Ballot Name: Approval of ANSI/ASB Standard 020
Ballot URL: https://workspace.aafs.org/higherlogic/ws/groups/DNA_CB/ballots/ballot?id=52
Document Number: ASB Standar d 020
Document Title: Standards for Validation Studies of DNA Mixtures, and Development and Verification of a Laboratory's Mixture Interpretation Protocol

Note: a specific Proposed Resolution must accompany each comment or it cannot be considered.

#	Section	Type of Comment	Comments	Proposed Resolution	Final Resolution
1	3.2, 4.4	T	The definition of consistency ("within an acceptable limited range of variation") could be widely interpreted and make this standard hard to enforce.	Potentially examples could be given as to acceptable versus unacceptable variation. For instance, one analyst resolving a partial major contributor at 13 loci vs another resolving at 14 loci due to a difference of opinion on mixture ratio is acceptable. This would be opposed to one analyst resolving a partial major contributor and another saying a profile is unresolvable and using CPI on the entire mixture is unacceptable variation.	Reject: Statement 4.1 refers to supporting normative information in Annex B which states: "The laboratory shall define the acceptable range of variability in the interpretation of DNA mixtures for use in the evaluation of the consistency within the laboratory." According to the definition, "consistency" is to be "defined by the laboratory protocol and validation data." This means that the laboratory should have and be able to provide validation data to support its definition of consistency and the defined acceptable range of variation, if any, permitted. The range of variation acceptable will necessarily depend on the portion of the protocol being verified, but ultimately any statement of inclusion vs. exclusion of a known individual should not vary within the laboratory. Variation beyond the accepted defined range would constitute an inconsistency and require additional studies and/or revision of the draft protocol. Additional guidance regarding "consistency" is under development in another document at the time these responses were made.
2	3.2; 4.4; and 4.4.2	T	The definition of "consistency" (3.2) and its use in 4.4 ("generate reliable and consistent intepretations and conclusions") is too vague. It gives no guidance on howa lab is to determine consistency. One lab's estimation of consistency under this definition could be radically different than another.	Delineate methods, i.e. statistical or other, to determine consistency.	Reject: See response in Comment #1
3	4.3.2	E	Size of font for "4.3.2" appears to be smaller than font for other numbers	verify and adjust font size if it is inconsistent with "4.3.1" and "4.3.3"	Accept
4	Annex C	E	Confirm web address "https://www.gov.uk/government/organisations/forensic-science-regulator" Does not work as a hyperlink. Copy-paste of the site gives a 404 error.	verify web address is correct, and include hyperlink	Reject: Link works fine in word and PDF version. Copy and paste method also worked fine.
5	Annex C	E	Confirm web address "http://www.ifsa-forensics.org/wp-content/uploads/2016/09/DNA_MRD_English.pdf" Does not work as a hyperlink. Copy-paste of the site gives a 404 error.	verify web address is correct, and include hyperlink	Reject: Link works fine in word and PDF version. Copy and paste method also worked fine.
6	Annex C	E	#7 does not include a hyperlink for the website address	Include hyperlink for "https://www.swgdam.org/publications" for #7.	Rejected: Hyperlink in PDF was tested and was shown to be functioning.
7	Annex C	E	Confirm web address "https://www.fbi.gov/file-repository/quality-assurance-standards-for-forensic-dna-testing-laboratories.pdf" Does not work as a hyperlink. Copy-paste of the site gives a 404 error.	verify web address is correct, and include hyperlink	Accept: Will change link to https://www.swgdam.org/publications.
8	4.4.3	T	why make this retroactive by including existing interpretation. These protocols are reviewed annually at a minimum and often changed based on current ideologiy/new guidelines.	remove existing from the standard	Reject: No statement regarding retroactive is present in the document. The goal is for all interpretation protocols in use in a laboratory to meet this stated set of requirements. Laboratories should verify that their current existing protocols work appropriately and have the support of relevant validation studies. If the laboratory discovers that its existing protocol has deficiencies, it would be incumbent upon the laboratory to explore the extent of deficiencies and address resolution and amendment of any previously reported casework.
