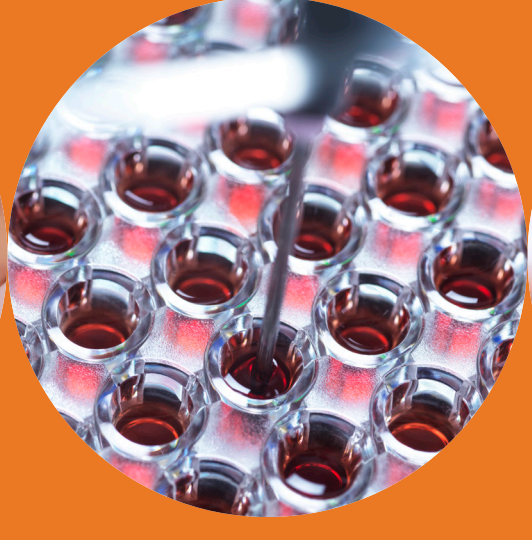
Relationship Testing

TECHNICAL REPORT FOR TESTING IN 2021

As compared to reported data from 2019 and 2020


ABSTRACT

AABB surveyed facilities accredited for Relationship Testing (RT) Activities for data of interest to the RT community. Data was collected for the calendar year of 2021 and compared to data collected from the two prior calendar years. The total volume of cases tested and reported in 2021 was 398,448. Of those tests, 50.39% were legal chain of custody cases for non-immigration purposes and demonstrated an exclusion rate of 21.36%. Of the total case volume, 3.5% were for immigration, visa, passport, or citizenship cases with an exclusion rate of 4.24% and 46.1% were unaccredited cases lacking a chain of custody tested for curiosity and showed an exclusion rate of 30.07%. Of all samples collected, more than 97% were buccal swabs. DNA analysis of autosomal short tandem repeats comprised more than 99% of the tests performed. X- chromosome analysis was performed in addition to autosomal analyses on >26% of the cases. DNA Single Nucleotide Polymorphisms (SNPs) were tested on ~5% of cases and a small number of cases also received Y Chromosome or mitochondrial analysis. Of the laboratories surveyed, 47.4% incorporate apparent mutations into the combined likelihood ratio by dividing mutation rate by the average probability of exclusion. 52.6% 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 40 loci are presented.

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 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).

In addition to full-service laboratories, AABB also accredits facilities that perform sample collection and report verification activities only. 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 volume reported for cases tested in 2021 was 398,448. The 2021 survey participation rate of 95% is an improvement over previous years, the reported volumes are estimated to be slightly less than the actual number of cases tested by AABB Accredited laboratories. For previously accredited facilities or facilities that ceased operations during the 2021 calendar year, data was not obtained. Testing volumes have increased overall, with a 29% increase in non-immigration legal testing and 18% increase in Immigration testing over the prior year. Non-legal testing saw an 8% increase over the prior year.

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

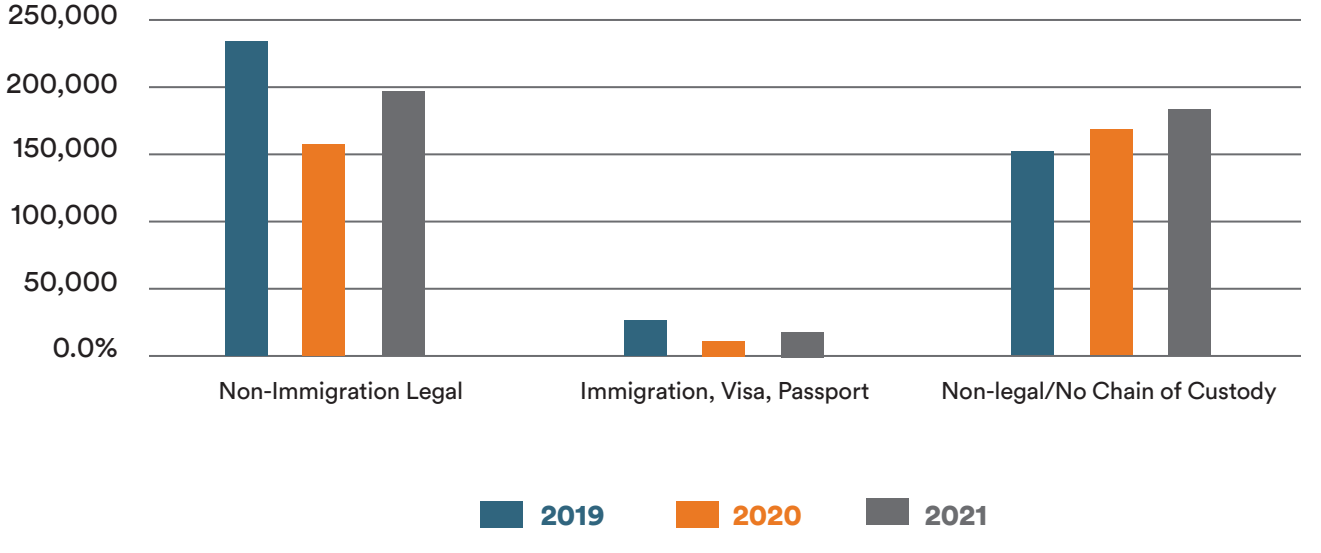
accredited laboratories from performing these types of tests but reminds laboratories that they cannot claim or advertise that their "non-legal" testing meets *AABB RT Standards*. This includes reports that state the "testing" meets the *RT Standards* and only the chain of custody is lacking. Laboratories must conform in all aspects and cannot choose standards to which they will adhere.

