Relationship Testing

TECHNICAL REPORT FOR TESTING IN 2022

As compared to reported data from 2020 and 2021

CORRECTION TO THE 2022 RELATIONSHIP TESTING TECHNICAL REPORT

Tables 8a and 8b have been corrected for a math error in the calculation of the mutation rates. Table 8a has been revised for transparency and includes information on how the mutation rates were calculated from the data obtained.

ABSTRACT

AABB surveyed facilities accredited for Relationship Testing (RT) activities regarding data of interest to the RT community. Data were collected for the calendar year 2022 and compared to information collected from the two prior calendar years. There were 386,048 cases tested and reported in 2022. Of those tests, 51% were legal chain of custody cases for non-immigration purposes, with an exclusion rate of 20.8%. Of the total case volume, 7% were for immigration, visa, passport, or citizenship cases with an exclusion rate of 3.5%, and 42% were unaccredited cases lacking a chain of custody tested for curiosity and showed an exclusion rate of 29.8%. Of all samples collected, more than 97% were buccal swabs. DNA analysis of autosomal short tandem repeats comprised more than 97% of the tests performed. X- chromosome analysis was performed in addition to autosomal analyses on >26% of the cases. DNA Next Generational Sequencing (NGS) was performed on 2.2% of cases, and a small number of cases also received Y Chromosome or mitochondrial analysis. Of the laboratories surveyed, 65% incorporate apparent mutations into the combined likelihood ratio by dividing the mutation rate by the average probability of exclusion. Mutation data were collected from the surveyed laboratories, and mutation frequencies for 41 loci are presented.

INTRODUCTION

AABB is an international, not-for-profit association representing institutions and individuals involved in relationship testing. The AABB Relationship Testing Laboratories Accreditation Program is based on member-developed standards. It provides for the assessment and accreditation of facilities performing relationship testing activities, including methods for forensic investigative genetic genealogy DNA analysis. The Accreditation Program assesses the quality and operational systems within a facility to verify compliance with applicable standards and offer objective, independent feedback to affirm sound practices and provide guidance on areas of improvement.

PREFACE

This survey provides information on the state of the relationship testing community, tries to ask questions that may be of interest, and tracks trends in testing. Evaluation of the 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).

In addition to full-service laboratories, AABB accredits facilities that only perform sample collection and report verification activities. These facilities are required to send their collected samples to an AABB-accredited laboratory for testing. The testing laboratories include these sample counts in their reported data. Any data submitted by AABB-accredited facilities that perform only collection and report verification activities are excluded from counts to avoid duplication of data submitted by the testing laboratory.

ANNUAL VOLUME OF TESTING

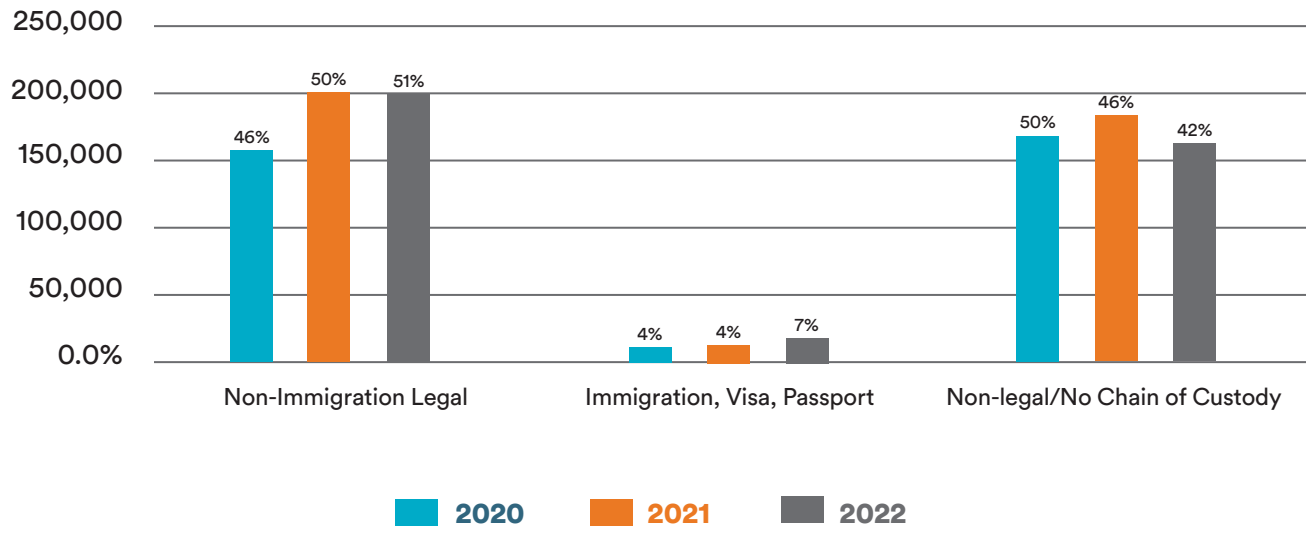
The total volume reported for cases tested in 2022 was 386,048. Data were not obtained for facilities that withdrew accreditation or ceased operations during the 2022 calendar year. Therefore, the reported volumes for 2022 are estimated to be slightly less than the actual number of cases tested by AABB-accredited laboratories.

