not achieve rank 1 in the HCT, the Stud Book will be required to retype the full foal crop of the last 2 years, or since the last HCT in which their lab scored Rank 1, using a laboratory that scored Rank 1.

Duty Lab Responsibilities

- A list of the current ISAG recommended microsatellite DNA markers will be made available to participants on the ISAG website, including primers for STRs and flanking DNA sequence. The current Standing Ctte is taking care of this now. In the meantime, see: <http://www.cstl.nist.gov/strbase/horseSTRs.htm> - by Leanne van Goor (VHL) and the table below.

Locus	Chrom. Location	Repeat structure	Repeat sequence	Original reference	Primer sequences (Forward and Reverse)	Amplicon length (bp)
AHT4	24q14	Compound	(AC) _n AT(AC) _n	Binns et al. (1995)	F: AACCGCCTGAGCAAGGAAGT R: CCCAGAGAGTTTACCCT	144-164
AHT5	8	Simple	(GT) _n	Binns et al. (1995)	F: ACGGACACATCCCTGCCTGC R: GCAGGCTAAGGAGGCTCAGC	126-144
ASB2	15q21.3-q23	Simple	(GT) _n	Breen et al. (1997)	F: CCACTAAGTGTCTGTTTCAGAAGG R: CACAACCTGAGTTCTCTGATAGG	216-250
ASB17	2p14-p15	Simple	(AC) _n	Breen et al. (1997)	F: ACCATTCAGGATCTCCACCG R: GAGGGCGGTACCTTTGTACC	87-129
ASB23	3q22.1-q22.3	Simple and Compound	(TG) _n and (TG) _n TT(TG) ₄	Irvin et al. (1998)	F: GAGGGCAGCAGGTTGGGAAGG R: ACATCCTGGTCAAATCACAGTCC	175-211
CA425 UCDEQ425	28q18	Simple	(GT) _n	Eggleston-Stott et al. (1997)	F: AGTGCCCTCGTTAAITCA R: CTCATGTCGGTTGTCTC	226-246
HMS1	15	Simple	(TG) _n	Guerin et al. (1994)	F: CATCACTTTCATGTCTGCTTGG R: TTGACATAAATGCTTATCCTATGGC	170-186
HMS2	10	Compound	(CA) _n (TC) ₂	Guerin et al. (1994)	F: CTGTCAGTCGAATGTGTATTAATG R: ACGGTGGCAACTGCCAAGGAAG	222-248
HMS3	9	Compound	(TG) ₂ (CA) ₂ TC(CA) _n and (TG) ₂ (CA) ₂ TC(CA) _n GA(CA) ₅	Guerin et al. (1994)	F: CCATCCTCACTTTTTCACTTTGTT R: CCAACTCTTTGTACATAACAAGA	148-170
HMS6	4	Simple	(GT) _n	Guerin et al. (1994)	F: GAAGCTGCCAGTATTCAACCATTG R: CTCCATCTTGTGAAGTGAACTCA	151-169
HMS7	1q25	Compound	(AC) ₂ (CA) _n	Guerin et al. (1994)	F: TGTGTTGAAACATACCTTGACTGT R: CAGGAAACTCATGTTGATACCATC	165-185
HTG4	9	Complex	(TG) _n AT(AG) ₅ AAG(GA) ₅ ACAG(AGG) ₃	Ellegren et al. (1992)	F: CTATCTCAGTCTTGATTGCAGGAC R: CTCCCTCCCTCCCTCTGTTCTC	127-139
HTG6	15q26-q27	Simple	(TG) _n	Ellegren et al. (1992)	F: GTTCACTGAATGTCAAATTCCTGCT R: CCTGCTTGGAGGCTGTGATAAGAT	84-102
HTG7	4	Simple	(GT) _n	Marklund et al. (1994)	F: CCTGAAGCAGAACATCCCTCCTTG R: ATAAAGTGTCTGGGCAGAGCTGCT	118-128
HTG10	21	Simple and Compound	(TG) _n and TATC(TG) _n	Marklund et al. (1994)	F: TTTTATTCTGATCTGTCACATTT R: CAATCCCCGCCCCACCCCGGCA	95-115
LEX3	Xq	Simple	(TG) _n	Coogler et al (1996)	F: ACATCTAACCAGTGCTGAGACT R: GAAGGAAAAAAGGAGGAAGAC	142-164
VHL20	30	Simple	(TG) _n	Van Haeringen et al. (1994)	F: CAAGTCCTCTTACTTGAAGACTAG R: AACTCAGGGAGAATCTTCCTCAG	87-105

- This information should be available to ISAG Institutional Members only and via FASS ISAG log on.
- The workshop recommended that the following sentence should be removed from the ISAG Guidelines for Comparison Test: “Additional questions for genotyping can be directed to the Duty Laboratory.”

