ISAG CONFERENCE 2017, Dublin, Ireland

Equine Genetics and Thoroughbred Parentage Testing

STANDING COMMITTEES / WORKSHOPS Information will be posted online

Organised by a standing committee yes

Date and meeting time: Tuesday, July 18, 9:00 AM - 1:00 PM

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

Agenda / programme

9:00 AM		**Welcoming Remarks.**
9:10 AM		**Horse Comparison Test.**
10:00 AM		**Donkey Comparison Test.**
10:30 AM		**Horse SNP Comparison Test.**
11:00 AM		**Coffee/Tea Break.**
11:30 AM	71554	**Genetic diagnosis of sex chromosome aberrations in horses based on analysis of microsatellite and X- and Y-linked markers.** J.A. BOUZADA*, J.M. LOZANO, M.R. MAYA, A. TRIGO, I. BONET, F. CASTILLO, J. FERNÁNDEZ-LEÓN, T. MAYORAL, E. ANADÓN, and L.B. PITARCH, *Laboratorio de Genética y Control, Ctra. M106, km. 1,4, 28110 Algete, Madrid Spain.*
11:45 AM	69688	**RATE OF SEX REVERSAL CASES IN HORSES OF ARGENTINA.** Maria Martinez*, Monica Costa, Belen Elguero, and Cecilia Ratti, *Laboratorio de Genética Aplicada, Sociedad Rural Argentina, Juncal 4431, 2º. Buenos Aires. Argentina.*
12:00 PM	71427	**Characterization of equine STR panel "15 TKY system" by imputation from dense SNP genotypes in a Thoroughbred population.** M Kikuchi*, H Kakoi, T Tozaki, K Hirota, and S Nagata, *Laboratory of Racing Chemistry, Utsunomiya, Tochigi, Japan.*
12:15 PM	71443	**Preliminary results of genetic monitoring of the occurrence of three genetic diseases (CA, SCID; LFS) in Arabian horses from Poland.** M Bugno-Poniewierska*[1], M Stefaniuk-Szmukier[2], A Piestrzynska-Kajtoch[1], A Fornal[1], and K Ropka-Molik[1], [1]*National Research Institute of Animal Production, Department of Animal Genomics and Molecular Biology, 1 Krakowska street, 32-083 Balice n. Krakow, Poland,*, [2]*University of Agriculture, Department of Horse Breeding, al. Mickiewicza 24/28, 30-059 Kraków; Poland.*
12:30 PM		**Election of Committee Members and Any Other Business.**

Number of participants at meeting: 71

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

1. Horse STR Comparison Test 2016-2017

Duty Laboratory: Dr. Cindy Harper, University of Pretoria, South Africa (Not present)

***Samples*:** 20 DNA samples representing 4 breeds – Thoroughbred (9), Nooitgedacht (8), Arabian (2), Basuto Pony (1). Extractions were done with phenol-chloroform procedure and 50 ul aliquots provided to participants. DNA concentrations were not normalized but ranged between 50-170 ng/ul. No problems with sample quality were reported by participants. Two reference DNA genotypes were provided for ISAG panel only.

***Participants*:** 100 laboratories applied for the test and 96 (44 countries) reported results. A few labs missed communications regarding change of deadline. Results were due March 31, 2017 but deadline was extended to April 30 as initially stated in report form. Among the 96 participants, 14 (15%) were new laboratories (not in 2013-2014 or 2015-2016 tests).

Summary of Results:
ISAG Panel*:* Overall agreement among labs was very good. Average genotype concordance for the ISAG panel (12 STRs) was 97.5% and ranged from 93% (HMS3) to 99.4% (HMS6). Four STRs, ASB23, HMS7, HTG10, and HMS3 had the lowest concordance rates. The table below summarizes concordance rates and common types of errors. In general, concordance rates were similar to 2015-2016 comparison test. First time participants, without much experience with markers and the standardized nomenclature, and uncommon variants not previously seen by some labs, accounted in part for reduced concordance.

Concordance of results for ISAG core panel

Locus	Percent Consensus	Incidence
HMS6	99.42	7 labs, genotyping and binning
ASB2	99.36	7 labs, genotyping
VHL20	99.36	7 labs, genotyping and binning
HMS2	98.73	8 labs, genotyping and binning
AHT5	98.60	11 labs, genotyping
HTG4	98.15	9 labs (1 lab = 18 miscalls)
AHT4	98.09	6 labs (2 labs with >10 miscalls)
ASB17	97.08	15 labs (1 lab with 11 miscalls)
ASB23	96.93	11 labs (“U” allele)
HMS7	96.24	49 labs (#16, “N”)
HTG10	95.49	23 labs, binning and “L”
HMS3	93.05	33 labs, binning and “M”, “P”

Historical problems with some variants in HMS7, HTG10 and HMS3 continued to occur. Topical examples of problematic variants were discussed.

Regarding two parentage questions, 99% of labs answered question 1 correctly (qualification) and 72% answered question 2 correctly (exclusion) but all recognized the mismatch in HMS3. Questions regarding ISAG standards for parentage analysis and how to deal with single-locus mismatches led to suggestion to circulate text from the 2012 Workshop that details the recommendations. The SC chair will follow-up on this request.