9	all	T	The FBI QAS document is being revised and the validation section is being changed. Has anyone looked at those standards to see if there are conflicts with these standards? Do we really need two agencies mandating standards for the same topic?	Confer with the committee revising the QAS document to see if there are conflicts and/or duplications since the QAS document is already used in accreditation standards. It would also be good to check with the SWGDAM minture interpretation guidelines for conflicts as well since that is not a "standard" but had been adopted as "good laboratory practice".	Noted. There are joint members on the OSAC, ASB and SWGDAM committees specifically tasked with ensuring that the standards issued from each group are compatible and complementary to each other.
10	2	E	The comma after "documents" should be a period	Replace with period	Accept
11	3	E	3.1, case-type samples. This term is not used anywhere in the document	Delete the term and definition	Accept
12	3	E	If case-type samples is inserted in the document somewhere and this definition stays, the last sentence has nothing to do with a definition	Delete last sentence	Accept: Delete paragraph 3.1 since the definition is not used.
13	4	E	4.3.3-the statement ends with "including the following:", but there is only one parameter and not a list	Reword to "…for samples containing mixtures of DNA, including criteria for establishing…"	Accept
14	Annex B	T	There are a number of "shall" statements in Annex B. If they are important enough to be a "shall", perhaps they belong in the standards themselves rather than in the Annex.	Evaluate each "shall" statement in the Annex and consider whether they should be placed into the Requirements in the appropriate section.	Rejected. No revision made to the document after review. An Annex can be normative. The annex is specifically referenced in statement 4.1. The 'shall' statements have been included purposefully.
15	Annex C	E	reference 8 has the year, which ties that version of the QAS to the standards	Delete the year so that whatever version is current applies	Accept. Delete 2011 so the latest version is used. Link was updated.

16		E	This is a general comment on the overall standard. This standard presents an opportunity to eliminate confusion as to how laboratories should conduct validation studies. The strength of this standard lies in the annex documents which provide specific instructions as to the choice of samples to be used in a validation and clarify that moving forward, interpretation guidelines should not be extrapolated beyond the range of the validation studies. This is the first place where it's explicitly stated that degraded samples need to be accounted for in a validation, although laboratories have commented on degraded samples for years. Additionally, mixture verification beyond the scope of the initial validation can lead to better interpretation guidelines.	No resolultion needed.	Noted.
17	4.2.1	T	Laboratories encounter a wide variety of samples from a number of different people and conditions. It has been demonstrated that there can be drastic changes in a sample between amplifications. While laboratories cannot account for all the variations that may occur, laboratories can perform a robust validation study that can take a significant number of these occurrences into account and help inform decision making. There is limited value to a validation constructed of samples from a limited number of contributors, a small sample size, and samples that were not repeated.	Annex B should require validation samples that resemble the types of samples seen in casework. Therefore the samples should include: (1) a variety of samples with multiple contributors based on the number of contributors the lab intends to interpret; (2) a pool of participants that demonstrate the diversity of the United States; (3) mixtures created from related individuals; (4) mixtures created both from individuals that are of different ethnicities and from individuals of the same ethnicity; (5) a range of mixture ratios; and (6) degraded samples. All validation samples should be run in replicate to evaluate stochastic effects between amplifications. The evaluation of multiple mixed samples from related individuals, degraded samples, and mixtures from the same and different ethnicities would ensure well informed mixture interpretation protocol.	Reject. (1) Testing of multiple contributor DNA mixtures is discussed in Annex B; (2) and (4) This should not be relevant as no studies have shown that ethnicity affects the types of profiles generated. This may only be relevant for statistical analysis which is beyond the scope of this document. The laboratories have the option of performing these types of mixture studies or reviewing data generated by other laboratories, and may include this in their verification step for this standard and/or in the evaluation of their protocols for statistical analyses; (3) A relationship validation is outside the scope of this document; however laboratories are not discouraged from performing those studies as part of their validation. An example of high allele sharing using DNA from close relatives has been added to Annex B Section 4.2 in response to another comment; (5) and (6) A range of mixtures ratios and testing of degraded samples are specifically included in Annex B. The generation of validation data and its evaluation for developing interpretation protocols addressing stochastic effects and verification of the protocol are required under all sections of this standard. Please also see response to #33 below. No modifications to the document were made in response to this comment.