There are three general categories of testing: legal cases where samples are collected with a documented chain-of-custody, legal cases intended to support a petition for U. S. immigration, and "non-legal" cases lacking a chain-of-custody generally performed for curiosity. A notable increase in immigration testing was reported over the prior year.

In addition to the 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 Laboratories (Standards)*. The tested individuals, without a proper witness, generally self-collect these "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 to all aspects of the *Standards* and cannot selectively choose the requirements to follow. Volumes are presented in Figure 1.

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

FIGURE 1. CASES REPORTED BY TYPE



LABORATORIES BY SIZE

Table 1 indicates size by the volume of cases reported for the 21 total responding laboratories.

TABLE 1. LABORATORY SIZE BY THE VOLUME OF CASES REPORTED

Number of Cases Reported	Percentage of RT Laboratories		
	2020	2021	2022
<100	38.9%	26.0%	9.5%
100-1,000	16.7%	26.0%	42.9%
1,001-10,000	22.2%	26.0%	28.6%
10,001-100,000	11.1%	11.0%	9.5%
>100,000	11.1%	11.0%	9.5%

EXCLUSION RATE

We asked laboratories to report parentage exclusions, or hypotheses not supported for non-parentage cases, and the number of cases for which a conclusion could not be reached, separately by case type. The observed rate of exclusion varies significantly depending on the type of case, as shown in Table 2.

TABLE 2. 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	20.82%	3.54%	30.07%
Inconclusive	0.41%	0.17%	0.45%

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, we can only conclude that, of the people who needed a relationship test, some percentage of those tests either exclude or do not support the tested relationship. 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* set the minimum CRI for parentage cases at 100. An index of 100 is meaningful, 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 higher thresholds for internal use is not inappropriate, claiming that lower values are not meaningful is inappropriate. The minimum acceptable CRI for parentage cases, by policy, exceeds the AABB *Standards* for 50% of the laboratories.

Data were 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 is defined in the *Standards* for two-party nonparentage comparisons.

TABLE 3. LABORATORIES' MINIMUM COMBINED LIKELIHOOD RATIOS (% OF LABORATORIES USING A CRI AS THEIR MINIMUM) FOR STANDARD TRIOS, ONE PARENT (MOTHER OR FATHER NOT TESTED), FAMILY STUDIES >2 PARTIES.

Minimum Combined Likelihood Ratios	Trio	One Parent	Family Study >2 parties*
Whatever is obtained			19.0%
10			42.9%
15			4.8%
80			4.8%
100	57.1%	57.1%	
200	4.8%	4.8%	
1,000	19.0%	19.0%	9.5%
2,500	4.8%	4.8%	
10,000	9.5%	14.3%	
20,000	4.8%		

*Note: For family studies, the sum of percentages is less than 100%, as some labs limit their testing to parentage only.