- There was some discussion about shipping samples for comparison tests. It was suggested that FASS or ISAG could have an account with one or possibly a few courier service companies and the duty labs would use this/these to ship samples. FASS would administer the payment by participants for the sample transport. If labs do not pay Transport fee they will not receive HCT results and certificate. The current Standing Ctte will put this question forward to the ISAG Executive Ctte.
- Health certificate has to be in English or include an English translation on the same document.
- Duty Lab will need to work/consult with Standing Ctte more often during organization of test.

HCT Samples

A vote was taken on sending a total of 20 HCT samples including the reference sample(s). Results were 25 in favour, 0 against and 0 abstain.

A vote was taken on sending a total of 20 HCT samples including either one (1) or two (2) reference samples. Results were 14 in favour of two reference samples, 11 in favour of 1 reference sample, 0 abstain. Participants thus voted to have two reference samples in each test, i.e. 18 unknowns and two reference samples.

A vote was taken to have the Duty Lab send the 2 reference samples to two members of the Standing Ctte to confirm sample quality and genotype prior to distribution to participants. Results were 20 in favour, 0 against, 0 abstain. Participants voted to approve this as QC check.

New Guidelines for Standing Committees:

- Election is for 4-Year Term, candidates may be re-elected multiple times.
- Standing Committee will produce a Standard Operating Procedure (SOP) for the HCT.
- The SOP can be tailored to suit the requirements of the HCT.

4. ISAG Conferences in 2016 and 2017

It was agreed at the business meeting in Cairns in 2012 that ISAG conferences should move to odd years so the conferences would not conflict with the World Congress on Genetics Applied to Livestock Production (WCGALP). The first ISAG conference on the odd year is in 2017. The question thus arose if a comparison test was required for the 2016 and 2017 conferences. There was a unanimous vote to run the HCT for 2015-2016 and 2016-2017 (24 votes in favor).

Volunteers as duty labs:

- 2015-2016: VGL UC Davis – Cecilia Penedo
- 2016-2017: OPVGL South Africa – Cindy Harper /Annette Ludwig in partnership with Pranisha Soma (Pranisha@arc.agric.za), Animal Genetics Division, Agricultural Research Council, Irene, South Africa.

5. SNP Panel for Horse Parentage Analysis

Development of a SNP Panel for Horse Parentage

The use of SNP for parentage testing of horses was discussed. Horse breeders know about SNPs and that these are currently used for genomic selection in cattle, for example. They see the future possibility of using SNPs for genomic selection also in the horse and to do the parentage testing at the same time. Research about genomic selection in horses is now ongoing in some countries.

Representatives from eight labs present at the workshop expressed interest in working on the panel of parentage SNPs published by Teruaki Tozaki and possibly performing a Horse Parentage SNP comparison test in the future. Ernie Bailey informed the group that a wide range of SNP's should be available soon when testing was done on many breeds using the Equine 670K chip. Some delegates expressed concern on the investment required to switch to SNP parentage verification and also concerns were raised in relation to the requirement to SNP type breeding stock.

The transfer from microsatellites to SNPs for parentage testing has already been done for cattle and we should consider experiences from that species. For one, cattle had better genomic resources to allow development of STR imputation chip to facilitate transition to SNP testing for major dairy and beef breeds undergoing genomic selection. About two sets of 100 SNP parentage markers for cattle are being fine-tuned for use across breeds and subspecies. Given the different situations between cattle and horse, it is more likely that NGS would be used for horse parentage before completing a process similar to what has been applied to cattle. We will be better off looking toward a change to NGS to allow combined STR and SNP typing as the future for horse parentage.