18	4.2.2	E	It is unclear what is meant by the phrase "dynamic range of the detection platform." Is this phrase meant to address a range of mixture ratios only?	Provide clarity and examples of what is meant by the dynamic range of the detection platform in Annex B.	Accept. This phrase is intended to cover all options permitted in the laboratory for the use of the specific detection platform with the range of samples tested in the laboratory. For example, a laboratory using capillary electrophoresis for detection of results must validate all permitted injection times, voltages, sample input volumes, etc. and have appropriate verified interpretation protocols for all available parameters. An additional statement has been added to Annex B for clarification.
19	4.2	T/E	The use of degraded samples should not be relegated to Annex B and ommitted from section 4.2. If the use of degraded samples is as important as is stated in Annex B, it seems appropriate to include a statement in section 4.2 to emphasize their importance. Additionally, greater description should be given to samples typically encountered in casework. If there is a common understanding regarding the types of samples commonly encountered in casework, explicitly describing them would avoid confusion.	Add a statement 4.2.5 which describes the type of samples (e.g degraded etc.) that should be used in validation testing.	Rejected. This is addressed in requirement 4.2.1 and in Annex B ("If the laboratory intends to interpret DNA mixture data resulting from testing degraded DNA, the laboratory shall conduct internal validation studies with mixtures of degraded DNA and/or use documented data generated from other source(s) using the same testing system and parameters as support for the interpretation and comparison protocol.") The requirement to perform validation studies with degraded samples would not be necessary for laboratories not accepting and testing samples likely to be degraded or not reporting results from degraded samples.
20	4.3	T/E	It is fair to assume that the samples used and the development of an interpretation protocol outlined in this standard would be used to evaluate probabilistic genotyping software. However, the standard lacks a statement that addresses the development of an interpretation protocol for probabilistic genotyping software. With an overwhelming number of laboratories moving towards the use of software for interpretation, the relevance of this standard will fade as traditional methods of manual interpretation become less common. The proposed standard for the validation of probabilistic software offers no guidance on the samples that should be used in a validation or what considerations need to be accounted for in an interpretation protocol. A stronger, explicit connection needs to be made between this standard and the probabilistic genotyping standard.	Section 4.3 needs a statement added to address the development of a mixture interpretation protocol for probabilistic genotyping software. This standard should be a normative reference for the Validation Standards for Probabilistic Genotyping Systems and should be mentioned in section 4.3 of said standard.	Accept with modification as an Editorial revision not a Techcnical revision. A statement has been added in Annex A regarding the validation of probabilistic genotyping software. While this standard may be used for the validation of probabilistic genotyping software, see ASB DNA-related standards for more information on this and related topics.
21	4.4.2	T	Verification is an essential step to add to the validation process and it is important that the individuals performing the validation are not the only ones involved in the verification process.	Recommend in Annex B that the verification step should be performed by individuals other thanthose involved in the actual validation.	Accept. The following statement has been added to section 4.4: "Verification of the protocol shall be performed by individuals in a blinded manner without knowledge of the expected results."
22		T	This proposed standard fails to lay out requirements with sufficient specificity to ensure minimal quality. Several examples are listed below.	Further revisision and drafting of the standard with additional, detailed requirements is necessary.	Noted.