TECHNOLOGY USE

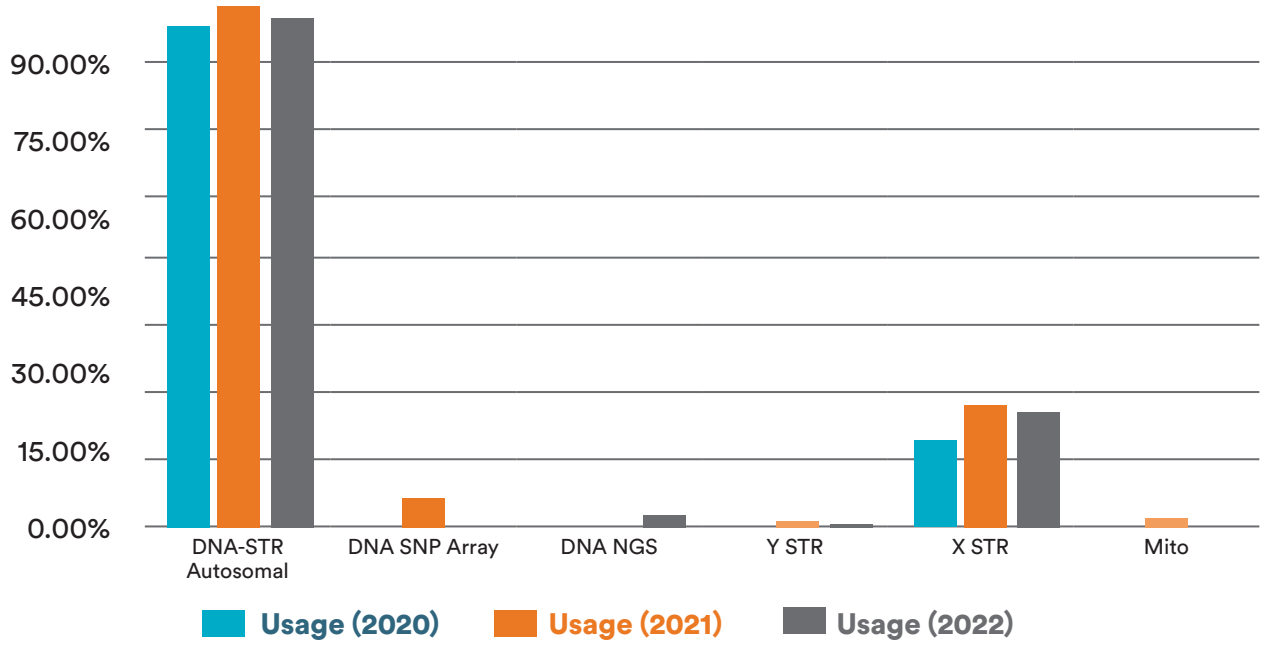
Laboratories reported the technologies used to determine relationship in cases tested. Short Tandem Repeat (STR) testing is still the primary method used across all laboratories. An increase in additional technologies used for testing was observed in 2022. More than one technology may be used to resolve an individual case.

Table 4 shows the technology used to resolve the reported cases.

TABLE 4. THE TECHNOLOGY USED IN CASES REPORTED

Technology / Method	Usage (2020)	Usage (2021)	Usage (2022)
DNA-STR Autosomal	97.12%	99.83%	97.79%
DNA SNP Array	none	4.86%	none
DNA NGS	none	none	2.21%
Y STR	0.19%	1.12%	0.35%
X STR	19.80%	26.92%	26.34%
Mito	0.01%	1.81%	0.0002%

FIGURE 2. THE TECHNOLOGY USED IN CASES REPORTED



SAMPLE SOURCE

Cases reported for 2022 include non-legal cases and samples collected without a chain of custody, any of the following sample types lacking a chain of custody or not meeting the requirements for identification in the *Standards* would not be appropriate for an AABB-accredited legal relationship test. Buccal swabs account for >97% of the samples. Various other samples were also reported (See Table 5).

TABLE 5. SAMPLE SOURCE

Sample Type	Percentage Total
Buccal Swabs	97.183%
Liquid Blood	1.867%
Dried Blood Spots	0.836%
Hair root	0.064%
Bone	0.015%
Amniotic fluid	0.009%
Cells, Slides, Urine, Sperm, Pellet	0.009%
FTA – Saliva	0.005%
Tissue	0.004%
Paraffin block	0.003%
DNA extracts	0.003%
Chorionic Villi	0.001%

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. The *Standards* do not specify the method for calculation when an apparent mutation is observed, although guidance is given on commonly accepted methods of calculation. Some labs reported using more than one method.