6. Standardised Nomenclature for Disease and Colour Markers

Many labs provide testing for disease and coat color genes but few report results for these tests on the HCT. The Standing Ctte did not find compelling need for international standardisation of nomenclature. It is more important that each lab provides adequate explanation of test results to clients.

7. ISAG Horse Comparison Test 2013-2014

A) Report from HCT 2013-2014 Duty Laboratory, Sofia Mikko, Dept. of Animal Breeding and Genetics, Swedish University of Agricultural Sciences.

Preparing and sending samples

- 94 laboratories requested samples. Duty lab prepared 115 sample sets to accommodate extra participants.
- Samples: 16 Standardbred, 4 Swedish Warmblood, 1 Standardbred reference sample.
- DNA was prepared from whole blood using a QIA Symphony robot, dissolved in 30ul ATE and normalized to 50ng/ul.

Problems found by HCT duty lab

- Late enrolment (4)
- Cancellation before sending out samples
- Invalid courier service accounts (17)
- Change of courier service or shipping method after application (17)
- Change of contact person
- Import permit needed but not provided with application, or wrong documents submitted
- Non-delivered sample packages returned to duty lab
- Samples not released by Customs (1)
- Extensive email contact with 35 participants to sort out problems around shipping time.

Suggestions for future comparison tests

- Participating laboratories book and arrange the shipment themselves

or

- FASS provides the Duty Lab with a courier account, pays for shipment of CT Samples and recovers costs from participants.

B) Discussion of 2013-2014 HCT Results

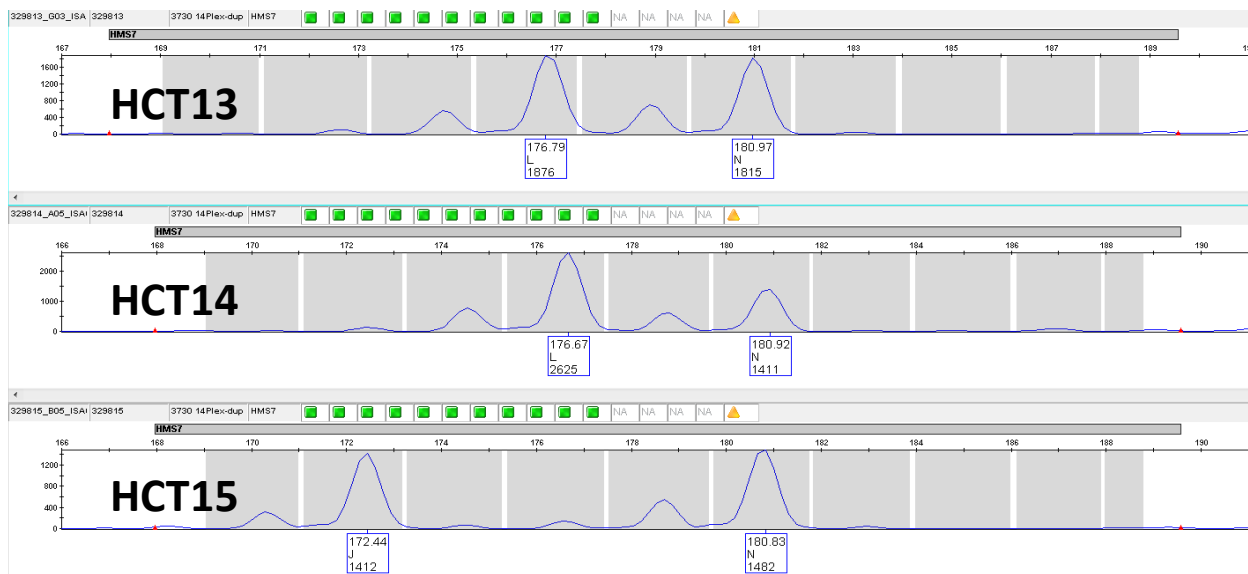
Overall concordance of results was good for both the ISAG and extra panels. With respect to the ISAG panel, 45% of laboratories with 100% agreement, 30% with 98-95%, 14% with 97-95% and 11% with less than 95%. Issues with allele classification were discussed for HMS7 (primary panel) and TKY374 (extra panel).

HMS7 results of sample #14 (39 of 85 labs failed to detect “N” allele).

