



XVI JORNADAS DO GHEP-ISFG

Vienna (Austria), 29 - 30 August 2011

M7

GHEP-ISFG 2011 Exercise

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Background

Módulo forense /Forensic module

M7: sangre no humana (perro)/*Non human blood (dog)*

M8*: mezcla de sangre de dos donantes y esperma de un tercer donante: 2,5 ml sangre D2 + 1 ml sangre D4 + 4,5 ml de dilución 1:1 de esperma D6/*Mixture of blood from two donors and sperm from one donor: 2.5 ml blood D2 + 1 ml blood D4 + 4.5 ml dilution 1:1 of sperm D6*

M9: cabello de mujer (D5) contaminado con saliva (D2)/*Hair from a female (D5) contaminated with saliva (D2)*

M7 – Non-human blood (dog)

Participating Laboratories - 45

Results summary for M7

Inconclusive - 13

Non-human - 28

Human - 4

Inconclusive – 13 (29%)

- No results with human genetic markers
- Possibly non-human or presence of inhibitors

23169 most likely blood stain of non-human origin

23229 La muestra M7 a pesar de haber sido extraída dos veces, no ha dado lugar a ningún resultado para la batería de análisis de rutina del laboratorio. No pueden aportarse datos.

23236 La muestra M7 no amplificó para ninguna de las plataformas de STR utilizadas por nuestro laboratorio. Este resultado plantea que dicha muestra contiene ADN de origen NO HUMANO, o que contiene inhibidores para la amplificación por PCR los cuales no pudieron ser eliminados mediante la extracción orgánica a la que se sometió a la muestra.

Non-human – 28 (62%)

Determine species
(animal)
12 (43%)

Not determine species
(animal)
16 (57%)

Mass Spect.
1

mtDNA
9

STRs
2

Preliminary
9

Human
Quantif.
5

P+HQ
2

Cyt B
7

16s
1

Dog CR
3

Cyt B + Dog CR – 2
Prelim + Cyt B – 1
Prelim + Cyt B + STRs – 1
No info - 1

Methods described for determining species and dog genetic profiling



Mass Spectrometry - ESI-MS (electrospray ionization mass spectrometry)

Comparison of hemoglobin subunit masses between Canis familiaris and Human

23262 Não foi detectada na amostra M7 a presença de DNA humano em quantidade mínima para os exames. A análise do espectro de massas da amostra utilizando ESI-MS indicou compatibilidade com as massas das subunidades da hemoglobina de Canis familiaris. Não foram observadas nenhuma das subunidades de hemoglobina humana.

Cyt B

+

16s

Int J Legal Med. 2000;114(1-2):23-8.

Species identification by means of the cytochrome b gene.

Parson W, Pegoraro K, Niederstätter H, Föger M, Steinlechner M.

Int Congress Series. 2006, 1288: 103-105.

Analysis of inter-specific mitochondrial DNA diversity for accurate species identification.

Pereira F, Meirinhos J, Amorim A, Pereira L.

23269 M 7: La muestra M 7 pertenece a una muestra de sangre de *Canis lupus familiaris*. Fue determinado por secuenciación de la región 16S del ADN mitocondrial.

23228 Muestra M7 Se realizó una búsqueda mediante la herramienta BLAST de la secuencia del gen citocromo b del ADN mitocondrial obtenida. Esta secuencia fue identificada como perteneciente a la especie *Canis lupus familiaris*.

23222, 23228, 23233, 23234, 23257, 23269, 23272, 23239

Dog mtDNA CR

Int J Legal Med. 2007 Sep;121(5):411-6.

Molecular characterization of the canine mitochondrial DNA control region for forensic applications.

Eichmann C, Parson W.

Forensic Sci Int Genet. 2009 Dec;4(1):49-54.

Forensic analysis of dog (*Canis lupus familiaris*) mitochondrial DNA sequences: an inter-laboratory study of the GEP-ISFG working group.

van Asch B, Albarran C, Alonso A, Angulo R, Alves C, Betancor E, Catanesi CI, Corach D, Crespillo M, Doutremepuich C, Estonba A, Fernandes AT, Fernandez E, Garcia AM, Garcia MA, Gilardi P, Gonçalves R, Hernández A, Lima G, Nascimento E, de Pancorbo MM, Parra D, Pinheiro Mde F, Prat E, Puente J, Ramírez JL, Rendo F, Rey I, Di Rocco F, Rodríguez A, Sala A, Salla J, Sanchez JJ, Solá D, Silva S, Pestano Brito JJ, Amorim A.