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

TABLE 1. CASES REPORTED BY TYPE

Case Type	2019		2020		2021	
	Reported	% Total	Reported	% Total	Reported	% Total
Non-Immigration Legal	236,516	57.56%	155,672	46.16%	200,794	50.39%
Immigration, Visa, Passport	23,602	5.74%	11,860	3.52%	13,960	3.50%
Non-legal / No Chain of Custody	150,813	36.70%	169,726	50.33%	183,694	46.10%

CHART 1. CASES REPORTED BY TYPE



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	2020	2021
<100	19.05%	38.89%	26%
100-1,000	33.33%	16.67%	26%
1,001-10,000	33.33%	22.22%	26%
10,001-100,000	4.76%	11.11%	11%
>100,000	9.52%	11.11%	11%

EXCLUSION RATE

For the 2021 report, 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 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	21.36%	4.24%	30.07%
Inconclusive	0.34%	0.14%	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. 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 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 58% of the laboratories.

For the 2021 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 are defined in the standards for two party non parentage comparisons.

TABLE 4. 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.

CRI	Trio	One Parent	Family Study >2 parties
whatever is obtained			5.3%
10			47.4%
80			5.3%
100	42.1%	42.1%	10.5%
200	10.5%	10.5%	5.3%
1,000	21.1%	21.1%	10.5%
2,500	5.3%	5.3%	
10,000	15.8%	21.1%	
20,000	5.3%		

TECHNOLOGY USE

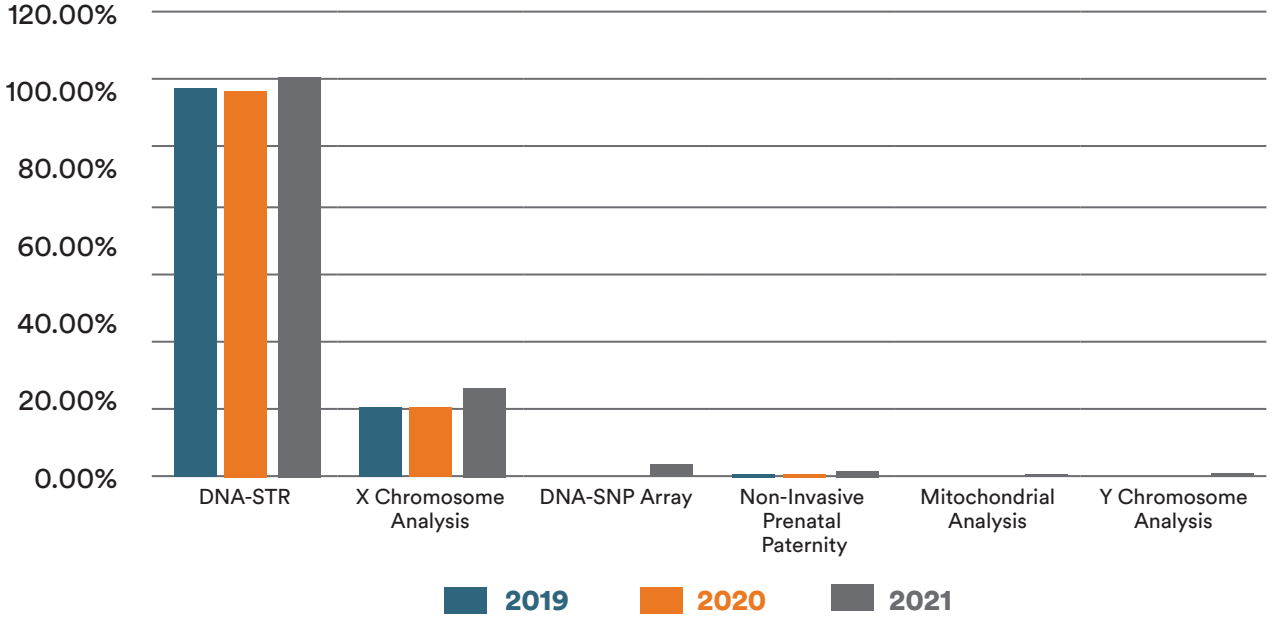
Laboratories reported the technologies used to determine relationship in cases tested. Short Tandem Repeat (STR) testing is still the primary method used across all laboratories. An increase in additional technologies used for testing was observed in 2021. More than one technology may be used to resolve an individual case.

