ISAG CONFERENCE 2016, Salt Lake City, USA

Equine Genetics and Thoroughbred Parentage Testing Workshop

Organised by a standing committee yes

Date and meeting time: July 25, 2016, 8:30AM -12 Noon

Chair, name and contact email: Cecilia Penedo, mctorrespenedo@ucdavis.edu

Agenda / programme attached

8:30 – 8:35: Welcome – Opening remarks
8:35 – 9:35: Horse Comparison Test
9:35 – 10:00: ISBC Directives and Lab Performance – Improvement of the Process
10:00 – 10:30: Coffee Break
10:30 – 11:00: Poster presentation - Development and Evaluation of a Set of 100 SNP Markers for DNA Typing in the Domestic Horse. Heather Holl et al. (ID 14744)
11:00 – 11:30: Donkey Comparison Test
11:00 – 11:30: Election of Committee & Any Other Business

Number of participants at meeting: 70-80

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

Several procedural changes were implemented for the 2015-2016 Horse Comparison Test (HCT). The test was organized by FASS that processed applications, collection of Customs documents and liability exemption, preparation of shipping labels for Duty Laboratory. Reports were directly uploaded onto ISAG website and results were compiled Jeremy Holzner at FASS, with assistance from Lee Millon, UC Davis.

Updated rules and policies governing the conduct of ISAG comparison tests are available online (http://www.isag.us/docs/Rules_CT.pdf?v2, http://www.isag.us/docs/Policy_CT.pdf?v2). On the policy document, item 27 specifies the process for revision of compiled results and item 28 specifies the process to request correction of concordant genotyping upon presentation of relevant supporting evidence. Participants were encouraged to read the new documents.

Horse Comparison Test
The Duty Lab was the Veterinary Genetics Laboratory, U.C. Davis, USA that prepared DNA for 20 horses (7 Thoroughbreds, 5 Quarter Horses, 2 Quarter Horse crosses, 2 Standardbreds, 1 Appaloosa, 1 Oldenburg, 1 Paint, 1 Percheron). Reference core panel genotypes for two samples were independently verified by two other labs (VHL, The Netherlands and Maxxam, Canada). Samples consisted of 100 ul DNA at 50 ng/ul diluted in light TE.

Delivery of packages was mostly without incident, with 3 requests for a new full set of samples and 2 requests to replace sample #1 because of missing DNA. From 104 applications,

results were returned by 95 laboratories among which 76 also participated in the 2013-2014 HCT. Participating labs represented 44 countries.

Draft results considered all discrepancies in ISAG core panel and extra markers from concordant types as "errors", including missing results, genotype format and genotype errors. There were no formatting discrepancies in the ISAG core panel but some were present in the set of extras markers to be corrected for the final report.

Concordance of results for the ISAG core panel was very good (98 – 99.7%) for 8 markers (AHT4, ASB17, ASB23, HMS2, HMS6, HMS7, HTG4, VHL20) with 2-8% of labs accounting for discrepancies. For 4 markers (AHT5, ASB2, HM3, HTG10) concordance was good (95.1 – 97.1%) but more labs (16-28%) presented discordant results. Overall, 55% of the participants had 100% concordance, 21% had 98-99%, 9% had 95-97% and 15% were below 95%.

Concordance of results for ISAG core panel

Locus	% Consensus	Incidence/Error type
HTG4	99.65	2 labs, genotyping and binning
AHT4	99.47	4 labs, genotyping and binning
HMS7	99.35	4 labs, genotyping and binning
HMS2	99.30	6 labs, genotyping and binning
ASB17	99.28	9 labs, genotyping and binning
HMS6	99.24	6 labs, genotyping and binning
ASB23	98.88	6 labs, genotyping and binning
VHL20	98.00	8 labs, genotyping and binning
ASB2	97.11	27 labs, genotyping, binning, detection of "B" allele
AHT5	96.21	16 labs, genotyping and binning
HTG10	95.17	16 labs, genotyping and binning, detection of "L" allele
HMS3	95.12	21 labs, genotyping and binning, detection of "M" allele

Concordance for the backup panel (14 TKY markers, TKY337 excluded) was also very good (96.7-99.7%) but only 9-24 laboratories returned results. Concordance for 6 extra markers (HMS1, HTG6, HTG7, LEX33, LEX3, CA425) was also high (average 99.1%) after exclusion of 1 lab for HMS1 and 1 lab for CA425 for what appeared to be related to incorrect primers used.

