APPENDIX 1. Mock report for the case putative father/child duo

REPORT
Paternity Test
nº xx/ yyyy

The XXXX asked for the genetic testing on the possible paternity relationship of

So-and-so

relatively to

Child

through the request ref. xxx (copy attached).

Technical procedures

On the day[th] of Month, Year, both individuals were present at the Institute/Laboratory xxx where they were identified by presentation of ID documents and filled and signed the identification forms x and y (copy attached, containing photographs).

Blood/saliva samples were taken; storage, and subsequent treatments and analyses were performed in the same way and under the same conditions. Genetic profiles were made according to the specifications described in Annex 1 and correspond to at least two results obtained independently by two experts.

Results

See Table 1.

Conclusions

Assuming the conditions described in Annex 2, the results obtained show that the genetic profile configuration is **241 756 832** times (rounded up to unities) more likely assuming the hypothesis that So-and-so is the **biological father of Child** than under the hypothesis of the individuals being **genetically unrelated** (results per marker presented in Table 1).

Date and signatures

REPORT
Paternity Test
nº xx/ yyyy
Table 1

Genetic systems	So-and-so	Child	Likelihood Ratios*
CSF1PO	12-13	10-12	0.804
D2S1338	24-25	19-24	2.488
D3S1358	14-16	14-15	2.420
D5S818	8-13	8-11	36.761
D7S820	10-12	10	1.889
D8S1179	11-12	11-14	2.663
D13S317	11-13	12-13	2.434
D16S539	11-13	13-14	1.376
D18S51	12-18	13-18	3.776
D19S433	13.2-15.2	13-13.2	32.048
D21S11	28-29	28-33.2	1.702
FGA	19-23	23	3.398
Penta D	2.2-8	8-11	12.137

Penta E	5-12	8-12	1.280
TH01	7-9	9	2.493
TPOX	8-11	8-11	1.369
VWA	16	16-18	2.171

 * Rounded up to 3 decimals.

Date and signatures

Genetic systems and methods

Genetic systems (or loci; singular: locus)

locus	Typing kit
CSF1PO	a,b
D1S1656	b
D2S1338	b
D2S441	b
D3S1358	a,b
D5S818	a
D7S820	a
D8S1179	a,b
D10S1248	b
D12S391	b
D13S317	a
D16S539	a,b
D18S51	a,b
D19S433	b
D21S11	a,b
D22S1045	b
FGA	a,b
Penta D	a
Penta E	a
TPOX	a
TH01	a,b
VWA	a,b

Methods

Genomic material (DNA) was extracted according to (ref), amplified through PCR and analysed after capillary electrophoresis in an automatic sequencer (*equipment model and manufacturer*) according to the instructions from the manufacturers' kits a and b (*names and manufacturers*). All procedures are described in the Internal Forms *xxx*. Considering the database X (*reference or description of the population sample used*) for allele frequencies the *a priori* probability of two unrelated individuals share at least one allele for all the analyzed markers is equal to 1.065e-06. If the individuals are assumed as second degree relatives (grandparent-grandchild, half-siblings or avuncular) such probability equals 0.00338 (rounded up to 5 decimals).

REPORT
Paternity Test
nº xx/ yyyy
Annex 2

THEORETICAL, STATISTICAL AND PROBABILISTIC FRAMEWORK

The approach used to weight the evidentiary value of the results compares:

A – the probability of the observations (genetic profiles of the two individuals) assuming paternity,

and

B – the probability of the same observations assuming the two individuals are unrelated.

These hypotheses were, a priori, considered as equally likely by the requesting parties.

The comparison A/B takes the form of a Likelihood Ratio (LR, sometimes also designated as paternity index, PI) which therefore measures how much the observed results are more likely under the hypothesis of paternity relatively to the alternative hypothesis of no biological relationship.

The calculations are performed assuming that:

1. The putative father has no monozygotic ('identical') twin(s).
2. The putative father and the real father are genetically unrelated with the mother of the child.
3. The putative father is either the true father or genetically unrelated with him.
4. The tested individuals are assumed to belong to the population sampled for the estimation of gene frequencies (*reference and/or description of the population sample used, including sampling criteria*).
5. No gametic association (linkage disequilibrium) exists between the analyzed loci.

Calculations were performed using a home developed software and confirmed using Software XXX, v. XXX, date, available at XXXX.

All procedures are described in the Internal Forms XXX and YYY.

