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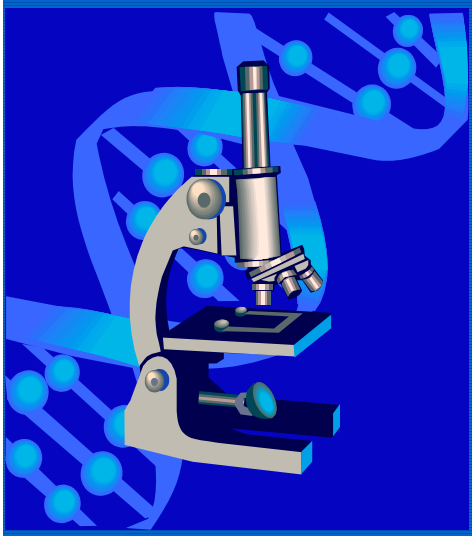

In This Issue

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Pre-analytical Errors and Problems in Relationship Testing

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Introduction

Lab quality control activities mostly focus on *analytical* inaccuracy and imprecision and on the *post-analytical* (e.g. turnaround time of reports). In fact, the vast majority of so-called "lab errors" are committed before a labeled specimen (a "sample") ever reaches a laboratory. Data from hospital laboratories indicates that >90% of errors are committed as *pre-analytical* ones. These include using the wrong preservative, poor storage conditions, sample adulteration or contamination, cell lysis and specimen mislabeling. Control of pre-analytical errors is difficult because people who are not lab employees often provide and oversee specimen collection, labeling, packaging and shipping.

Pre-analytical errors also affect relationship-testing (RT) laboratories and can affect court decisions, cause family disruptions and lead to other adverse events. This newsletter will describe pre-analytical problems of RT labs and suggest some possible solutions.

Case History

An RT laboratory tested buccal samples DNA from an alleged father (AF) who is a U.S. citizen and an alien child who hoped to immigrate to the United States. The AF's sample was collected in a physician's office and was mailed to the RT lab for a one-parent test of paternity. Several STR loci demonstrated genetic inconsistencies with parentage and the paternity index (PI) was calculated as zero. The AF was excluded from paternity and the immigration petition was denied.

The AF subsequently paid to have the child's sample and his own sample recollected and tested at another laboratory. This time, there were no inconsistencies with parentage in 14 independent STR tests and the PI was more than 200/1 (posterior P >99.5% given a prior P =50%). The first lab's director was notified of the discrepant results.

Which set of results is correct?

If mislabeling of the AF's first specimen occurred in the physician's office, then another person's buccal specimen was in the collection tube. It is nearly impossible for a random person to have a DNA profile that would be consistent with the paternity of the child.

On the other hand, *a mislabeled specimen from an unrelated person would very probably have a DNA profile that is genetically inconsistent with the paternity of the child*. The test results indicating paternity are almost certainly correct.

What is a "WBIT" error and how has it been addressed?

In the described case, the collection tube was mislabeled with the name of the AF in the case, but the tube did not contain the AF's specimen. In blood banks, which collect blood samples for compatibility tests, this kind of mislabeling problem has been named the "Wrong *Blood* In Tube" (WBIT) error. (Other sample identification errors causing sample rejection include: label illegibility, misspelling of the name of the person to be tested, etc.). Unless it is controlled in some way, the WBIT error has a frequency of between 1 per 1,000 and 1 per 2,500 specimen collections. The WBIT can have dire clinical consequences for the patient as well as malpractice risk consequences. Therefore, if the patient has already been blood-typed during an earlier medical encounter, the previously recorded results (ABO type) on file must be observed and found identical to the current one. (If there is no record of ABO typing, the blood bank performs a duplicate sample collection followed by an "ABO recheck", a duplication of the ABO blood grouping test. The two blood collections are carried out by 2 different people or by 1 person at 2 different times and the duplicates must show identical test results.)

How can RT labs improve specimen collection to avoid this kind of error?

