Relationship Testing

TECHNICAL REPORT FOR TESTING IN 2019

ABSTRACT

AABB surveyed facilities accredited for Relationship Testing (RT) activities for data of interest to the RT community. The total volume of cases tested and reported in 2019 was 410,931. Of those, 58% were legal chain of custody cases for nonimmigration purposes and demonstrated an exclusion rate of 22.62%. Of the total case volume, 6% were for immigration, visa, passport or citizenship cases with an exclusion rate of 5.91%; 37% were unaccredited cases lacking a chain of custody tested for curiosity and showed an exclusion rate of 30.86%. Of all samples collected, more than 98% were buccal swabs. DNA analysis of autosomal short tandem repeats made up for 98% of the tests performed. X-chromosome analysis was performed in addition to the autosomal analyses on 20% of the cases, a small number of cases also received Y-chromosome or mitochondrial analysis. Of the laboratories surveyed, 68.4% incorporate apparent mutations into the combined likelihood ratio by dividing mutation rate by the average probability of exclusion. Twenty percent of the laboratories use a method that considers the short tandem repeat differences. Mutation data was collected from the surveyed laboratories and frequencies of mutation for 31 loci are presented.

PREFACE

This survey provides information on the state of the RT community, tries to ask questions that may be of interest and tracks trends in testing. Evaluation of these data was anonymous. AABB scientific staff reviewed the raw data and provided only anonymized aggregate data and tables for review by the Relationship Testing Standards Committee (RTSC) and the Relationship Testing Accreditation Committee (RTAC). Data from AABB-accredited facilities that perform only collection and report verification activities are excluded from this report to avoid any duplication of data submitted by the testing laboratory.

ANNUAL VOLUME OF TESTING

The volume reported for cases tested in 2019 was 410,931. Because some laboratories did not provide data, this is an underestimate of the actual number of cases tested by AABB-accredited laboratories. In addition to volume of accredited tests, laboratories were asked if they tested cases where the chain of custody did not meet the requirements of the *Standards for Relationship Testing*. The tested individuals, without a proper witness (see Standards), generally self-collect these so called "non-legal" tests. AABB has taken the position that it cannot prohibit accredited laboratories from performing these types of tests but reminds laboratories that they cannot claim or advertise that their "non-legal" testing meets AABB standards. This includes reports that state the "testing" meets the standards and only the chain of custody is lacking. Laboratories must conform in all aspects and cannot choose standards to which they will adhere.

Table 1 indicates the volumes of cases reported by case type.

TABLE 1. CASES REPORTED BY TYPE

Case Type	Cases Reported	% Total 2019
Non-Immigration Legal	236,516	57.56%
Immigration, Visa, Passport	23,602	5.74%
Non-legal / No Chain of Custody	150,813	36.70%

LABORATORIES BY SIZE

Table 2 indicates the size of the various responding laboratories by volume of cases reported.

TABLE 2. LABORATORY SIZE BY THE VOLUME OF CASES REPORTED

Number of Cases Reported	Percentage of RT Laboratories
	2019
<100	19.05%
100-1,000	33.33%
1,001-10,000	33.33%
10,001-100,000	4.76%
>100,000	9.52%

EXCLUSION RATE

For the 2019 report, we asked laboratories to report exclusions, or hypotheses not supported for non-parentage cases, separately by case type. The observed rate of exclusion varies significantly depending on the type of case as shown in Table 3.

TABLE 3. EXCLUSIONS REPORTED BY CASE TYPE

Exclusions(or hypothesis not supported)	Non-Immigration Legal	Immigration, Visa, Passport	Non-legal / No Chain of Custody
Average Exclusion Rate	22.62%	5.91%	30.86%
Standard Deviation	11.70%	7.09%	13.01%
Median Exclusion Rate	21.02%	3.59%	31.21%
Range	0-43.48%	0-27.01%	0-50%
Inconclusive	0.0026%	0.0085%	0.0007%

MISCONCEPTIONS IN PATERNITY TESTING – EXCLUSION RATE

AABB has observed misinterpretation of data reported for exclusion rates in previous reports. It is important to clarify what the exclusion rate does not represent. An exclusion rate of 30% does not mean that 30% of fathers are raising children that are not biologically theirs. From the data, it can only be concluded that, of the people who needed a relationship test, some percentage of those tests either exclude or do not support the tested relationship. Additionally, there are many situations in which the relationship was never in question, but a DNA test was necessary to provide proof of relationship for legal reasons.

COMBINED RELATIONSHIP INDEX (COMBINED LIKELIHOOD RATIO)

The laboratories were asked to indicate what combined relationship index (CRI) they considered acceptable for cases with a standard trio (mother, child, father), single parent cases (mother [or father] not tested cases), and family study / reconstruction cases of more than two tested parties (cases where the disputed parent is missing and other relatives are used to evaluate parentage).