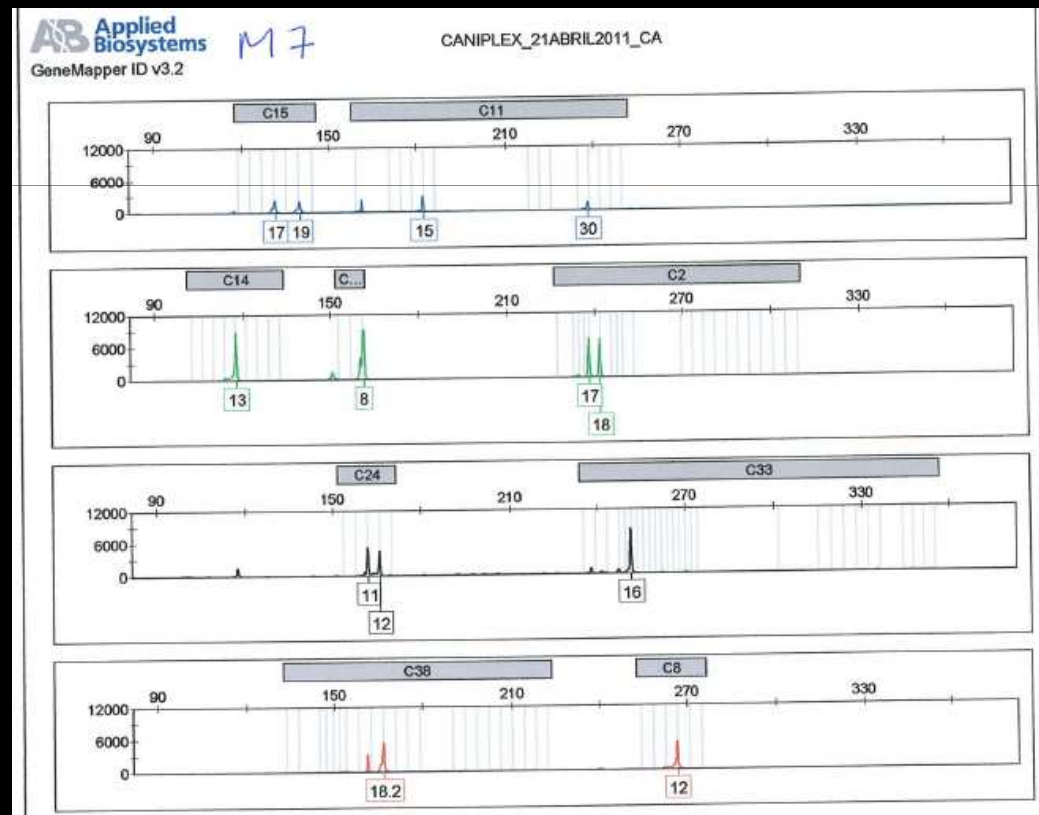
23222, 23224, 23239

STRs

Electrophoresis. 2009 Jan;30(2):417-23.

A new autosomal STR nineplex for canine identification and parentage testing.

van Asch B, Alves C, Gusmão L, Pereira V, Pereira F, Amorim A.



