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In This Issue

Standards for Relationship Testing Laboratories, 15th Edition, Take Effect Jan. 1

The 15th edition of Standards for Relationship Testing Laboratories will take effect Jan. 1, 2022, and includes a number of changes and additions.

The 15th edition is available in the AABB Store in an electronic format, print format, or as a bundle of both versions. A bundle of 15th edition (in print) and the Guidance for Standards for Relationship Testing Laboratories is also available. Organizations interested in adopting the Standards can participate in a complimentary two-week trial of the electronic version of the 15th edition in the Standards Portal.

For those implementing the 15th edition of Relationship Testing Standards, the RT Standards Committee has created a document titled, "The Significant Changes to the 9th edition of Standards for Relationship Testing Laboratories."

Individuals with questions regarding the Standards should contact standards@aabb.org.

What's New in the 15th Edition of Standards for Relationship Testing Laboratories

The RT Standards contains requirements to be implemented by AABB-accredited

relationship testing laboratories. Failure to meet the requirement would constitute a nonconformance under the AABB Accreditation Program.

1.2.2.1 The laboratory director shall have responsibility and authority for all policies, processes, and procedures and to stop or suspend laboratory operations.

This standard was changed to include the authority to stop or suspend laboratory operations to the responsibilities of Laboratory Director.

1.6 Assessment of Risk: The facility shall have a process in place to perform risk assessments for activities at defined intervals.

1.6.1 Mitigation strategies shall identify, assess, and address the level of risk associated with activities performed in the facility.

These standards are new to the proposed edition and new to the quality template. The standards were first introduced in the 10th edition of CT Standards and will now be put in all sets of AABB Standards. Guidance will be published with examples of various Risk Assessment Methods.

3.5.6 The laboratory shall have processes in place to minimize the risk and impact of an internal or external data breach.

This standard is new to the proposed edition and new to the quality template. This standard was first introduced in the 32nd edition of BBTS Standards and will now be put in all sets of applicable AABB Standards.

4.4.1 The laboratory shall have a process to review promotional materials of contracted third-party administrators at defined intervals to ensure that the information contained therein comply with these RT Standards.

The facility is expected to have a process to review websites and other promotional materials for each of their contracted third-party administrators for compliance with the written agreement. The interval of review should be defined in the process and records maintained.

5.1.5.2 The laboratory shall release an identifiable sample and/or profile of an individual only for purposes relevant to the actual relationship testing for which the sample was submitted.

The clause “and/or profile” was added to this standard to ensure use of profiles are only for the purpose relevant to relationship testing.

5.1.5.2.1 If additional relationship is requested to be evaluated, a court order or the written permission of the individual(s) who furnished the sample, or the individual(s) with legal authority to provide consent, is required.

This standard was added to ensure that permission is obtained prior to evaluating additional relationships for a sample.

5.2.3.5 Samples intended for immigration, visa, passport, and citizenship testing cases for the United States of America shall be accepted only if the case is **initiated directly between the petitioner and a facility accredited by AABB for relationship testing activities**. **Records of the initiation of this service by the petitioner shall be maintained in the facility’s records.**

The element in bold is new to this standard and was added for clarity. The requirement ensures that the facility maintains records of the direct communication between the accredited facility and the petitioner for the initiation of relationship tests for U. S. immigration purposes This activity is typically occurring but was a blind spot in the Standards.

5.2.4.1 Printed name, alleged relationship, and date of birth of each individual tested and untested person(s) signing consent for a minor child or legally incompetent adult.

5.2.4.8 Original or legible photocopies of at least one of the following items for each individual tested and untested person(s) signing consent for a minor child or legally incompetent adult:

1. Valid government-issued photo identification (ID).
2. Photograph that is suitable for positive identification.

The clause "and untested person(s) signing consent for a minor child or legally incompetent adult" was added to these standards to ensure that identification records are obtained for the person providing consent.

5.2.4.8.1 For cases intended for immigration, visa, passport, and citizenship for the United States of America, the following shall be submitted:

1. For an adult being tested, a legible copy of the government-issued photo ID and a photo suitable for positive ID.
2. For a child being tested, a copy of the government issued photo or ID or the birth certificate and a photo suitable for positive ID.

If these are not available, the collector shall record the reason for the absence of documentation.

This standard was changed in consultation with the U. S. Department of State to reflect its internal policies and procedures.