Supplementary Extension of

Formulation and communication of evaluative forensic science expert opinion.
A GHEP-ISFG contribution to the establishments of standards

On this Supplementary Extension we address the case where, after the corresponding report of a standard identity / paternity case was issued to the court, it is returned with the information that the man refuses the identity / paternity and claims that the true donor of the sample / father is in fact his meanwhile deceased full brother. In this extension we will discuss how to deal with this new information for a situation in which, under the assumption of one of the hypotheses of kinship, the alleged father is not available for testing

Thus, keeping as framework the typical identity and paternity cases previously issued, the paradigmatic examples here considered are situations where, after the emission of a first report where such information was not available, the possibility of the real donor of the sample (case i.) / real father of the child (case ii.) to be an unavailable full-brother of the tested individual must be evaluated.

Note that when kinship analyses are computed, experts have to acknowledge the existence of kinship classes of pedigrees, clearly transmitting in the emitted report that unassociated markers can only distinguish between classes of pedigrees, not between specific genealogies, see (Thompson, 1976), (Weir et al., 2006), and (Pinto et al. 2010), for example. Inconceivable pedigrees (considering other sources of information than genetics) belonging to the same kinship class as the questioned one can be, thus, a priori discarded. Indeed, for analyses in paternity framework where two or, eventually, three individuals (putative father, child and, possibly, the mother of the child) are analyzed, it is equally likely the real father of the child to be (a.) a full-brother, (b.) the father or (c.) a son, of the tested alleged father, since the three pedigrees: (a.) avuncular, (b.) half-siblings and (c.) grandparent-grandchild, belong (and compose) a specific autosomal kinship class of pedigrees and are, therefore, indistinguishable through autosomal unassociated markers (see Figure 1). Thus, if we want to quantify the possibility of the tested individuals being related as uncle – nephew, we have to discard a priori (and based upon sources of information other than genetics) the possibility of the real father of the child to be the father or a son of the tested man (in which cases the individuals are related as half-siblings and grandfather – granddaughter, respectively).

Figure 1: Second degree pedigrees belonging (and composing) a specific kinship class. Note that, regardless of the amount of unassociated autosomal data analyzed, the pedigrees are (theoretically) indistinguishable. Indeed, $LR = \frac{P(G|H_1)}{P(G|H_2)} = \frac{P(G|H_1)}{P(G|H_3)} = \frac{P(G|H_2)}{P(G|H_3)} = 1$, for any genetic configuration G and assuming the a priori probabilities $P(H_i)$, for i = 1, 2, 3, as equally likely.

H_1: Avuncular H_2: Half-siblings H_3: Grandparent / Grandchild
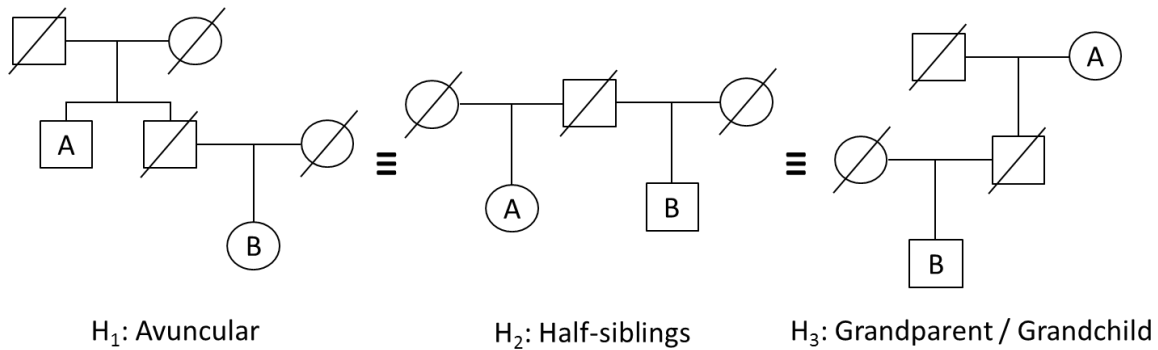
On the other hand, for analyses in identity context, and for the particular case here considered: the real donor of the DNA evidence can be a full brother of the analyzed individual, the interpretation of the statistical analyses is simpler since, assuming two individuals, the pedigree "full-siblings" composes itself a kinship class (Pinto et al. 2010). Nevertheless, caution has to be taken if a different pedigree connecting the real donor and the tested individual is at stake. For example, if the real donor A of the sample recovered from the crime scene is possibly (a.) the father, or (b.) a son, of the analyzed individual B, statistical calculations cannot weigh differently the two possibilities: (a.) A is father of B, and (b.) A is son of B.