23232

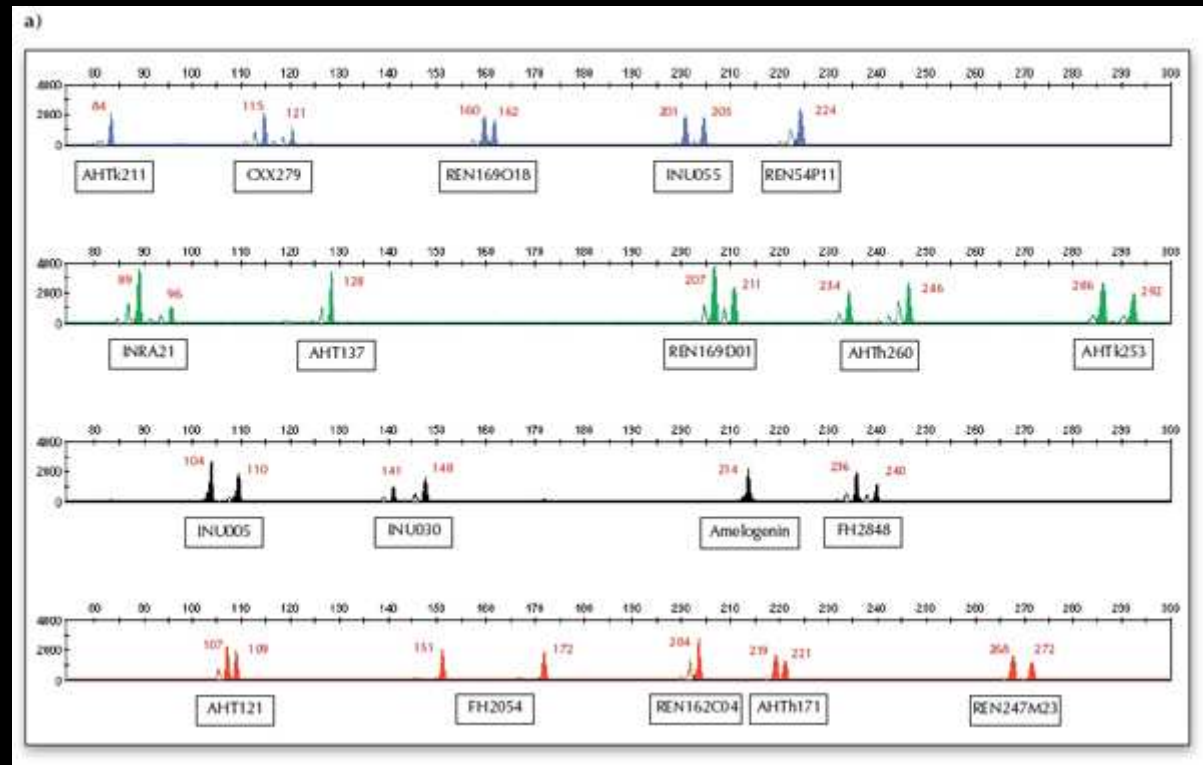
STRs

Canine Genotypes™ Panel 1.1

Microsatellite assay for dog parentage testing and identification

http://diagnostics.finnzymes.fi/canine_genotypes.html

18 STR loci



23228

Human – 4 (9%)

Laboratories report...

STR data (Aut, Y, X) - 3

mtDNA data - 1

23230, 23245, 23263, 23358

23230

23230 M7 es una mezcla de M1 o M3 y otro individuo con las mutaciones 16069C y 16126C.

23230 M7 es una mezcla de M1 o M3 y otro individuo con las mutaciones 16069C y 16126C. M1 y M3 corresponden al haplogrupo T1, y el individuo restante pertenece al haplogrupo J.

Only reports mtDNA data for M7

23230

M7	15-426, 15975-16418	73G 263G 309.1C 315.1C 16069M 16126C 16163R 16186Y 16189Y 16294Y 16390R
M8	17-426, 15975-16418	73R 152Y 195Y 263G 309.1C 315.1C 16069Y 16093Y 16192Y 16224Y 16270Y 16304Y 16311Y 16318W 16319R
M9	17-426, 15975-16418	73G 263G 309.1C 315.1C 16126C 16163G 16186T 16189C 16294T 16390A

Precinto	Muestra	DR/FR1	DR/FR2	DR/FR3	DR/FR4	Ciclos
<i>Code</i>	<i>Sample</i>					<i>Cycles</i>
23230	M7	L15975	H16418	L15	H429	40
23230	M8	L15975	H16418	L15	H429	40
23230	M9	L15975	H16418	L15	H429	40