5.3.11.2 For nonparentage cases where the genetic evidence does not support the alleged relationship, either by exclusions or a low likelihood ratio, phenotypes for parties in question shall be confirmed with an independent isolation. For closed systems, **Standard 5.4.2 applies**.

This standard was re-written for clarity. The standard is now written in a way that focuses on confirming the phenotypes of each tested party which is the intent.

5.5 Calculations: The laboratory shall have policies, processes, and procedures for the use of validated calculation methods used in relationship testing.

The committee rewrote this standard to match the typical method by which a standard is written. The intent and content have not changed.

5.5.2 When linked loci are used, the laboratory shall have policies, processes, and procedures for estimating and minimizing the effects of linkage on non-parentage cases.

This standard is new to the proposed edition and was added for completeness. These elements were introduced in a previous edition of Guidance and should not be unfamiliar to our member laboratories.

6.3A (5) Racial/ethnic background(s) used by the laboratory for calculations as designated by the participants or closest available frequency database.

The clause "as designated by the participants or closest available frequency database" was added for clarity. A generalized universal database should not be routinely used for calculations.

6.3A B (3d) When autosomal loci are not tested, the conclusion shall not overstate the relationship. An explanation on non-recombining haplotypes inheritance and limitations to these markers shall be provided.

The standard was amended to require an explanation when reporting non-autosomal results independent of or not combined with autosomal STR results. When autosomal loci are not tested, the laboratory must be careful to not overstate the significance of the testing. For example, two individuals sharing the same Y chromosome alleles can be said to have a possible common male ancestor, but it may be overstating the evidence to say they have the same father. A statement that explains the limitation of using non-recombining haplotypes (i.e. Y-STRs, X-STRs) in relationship testing must be included on the report if not combined with autosomal STR testing results.

6.3A B(3e) When autosomal likelihood ratios are not in agreement with non-recombining haplotypes (leading to a different conclusion) an explanation on non-autosomal inheritance and limitations to these markers shall be provided.

This standard is new to the 15th Edition. When results from non-recombining haplotypes (i.e., Y-STRs, X-STRs) lead to a different conclusion than the combined LRs for autosomal STRs, a statement with an explanation of the limitations of non-autosomal inheritance must appear on the report. For example, a CLR<10 for a paternity test combined with inclusionary Y-STR results may falsely include a first-degree male relative of the tested AF. In such cases it may be helpful to issue reports with a discussion of the autosomal and non-autosomal findings separately in addition to the combined relationship index (see standard 6.3.3).

6.3.2 Non-autosomal results, when tested for parentage, avuncular, full sibling, half sibling and grandparent, shall be incorporated with autosomal results into the combined relationship index. **In addition to the combined relationship index, the laboratory shall have the opportunity to discuss autosomal and non-autosomal findings separately.**

The addition in bold was added to this standard for clarity. This will ensure the standard reflects current practice.

Common Nonconformances Written to the 14th Edition of Standards for Relationship Testing Laboratories

1.1.1 Compliance with these RT Standards and applicable laws and regulations.

1.1.2 Defining, documenting, implementing, maintaining, and improving the quality system.

The facility is expected to have policies, processes, or procedures (PPP) for each element of the standard. Internal assessment schedules should ensure compliance with new editions of the standard by the implementation date.

1.1.6 Executive management shall have responsibility and authority for: Ensuring adherence to quality and operational policies, processes, and procedures.

Standard 1.1.6 is cited when there is a written policy, but the assessor observed evidence that the policy is not being followed.

4.3.3 There shall be written agreements between laboratories and third-party administrators that define the following:

1. Collection requirements.
2. Responsibility for testing process.
3. Reporting of test results.
4. Appropriate marketing materials and claims.
5. Use of laboratory's name and accreditation status.
6. Unless accredited for collection or verification activities by AABB, third party administrators are prohibited from initiating cases for United States of America immigration, visa, passport, and citizenship testing.

Facilities that accept cases from third-party administrators must have a written agreement that addresses elements 1) through 6). The agreement covers the requirements, expectations, and responsibilities of both parties. The intent of this standard is not to require an agreement for the occasional individual case, but to address situations where a facility is conducting ongoing testing for a third-party administrator, even at low levels or in trial or probationary periods. Regular monitoring to ensure that third-party administrators continue to meet the requirements of the agreement is also required, see Standard 4.4. If third-party administrators refuse to comply with the agreement, action must be taken, including ceasing to accept cases from that supplier and/or ceasing to schedule collections at its facility.

4.7.1 The facility shall have policies, processes, and procedures to evaluate and respond to possible altered or fabricated documents.

