RELATIONSHIP TESTING NEWS

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In This Issue

Proposed Parentage Calculations in Cases with a Chimera

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This article is part 2 of 3, continued from the Volume 13, Number 1 Edition of RT News.

A chimera's recognition requires 1) observing more than two alleles per locus at independent loci and 2) excluding contamination of a normal sample by allogeneic cells or their DNA.

II. Paternity Testing in Cases with Chimera

Just because an alleged father (AF) or child is discovered to be a chimera, there is no reason to forego genetic and statistical analyses that can provide a probability of paternity. This article describes two proposed methods for determining paternity, one when the child is a chimera and the other when the alleged father is.

In a chimera, tests for alleles of microsatellite (STR) loci may reveal 1-4 *visible* alleles per locus. If no (0) alleles are duplicated, all four are visible. If all four alleles are identical, only one allele will be observed and three will be invisible. If one allele of four is a duplicate, three alleles will be visible. If two alleles are duplicates, two will be visible and two will not.

Calculating the probability that a chimeric AF transmitted two POAs, one visible and one "invisible" is complicated: a duplicate allele could be identical to any one, two or three visible alleles in his phenotype. In a child chimera, the problem of duplicate alleles is further complicated – one or both maternal and paternal alleles of the child chimera may be identical. The child's alleles might have been inherited from either parent and its obligate alleles (MOAs and POAs) are unclear.

III. Review of the Logic of Ordinary Paternity Cases

An ordinary child has two alleles per locus, one necessarily inherited from each parent. When testing a trio in a paternity case, the maternal obligate allele (MOA) is a locus allele found in both the mother and child. The remaining allele in the child's phenotype must have been inherited from the child's biologic father (BF) and is the paternal obligate allele (POA). If mother and child share both alleles at a locus, then both the MOA and POA consist of the two alleles.

If an AF is homozygous, the probability he would transmit the POA to his child is 1.0. If the AF is heterozygous, the probability he would transmit the POA is 0.5. The probability that 'the random man' (RM) transmitted the POA is simply the POA's frequency in AF's population. ('The RM' represents all homozygous and heterozygous men in AF's ethnic population who could transmit the POA to the child.) If the POA consists of two alleles per locus, the transmission probability is the sum of their frequencies. (The statistical addition rule applies because the AF or RM must transmit either one allele or the other to his child, two mutually exclusive genetic events.) The conditional probability that the child inherits the POA from the AF is divided by the conditional probability that the child inherits the POA from the RM. The quotient is the paternity likelihood ratio (LR), which is reduced to an odds ratio with a denominator of 1.0 and the odds ratio is termed the 'paternity index'.

IV. Statistical Logic for a Paternity Case With a Child Chimera

There are two maternal and two paternal alleles at each locus of a chimeric child. (Two ova and two sperm cells each carried one allele of a chimeric child's four per locus.) A homozygous parent (P/P) would transmit the same allele (P) to a chimeric child in all gametes with a probability of 1.0. A heterozygous parent (P/Q) would transmit either allele (P or Q) to the chimeric child with a probability of 0.5. The probabilities that an ordinary AF transmitted two alleles to the chimeric child are:
[P & P] = 0.5 x 0.5 = 0.25 and that AF transmitted [Q & Q] = 0.25. The sum of probabilities that AF transmitted either [P & Q] or [Q & P] = 0.25 + 0.25 = 0.5.

Scenario 1. A child chimera exhibits 2 visible POAs per locus

A minority of a child chimera's loci exhibits two different paternal alleles per locus [P & Q]. A heterozygous AF (P/Q) would transmit [P & Q] with a probability of 0.5 and a heterozygous RM would transmit [P & Q] with a probability of 0.5(2pq), where p and q are

the respective frequencies of alleles P and Q in AF's ethnic population and 2pq is the frequency of P/Q heterozygotes in a population that approximates Hardy-Weinberg equilibrium. (AF and RM must be heterozygotes to have transmitted two different alleles to a child chimera.)