23230 reports consensus results for all other samples

23245, 23263, 23358

All report STR data for M7

23245 La muestra M7 presenta un perfil masculino único diferente al de las muestras M1, M2, M3 y M4.

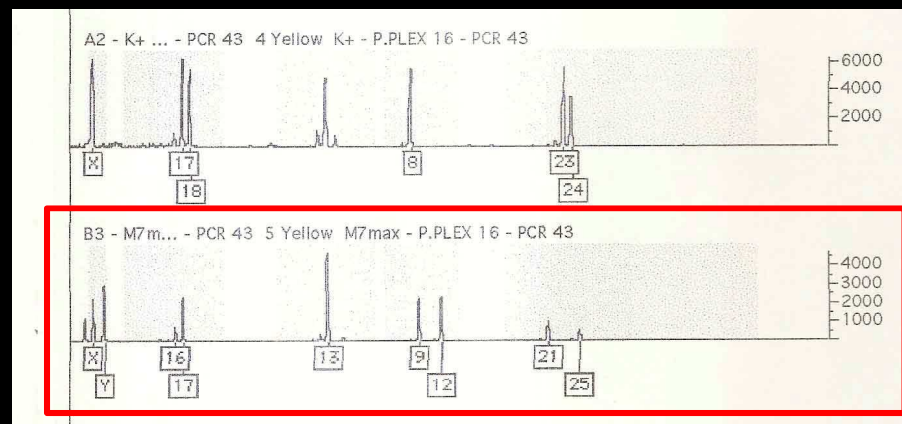
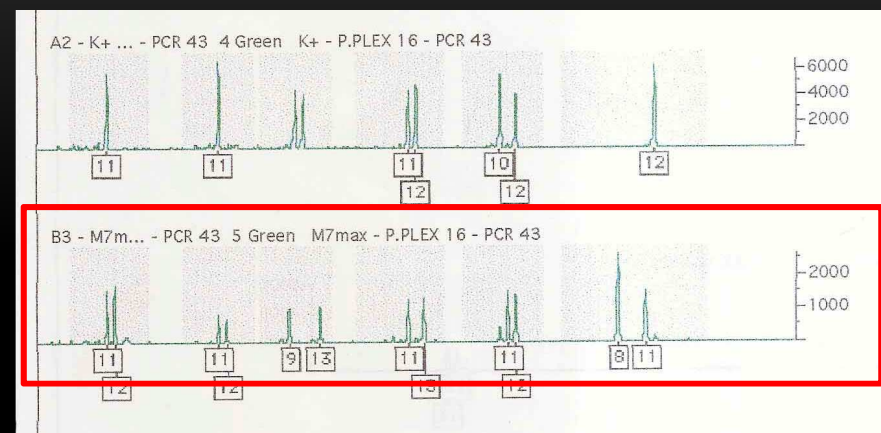
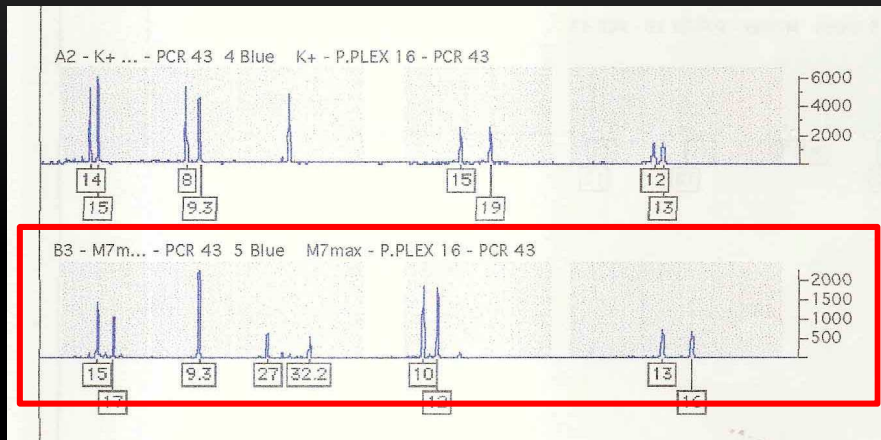
23263 No se pudo obtener una secuencia de ADN mitocondrial con la calidad suficiente para ser analizada de la muestra 7.