Facilities have occasionally seen final reports and other documents that are altered or fabricated. Facilities should have mechanisms in place to readily identify these altered or fabricated documents. A written policy is required even if the facility has never encountered a possible altered or fabricated document.

5.1.2 The laboratory shall participate in a proficiency testing program for each genetic system used for reporting test results.

Every locus used to test case samples, even those only used occasionally, must be included in proficiency testing 3 times per year.

5.1.2.4 Proficiency Testing Program
When no formal graded external proficiency testing program is available for any of the genetic systems used to report test results, the laboratory shall use one of the following methods:

1. Test on a monthly basis known samples from when graded proficiency testing was available.
2. Test on a monthly basis a standard trio of samples developed from persons of an undisputed relationship.
3. Participate three times a year in a sample exchange program.

Pandemic-related shipping issues left some facilities without access to a formal graded external proficiency test or able to participate in sample exchanges. When an external PT is not available, even for one cycle, a monthly internal proficiency test should be performed.

5.1.2.6 Proficiency testing, whether graded or not graded, shall be representative of the cases the laboratory performs, including standard trios, single parent, and family studies (reconstruction cases).

Some calculations may not be routinely available from a proficiency provider. An example is single parent cases. One mechanism of checking the single parent studies is to recalculate tested trios and compare the results. Typically a lower combined paternity index is expected when a parent is missing. Hand calculating a single parent case to evaluate the laboratory's computer calculations would also be documentation of checking this single parent calculation.

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

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 RT 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 United States of America 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.

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 document the reason no identification was available. When collecting for other countries' equivalent of USCIS or DOS activities, check to see if there are different requirements than in the United States of America.

5.3.8 Two-Party Comparisons of Full Sibling, Half Sibling, Avuncular, and Single Grandparent Likelihood Ratios
The laboratory shall have policies, processes, and procedures for two-party comparisons of full sibling, half sibling, avuncular, and single grandparent likelihood ratios.

A written policy is a requirement of this standard.

6.3.3 The laboratory shall have a process to ensure that relationship testing services and test reports meet these RT Standards before distribution or delivery.

Final inspection is the last opportunity to confirm that the relationship testing report has met all requirements and is ready for issue. Prior to release, the laboratory is expected to confirm that all in-process inspections and tests were performed and verify that customer requirements are satisfied. Only final reports that have met these requirements should be signed.

6.4.2 The facility shall distinguish between AABB accredited and non-accredited activities with respect to all claims in promotional, marketing, and educational materials in which AABB trademarks are used.

The AABB logo should never appear on a facility website in association with unaccredited activities unless it is clearly identified as an unaccredited activity.

9.1.6 Corrective Action: The laboratory shall have a process for corrective action that includes the following elements: … An assessment of risk.

9.2.1 Preventive Action: The laboratory shall have a process for preventive action that includes the following elements: … An assessment of risk.

An assessment of risk should include identification of risks in the laboratory, an evaluation of the impact and severity of the risks and potential for occurrence, and implementation of controls to mitigate potential adverse events. Standards 9.1.6 and 9.2.1 require an assessment of risk for every corrective and preventive action.

A Brief History of Biological Relationship Testing

Since very little relationship testing is done using blood samples in the 21st century, we have lost sight of the beginnings of the process. One of the early high-profile cases involving genetic testing for parentage establishment took place in 1942, when the silent film star Charlie Chaplin was accused of fathering a child, Carol Ann, with a relatively unknown actress by the name of Joan Berry. At the trial that ensued, the charge was dismissed due to a deadlocked jury but in a second trial a year later, the jury voted 11 to 1 that he was the biological father of Ms. Berry's child, even though the results of a blood test indicated otherwise.

One of the few tests that could be used for parentage establishment at the time was a test for the Landsteiner, or ABO, red blood cell antigens. The A and B genes are co-dominant, meaning that neither will mask the presence of the other. If an individual has a gene for both A and B, that person will be blood type AB. If the person has neither, the blood type will be O. Type A and type B, however, are a bit more complicated. O is, effectively, just the absence of A or B. Type A cells can be either AA or AO and type B cells may similarly be BB or BO. Ms. Berry was identified to the jury as being Type A and her child was Type B. The gene for the B phenotype must have come from the child's biological father; Chaplin was Type O and thus could not have been Carol Ann's father. However, the blood test results were deemed inadmissible and not presented to the jury. The verdict in *Berry v. Chaplin* and other cases formed the basis for the reform of paternity laws in a number of states in the U.S.A.[1]

Over the next several decades, other tests involving factors in blood were employed in paternity cases. Some of those tests involved cellular antigens, others were genetically determined traits in blood serum. Initially, their strongest power was in their ability to exclude a man who had been accused falsely in a paternity matter. Having determined the likely contribution of the mother to the child's typing allowed, by a process of Mendelian elimination, what the contribution by the alleged father must have been. If, like Chaplin, the man did not have the requisite genetically determined factor, it followed that he could not be the biological father.