Due to the sharing of identical-by-descent alleles being not required for other kinships than identity and paternity between non-inbred individuals, statistical calculations are likely to be less powerful for these pedigrees and extra statistics can be added to the report. Namely, it can be useful to present the probability of a pair of 2nd degree relatives (for case i.) and a pair of full-siblings (for case ii.) sharing identical alleles (both alleles for case i. and at least one allele for case ii.) for all the analyzed markers. However, it should be clearly communicated to the court that it is an *a priori* statistical parameter, only dependent of the allele frequencies in the population and not related with the analyzed individuals at that specific case.

TECHNICAL, STATISTICAL AND PROBABILISTIC FRAMEWORK

Note that techniques, methods, assumptions and even genetic material are the same for this second analysis and no extra genetic analyses in wet laboratory are required. Indeed, only the theoretical framework of the case has changed and solely new statistical calculations are required. As previously only autosomal, unlinked and unassociated markers, expected to be in conformity with Hardy-Weinberg expectations, are considered. Reliable estimates for allele frequencies and mutation rates are assumed to be available.

Particularly for the cases where other possibilities beyond identity and paternity are involved, experts should consider the possibility of including in the report parameters concerning the set of analyzed markers (and thus independent of the profiles of the individuals of a specific case).

In the specific cases here considered it should be presented the probability of a pair of unrelated individuals and a pair of full-siblings have the same (codominant) genotype for all the analyzed markers (case i.) and the probability of a pair of unrelated individuals and a pair of 2nd degree relatives share at least one allele for all the markers (case ii.); for algebraic formulae see (Weir et al., 2006) and (Pinto et al., 2013). Reinforcing: these statistics should be presented in the report together with the description of genetic systems and methods, to stress the independence between them and the specific case analyzed.

Hypotheses of kinship A and C, here considered are as follows and assumed to be exhaustive and mutually exclusive, according to the court's new scenario.

Identity case

A: The profiles correspond to the same donor;

C: The profiles correspond to a pair of individuals related as full-siblings.

Paternity case (duo)

  A. The profiles correspond to a pair of individuals related as father/child.
  C. The profiles correspond to a pair of individuals related as avuncular.

Paternity case (trio)

  A. The profiles correspond to individuals related as father/ mother/child.
  C. The profiles correspond to individuals related as paternal uncle/ mother/child.

Hypotheses A and C of kinship are assumed to be mutually exclusive.

For each case, the quantitative evaluation presented in the report is as follows:

  1. The probability of the observations (genetic profiles) under the specific hypothesis A;
  2. The probability of the observations (genetic profiles) under the specific hypothesis C;
  3. A Likelihood Ratio between probabilities 1. and 2.

MODEL REPORT EXAMPLE

As in the main part of this work, a mock report, assuming now the two kinship hypotheses: putative father/child, avuncular/nephew-niece, for duo case is presented (Appendix 2.).

REFERENCES

Pinto N, Silva PV, Amorim A (2010) General derivation of the sets of pedigrees with the same kinship coefficients. Hum Hered 70: 194–204.

Pinto N, Gusmão L, Egeland T, Amorim A (2013) Paternity exclusion power: comparative behaviour of autosomal and X-chromosomal markers in standard and deficient cases with inbreeding. Forensic Sci Int Genet 7(2):290-5

Thompson E (1975) The estimation of pairwise relationships. Ann Hum Genet 39:173–188.

Weir BS, Anderson AD, Hepler AB (2006) Genetic relatedness analysis: modern data and new challenges. Nat Rev Genet 7: 771–780.

APPENDIX 2. Mock report for a duo case where a first paternity report was returned with the new information that the putative father claims that the true father is his (unavailable) brother.

REPORT
Kinship Test
nº xx/ yyyy

The XXXX asked for the genetic testing on the possible avuncular relationship of

So-and-so

relatively to

Child

through the request ref. xxx (copy attached), assuming that the alternative kinship is paternity.

Technical procedures

On the day[th] of Month, Year, both individuals were present at the Institute/Laboratory xxx where they were identified by presentation of ID documents and filled and signed the identification forms x and y (copy attached, containing photographs). Blood/saliva samples were taken; storage, and subsequent treatments and analyses were performed in the same way and under the same conditions. Genetic profiles were made according to the specifications described in Annex 1 and correspond to at least two results obtained independently by two experts.