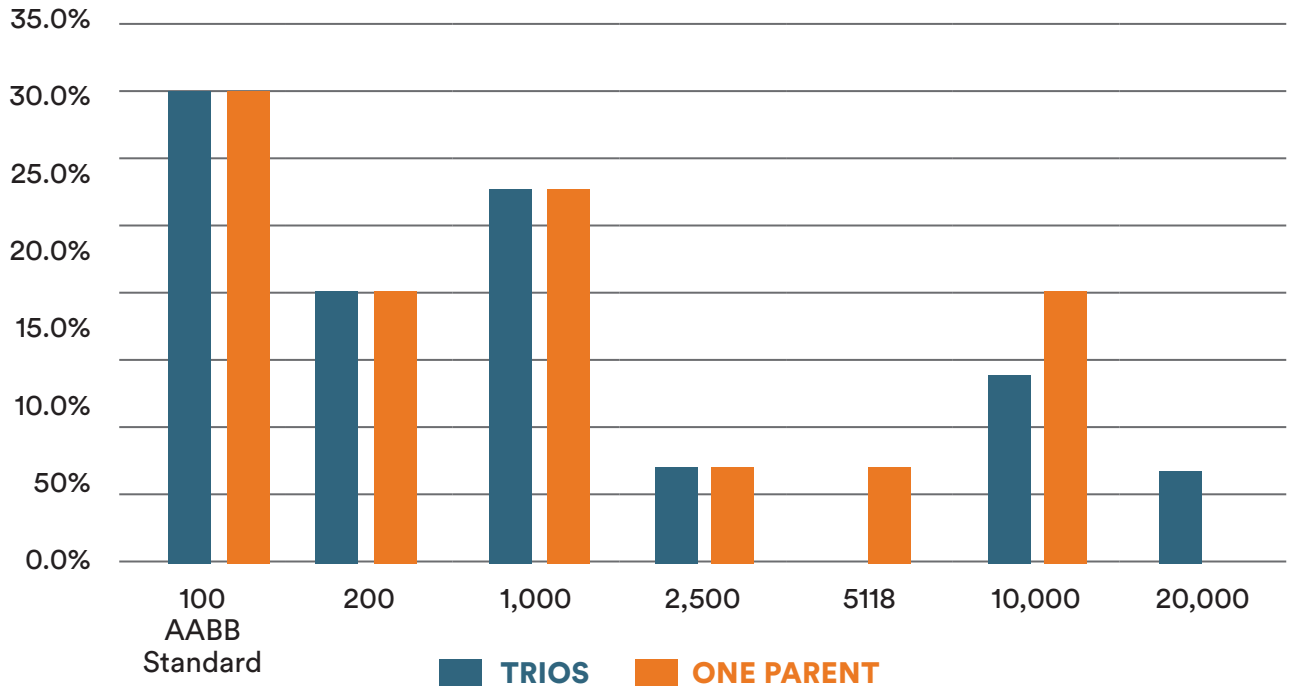
The *AABB Standards for Relationship Testing Laboratories* sets the minimum CRI (W) for parentage cases at 100. An index of 100 is reliable, but indices of higher values can be obtained using current methods. There has been a tendency for laboratories to set much higher values as a minimum likelihood ratio, such as 10,000 to 1 and 100,000 to 1 for some of their tested hypotheses, but not all (such as family study/reconstruction cases). Although setting these higher standards for internal use is not inappropriate, it is inappropriate to claim lower values are not reliable. The minimum acceptable CRI for parentage cases, by policy, is in excess of the AABB standard for 70.6% of the laboratories. One laboratory reporting using a lower CRI for single parent cases than that used for standard trios.

For the *2019 RT Technical Report*, data was not collected on minimum CRI for two party comparisons of full siblings, half siblings, avuncular, and single grandparentage likelihood ratios. Beginning with the 13th Edition of the *AABB Standards for Relationship Testing Laboratories*, minimum CRI standards are defined for two party non parentage comparisons.