23	4.2.3; 4.3.3	T	There should additional requirements concerning the number of contributors testing. Given the substantial and well-documented difficulties in accurately specifying the number of contributors, see, e.g., David Paoletti et al. Empirical Analysis of the STR Profiles Resulting from Conceptual Mixtures, J. Forensic Sci., Nov. 2005, Vol. 50, No. 6, the lab should be required to test mixtures with a number of contributors which exceeds the limit which will be applied in casework ("n +1" or "n +2").	Create a requirement that n + 2 contributors be tested during validation.	Reject. The requirement to test n+2 number of contributors may not be a necessary or appropriate requirement for all laboratories. While laboratories, especially if routinely interpreting complex mixtures, are encouraged to perform validation studies with additional contributors beyond what they intend to interpret, this knowledge may be sufficiently gained by some laboratories with limited testing situations from the literature or by reviewing data from other laboratories to establish a limited range of samples to be interpreted. For example, a laboratory testing primarily bones, blood stains and sexual assault samples as their only mixed samples would not likely benefit from performing validation studies with four or five person mixtures.

24	4.2.	T	No specification of minimum number of mixed samples to be used in the validation.	Add a requirement for the minimum number of samples in the validation, to be categorized as necessary, e.g., how many samples for each set of 3-person contributors, 4-person contributors, etc.	Rejected with modification. It is not possible to establish a minimum number of samples to test as this will be dependent on the DNA test used, the testing parameters used by the laboratory, the types of samples tested, etc. For example, a laboratory may choose to test only a small number of four person contributor mixtures to decide that they do not intend to interpret profiles likely to contain four or more contributors, whereas laboratories planning to interpret profiles from four or more contributors should have a large sample set of multi-contributor mixtures in their validation studies sufficient to develop and appropriately verify robust protocols. New text added to Annex B: " Repeated testing and data analysis are critical to the understanding of variability. While specific requirements for the minimum number of studies and sample sets used for the validation studies and the verification process are not detailed in this standard, the laboratory shall perform sufficient replicate studies to address the variability inherent to the various aspects of DNA testing, data generation and the analysis and interpretation of the data."
25	4.4.	T	No specification of minimum number of mixed samples to be used in the verification.	Add a requirement for the minimum number of samples to be used in the verification of the mixture protocols, categorized as necessary, e.g., how many samples for each set of 3-person contributors, 4-person contributors, etc.	Reject with modification. See comment for #24
26	4.3.2	E	Need to define stochastic effects.	Define stochastic effects; specify Alleleic drop-out, peak height imbalance, exaggerated stutter, and drop in.	Accept. Definition added.
27	4.3.2	T	Include mixture ratios as a limitation.	Add "ratio of the contributors".	Reject but noted. The list provided is not exhaustive. Samples with varying ratios of DNA from contributors would necessarily be a part of the validation studies required to address this standard and particularly when assessing stochastic effects with DNA mixtures, which is listed as a limitation. If estimated mixture ratios are used in the interpretation of mixed DNA profiles according to the laboratory protocol, then validation studies would need to be available to support how the mixture ratios are used and that process verified according to other requirements in this standard. Refer to 4.2.4, 4.3, and Annex B.
28		T	The language of the standard coupled with Annex B provides little concrete guidance for lab's attempting to design mixture validations studies and interpretation protocols. Given the lack of such guidance including I foresee that lab's will be able to satisfy this standard while conducting inadequate validation studies. One example is not requiring labs to conduct validation on mixtures with "n+1" number of contributors which the lab intends to interpret.		Rejected; no resolution suggested. We would like to reiterate that this standard applies to any type of DNA testing technology and methodology used, including but not limited to, STR testing, DNA sequencing, SNP testing, haplotype testing, traditional and rapid protocols, etc., where mixtures of DNA may be encountered, analyzed and interpreted. It is recognized that each laboratory performing DNA testing has individual case- and sample-acceptance policies and uses different technologies, methods, and protocols to generate DNA data. Specific studies conducted and approaches used, the type of data evaluated, and the details of the protocols will vary between laboratories. It is the responsibility of the individual laboratory to perform the range of studies appropriate to cover the breadth of samples accepted and tested for the technologies and methodologies used. These studies should be maintained and available for review. It is anticipated that accrediting or other agencies that adopt these requirements may provide more specific clarification as it applies to a specific technology or use. Also see response to #23 above.