TABLE 6. REPORTED MUTATION CALCULATION METHODS

Mutation calculation method	Usage (2020)	Usage (2021)	Usage (2022)
Mutation rate/average probability of exclusion	66.7%	47.4%	65.0%
Using a stepwise mutation model – Brenner's Method	26.7%	52.6%	40.0%
Standard PI using the mutation rate as the disputed parent's transmission chance	6.7%	15.8%	15.0%
Using a stepwise mutation model – Familias	none	5.3%	5.0%
Fimmer's Method	6.7%	5.3%	none
Use the mutation rate as the PI	6.7%	none	none

Note: the sum of percentages is greater than 100%, as some labs use more than one method.

MUTATION FREQUENCIES

For the 2022 survey, laboratories were asked to provide counts of apparent mutations for trio cases tested with the mother, child, and alleged father. Data were requested in the 2022 survey to calculate separate maternal and paternal rates of mutation. Combined mutation rates additionally include data where the source of the mutation is undetermined. Null alleles were excluded from the total counts. Table 8a presents the mutation rates of 41 loci, arranged in alphanumeric order for convenient reference. The 2021 combined mutation rates are included for comparison. Table 8b sorts the data by combined mutation rate from largest to smallest. For loci with no observed mutations in 2022, the 2021 mutation rate is used and indicated in the table.

The mutation rates are calculated using pooled data from many population groups. Since mutation events are relatively rare, there are insufficient data collected from this survey to allow the calculation of mutation rates for specific population groups. The mutation rates presented may be applied generally to any calculation involving an apparent mutation event.

Laboratories reported testing at 17 loci for which no mutations were observed (see Table 9). If an apparent mutation is observed for which the mutation rate is not yet known, it may be estimated. One estimate may be the average mutation rate for other loci evaluated with similar methods.

Five laboratories reported confirmed double mutations (i.e., two tested loci out of a minimum of 20 exhibited alleles with mutations between parent and offspring). Two laboratories observed a triple mutation (see Table 7).

TABLE 7. MULTIPLE MUTATIONS OBSERVED

	number observed	% total testing volume
Double mutations	243	0.0654%
Triple mutations	9	0.0024%

TABLE 8a. MUTATION RATES (2022, SORTED ALPHANUMERICALLY)

Locus	Maternal			Paternal			Combined				Sum of Total Trios Reported
	Apparent Mutations Observed	Maternal Meioses	Mutation Rate = Mutations/ Maternal Meioses	Apparent Mutations Observed	Paternal Meioses	Mutation Rate = Mutations/ Paternal Meioses	Undetermined (mutation could be maternal or paternal)	Total Apparent Mutations Observed	Total Meioses	Mutation Rate = Total Mutations/ Total Meioses	
CSF1PO	20	250848	0.000080	214	250848	0.000853	36	270	501768	0.000538	250884
D10S1248	31	248552	0.000125	128	248552	0.000515	24	183	497152	0.000368	248576
D12S391	33	57996	0.000569	270	57996	0.004655	30	333	116052	0.002869	58026
D13S317	44	255448	0.000172	212	255448	0.000830	19	275	510934	0.000538	255467
D14S1434	1	2119	0.000472	*	2119	*	*	1	4238	0.000236	2119
D16S539	34	254992	0.000133	132	254992	0.000518	22	188	510028	0.000369	255014
D17S1301	1	2064	0.000484	1	2064	0.000484	*	2	4128	0.000484	2064
D18S51	56	254465	0.000220	296	254465	0.001163	21	373	508972	0.000733	254486
D19S433	61	253807	0.000240	111	253807	0.000437	8	180	507630	0.000355	253815
D1S1656	27	249426	0.000108	162	249426	0.000649	13	202	498878	0.000405	249439
D21S11	118	254130	0.000464	200	254130	0.000787	25	343	508310	0.000675	254155
D22S1045	10	252088	0.000040	37	252088	0.000147	7	54	504190	0.000107	252095
D2S1338	22	252486	0.000087	193	252486	0.000764	16	231	505004	0.000457	252502
D2S441	19	249802	0.000076	60	249802	0.000240	10	89	499624	0.000178	249812
D3S1358	14	255596	0.000055	170	255596	0.000665	31	215	511254	0.000421	255627
D3S4529	*	2104	*	1	2104	0.000475	*	1	4208	0.000238	2104
D5S818	27	255538	0.000106	158	255538	0.000618	34	219	511144	0.000428	255572
D6S1043	19	55325	0.000343	97	55325	0.001753	13	129	110676	0.001166	55338
D6S474	12	2107	0.005695	18	2107	0.008543	*	30	4214	0.007119	2107
D7S820	24	251556	0.000095	145	251556	0.000576	19	188	503150	0.000374	251575
D8S1115	1	1879	0.000532	2	1879	0.001064	*	3	3758	0.000798	1879
D8S1179	30	254847	0.000118	230	254847	0.000903	32	292	509758	0.000573	254879
D9S1122	*	2116	*	1	2116	0.000473	*	1	4232	0.000236	2116
DXS101	12	27239	0.000441	57	27239	0.002093	6	75	54490	0.001376	27245
DXS6810	6	24076	0.000249	30	24076	0.001246	16	52	48184	0.001079	24092
DXS9895	11	23574	0.000467	20	23574	0.000848	8	39	47164	0.000827	23582
DYS392	0	25835	*	9	25835	0.000348	0	9	51670	0.000174	25835
FGA	60	254171	0.000236	435	254171	0.001711	36	531	508414	0.001044	254207
HPRTB	8	25410	0.000315	82	25410	0.003227	15	105	50850	0.002065	25425
LPL	*	1027	*	3	1027	0.002921	0	3	2054	0.001461	1027
Penta D	2	7261	0.000275	11	7261	0.001515	1	14	14524	0.000964	7262
Penta E	4	7248	0.000552	31	7248	0.004277	0	35	14496	0.002414	7248
SE33	127	187465	0.000677	481	187465	0.002566	*	608	374930	0.001622	187465
TH01	4	254940	0.000016	17	254940	0.000067	1	22	509882	0.000043	254941
TPOX	5	252651	0.000020	25	252651	0.000099	6	36	505314	0.000071	252657
vWA	31	253210	0.000122	327	253210	0.001291	59	417	506538	0.000823	253269