Does not comment the STR data obtained

23358 La muestra 7 corresponde a un individuo de sexo femenino

All report consensus results for other samples except 23263 (especially bad in mtDNA)

23245

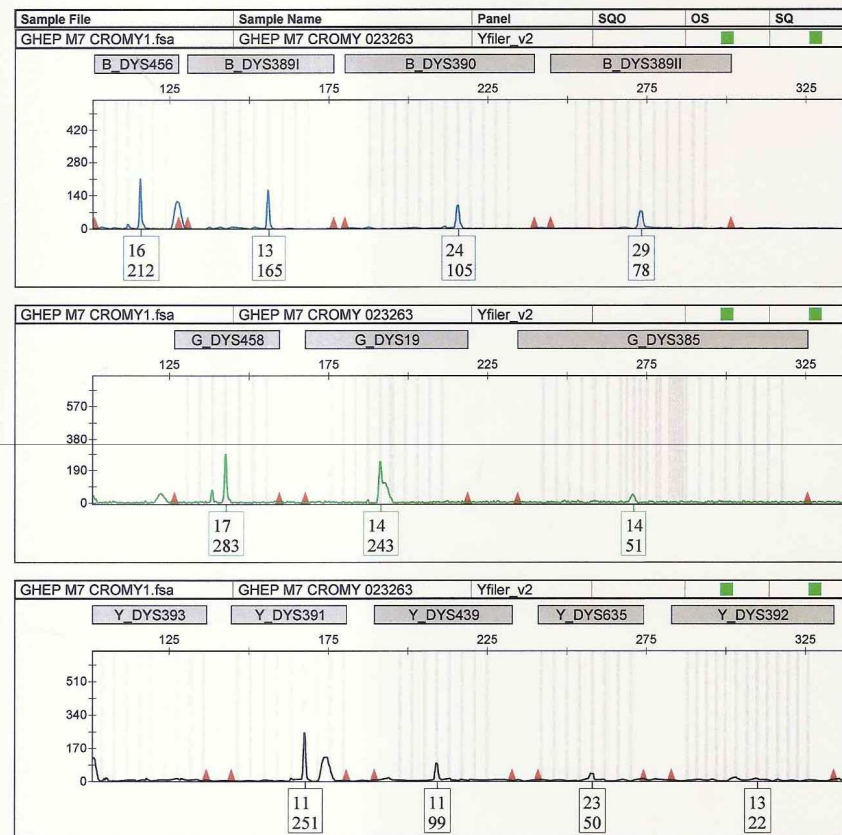


23263

23263 47

Applied
Biosystems
GeneMapper ID v3.2

CC GHEP-ISFG 2011



23358



23245, 23263, 23358

These 3 labs only report STR data for M7

Apparently complete profiles

Profiles are not shared between labs – distinct profiles

Profiles are not shared with any of the other CQ samples

Contamination / sample switch with routine casework

Summary for M7 results

- ★ Nearly a third (29%) of participating (forensic?) laboratories are not equipped for species determination of unknown samples. Most laboratories base their conclusions on suspicions (due to non-amplification of human specific markers).
 - ★ The remaining two thirds (62%) are able to confirm non-human origin of the samples; nearly half of these (43%) can identify the species.
 - ★ The method of choice for 75% labs who identify species is analysis of Cytochrome b region of mtDNA.
 - ★ Nearly 60% of labs which confirm non-human origin of the sample, base their conclusion solely (?) on preliminary analysis results.
 - ★ Finally, there are 4 labs (9%) which claim M7 is of human origin by reporting human genetic marker results.
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Species identification

Review of methods, e.g.

Recent Pat DNA Gene Seq. 2008;2(3):187-99.

Identification of species with DNA-based technology: current progress and challenges.

Pereira F, Carneiro J, Amorim A.

Forensic Sci Med Pathol. 2010 Sep;6(3):195-206.

DNA typing in wildlife crime: recent developments in species identification.

Tobe SS, Linacre A.

Current methods, e.g.

Nucleic Acids Res. 2010 Dec;38(22):e203.

Identification of species by multiplex analysis of variable-length sequences.

Pereira F, Carneiro J, Matthiesen R, van Asch B, Pinto N, Gusmão L, Amorim A.

Am J Forensic Med Pathol. 2011 Jun;32(2):180-2.

Discrimination between human and animal DNA: application of a duplex polymerase chain reaction to forensic identification.

Tozzo P, Ponzano E, Novelli E, Onisto M, Caenazzo L.