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

TABLE 5. TECHNOLOGY USED IN CASES REPORTED

Technology / Method	Usage (2019)	Usage (2020)	Usage (2021)
DNA-STR	98.02%	97.12%	99.83%
X Chromosome Analysis	20.47%	19.80%	26.92%
DNA-SNP Array	none	none	4.86%
Non-Invasive Prenatal Paternity	1.30%	1.88%	2.31%
Mitochondrial Analysis	0.01%	0.01%	1.81%
Y Chromosome Analysis	0.20%	0.19%	1.12%
DNA-NGS	none	none	none

CHART 5. TECHNOLOGY USED IN CASES REPORTED



SAMPLE SOURCE

Laboratories reported approximately 984,292 samples used for casework in 2021. 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

Sample Type	Percentage
Buccal Swabs	97.695%
Liquid Blood	1.272%
Dried Blood Spots	0.813%
Other Samples (type unspecified)	0.112%
Hair	0.055%
Contact Article	0.018%
Amniotic Fluid	0.010%
Bone	0.008%
DNA Extracts	0.006%
Tissue	0.005%
Paraffin Block	0.004%
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 for Relationship Testing Laboratories* 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 (26%) reported using more than one method.

TABLE 7. REPORTED MUTATION CALCULATION METHODS

Mutation calculation method	Usage (2019)	Usage (2020)	Usage (2021)
Mutation rate/average probability of exclusion	68.40%	66.70%	47.40%
Standard PI using the mutation rate as the disputed parent's transmission chance	5.30%	6.70%	15.80%
Use the mutation rate as the PI	5.30%	6.70%	none
Using a method that takes into account STR repeat differences			
Brenner's Method	21.10%	26.70%	52.60%
Familias	5.30%	none	5.30%
Fimmer's Method	10.50%	6.70%	5.30%

Note: the percentages do not add to 100% as some labs used more than one method.

MUTATION FREQUENCIES

Laboratories were asked to report counts of apparent mutation. Null alleles were excluded from the total counts. Combined mutation rates for 40 loci are presented in Table 8. Limitations on the data provided allowed for calculation of separate maternal or paternal mutation rates for only a few loci. Where separate mutation rates for maternity and paternity could not be calculated, it should be noted that the 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. With two party cases apparent mutations between the untested parent and child will be missed resulting in a lower mutation rate. Data was requested in the 2021 survey to calculate separate maternal and paternal rates of mutation. An apparent misunderstanding on how to provide the separated data for meioses resulted in limitations to the number of loci for which the separate rates could be calculated. Further changes will be made to the method of data collection to obtain a more complete picture of the 2022 data.

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 9a as a percentage of the total count of mutations for each increment in step. Tables 9b and 9c present the observed mutations that could be confirmed as paternal and maternal, respectively. Confirmed double mutations were reported by 6 laboratories and 3 laboratories observed a triple mutation.

TABLE 8. COMBINED MUTATION RATES (2021)