Excluding an accused man was a clear way to determine non-paternity, but it was apparent that just because a man was not excluded did not mean that he WAS the father. Approximately 40% of people have Type A blood, either AA or AO genetically[2]. Since a blood test does not determine zygosity, only the phenotype A, an ABO test for paternity of a child who must have inherited an A from their father would not exclude about 40% of the general male population. Type O is similarly common. Other blood-associated characteristics could be, and were, used in combination with ABO and their combined ability to exclude a falsely accused man improved matters somewhat but the occurrence of some of those characteristics in the population was even higher than A and O. The most common Rh

antigen, for example, is present in from 85% to 99% of people, depending on their racial ancestry. Since most of the commonly used tests are for genetically independent markers, the overall probability of paternity by the tested man rather than another random individual can be obtained from the multiplication together of the individual likelihoods. A typical probability of paternity with the most commonly used red cell markers might yield a value in most cases in the range of 90 – 95%.

Recognition of a highly polymorphic glycoprotein on the white blood cells led to typing of the Human Leukocyte Antigens system. The genes for HLA-A, HLA-B, and HLA-C are on chromosome 6. The genes for B and C are relatively closely linked, with HLA-A being some distance away. Because of the polymorphism of the HLA-A and HLA-B genes, they were very useful in relationship testing, although some calculation adjustment for their chromosomal linkage was necessary. An HLA analysis alone could have the power of exclusion similar to an analysis with a single DNA probe for a later used type of DNA analysis of restriction fragments. Until then, combination of HLA-A and HLA-B with the other available blood markers could lead to a high and legally useful paternity index.

Blood cell and serum markers relied on the detection of the various types by serological antibody testing. ABO typing is relatively straightforward, so much so that it is often taught in basic laboratory classes. HLA typing, much more capable of excluding a falsely excluded alleged father, is also more difficult to interpret. Now typeable by DNA methodologies, the original method used antisera to the various types. Antisera have a certain level of instability and probably limited shelf lives. The types are polymorphic and also can be cross-reactive with each other. HLA-A and HLA-B, usually typed together because they're linked, are still subject to a level of crossing over which had to be taken into account. The method also requires liquid blood, considered to be a hazardous material by transporting entities.

DNA testing for relationship establishment began in the mid- to late-1980s. The methodology made use of the presence in the genome of repeating stretches of nucleotides, about 10 to 100 nucleotides in size each, linked end to end with the repeats varying in number in individuals within a population. These regions are referred to as Variable Number of Tandem Repeat regions, or VNTRs. It was possible in the 80s and 90s to estimate the lengths of VNTRs by partially digesting DNA from the white cells with enzymes that cut the human DNA at specific nucleotide sequences. The DNA digests were then separated according to size by electrophoresis on an agarose gel, alongside size standards. After transferring the electrophoresed DNA digest to a fabric membrane by a process called Southern blotting, the membrane was treated with a radioactive DNA probe that would bind to complementary fragments on the membrane. Exposure of the probed membrane to X-ray film produced an autoradiograph which allowed the visualization of the labeled genomic regions, whose sizes could then be determined by comparison of their migration through the gel with the size standards in adjacent electrophoretic lanes. The fragments owed their differences in length to the number of tandem repeats between the restriction fragment cleavage sites. The greater the number of repeats, the larger the fragment and the more slowly it migrated into the electrophoretic gel. Because the DNA fragments were generated through the digestion of DNA by bacterial restriction enzymes, they became known as Restriction Fragment Length Polymorphisms or RFLPs.