On the day[th] of Month, Year, a report was issued statistically comparing the probabilities of the genetic configuration of the individuals assuming the hypotheses: A - The two profiles correspond to a pair of individuals related as parent/child, and B - The two profiles correspond to a pair of genetically unrelated individuals. A result favoring paternity was obtained.

On the day[th] of Month, Year, the up mentioned report was returned with the information that So-and-So refuses the paternity, claiming that the true father is in fact his meanwhile deceased full brother. New statistical calculations accommodating this information and the hypotheses: A - The two profiles correspond to a pair of individuals related as parent/child, and C - The two profiles correspond to a pair of individuals related as avuncular, were computed.

Results

See Table 1.

Conclusions

Assuming the conditions described in Annex 2, the results obtained show that the genetic profile configuration is **306** times (rounded up to unities) more probable assuming the hypothesis that So-and-so is the **biological father** of Child than under the hypothesis of the individuals being related as **avuncular** (results per marker presented in Table 1).

Date and signatures

REPORT

Paternity vs. Avuncularity Test
nº xx/ yyyy
Table 1

Genetic systems	So-and-so	Child	Likelihood Ratios*
CSF1PO	12-13	10-12	0.891
D2S1338	24-25	19-24	1.427
D3S1358	14-16	14-15	1.415
D5S818	8-13	8-11	1.947
D7S820	10-12	10	1.308
D8S1179	11-12	11-14	1.454
D13S317	11-13	12-13	1.418
D16S539	11-13	13-14	1.158
D18S51	12-18	13-18	1.581
D19S433	13.2-15.2	13-13.2	1.939
D21S11	28-29	28-33.2	1.260
FGA	19-23	23	1.545
Penta D	2.2-8	8-11	1.848
Penta E	5-12	8-12	1.123
TH01	7-9	9	1.427
TPOX	8-11	8-11	1.156
VWA	16	16-18	1.369

* Rounded up to 3 decimals.

Annex 1

Genetic systems and methods

Genetic systems (or loci; singular: locus)

locus	Typing kit
CSF1PO	a,b
D1S1656	b
D2S1338	b
D2S441	b
D3S1358	a,b
D5S818	a
D7S820	a
D8S1179	a,b
D10S1248	b
D12S391	b
D13S317	a
D16S539	a,b
D18S51	a,b
D19S433	b
D21S11	a,b
D22S1045	b
FGA	a,b
Penta D	a
Penta E	a
TPOX	a
TH01	a,b
VWA	a,b

Methods
Genomic material (DNA) was extracted according to (ref), amplified through PCR and analysed after capillary electrophoresis in an automatic sequencer (*equipment model and manufacturer*) according to the instructions from the manufacturers' kits a and b (*names and manufacturers*). All procedures are described in the Internal Forms *xxx*. Considering the database X (*reference or description of the population sample used*) for allele frequencies the *a priori* probability of two unrelated individuals share at least one allele for all the analyzed markers is equal to 1.065e-06. If the individuals are assumed as second degree relatives (grandparent-grandchild, half-siblings or avuncular) such probability equals 0.00338 (rounded up to 5 decimals).

THEORETICAL, STATISTICAL AND PROBABILISTIC FRAMEWORK

The approach used to weight the evidentiary value of the results compares:

A – The probability of the observations (genetic profiles of the two individuals) assuming paternity,

and

C – The probability of the same observations assuming avuncularity.

These hypotheses were, a priori, considered as equally likely by the requesting parties.

The comparisons A/C take the form of likelihood ratios (LRs) which therefore measure how much the observed genetic results are more probable under the hypothesis of paternity relatively to the alternative hypothesis of avuncularity.

The calculations are performed assuming that:

1. The putative father has no monozygotic ('identical') twin(s).
2. The probabilities of the hypotheses of the putative father to be the father or son of the real father are *a priori* considered as nil.
3. The putative father and the real father are genetically unrelated with the mother of the child.
4. The putative father is either: (a.) the true father, or (b.) a full-brother of him.
5. The tested individuals are assumed to belong to the population sampled for the estimation of gene frequencies (*reference and/or description of the population sample used, including sampling criteria*).
6. No gametic association (linkage disequilibrium) exists between the analyzed loci.

Calculations were performed using a home developed software and confirmed using Software XXX, v. XXX, date, available at XXXX.

All procedures are described in the Internal Forms XXX and YYY.