TABLE 4A. LABORATORIES' MINIMUM COMBINED LIKELIHOOD RATIOS (% OF LABORATORIES USING A W AS THEIR MINIMUM) FOR STANDARD TRIOS, ONE PARENT (MOTHER OR FATHER NOT TESTED), FAMILY STUDIES >2 PARTIES

W	Trio	One Parent	Family Study >2 parties
whatever is obtained			5.88%
10			35.29%
80			5.88%
100	29.41%	29.41%	23.53%
200	17.65%	17.65%	
1,000	23.53%	23.53%	11.76%
2,500	5.88%	5.88%	
5,118		5.88%	
10,000	11.76%	17.65%	
20,000	5.88%		

TABLE 4B. LABORATORIES' MINIMUM COMBINED LIKELIHOOD RATIOS (% OF LABORATORIES USING A W AS THEIR MINIMUM) FOR STANDARD TRIOS AND ONE PARENT CASES



TECHNOLOGY USE

Table 5 provides a breakdown of the technology used to resolve the reported cases.

TABLE 5. THE TECHNOLOGY USED IN CASES REPORTED IN 2019

Technology / Method	Utilization
DNA-STR	98.02%
X Chromosome Analysis	20.47%
Non-Invasive Prenatal Paternity	1.30%
Y Chromosome Analysis	0.20%
Mitochondrial Analysis	0.01%
DNA-SNP Array	none
DNA-NGS	none

SAMPLE SOURCE

Laboratories reported approximately 983,037 samples used for casework in 2019. This total includes non-legal cases and samples collected without a chain of custody, any of the following sample types lacking a chain of custody or do not meet the requirements for identification in the *Standards for Relationship Testing Laboratories* would not be appropriate for an AABB-accredited legal relationship test. Buccal swabs account for ~98% of the samples. Various other samples were also reported (See Table 6).

TABLE 6. SAMPLE SOURCE IN 2019

Sample Type	Percentage
Buccal Swabs	98.256%
Liquid Blood	0.611%
Dried Blood Spots	0.554%
Tissues, body fluids, teeth, cell pellets	0.480%
Hair	0.045%
Fingernails or swabs other than buccal	0.037%
Bone	0.006%
Amniotic Fluid	0.006%
Paraffin Block	0.002%
received DNA extracts	0.001%
Chorionic Villi	0.001%
abandoned DNA (toothbrush, etc.)	0.0004%

NULL ALLELES

Apparent null alleles were reported and are summarized in Table 7. The frequency of the null phenotype is separated from the mutation rate, as these frequencies do not represent the frequency of the null allele. Laboratories should be careful in evaluating case with potential null alleles. The use of alternative primers may resolve these cases. Note that the frequencies of reported apparent null alleles are not the same as the frequency of the null allele, which cannot be directly determined from the data collected for this report.

TABLE 7. APPARENT NULL ALLELES AS A PERCENTAGE OF CASE VOLUME

Locus	Number Observed Apparent Null Alleles	% Case Volume
CSF1PO	3	0.0012%
D10S1248	5	0.0020%
D12S391	6	0.0023%
D13S317	29	0.0112%
D14S1434	0	0.0000%
D16S539	1	0.0004%
D17S1301	0	0.0000%
D18S51	11	0.0042%
D19S433	42	0.0165%
D1S1656	17	0.0066%
D21S11	9	0.0035%
D22S1045	15	0.0058%
D2S1338	0	0.0000%
D2S441	8	0.0032%
D3S1358	5	0.0019%
D3S4529	0	0.0000%
D5S818	5	0.0019%
D6S1043	0	0.0000%
D6S474	0	0.0000%
D7S820	5	0.0020%
D8S1179	4	0.0015%
D9S1122	0	0.0000%
F13A01	0	0.0000%
F13B	0	0.0000%
FESFPS	0	0.0000%
FGA	11	0.0043%
Penta D	0	0.0000%
Penta E	2	0.0008%
SE33	157	0.0627%
TH01	5	0.0020%
TPOX	11	0.0044%
vWA	16	0.0063%

MUTATION CALCULATION

Single inconsistencies are routinely seen in the testing of parentage cases. If a laboratory concludes that the inconsistency is a mutation, then the mutation result must be incorporated into the reported results. Laboratories were asked how they calculated the parentage index (PI) for these loci. Most commonly, laboratories use the mutation rate divided by the average probability of exclusion. Some labs reported using more than one method.

TABLE 8. REPORTED MUTATION CALCULATION METHODS FOR 2019

Mutation calculation method	Usage
Mutation rate/average probability of exclusion	68.42%
Using a method that takes into account STR repeat differences (Brenner's Method)	21.05%
Fimmer's Method	10.53%
Familias	5.26%
Standard PI using the mutation rate as the disputed parent's transmission chance	5.26%
Use the mutation rate as the PI	5.26%

MUTATION FREQUENCIES

Laboratories were asked to report apparent mutation counts. Null alleles were excluded from the total counts. Limitations of the data collected allowed for a combined mutation rate only, presented in table 9. Because separate mutation rates for maternity and paternity could not be calculated, it should be noted that a combined mutation rate is likely an over-estimation of the frequency of maternal mutation.