Participants were reminded of the 1997 publication (Eggleston-Stott et al. Animal Genetics 28:438-440) regarding this null allele. Recommendations were made to use an alternative primer (or a mixed-based primer) to amplify the null allele. Also, commercial STR kit corrects for this problem. With respect to the current HCT, a motion was put to a vote to exclude HMS7 results for sample 14 in the calculation of performance. Voting results were 14 to exclude, 6 against exclusion, 3 abstentions. Computer Lab will provide adjusted lab performance rating. It was further discussed that the Standing Cttee will not make allowances for similar problem in future HCTs.

This figure below shows DNA profiles for 2013-14 HCT samples HCT13, HCT14 and HCT15. The “N” allele in HCT14 is detectable using the solutions mentioned above. The heterozygous status of Sample HCT14 at HMS7 was further confirmed by sequencing this sample.

Locus	HCT01	HCT02	HCT03	HCT04	HCT05	HCT06	HCT07	HCT08	HCT09	HCT10	HCT11	HCT12	HCT13	HCT14	HCT15	HCT16
HMS7	LN	L	L	LN	JN	JN	NO	LN	N	LN	JO	N	LN	LN	JN	N
HMS7	LN	L	L	LN	JN	JN	NO	LN	N	LN	JO	N	LN	L	JN	N
HMS7	LN	L	L	LN	JN	JN	NO	LN	N	LN	JO	N	LN	LN	JN	N
HMS7	LN	L	L	LN	JN	JN	NO	LN	N	LN	JO	N	LN	L	JN	N



TKY374 (rounding of alleles at low end of allele range)

Participants were reminded of differential migration of amplicons across the range of alleles and need to adjust bin sizes. An example was given for allele F samples 18 and 20, which were misclassified as “G” by two laboratories.

C) Certificates of Participation and Performance in the 2013-2014 ISAG Horse Comparison Test.

Certificates will be issued by the ISAG Secretary sometime after the Conference.

D) Current ISAG Recommendations for:

- A. Equine Identification and Parentage Analysis Standards
- B. Transfer of Equine DNA Profiles (Genotypes) and/or Parentage Analysis Results Between ISAG Member Laboratories

These recommendations have not changed since the 2012 Conference and will be placed on the ISAG website, accessible by Institutional members only.

8. Election New Members: Equine Genetics and Parentage Standard Committee

Delegates elected a new committee. The former chair Ann Trezise stepped down. The members of the new committee are:

Name	Organization	Email	Role/Term of Service
Cecilia Penedo	VGL UC Davis , USA	mctorrespenedo@ucdavis.edu	Chair: 2014-2017 Member: 2014-2017
Romy Morrin-O'Donnell	Weatherbys, Ireland	rmorrin@weatherbys.ie	Member: 2014-2017 2006-2010 & 2010-2014
Sofia Mikko	SLU, Sweden	sofia.mikko@hgen.slu.se	Member: 2014-2017, 2006-2010 & 2010-2014
Rosina Fossatti	Genia, Uruguay	fossatti@genia.com.uy	Member 2014-2017
Lee Millon	VGL-UCD, USA	lvmillon@ucdavis.edu	Results Lab: since 2008
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
Hironaga Kakoi	Laboratory of Racing Chemistry, Japan	h-kakoi@lrc.or.jp	Member 2014-2017
Marcela Martinez	Lab. De Genetica Aplicada, Argentina	mmartinez@sra.org.ar	Member 2014-2017

Duty laboratory for the next comparison test with contact details

Cecilia Penedo	VGL UC Davis , USA	mctorrespenedo@ucdavis.edu	Duty Lab 2015-2016
Cindy Harper	OPVGL South Africa	cindy.harper@up.ac.za	Duty Lab 2016-2017
Pranisha Soma	Agric. Res. Council	Pranisha@arc.agric.za	Duty Lab 2016-2017

Computing laboratory for the next comparison test with contact details

FASS and Standing Committee with advice from Lee Millon (past Results Lab)	VGL-UCD, USA	lvmillon@ucdavis.edu
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Report prepared by the members of the 2010-2014 and 2014-2017 Equine Genetics & Parentage Standing Committee.

SIGNATURES

Chair
Ann Trezise

Duty laboratory
Sofia Mikko

Computing laboratory
Lee Millon