* No mutations observed

TABLE 8b. COMBINED MUTATION RATES (2022*, SORTED BY MUTATION RATE)

Locus	combined mutation rate	*
D6S474	0.007119	
D12S391	0.002869	
Penta E	0.002414	
HPRTB	0.002065	
SE33	0.001622	
LPL	0.001461	
Penta C	0.001379	2021
DXS101	0.001376	
D6S1043	0.001166	
DXS6810	0.001079	
FGA	0.001044	
Penta D	0.000964	
DXS9895	0.000827	
vWA	0.000823	
D8S1115	0.000798	
D18S51	0.000733	
F13B	0.000713	2021
F13A01	0.000697	2021
DYS391	0.000686	2021
D21S11	0.000675	
D8S1179	0.000573	
D13S317	0.000538	
CSF1PO	0.000538	
D17S1301	0.000484	
D2S1338	0.000457	
D5S818	0.000428	
D3S1358	0.000421	
D1S1656	0.000405	
D7S820	0.000374	
D16S539	0.000369	
D10S1248	0.000368	
D19S433	0.000355	
D3S4529	0.000238	
D9S1122	0.000236	
D14S1434	0.000236	
D2S441	0.000178	
D9S2157	0.000178	2021
DYS392	0.000174	
D22S1045	0.000107	
TPOX	0.000071	
TH01	0.000043	

*For loci with no observed mutations in 2022, the 2021 mutation rate is used and indicated in the table.

TABLE 9. TESTED LOCI WITH NO OBSERVED MUTATIONS (2022)

Locus	Total Meioses
D9S2157	>1000
F13A01	>1000
F13B	>1000
FESFPS	<100
Penta C	>1000
DYS19	<100
DYS385AB	<100
DYS389I	<100
DYS389II	<100
DYS390	<100
DYS391	100-1000
DYS393	<100
DYS439	<100
DYS456	<100
DYS458	<100
DYS635	<100
YGATAH4	<100

CONCLUSION

AABB surveyed facilities accredited for Relationship Testing activities for data of interest to the RT community. A notable trend in the data is the increased volume of testing for immigration, visa, and passport cases compared to 2021. For the 2022 report, we provided separate maternal and paternal mutation rates for many loci in addition to a combined mutation rate. Apparent mutations were observed for the previously unreported locus, LPL, and the mutation rate is now provided.

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