Scenario 2. A child chimera exhibits 1 visible paternal allele per locus

Most loci of a chimera exhibit only one visible POA per locus because it is common for a second paternal allele to be an invisible duplicate of another allele in the child chimera's phenotype. The single visible POA in the chimera is treated as the POA in an ordinary case: If AF is heterozygous for the POA, the probability that he would transmit it is 0.5; and the probability that the RM would transmit the POA is the POA's frequency (p). The likelihood ratio (LR) comparing the probabilities that a chimeric child would inherit the POA from the AF and from the RM is: LR = 0.5/p.

Note that the probability that the AF could transmit an invisible copy of the POA could be calculated but isn't. Suppose both maternal alleles (Q & R) are observed in the child chimera's phenotype (P, Q, R) and there is one visible POA (P). The child's invisible paternal allele could be a duplicate of any one of the three visible alleles (P, Q, R). If AF is a POA heterozygote (P/S) and if he is the child's true father, the probability that he twice transmitted the P allele is 0.5 (see Scenario 1). If RM is the child's father, the probability that he transmitted the invisible POA is the sum of population frequencies (p + q + r) of the three visible alleles (P, Q, R) in the child's phenotype. The probability that a chimera inherited (P & P) from RM = p^2; the probability that a RM transmitted (P & Q) = 2pq; and the probability that a chimera inherited (P & R) from RM = 2pr. The total probability that the RM transmitted the visible P allele and an invisible P or Q or R allele is the sum of the probabilities:
$p^2 + 2pq + 2pr$. The LR = $0.5/(p^2 + (0.5)2pq + (0.5)2pr$. LR = $0.5/(p^2 + 1/2pq + 1/2pr)$.

The simpler method (LR = 0.5/p) ignores possible inheritance of an invisible allele and determines the probability that a RM would transmit only the visible paternal allele. While the more complicated method (LR = $0.5/(p^2 + 1/2pq + 1/2pr)$) would raise the LR, its complex calculation hardly seems worthwhile. Using the certainty of inheriting the visible POA yields an LR with a magnitude identical to one in a case with an ordinary child and if the usual number of STR loci is examined, the combined LR should be as persuasive as a typical paternity case. The complicated method also requires time and effort to find the frequencies of invisible alleles and perform the more elaborate calculations.

Scenario 3. A child chimera exhibits 0 visible POAs/locus

A child chimera only infrequently exhibits no visible POA in its phenotype because locus alleles (e.g., P, Q) are identical to those in the child's mother (P, Q). The chimeric child's two inherited paternal alleles may be [P & P], [Q & Q], [P & Q] or [Q & P].

If the AF is the chimera's biologic father and the child exhibits two visible paternal alleles at a locus, the transmission sequences [P & Q] or [Q & P], the probability that AF transmitted them is 0.5 (as described in Scenario 1). If RM is the chimera's father, [P & Q] were transmitted with a probability = 0.5(2pq). The paternity LR = 0.5/(0.5)(2pq) = 1/2pq.

Table 1. Probabilities that a child chimera inherited POAs from an AF vs a RM.

Locus	Phenotypes of A Paternity Test Trio:			Probability POA Inherited		
	Mother	Child	AF	From AF	From RM	
1	R	**P, Q**, R	P, Q	0.5	(0.5)2pq	1
2	R	**P**, R	P, Q	0.5	p	(
3	R, S	**P, Q**, R, S	P, Q	0.5	(0.5)2pq	1
4	P, Q	**P, Q**	P, Q	1.0	p + q	1
5	P, Q	**P, Q**	P	1.0	p	1
6	P	**P**	P	1.0	p	1/

*__Boldface__ = POA

Part V, Statistical Logic for a Paternity Case With an AF Chimera" will be addressed in the next Newsletter

Common Nonconformances of the 13th ed of Standards

1.2 Laboratory Director Qualifications and Responsibilities

New Guidance

Individuals who are in a designee position but who have completed training and wish to move into a director position must provide documentation of their training experience that is signed by the Director who has performed the training.

1.2.2 Laboratory Director Designee

New Guidance

Documentation of training is required; the laboratory director should provide signed approval that the designee is qualified to perform the delegated task.

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1.4 Staffing Changes

New Guidance

Documentation must be approved by AABB prior to assuming the position

4.5 Receipt, Inspection, and Testing of Incoming Critical Supplies and Samples

Incoming reagents, samples, materials, equipment, and products shall be inspected and tested before reporting of results. The laboratory shall ensure that:

1) Each lot shall be tested.
2) Each shipment, regardless of lot, shall be tested.
3) Each lot within a shipment shall be tested.