Locus	Total Observed Mutations	Total Meioses	Combined Mutation Rate	2019 Combined Mutation Rate (for comparison)	Maternal Mutation Rate	Paternal Mutation Rate
CSF1PO	376	354561	0.001060	0.0008198	0.001353	0.000713
D10S1248	229	350608	0.000653	0.0007281		0.002153
D12S391	154	110249	0.001397	0.0005065		0.003357
D13S317	396	355160	0.001115	0.0012969		0.004790
D14S1434	2	6103	0.000328	0.0002546		
D16S539	267	355866	0.000750	0.0009869		0.002216
D17S1301	5	5345	0.000935	0.0006061		
D18S51	544	357320	0.001522	0.0022276	0.000550	0.001544
D19S433	167	354446	0.000471	0.0009637	0.001471	
D1S1656	295	350530	0.000842	0.0011421		0.001483
D21S11	422	355866	0.001186	0.0016658		0.001493
D22S1045	73	352482	0.000207	0.0001986		
D2S1338	368	351025	0.001048	0.0012062		
D2S441	159	352121	0.000452	0.0005017		0.001682
D3S1358	326	355982	0.000916	0.0013805		0.002214
D3S4529	2	5971	0.000335	0.0002566		
D5S818	324	356858	0.000908	0.0011063		
D6S1043	59	107446	0.000549	0.0081081		
D6S474	56	6080	0.009211	0.0096618		
D7S820	232	354692	0.000654	0.000961		0.000871
D8S1115	2	3770	0.000531			0.002528
D8S1179	416	356545	0.001167	0.0014148		
D9S1122	3	6090	0.000493	0.0003176		
D9S2157	1	5627	0.000178			
DXS101	23	107446	0.000214			
DXS6810	47	107446	0.000437			
DXS9895	18	107446	0.000168			
DYS391	1	1458	0.000686			0.000742
DYS392	7	107446	0.000065			
F13A01	1	1435	0.000697	0.000677		
F13B	1	1403	0.000713	0.000289		
FGA	820	356010	0.002303	0.0028628		
HPRTB	51	107446	0.000475			
Penta C	2	1450	0.001379			
Penta D	7	112632	0.000062	1.963E-05		
Penta E	15	112582	0.000133	3.534E-05		
SE33	1701	234516	0.007253	0.0058378		
TH01	16	348891	0.000046	3.199E-05		
TPOX	45	348257	0.000129	0.0001203		
vWA	631	355464	0.001775	0.0023801		

TABLE 9a. PERCENTAGE OF THE TOTAL COUNT OF MUTATIONS FOR EACH INCREMENT (+/-) IN STEP.

Total Observed Mutations				
Locus	**+/- <1 Step**	**+/- 1 Step**	**+/- 2 Step**	**+/- >2 Step**
CSF1PO	*	98.14%	1.86%	*
D10S1248	*	94.76%	2.62%	2.62%
D12S391	0.65%	97.40%	1.30%	0.65%
D13S17	*	100.00%	*	*
D13S317	*	94.19%	2.02%	3.79%
D14S1434	*	100.00%	*	*
D16S539	*	98.88%	1.12%	*
D17S1301	*	100.00%	0.00%	0.00%
D18S51	0.18%	97.61%	0.74%	1.47%
D19S433	5.99%	85.63%	3.59%	4.79%
D19S443	*	100.00%	*	*
D1S1656	2.71%	90.85%	1.69%	4.75%
D21S11	0.71%	96.21%	1.18%	1.90%
D22S1045	*	72.60%	9.59%	17.81%
D2S1338	*	93.75%	3.26%	2.99%
D2S441	3.14%	87.42%	0.63%	8.81%
D3S1358	*	98.16%	1.23%	0.61%
D3S4529	*	50.00%	50.00%	0.00%
D5S818	*	98.15%	1.54%	0.31%
D6S1043	*	100.00%	*	*
D6S474	*	55.36%	26.79%	17.86%
D7S820	*	97.84%	1.72%	0.43%
D8S1115	*	100.00%	*	*
D8S1179	*	98.08%	1.44%	0.48%
D9S1122	*	100.00%	*	*
D9S2157	*	100.00%	*	*
DXS101	*	82.61%	8.70%	8.70%
DXS6810	*	100.00%	*	*
DXS9895	5.56%	94.44%	*	*
DYS391	*	100.00%	*	*
DYS392	*	85.71%	14.29%	*
F13A01	*	100.00%	*	*
F13B	*	*	100.00%	*
FGA	*	97.56%	1.83%	0.61%
HPRTB	*	98.04%	1.96%	*
Penta C	*	100.00%	*	*
Penta D	*	100.00%	*	*
Penta E	*	80.00%	13.33%	6.67%
SE33	0.18%	92.83%	2.00%	5.00%
TH01	6.25%	81.25%	12.50%	*
TPOX	*	82.22%	2.22%	15.56%
VWA	*	97.62%	0.63%	1.74%