Previously published mutation data was limited to trios, cases tested with the mother, child, and alleged father. The data collected for 2019 combined trios and two-party cases in the number of total meiosis. Current protocols do not allow for the mutation rates of trio cases to be calculated separately. Thus, when comparing the new data to previous studies, the mutation rates may seem lower. With two party cases apparent mutations between the untested parent and child will be missed resulting in a lower mutation rate. A change in data collection method is in progress to address these issues in the *2021 RT Technical Report.*

For step counts, it was assumed that the mutation involved the closest allele. Because of the difficulty in determining directionality of the change in allele, the data is presented in Table 10 as a percentage of the total count of mutations for each increment in step. Apparent mutations of plus/minus a full repeat are detailed in table 11. Confirmed double mutations were reported by 4 laboratories and one lab observed a triple mutation.

TABLE 9. COMBINED MUTATION RATES

Locus	Total Meioses	Total Mutations	Combined Mutation Rate
CSF1PO	257,381	211	0.000820
D10S1248	254,102	185	0.000728
D12S391	258,646	131	0.000506
D13S317	258,313	335	0.001297
D14S1434	3,927	1	0.000255
D16S539	259,405	256	0.000987
D17S1301	3,300	2	0.000606
D18S51	259,471	578	0.002228
D19S433	254,223	245	0.000964
D1S1656	255,666	292	0.001142
D21S11	258,733	431	0.001666
D22S1045	256,754	51	0.000199
D2S1338	255,350	308	0.001206
D2S441	251,122	126	0.000502
D3S1358	257,870	356	0.001381
D3S4529	3,897	1	0.000257
D5S818	258,528	286	0.001106
D6S1043	370	3	0.008108
D6S474	3,933	38	0.009662
D7S820	252,859	243	0.000961
D8S1179	259,392	367	0.001415
D9S1122	9,447	3	0.000318
F13A01	1,477	1	0.000677
F13B	3,460	1	0.000289
FGA	258,491	740	0.002863
Penta D	254,654	5	0.000020
Penta E	254,654	9	0.000035
SE33	250,265	1,461	0.005838
TH01	250,062	8	0.000032
TPOX	249,385	30	0.000120
vWA	255,029	607	0.002380

TABLE 10. PERCENTAGE OF THE TOTAL COUNT OF MUTATIONS FOR EACH INCREMENT (+/-) IN STEP.

Locus	+/- 1 Step	+/- 2 Step	+/- >2 Step
CSF1PO	99.53%	0.47%	0.00%
D10S1248	98.92%	1.08%	0.00%
D12S391	96.18%	2.29%	0.76%
D13S317	99.10%	0.90%	0.00%
D14S1434	100.00%	0.00%	0.00%
D16S539	96.48%	2.73%	0.39%
D17S1301	100.00%	0.00%	0.00%
D18S51	97.23%	1.90%	0.87%
D19S433	90.61%	4.49%	1.22%
D1S1656	95.55%	3.77%	0.00%
D21S11	98.14%	1.62%	0.00%
D22S1045	92.16%	7.84%	0.00%
D2S1338	98.38%	0.97%	0.65%
D2S441	95.24%	3.17%	0.79%
D3S1358	98.03%	1.97%	0.00%
D3S4529	100.00%	0.00%	0.00%
D5S818	99.30%	0.35%	0.35%
D6S1043	100.00%	0.00%	0.00%
D6S474	100.00%	0.00%	0.00%
D7S820	100.00%	0.00%	0.00%
D8S1179	98.91%	0.54%	0.00%
D9S1122	100.00%	0.00%	0.00%
F13A01	100.00%	0.00%	0.00%
F13B	100.00%	0.00%	0.00%
FESFPS	100.00%	0.00%	0.00%
FGA	97.97%	2.03%	0.00%
Penta D	100.00%	0.00%	0.00%
Penta E	100.00%	0.00%	0.00%
SE33	98.56%	1.03%	0.21%
TH01	87.50%	0.00%	0.00%
TPOX	93.33%	3.33%	0.00%
vWA	99.67%	0.33%	0.00%

TABLE 11. +/- FULL REPEAT MUTATIONS

Locus	from	to	observed
D19S433	14	13.2	2
	15	14.2	1
	14	14.2	3
	12	11.2	1
	14.2	14	1
	13.2	13	1
D12S391	19.3	19	1
D1S1656	18.3	19	1
	16	15.3	1
D21S11	32.2	31	1
D2S441	12	11.3	1
TH01	9.3	8	1
SE33	19	20.2	1
	22.2	21.1	1
	25.2	25	1
	28.2	28	1
TPOX	8	7.3	1

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