New Guidance

In the 13th edition of standards, three subparagraphs were added to emphasize that inspections are not just the lot number or shipment. While any lot received is inspected and tested, if it comes in a different shipment that lot will require inspection and testing regardless of previous inspection. Likewise, if multiple lots come in one shipment, each lot in the shipment needs to be inspected and tested.

5.2.2.2

The laboratory shall have policies, processes and procedures to ensure that collectors are trained. Standard 2.1.2 applies.

New Guidance

The committee added new standard 5.2.2.2 in an effort to clarify that laboratories are responsible for using trained collectors. Options to ensure compliance are for the laboratories to use collectors with an AABB RT Collection certificate of training, a list of registered collectors is provided to all accredited RT facilities via email quarterly.

If an accredited collection facility is not used, the laboratory shall provide training instructions to the collection facility to ensure that the samples are collected in accordance with these Standards. Both the trainer and the trainee should acknowledge in writing that the training for a particular task has occurred. Documentation should indicate that the trainee adequately understood the training prior to working on client samples.

5.2.4.8.1

For cases intended for immigration, visa, passport, and citizenship, both a photo suitable for positive ID and a legible copy of the government issued photo ID shall be submitted for each tested individual. If these documents are not available, the collector shall document the explanation.

New Guidance

The United States Citizenship and Immigration Services (USCIS) and Department of State (DOS) have requested that a photocopy of the ID used be provided. If a document is not available the collector must thoroughly document the reason no identification was available.

5.3.8 Two Party Comparisons of Full Siblings, Half Siblings, Avuncular, and Single Grandparentage Likelihood Ratios

The laboratory shall have policies, processes and procedures for two party comparisons of full siblings, half siblings, avuncular, and single grandparentage likelihood ratios.

5.3.8.1

Before reporting an inconclusive result, the laboratory shall use a minimum test battery of at least 20 autosomal Short Tandem Repeat (STR) loci when testing.

5.3.8.2

Likelihood ratios greater than 10 shall be considered genetic evidence supporting the tested relationship.

5.3.8.3

Likelihood ratios of 0.1 through 10 shall be considered inconclusive for the tested relationship.

5.3.8.4

Likelihood ratios less than 0.1 shall be considered genetic evidence not supporting the tested relationship.

5.3.8.5

The laboratory shall report the estimate of the percentage of individuals of known relationship that may have a combined likelihood ratio that is inconclusive, or supportive, or not supportive of the tested relationship for the laboratory's test protocol at the combined likelihood ratio reported for the case work.

New Guidance

Standard 5.3.8 was added at the request of the DOS, DHS Headquarters, and USCIS. A recent ruling concluded that sibling testing "should be accepted and considered to be probative evidence of the relationship" (Matter of Ruzku, 26 I&N Dec. 731 (BIA 2016)). Sibling relationships had not been accepted prior to this ruling by USCIS and DOS. Thus, these government agencies requested AABB to provide standards to assist in the interpretation of these sibling test results. Note that standard 5.3.8 only applies to two party cases. Cases with three or more individuals being evaluated, for example two acknowledged siblings compared to an individual whose relationship is disputed, do not fall under standard 5.3.8. If the results from two parties are inconclusive for the claimed relationship then the laboratory should request samples from additional biological family members in order to support or not support the claimed relationship.

Another problem was the variability on what the various laboratories reported as inconclusive or evidence of a relationship. This variability was also seen in paper challenges in proficiency testing. In order to establish standards, the committee met with the DOS, DHS Headquarters and USCIS personnel. Multiple scientific studies were reviewed. Once the initial standards were written, they were put out for several months of public comment. The final standards took into account these comments.