*no mutations observed

TABLE 9b. PERCENTAGE OF THE TOTAL COUNT OF MUTATIONS FOR EACH INCREMENT (+/-) IN STEP

Paternal Mutations				
Locus	+/- <1 Step	+/- 1 Step	+/- 2 Step	+/- >2 Step
CSF1PO	*	87.50%	1.33%	*
D10S1248	*	79.04%	0.87%	2.18%
D12S391	*	70.13%	*	*
D13S17	*	100.00%	*	*
D13S317	*	81.31%	1.26%	3.03%
D14S1434	*	100.00%	*	*
D16S539	*	75.66%	0.75%	*
D17S1301	*	100.00%	*	*
D18S51	0.18%	86.76%	0.37%	1.29%
D19S433	4.79%	67.66%	3.59%	2.99%
D19S443	*	*	*	*
D1S1656	2.71%	82.03%	1.36%	4.07%
D21S11	*	68.96%	0.47%	0.95%
D22S1045	*	60.27%	6.85%	17.81%
D2S1338	*	86.14%	1.90%	1.90%
D2S441	1.89%	73.58%	0.63%	8.18%
D3S1358	*	85.28%	1.23%	0.31%
D3S4529	*	50.00%	*	*
D5S818	*	82.41%	0.62%	0.31%
D6S1043	*	71.19%	*	*
D6S474	*	39.29%	21.43%	12.50%
D7S820	*	87.07%	1.72%	0.43%
D8S1115	*	100.00%	*	*
D8S1179	*	88.70%	0.72%	0.48%
D9S1122	*	100.00%	*	*
D9S2157	*	100.00%	*	*
DXS101	*	73.91%	8.70%	8.70%
DXS6810	*	57.45%	*	*
DXS9895	*	66.67%	*	*
DYS391	*	100.00%	*	*
DYS392	*	85.71%	14.29%	*
F13A01	*	100.00%	*	*
F13B	*	*	*	*
FGA	*	88.90%	1.46%	0.37%
HPRTB	*	88.24%	1.96%	*
Penta C	*	50.00%	*	*
Penta D	*	85.71%	*	*
Penta E	*	80.00%	13.33%	6.67%
SE33	0.18%	86.24%	1.70%	4.06%
TH01	6.25%	43.75%	6.25%	*
TPOX	*	68.89%	2.22%	11.11%
VWA	*	87.96%	0.63%	0.95%

*no mutations observed

TABLE 9c. PERCENTAGE OF THE TOTAL COUNT OF MUTATIONS FOR EACH INCREMENT (+/-) IN STEP.

Maternal Mutations				
Locus	**+/- <1 Step**	**+/- 1 Step**	**+/- 2 Step**	**+/- >2 Step**
CSF1PO	*	6.12%	0.53%	*
D10S1248	*	11.79%	1.31%	0.44%
D12S391	*	14.94%	1.30%	0.65%
D13S17	*	*	*	*
D13S317	*	8.84%	0.76%	0.76%
D14S1434	*	*	*	*
D16S539	*	14.23%	0.37%	*
D17S1301	*	*	*	*
D18S51	*	8.82%	0.37%	0.18%
D19S433	1.20%	14.37%	*	1.80%
D19S443	*	100.00%	*	*
D1S1656	*	8.47%	0.34%	0.68%
D21S11	0.71%	25.36%	0.71%	0.95%
D22S1045	*	10.96%	2.74%	*
D2S1338	*	5.71%	1.36%	1.09%
D2S441	1.26%	8.81%	*	0.63%
D3S1358	*	9.20%	*	0.31%
D3S4529	*	*	50.00%	*
D5S818	*	9.88%	0.93%	*
D6S1043	*	16.95%	*	*
D6S474	*	16.07%	5.36%	5.36%
D7S820	*	4.74%	*	*
D8S1115	*	*	*	*
D8S1179	*	5.77%	0.72%	*
D9S1122	*	*	*	*
D9S2157	*	*	*	*
DXS101	*	4.35%	*	*
DXS6810	*	23.40%	*	*
DXS9895	*	22.22%	*	*
DYS391	*	*	*	*
DYS392	*	*	*	*
F13A01	*	*	*	*
F13B	*	*	100.00%	*
FGA	*	6.34%	0.37%	0.24%
HPRTB	*	3.92%	*	*
Penta C	*	50.00%	*	*
Penta D	*	14.29%	*	*
Penta E	*	*	*	*
SE33	*	6.58%	0.29%	0.94%
TH01	*	37.50%	6.25%	*
TPOX	*	13.33%	*	4.44%
VWA	*	6.50%	*	0.79%

*no mutations observed

CONCLUSION

AABB surveyed facilities accredited for Relationship Testing Activities for data of interest to the RT community. Some notable trends in the data are the overall decrease in testing in 2020, most likely due to decreased demand as a result of the Pandemic. The decline observed in 2020 for Immigration, Visa, Passport cases compared to 2019 was sustained in 2021. This could have been a result of temporary immigration processing halt introduced during the early pandemic. In 2021 we also observed increased use of two testing technologies: DNA-SNP Array and Non-Invasive Prenatal Paternity. Apparent mutations were reported for nine previously unreported loci and mutation rates are now provided.

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