The SC reviewed 4 requests for changes of genotype results (3) and sample order (1). The unanimous consensus was for no such corrections to be made. Quality control of genotype results is the responsibility of CT participants. Paragraphs 27 and 28 of the Rules for Conducting Comparison Tests for Animal DNA Test apply to copying errors in compilation or stipulation of "concordant" genotype as the correct genotype. Only clerical changes (extra spaces or characters) that do not change genotype calls were done for final version of compiled results.

Horse Extra Panel – TKY markers:
Even though reference genotypes were not provided for TKY panel markers, overall concordance was good averaging 97.2% and ranging from 88.2-100%. Fewer labs returned data for this panel, with 17-23 labs reporting results for 14 markers and 8 labs for TKY279. Most problems appeared related to lack of reference genotypes for proper binning of alleles.

Horse Extra Markers:
Among other markers reported, a set of 6 markers – CA425, HMS1, HTG6, HTG7, LEX3, LEX33 - were reported by 15-69 labs. Concordance rate was very good averaging 99% and ranging from 98.2-99.5%.

Diagnostic Markers:
Multi-lab comparisons were possible for 4 coat color (Agouti, Cream, MC1R, LWO) and two sex markers (AME, SRY), with 6 to 23 labs returning results. Concordance was high, averaging 99.6% and ranging from 98.7-100%.

For future horse comparison tests, the following recommendations are made:

1. Duty Laboratory needs to provide aliquots of 80-100 ul of DNA extracts normalized to 50 ng/ul (or 4000-5000 ng DNA in total) to allow for multiple testing, including SNP panels.
2. Duty Laboratory needs to provide reference genotypes for as many markers as possible. Reference genotypes are the consensus of 2-3 labs, including Duty Lab.
3. Duty Laboratory will not include report deadline in paperwork to participants. This information will come from ISAG/FASS.
4. Participants need to be prepared to run ISAG Panel STRs individually, especially HMS3, HMS7 and HTG10.

2. Donkey Comparison Tests 2016-2017

Duty Laboratory: Dr. Torsten Brendel, Eurofins Medigenomix GmbH, Ebersberg, Germany

Samples: 20 DNA samples representing 7 breeds - Ane de Provence (5), Ane du Cotentin (4), Baudet du Poitou (2), Ane D'Origine Constatee (2) , Ane Normand (3), Ane des Pyrenees (1), Bake (3). DNA was extracted from blood and hair roots (Bake) samples and was sent in a concentration of around 20ng/µl in a volume of 30µl to the participants. DNA extractions were done with Chemagic DNA Blood100 Kit, Chemagen (PerkinElmer)/ Maxwell 16 FFS Nucleic Acid Extraction Kit (Promega)

Participants: 25 laboratories (12 countries). After shipment and delivery of the packages three labs asked for a new set of samples and were provided with the requested samples.

Summary of Results:
Concordance of results for the markers ASB3, HMS18, HMS2, HTG10, HTG7, TKY312 and TKY343 was very good (above 97%). In marker AHT4 some labs struggled with the binning of the allele "z". The nomenclature system names alleles below A (lower end of range) with lower case and reverse order of alphabet. Alleles beyond Z (upper end of range), alleles are named with lower case letter starting with "a".

In marker HMS3, HMS7, TKY297 some labs had more general binning issues. In marker HMS6 some labs missed alleles. And as in the last year's CT the concordance of marker TKY337 suffered from a null allele caused by a mutation in the primer binding site of the original primers mentioned in the paper (*Population Study and Validation of Paternity Testing for Thoroughbred Horses by 15 Microsatellite Loci; Tozaki et al., 2001*). It was suggested to labs that had issues with the null allele to get in touch with labs that have modified their primers with regards to this problem.

Overall, more genotyping errors occurred with DNA extracted from hair samples than from DNA extracted from blood samples. During the workshop the overall opinion was that a possible lower DNA concentration of these samples could have led to this phenomenon. The parentage question was answered correctly by all labs.

Concordance of results for the ISAG core panel

Locus	Percent Consensus
AHT4	96.62
ASB23	98.84
HMS18	98.23
HMS2	99.07
HMS3	93.46
HMS6	96.76
HMS7	93.69
HTG10	98.73
HTG7	98.55
TKY297	92.78
TKY312	97.22
TKY337	89.89
TKY343	98.89

3. Horse SNP Comparison Test

Eight laboratories contributed data for the first horse SNP CT. Participation was voluntary. Candidate markers consisted of 53 SNPs validated for Thoroughbred parentage testing (Hirota et al. 2010, JVMS 72(6):719-726) and 101 SNPs from the panel selected by Etalon/Heather Holl and presented at the 2015-2016 workshop. Samples were the same as provided for the STR test. Results were compiled by Lee Millon and Cecilia Penedo from University of California, Davis and distributed to participants. Only 3 labs provided data for the 53-SNP marker set.

Platforms used: KASPar (1 lab, 10 SNPs only), Illumina SNP70 (2 labs), Illumina Infinium (1 lab), MassArray (3 labs), Affymetrix 670K (1 lab).