Several published and unpublished studies were reviewed that provide empirical data on the range of combined siblingship indices (likelihood ratios) encountered with individual pairs that are known to be full siblings, half-siblings, and unrelated. These studies are summarized below:

In a 2004 siblingship study (Thomas M. Reid, Caitlin A. Wolf, Christopher M. Kraemer, Susannie C. Lee, Michael L. Baird, and Richard F. Lee. Specificity of Sibship Determination Using the ABI Identifiler Multiplex System. Journal of Forensic Science, 2004, 49:1262-1664) using 15 autosomal loci, the combined full siblingship indices (vs. unrelated) for known full siblings ranged from 4.6 to over 1 billion and for random, unrelated individuals from 0.000000045 to 0.12. There was no overlap between the group of true siblings and the group of non-related individuals.

In the half siblingship study (Robert W. Allen, Jun Fu, Thomas M. Reid, and Michael Baird. Considerations for the interpretation of STR results in cases of questioned half-sibship. Transfusion (2007) 47:515-519) using 15 autosomal loci, the combined half siblingship indices (vs. unrelated) for known half siblings ranged from 0.1 to 3763 with a median likelihood ratio of 24. The combined half-siblingship indices for the unrelated pairs ranged from 0.0001 to 42 with a median likelihood ratio of 0.13. In this study, there was overlap between the two groups.

Unpublished AABB laboratory data was found to be consistent with these results and studies using synthetic data also provided similar results. The decision to use a likelihood ratio of 10 as a cut-off between inconclusive and evidence of a relationship was a balance between detecting true non-siblings and finding evidence of a relationship for true siblings. Both are a concern for laboratories and their clients. (See Chang En Pu & Adrian Linacre. Systematic evaluation of sensitivity and specificity of sibship determination by using 15 STR loci. Journal of Forensic and Legal Medicine 15 (2008) 329–334.; Rajiv I. Giroti, Sunita Verma, Kulwant Singh, Rohit Malik, Indu Talwar. A grey zone approach for evaluation of 15 short tandem repeat loci in sibship analysis: A pilot study in Indian subjects. Journal of Clinical Forensic Medicine 14 (2007) 261–265.; Chang En Pu & Adrian Linacre. Increasing the confidence in half-sibship determination based upon 15 STR loci. Journal of Forensic and Legal Medicine 15 (2008) 373–377.)
One concern may be the use of appropriate frequency tables and inbreeding. For example, it has been claimed that laboratories are using North American Black (African American) frequencies to calculate relationships for various native African populations. Laboratories are encouraged to develop appropriate tables or use published frequencies. Standards are available concerning the use of published frequencies, see Standard 5.5.3.2. If a laboratory cannot find or develop appropriate tables or believes a population has significant substructure, the laboratory may consider modifying the likelihood ratio calculations using the inbreeding coefficient theta (FST). (SS)

The new Standard 5.3.8.5 is a new requirement that was added over concern that the while the choice of a likelihood ratio of 10 as a cutoff is a balance between a false finding of a relationship and a false interpretation of no relationship, another measure of reliability of the test is needed. The standard indicates the measure is “percentage of individuals of known relationship that may have a combined likelihood ratio that is inconclusive, or supportive, or not supportive of the tested relationship.” One way these could be expressed is using the sensitivity or specificity of the test. Sensitivity is the percent of true full-sibs with CSI values greater than the threshold likelihood ratio. Sensitivity of the test is based upon one minus the percentage of false negatives. Rate of false negatives equaled the percentage of known relationship testing cases that would be excluded based upon any given cutoff point of combined likelihood ratio. Specificity is the percent random pairs with combined likelihood ratio less than threshold. The specificity of the test is based upon one minus the percentage of false positives. The rate of false positives equals the percentage of random pairs (known unrelated individuals) of DNA profiles where their combined likelihood ratio was greater than any recommended cut-off value. Variants of these or combinations are also acceptable but should be reflected in the laboratory’s standard operating procedures. Also recognizing that this information may not have been developed by a laboratory, the new standard indicates that the “laboratory shall report the estimate”. The word “estimate” was added giving laboratories the ability to use estimates of their work. For example, there are published data on the sensitivity and specificity of using 15 autosomal loci. These studies could be used to conservatively estimate the sensitivity and specificity of any laboratory’s testing of 15 autosomal loci or greater. Note that this language would be needed on any report regardless of a conclusion of no relationship, inconclusive, or a relationship is possible. If a definitive exclusion is obtained, such as, when appropriate, the additional testing of Y chromosome markers, the statement would not be needed. For examples of sensitivity and specificity, see Chang En Pu & Adrian Linacre. Systematic evaluation of sensitivity and specificity of sibship determination by using 15 STR loci. Journal of Forensic and Legal Medicine 15 (2008) 329–334. and Chang En Pu & Adrian Linacre. Increasing the confidence in half-sibship

determination based upon 15 STR loci. Journal of Forensic and Legal Medicine 15 (2008) 373–377.