The parentage question was answered correctly by 90% with sire and dam assignment; about 4% of labs identified only the dam or the sire and 2% of labs excluded all candidates.

Genotyping issues in AHT5, ASB2, HMS3 and HTG10 and TKY 337 were reviewed. Historic problems of allele dropout in HMS3, HTG10 and TKY337 were discussed. Alternate primers to improve detection of alleles with poor or no amplification for HMS3 and HTG10 are given below. (SC note: Alternate primers for TKY337 will be available for HCT2017, pending additional validation). Problems with amplification of AHT5 by 3 users of a commercial kit, and other genotyping errors, suggested need for additional, lab-specific optimization given that 79% of labs had perfect concordance.

Primer	Sequence	Approx. Size Range

HTG10-F	CCTAATGTCATATGGAAAGCCTTG	135-169
HTG10-R	TGGGCTTTTTATTCTGATCTGTCACATTT	
HMS3-F	ACATCAGTCAGAAGCTGCGAAC	257-283
HMS3-R	CCCCTCTTGCTCTAAAGCCCCA	

Participants were encouraged to review their data based on compiled results to improve accuracy of genotypes. The Duty Lab informed that DNA samples are still available that can be provided upon request and payment for shipment to assist with optimization of test. Regarding contents of the report, participants agreed that results were compiled correctly. Regarding preparation of the final report, the SC will work with FASS to correct formatting problems but preserve reported genotypes.

Comparisons for diagnostic markers were possible for 9 loci, with 6-16 labs returning results and high level of concordance as shown below.

Locus	Correct Answer Returned	% Consensus	Notes
AME	310/320	96.9 (99.7)	1 lab - 9 errors
SRY	115/116	99.1	
AGOUTI	259/260	99.6	
MC1R	279/280	99.6	
LWO	220/220	100	
CREAM	240/240	100	
GRAY	140/140	100	
LEOPARD	120/120	100	
SCID	80/80	100	Mutation not present

ISBC Directives and Lab Performance – Improvement of the Process

The International Studbook Committee (ISBC) relies on ISAG and the Equine Genetics and Thoroughbred Parentage Testing Standing Committee (EGTPT) recommendations to enact policies for all regional studbook authorities regarding performance requirements of laboratories that provide parentage testing services for Thoroughbreds. Current policy to strengthen international compliance requires Rank 1 status in biennial HCTs and sanctions laboratories with Rank 2 and below. Sanctions include interruption of service and retesting of horses up to 2 years back by a Rank 1 laboratory. The current system tied to the HCT cycle is not meeting expectations of all stakeholders and solutions need to be found.

The workshop discussion considered 3 possibilities not-mutually exclusive: 1) annual ISBC-organized test intercalated with ISAG HCT, 2) assembly by ISBC of an expert panel to investigate failure and guide corrective action, with support from EGTPT SC, 3) ISBC requirement for staff training at a qualified laboratory for at least 3-4 weeks.

Discussions lead to 2 motions that were put to vote, each with unanimous approval:

1. **Delegate authority to the standing committee to review requests for corrections of clerical errors in genotypes that are properly documented by the HCT participant and to make justified corrections for final compilation and ranking.**

Implementation of this decision removes the impact of clerical errors in performance ranking.

2. **If ISBC changes current rules to accommodate corrective action for sanctioned labs between HCTs, the SC will work with them to establish an expert panel to redress problems which may include, for example, additional testing and staff training.**

Composition of expert panel should include representatives of ISBC and impacted local studbook(s), 1 member each from ISAG Executive Committee and EGTPT SC, 1 service laboratory with good record of performance.