The RFLP methods had a higher ability to discriminate between individuals than did the older blood antigen analyses, largely because the sizes of the observed fragments were quite variable from one individual to another. Being on an individual's DNA, those size variations are transmitted from parent to child, so they can be used to determine the likelihood of a familial relationship. RFLP technology found wide acceptance in the scientific arena for the investigating the inheritance of a number of genetic traits as well as in the legal world for establishing parentage and investigating criminal cases. The method still required

blood as a source of the genetic material because of its relative insensitivity to the probe technique. It was also slow; the entire process, from DNA extraction to the final data acquisition would take several days. The DNA probes were radioactive, which was another drawback, requiring the handling radioactive isotopes. After collecting the data from one DNA probe, the process could be repeated by reprobing the original membrane with several others sequentially.[3]

There are other kinds of VNTRs besides RFLPs. Of particular use in the next generation of DNA testing methods are the Short Tandem Repeat (STR) loci. RFLPs can be quite large; the size range analyzed ranged from about 500 to several thousand bases. STRs, used for relationship testing, on the other hand, are generally four to six bases per repeat and the nucleotide region might be replicated a few dozen times per locus. The small sizes and polymorphism of the STRs allows replication of the genomic region by the Polymerase Chain Reaction, a biochemical procedure that allows the amplification in the laboratory of small quantities of DNA in a short amount of time. The amount of DNA for relationship analysis (or forensic evidence evaluation, a similar process) can be collected by a swab of the mouth or even of an item handled by the subject being tested. Dried swabs can be sent through ordinary mail or courier services, amplified in the laboratory in a few hours, with analysis taking about 30 minutes per sample. The DNA sample no longer requires blood. Gone are the days of needing a trained phlebotomist to obtain vials of blood or the resulting inconvenience of shipping potentially hazardous bodily fluid through the postal or parcel transport system.

[1] A search for the actual blood types of the parties in the Chaplin case may turn up different answers. The main point, however, is that Carol Ann's ABO type was incompatible for her to have been fathered by Chaplin.

[2] The blood markers discussed here have different rates of occurrence in different ethnic populations. Those differences are ignored here for the sake of brevity.

[3] A compilation of the method may be found in Sozer A. C., Kelly, C. M., and Demers, D. B., "Current Protocols in Human Genetics" (1998), 14.4.1-14.4.26.

In Search of RT Assessors

The AABB Relationship Testing Program is in need of a few volunteer assessors. AABB assessors are a vital part of the AABB Accreditation Program. AABB Assessors are highly skilled professionals from accredited organizations. They bring their invaluable experience, knowledge and insight to AABB facilities worldwide.

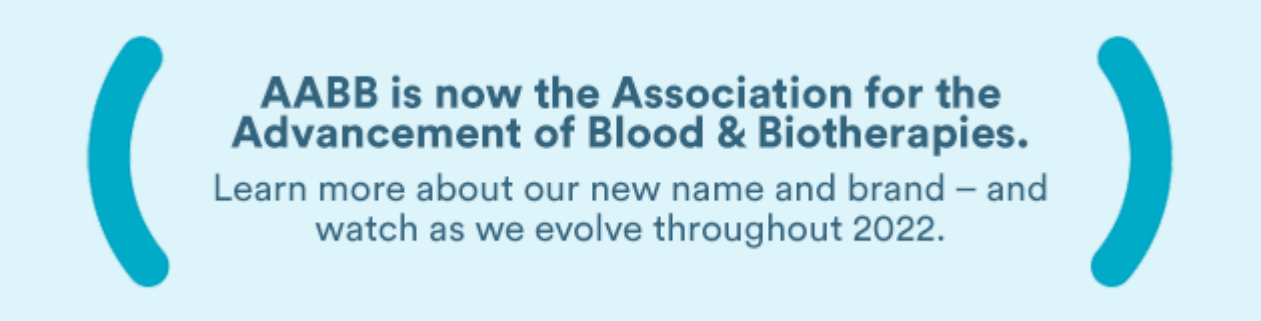
The AABB Accreditation Assessor Program provides the volunteer professional training in auditing techniques, quality program and operations, and the competitive, legal and ethical issues associated with assessments. With this training assessors are able to evaluate a facility's Quality and Operational systems to determine whether the service they provide is appropriate and in control. The program provides both the assessors and the facility educational exposure to new ideas with regard to quality systems. The AABB Accreditation Assessor Program has been granted accreditation by the International Society for Quality in Healthcare (ISQua).

Interested in becoming an assessor? Review the Assessor Qualifications, Requirements, Responsibilities documents carefully to determine if you qualify. Also review the financial and time commitment information. If you have the necessary work experience please submit an Assessor Application.

Ideas for Your Newsletter

Do you have a suggestion for a relevant topic, article, or announcement to be included in the

next edition of the Relationship Testing Newsletter? Send us an email at accreditation@aabb.org.



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