Summary of Results:
Genotype concordance (relative accuracy) was high (≥ 98.8%) among markers tested. Among markers tested, 2 SNPs with 3 alleles were identified by 1 lab: BIEC349712 (expected based on Hirota et al.'s paper) and BIEC2-1061845. Participants were encouraged to review their results for these two markers, and adjust allele calling rules to account for 3 alleles.

Lab Code	Percent Consensus
84414	99.42%
84422	100% (10 SNPs)
84460	99.94%
84486	99.95%
84764	98.96%
85067	99.62%
110956	98.94%
119135	98.76%

The pattern of discrepancies for 3 SNPs - BIEC2-183251, BIEC2-444978, BIEC2-1095371 – suggested that these markers may not be as robust as others, and sensitive to assay design, to warrant monitoring. Development of a secondary 100-SNP panel was suggested with general agreement from participants.

Other business and election of committee members

1. Participants were informed of communication by the Executive Committee (during Chairs meeting with Secretary) regarding increase of CT processing fee to $85.00.

2. The proposal for the Executive Committee to reorganize CT sample distribution to defray costs and avoid further financial losses to ISAG was discussed. The proposal considers the use of a central laboratory in Europe with access to worldwide couriers and competence to receive bulk DNA for all CTs, aliquot samples for safe transport, and make single shipments to labs according to applications. Ensuing discussions recognized positive aspects of the proposal but also raised questions regarding overall costs, how institutional membership fees are used by ISAG, and the diminishing role of CT workshop participants in the process. SC chairs requested via letter to the Secretary for additional information and detail regarding

proposal and finances. This topic is still under consideration at the EC level and no immediate change will be implemented.

3. Status of communications with ISBC and lab requirements for Thoroughbred testing. The SC submitted a proposal in 2016. A new ISBC Genetics Sub-Committee has been formed and will consider suggestions to implement a process that addresses their needs. Romy Morrin-O'Donnell is a member if this committee and will keep the SC informed on developments.

4. **2018-2019 Comparison Tests**: Horse and Donkey CTs will be organized. Dr. Torsten Brendel (Eurofins) volunteered as Duty Laboratory for both.

5. **Election of SC Members**:

   Leslie Bickel, University of California-Davis, was elected as new member of SC to replace Lee Millon who timed out. Dr. Torsten Brendel continues to join the SC in his capacity as Duty Lab.

Committee members (the new committee)

Chair	term of service	E mail address:
Cecilia Penedo	2017-2019 (2nd)	mctorrespenedo@ucdavis.edu

Other members	**term of service**	**E mail address:**
Romy Morrin-O'Donnell	2017-2021 (2nd)	rmorrin@weatherbys.ie
Rosina Fossatti	2017-2021 (2nd)	fossati@genia.com.uy
Lucie Genestout	2017-2021 (2nd)	lucie.genestout@labogena.fr
Hironaga Kakoi	2017-2021 (2nd)	h-kakoi@lrc.or.jp
Marcela Martinez	2017-2021 (2nd)	mmartinez@sra.org.ar
Leslie Bickel	2017-2021 (2nd)	labickel@ucdavis.edu
Torsten Brendel (Duty Lab)	2017-2019	TorstenBrendel@eurofins.com

COMPARISON TEST (2016-2017) **YES**

Duty laboratory name and email address

Dr. Cindy Harper, University of Pretoria, South Africa, cindy.harper@up.ac.za

Comments (issues rising)

Import permit for US laboratories. Problem resolved with DNA extraction protocol. Permits issued and samples shipped to all US applicants.

List of recommended markers with primer information

Horse:

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.

Donkey:
ISAG core panel for donkeys is: AHT4, HMS6, ASB23, HTG10, HMS3, HMS2, HTG7, HMS7, HMS18, TKY297, TKY312, TKY337, TKY343.

Primer sequences for first 8 STRs are the same as used in horse test. Sequences for last 5 STRs are:

HMS18-F	CAACAATGAAAATTTGTCCTGTGC
HMS18-R	GTAAATGAGTAGACAATCATGAGG
TKY297-F	GTCTTTTTGTGCCTCTGGTG
TKY297-R	TCAGGGGACAGTGGCAGCAG
TKY312-F	AACCTGGGTTTCTGTTGTTG
TKY312-R	GATCCTTCTTTTTATGGCTG
TKY337-Fv	TTTTGAGCAGAGCAGGGTTT
TKY337-Rv	CTTGTGCCCCTCATGTCTTT
or	
TKY337-Fg	CTCAAGAGGTCAATCAGAGGA
TKY337-Rg	CTCCAACTCTTCCACTCTGC
TKY343-F	TAGTCCCTATTTCTCCTGAG
TKY343-R	AAACCCACAGATACTCTAGA

Duty laboratory for the next comparison test with contact details

Dr. Torsten Brendel, Eurofins Medigenomix GmbH, Ebersberg, Germany,
TorstenBrendel@eurofins.com

SIGNATURES

Chair

Duty laboratory (Horse)

Duty laboratory (Donkey)