To address request from participants with limited genotyping experience with breeds other than Thoroughbreds, future HCTs documentation from Duty Lab will include information on range of alleles per marker. Allele ranges will be included in workshop reports for future reference.

SNP Testing

Discussion about transition to SNPs for horse genotyping followed the poster presentation by Dr. Heather Holl (ID 14744) on the selection of 100 SNPs from the Equine SNP50 public dataset and validation across 32 breeds for power in individual identification and parentage analysis. It was generally recognized that a change in technology will be needed in the near future but concerns with cost to implement transition, current costs of SNP testing, ability to effect a transition on a worldwide basis were raised. The need to develop additional SNP panels was also recognized. New technologies are emerging that may be better suited to support the switch and need to be explored.

There was agreement that laboratories should be given an opportunity to evaluate SNP panels for the next comparison test. Sequence information will be collected by the SC to be included with HCT documentation.

Donkey Comparison Test

Duty Lab: Dr. Rainer Schubbert, Eurofins, Germany
Number of participants: 10

DNA testing of donkeys and mules occurs in different countries but a standardized ISAG core panel for donkeys is lacking. The horse ISAG core panel is not sufficient for use in donkeys. To address this need, the first donkey comparison test was organized by Dr. Rainer Schubbert of Eurofins (Germany) with support from ISAG/FASS, EGTPT SC, and several laboratories that contributed information about markers. DNA samples were provided from 20 donkeys with reference genotypes for 2 samples and a core of pre-selected 13 markers (see below). Results were compiled and distributed by Eurofins. Concordance of results was high except for TKY337 which showed evidence of "null" allele with original primer sequences. (SC note: Alternate primers for TKY337 will be available for HCT2017, pending additional validation). Other few discrepancies were related to incorrect binning and failure to amplify some alleles (e.g. in HTG10-M, TKY297-G).

The motion **"Elect the 13 marker panel selected by Eurofins as the minimum core ISAG panel for donkeys with nomenclature that follows the horse standard"** was put to vote with unanimous approval. Use of this nomenclature accommodates parentage analysis of mules.

Additional resolutions were to conduct another donkey CT in 2017 and to identify additional markers that may be of interest for donkey testing, such as MHC-linked STRs to be provided by Dr. Doug Antczak. Announcement for the next CT will be posted by FASS. Eurofins volunteered as Duty Lab, with Dr. Torsten Brendel as the contact. Donkey CT results will be discussed in the EGTPT workshop.

Donkey ISAG Core Panel:

AHT4, HMS6, ASB23, HTG10, HMS3, HMS2, HTG7, HMS7, HMS18, TKY297, TKY312, TKY337, TKY343.

Except as noted above for TKY337, primer sequences are the same as those used for horses. Primers for HMS18 (not in horse ISAG core panel) are:

HMS18-F: CAACAATGAAAATTTGTCCTGTGC
HMS18-R: GTAAATGAGTAGACAATCATGAGG

Elections/Any Other Business

Next HCT will occur in 2017 with Duty Lab work by Dr. Cindy Harper from South Africa. Announcement and forms will be posted via ISAG/FASS in mid-September.

Late applications, submission of reports: Late applications continued to be a problem in 2015-2016 HCT with shipments occurring through February. Delays also occurred in reporting of results. **Deadlines for CTs in 2017 will be enforced**.