29	4& Annex B	T	The proposed standard fails to set forth requirements with enough specificity to guarantee minimal quality. A lab could check off each of these requirements, yet still have failed to adequately tested sufficient mixtures to ensure reproducible, accurate results.	Add additional requirements, see below	Noted. The goal of a well written, detailed protocol and an adequately performed verification check is to provide support that the interpretation and comparison protocol in use in a laboratory results in reproducible and correct interpretations and comparisons. Please see response to #28.
30	1	T	Lack of mandatory compliance for labs which have already conducted a mixture validation study, e.g., labs "are advised to review their previous validation…"	Substitute "shall" for "are advised to review"	Reject. Please see response to #8 above.
31	Annex B	T	One of the most continuously studied, yet unresolved issues is the accurate estimation of the number of contributors to a mixture. Because underestimating the number of contributors is so prevalent due to allele sharing and stochastic effects, the lab should be required to test mixtures containing at least one contributor more than the maximum that will be interpreted in case work (n +1).	Require n+ 1 contributors in the mixture validation	Reject. Please see response to #23 above.
32	4.2 & 4.4	T	There should be a requirement concerning minimum number of samples to be used during both the validation and verification	Insert requirement of minimum number of samples	Reject with modification. See comment for #24
33	4.3.2	T	insufficient requirements concerning testing of mixtures exhibit stochastic effects, even when read in conjunction with the note to §4.3.2	Add requirements concerning stochastic effects.	Rejected but noted. Stochastic effects result from the amplification of small amounts of DNA. Therefore sufficient data to address the issues of stochastic effects and the interpretation of DNA profile data likely impacted by stochastic effects should be obtained under requirement 4.2 of Annex B if the laboratory interprets any profiles generated from small amounts of DNA. Verification of the robustness of the laboratory protocol to address stochastic effects is mandatory under requirement 4.4 of Annex B.
34	4.4, 4.4.2	T	Too much latitude/not enough guidance is provided in how a laboratory is to determine what an acceptable range of variability is during the verification process	Include requirements about minimum tests a lab must conduct in determining what an acceptable range of variation is	Noted with modification. Please see response to #1 above. Discussed with submitter to resolve comment. Resolution was to add additional clarification to Annex B requiring inconclusive determinations to also be consistent. This is a clarification, not a technical change.

35	4.2/4.4	T	Touch samples need to be explicitly required during the validation/verification process as complex touch mixtures are among the most challenging for labs to interpret	Add requriement for touch mixtures	Reject. Laboratories choosing to test handled items certainly have the option to include “touch DNA” samples in their validation studies; however, since other options may be suitable for validating protocols for interpreting profiles resulting from these types of samples, it is not appropriate to make this a specific requirement at this time. Any laboratory testing these types of samples would need to have appropriate validation studies to support its interpretation protocol and provide verification of the protocol.
36	Annex B, 4.2	T	The requirement for validation in high-shared allele and low-shared allele situations as expressed with no guidance for what constitutes high or low. The requirement for low-sharing of alleles seems pointless as this does not cause issues.	High should be defined.	Accept with modification. The requirement to use mixtures with “high vs. low allele sharing” of contributors is intended to ensure that the laboratory collects and reviews data generated from a variety of mixtures, and not, for example, to specifically construct “easy” mixtures with limited allele shares (or other "easy" situations) for their validation and verification studies. The word "low" was deleted and an example of a familial relationship was provided. This is a clarification, not a technical change.