Also, note that while this standard uses the likelihood ratio, laboratories under other standards may need to report the Probability of the Relationship and the prior probability used. Reference Standard 6.3A, Requirements for Test Reports applies

7.1.4.2
If a laboratory issues an amended report, the laboratory shall distribute amended reports to all recipients of the original report.
New Guidance
It is essential that all parties receiving the initial report be notified of any amendments to the report. All amended reports should follow the same release procedures and processes as the original report. If the amended report is released to a third-party administrator, the third-party administrator must distribute the amended report to all parties.

COMING SOON

New Publication

Title : Relationship Testing 1.0
WENK R E

Bethesda, MD: AABB Press, 2018
Available at aabb.org - Fall 2018

New Accreditation Portal

This portal is designed to streamline the accreditation process and make it easier for members to access and track their accreditation information online.

FREE AABB Workshop

International Symposium on Human Identification
Phoenix, AZ
Sunday September 23rd, 2018 // 9:00 am - 12:00 pm

Topics include common nonconformances, the initial accreditation process, proficiency testing, and complex calculations.
There is no charge for this workshop, but pre-registration is requested atISHInews.org. Registration for the remainder of the Symposium is not required to attend the AABB workshop.

AABB / A2LA
Joint Assessment Program

Now Available

AABB and the American Association for Laboratory Accreditation (A2LA) have joined forces to offer the AABB/A2LA Accreditation Program to the relationship testing and forensics communities.

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Randy Querry, A2LA Accreditation Manager

1-301-644-3221 or rquerry@A2LA.org

USCIS POLICY UPDATE :

Acceptance of DNA Evidence for Sibling Relationships

There have been significant changes to USCIS policies regarding the acceptance of DNA evidence supporting sibling relationships. The news release is available on the USCIS website: https://www.uscis.gov/news/alerts/uscis-updates-policy-dna-evidence-support-sibling-relationships

The complete policy memorandum is available at: https://www.uscis.gov/sites/default/files/USCIS/Laws/Memoranda/2018/2018-04-17-PM-DNA-Evidence-of-Sibling-Relationships.pdf

VOLUNTEER OPPORTUNITIES

RT Accreditation Committee and RT Standards Committee

- Are you interested in ensuring that assessment/audit procedures are in consistent with AABB policies established by the AABB Accreditation Program Committee?
- Are you interested in working with U.S. Citizenship and Immigration Service and/or the Dept. of State as it relates to RT?
- Are you currently an AABB Member?
- Would you like to be involved in creating and revising the Relationship Testing Standards?
- Would you like to be involved in creating and revising the Guidance for the Standards?

If these issues are of interest to you or to get involved? Email us atnikkib@aabb.org.

Webinar Content

Would you like to repurpose your old talks or presentations?

If you have given a talk or presentation in the last 2-3 years on a topic that you think may be of interest to the relationship testing community, share your content as part of AABB's 2018 RT Webinar Series. If you decide to submit your content, you can choose to moderate the audio conference or we can assign a speaker for you.

For more information or to submit your content, email us atnikkib@aabb.org

Articles

Do you have an interesting case or question you would like to share through this newsletter?

Or is there a topic or issue you would like us to write about? Email us atnikkib@aabb.org

Misleading Claims of Accreditation and Logo Use

We are renewing our efforts to stop such practices and are actively searching out these organizations so that we can address this problem on a more global scale.
You can aid these efforts by bringing to our attention instances of logo misuse or misleading statements regarding accreditation. Please advise the Accreditation Department at accreditation@aabb.org by providing the offending Web site and briefly describing the issue. It would be particularly helpful if you copy and email the actual link from your browser's address bar, as some offending organizations maintain multiple Web sites.

The AABB Trademark Usage Guideline as well as Language for use by Third Party Collectors can found on the AABB Website.

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