Standing Committed Elections:
To synchronize terms of service with new ISAG conference calendar, the following motion was put to vote with unanimous approval: **Current SC members will continue to serve through 2017 with election of new members to happen at the next workshop in Dublin.**

Committee members (the new committee)

Chair	**term of service**	**E mail address:**
Cecilia Penedo	2014-2017 (1st term)	mctorrespenedo@ucdavis.edu
Other members	**term of service**	**E mail address:**
Romy Morrin-O'Donnell	2014-2017	rmorrin@weatherbys.ie
Sofia Mikko	2014-2017	sofia.mikko@hgen.slu.se
Rosina Fossatti	2014-2017	fossati@genia.com.uy
Lee Millon	2008-2017	lvmillon@ucdavis.edu
Lucie Genestout	2012-2017	lucie.genestout@labogena.fr
Hironaga Kakoi	2014-2017	h-kakoi@lrc.or.jp
Marcela Martinez	2014-2017	mmartinez@sra.org.ar
Cindy Harper (Duty Lab, horse)	2016-2017	cindy.harper@up.ac.za
Torsten Brendel (Duty Lab, donkey)	2016-2017	TorstenBrendel@eurofins.com

COMPARISON TEST (2015-2016) **yes**

Duty laboratory name and email address

Dr. Cecilia Penedo, mctorrespenedo@ucdavis.edu – horse

Dr. Rainer Schubbert, RainerSchubbert@eurofins.com - donkey

Comments (issues rising)

No Duty Lab issue to report. Organization by FASS ensured smooth and efficient processing of applications.

List of recommended markers for horses with primer information

Locus	ECA	Primer sequences		Amplicon length
AHT4	24	F: AACCGCCTGAGCAAGGAAGT	R: CCCAGAGAGTTTACCCT	144-164
AHT5	8	F: ACGGACACATCCCTGCCTGC	R: GCAGGCTAAGGAGGCTCAGC	126-144
ASB2	15	F: CCACTAAGTGTCGTTTCAGAAGG	R: CACAACTGAGTTCTCTGATAGG	216-250
ASB17	2	F: ACCATTCAGGATCTCCACCG	R: GAGGGCGGTACCTTTGTACC	87-129
ASB23	3	F: GAGGGCAGCAGGTTGGGAAGG	R: ACATCCTGGTCAAATCACAGTCC	175-211
CA425/UCDEQ425	28	F: AGCTGCCTCGTTAATTCA	R: CTCATGTCCGCTTGTCTC	226-246
HMS1	15	F: CATCACTCTTCATGTCTGCTTGG	R: TTGACATAAATGCTTATCCTATGGC	170-186
HMS2	10	F: CTTGCAGTCGAATGTGTATTAAATG	R: ACGGTGGCAACTGCCAAGGAAG	222-248
HMS3*	9	F: CCATCCTCACTTTTTCACTTTGTT	R: CCAACTCTTTGTCACATAACAAGA	148-170
HMS6	4	F: GAAGCTGCCAGTATTCAACCATTG	R: CTCCATCTTGTGAAGTGTAACTCA	151-169
HMS7	1	F: TGTTGTTGAAACATACCTTGACTGT **	R: CAGGAAACTCATGTTGATACCATC	165-185
HTG4	9	F: CTATCTCAGTCTTGATTGCAGGAC	R: CTCCCTCCCTCCCTCTGTTCTC	127-139
HTG6	15	F: GTTCACTGAATGTCAAATTCTGCT	R: CCTGCTTGGAGGCTGTGATAAGAT	84-102
HTG7	4	F: CCTGAAGCAGAACATCCCTCCTTG	R: ATAAAGTGTCTGGGCAGAGCTGCT	118-128
HTG10*	21	F: TTTTTATTCTGATCTGTCACATTT	R: CAATTCCCGCCCCACCCCCGGCA	95-115
LEX3	X	F: ACATCTAACCAGTGCTGAGACT	R: GAAGGAAAAAAAGGAGGAAGAC	142-164
VHL20	30	F: CAAGTCCTCTTACTTGAAGACTAG	R: AACTCAGGGAGAATCTTCCTCAG	87-105

*original sequence; can produce null allele. Alternative primers suggested elsewhere in report.

** original sequence; can produce null allele. Alternate sequence for consideration: TGTTSTTGAAACATACATTGACTGT.

Duty laboratory for the next comparison test with contact details
Horse: Cindy Harper, cindy.harper@up.ac.za

Donkey: Torsten Brendel, TorstenBrendel@eurofins.com

SIGNATURES

Chair **Duty laboratory**