RT labs that collect buccal instead of blood samples, experience a similar WBIT problem ("Wrong *Buccal*- cells In Tube"). To avoid this problem without resorting to duplicate collections, RT labs may try the following:

- *The lab should provide rigorous, written buccal swab collection procedures* for collection sites. The lab should insist upon a fixed order of steps in addition to how each step is done. An acceptable procedure order is: The buccal sample is first collected onto 4 swabs; the swabs are then <u>all</u> inserted into a collection tube; a blank label is affixed to the tube; the tested person is asked to spell his/her name; the name is written on the tube label; the correct steps are confirmed by the tested person and a witness. *Handouts to the person to be tested can spell out his/her role in the proper identification* of the collected specimen and labeling.
- *Swabs, identifier labels or tubes should never be left free (loose) in the specimen collection environment*. Loose labels containing a person's ID may find their way onto another person's specimen tube and cause a double mis-labeling. Loose swabs and tubes may have been contaminated with random DNA. Only the tested person, the collector and the witness should be present in the collection area. The collector and witness should be trained to prevent poor practices.

- *After a DNA analysis, an RT lab might review its files seeking records of the people who were tested*. If the same people and the same tests' results are recorded, then *old and new DNA profiles can be compared directly*. (In the case presented above, the AF had been tested using a different DNA multiplex and was found to be the biologic father of 2 other children. Stored DNA samples of those children and the rejected child were tested for sibship using the lab's current multiplex and the full-sibling indices were very high.)
- *When a tested person vehemently complains about the results, the lab should recommend a resampling and retesting.* If the testing was ordered by a third party, such as a lawyer or child support agency, the laboratory should discuss the case with the third party. It should be noted that simply repeating the DNA analyses but using the same lab sample, as required in AABB Standard 5.3.8, will only detect identification errors that occur within the lab (i.e., analytical errors).
- In routine paternity tests (i.e. for civil lawsuits), a mother and young child usually appear together for specimen collection. It may be necessary to collect their two specimens sequentially with mother and child in the same room. With the mother trying to control her obstreperous child, *it is easy for a specimen collector to exchange tubes or labels of the mother and child*. After DNA analysis, the inadvertent swap will cause an apparent exclusion from paternity. In truth, the AF has been excluded as a parent of the child's mother. A simple, inexpensive way to detect this particular WBIT error is to *always do a "mother-child switch check": Always reanalyze (e.g. by computer) the DNA data after the maternal and offspring profiles have been swapped. If the AF now appears included, it is likely that a mother-child sample switch has occurred.* A failure to exclude the AF is strong evidence that two WBIT errors have occurred at the time of specimen collection. Note that repeating the analytical steps from the isolation of DNA (AABB Std. 5.3.8) does not address the switch of mother and child specimens.
- Labs might *submit reports that disclaim responsibility for possible specimen collection errors* when collectors are not employees of the RT lab. (This would be analogous to a clinical lab providing the name and address of any reference laboratory that performs procedures or analyses not done by the primary lab.)

What other pre-analytical problems can falsely exclude parentage?

Properly collected *specimens* can present their own pre-analytical problems.

- Multiple mutations at tested loci could produce several genetic inconsistencies in a parentage case. Up to 3 mutations in 15-STR multiplexes have been reported in a single case.
- Silent (e.g. null) alleles at more than one locus, or in combination with mutations, could produce a false exclusion too. If a child shares a null allele with an alleged parent (e.g. an AF),

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each of the pair would demonstrate only one allele (1 STR peak) and that allele might not be present in the other person. This is termed "apparent opposite homozygosity" or AOH. An apparent single allele in a child that differs from the single allele in the AF is usually interpreted as evidence of exclusion. That evidence, however, is "*indirect*" or "secondary" because two apparently homozygous genotypes have been wrongly inferred from two single-peak phenotypes. In fact, the parent and child are each heterozygotes. Direct evidence of exclusion is better than indirect evidence. *Direct* evidence of exclusion is found when the alleged parent and child are both heterozygotes and share no alleles or when an alleged father has a 1-peak phenotype, but the child is heterozygous and the tested mother's single obligate allele is identified in the child.

- Rarely, a buccal or tissue sample contains a mixture of two or more cell populations. This can occur by fraud: In one documented paternity case, an AF's girlfriend kissed him, thereby transferring unrelated buccal cells into his oral cavity prior to specimen collection. Two cell population mixtures also occur when the tested person either possesses engrafted cells or is a genetic chimera. If sampling misses the minor cell population or if the minor proportion of cells is below the test's detection limit, then erroneous parentage exclusion will result.

Can pre-analytical problems cause false positive relationship results?

- Among civil paternity cases, a mother will occasionally attribute the paternity of her child to a first-degree relative of the true father (a full-sib, father or son of the AF). If testing is insufficient in polymorphism information content (PIC), which is a function of the number of loci examined and their heterozygosities, then the AF may not be excluded from paternity. Indeed, the AF may share with the child a number of paternal obligate alleles at several loci. If those alleles are infrequent in the AF's ethnic population, the paternity index will be high.
- In immigration cases of parentage or sibship, a true relative has been known to fraudulently substitute for an unrelated person. (Whereas AFs in civil paternity cases usually argue their non-paternity, AFs, alleged mothers and children in immigration cases all desire test outcomes that demonstrates parentage or sibship.) A variation on this impostor fraud is to substitute a true relative's samples for the impostor's. In most cases, the true relative has already immigrated to the U.S. Either method of this identity theft (or "DNA Recycling") requires the cooperation of the specimen collector or interference with chain-of-custody procedures.

For discussion of the probability of not excluding a related man see in the Guidance for Standards for Relationship Testing Laboratories, 11th Edition, Appendix 8. Non-Exclusion Probabilities for Related Brothers/Fathers of Included Tested Man.

Do pre-analytical histories and problems affect relationship test logic?

Even in the simple relationship test of one alleged parent and one child, there are a number of possible 'states of nature' (SON). Only one state is the true one:

1) The two people are related as parent-child.
2) The two are related as (first-degree) full-siblings.
3) The relationship is second-degree (half-sibs, uncle-child, grandparent-child).
4) More distant relatives constitute the pair.
5) The two are unrelated.

In most relationship testing laboratories, only parent-child & unrelated hypotheses states are routinely considered and only 2 alternate hypotheses are compared in a parentage index. A different hypothesis, however, might be the true SON. If an RT lab had more historical information about each case, more possible states of nature might be considered, better alternative hypotheses could be chosen and test results could improve.

Even if the chosen alternate hypotheses represent the two most likely states of nature, LRs do not account for the effects of pre-analytical problems in likelihood ratio calculations. Any LR is based solely on analytical phenotypes and does not account for pre-analytical problems. While a paternity index of zero implies a certainty that the alleged father is not the child's biologic parent, the truth is that he still could be. There is a small chance that a WBIT error has occurred, that genetic inconsistencies are actually the result of mutations and silent alleles, or that a tested person is a chimera. Presently, RT calculations neither consider this information nor do reports mention possible pre-analytical problems.

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WANTED

Audio Conference Content

Would you like to repurpose your old talks or presentations?

Share your content as part of AABB's 2015 RT Webinar Series.

Please let us know 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. Topics of interest may include but are not limited to calculations, new technologies, expert systems, court room basics, forensics, DNA etc. If you decide to submit your content, you can choose to moderate the webinar or we can assign a speaker for you.

For more information or to submit your content, contact Nikki Bass at nikkib@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 at nikkib@aabb.org

RTAPU or RTSPU Member

- 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?

If these issues are of interest to you, the **Relationship Testing Accreditation Program Unit** would like to have you as a member.

- 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, the **Relationship Testing Standards Program Unit** would like to have you as a member. To get involved, please contact Nikki Bass at the AABB National Office at nikkib@aabb.org.

GREAT RESOURCES

- AABB has launched a new certificate course for individuals who would like to be recognized as AABB-trained sample collectors for relationship testing purposes. Find more information at the following link: http://www.aabb.org/development/education/materials/Pages/RT-certificate-course.aspx

- Standards for Relationship Testing Laboratories for 11th Edition is now available. The publication contains a CD of the Guidance for Standards for Relationship Testing Laboratories, 11th Edition as well as a cross walk to identify the changes from the previous edition. Purchase a copy at the following link: http://marketplace.aabb.org/EbusPPROD/Default.aspx?TabID=54

- Purchase a copy of the previously held (Nov. 7th) audio conference titled Changes to the 11th Edition of Standards at the following link: http://www.softconference.com/aabb/slist.asp?c=5054#DTJanuary92013

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