

APPENDIX B

NDIS Standards for Acceptance of DNA Data

National DNA Index System (NDIS)

NDIS STANDARDS FOR ACCEPTANCE OF DNA DATA

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NDIS STANDARDS FOR ACCEPTANCE OF DNA DATA

Purpose

The concept for utilizing DNA profiles for forensic analysis was proposed by the Technical Working Group for DNA Analysis (TWGDAM), as described by Kirby (1990)¹. The Federal Bureau of Investigation Laboratory initiated development of the Combined DNA Index System, (CODIS), which contains separate files or indexes of DNA profile information. The main files in use at the local and state level are forensic and convicted offender. The DNA profiles in CODIS are used for law enforcement purposes only, and access is limited to criminal justice agencies performing DNA analysis (DNA Identification Act of 1994, 42 U.S.C. §14132). CODIS facilitates comparisons of DNA records to generate investigative leads. CODIS also provides functionality for use in assessing the statistical significance of a forensic DNA match.

The National DNA Index System (NDIS) is intended to be a single central repository of DNA records. These DNA records will be locally generated by NDIS participating laboratories in the United States. The centralized repository of DNA records will be used to generate investigative leads. System-wide standards have been established thereby ensuring that only reliable and compatible DNA profiles are contained in the NDIS files.

This document provides the NDIS standards for acceptance of DNA profiles. This version governs DNA data generated by Restriction Fragment Length Polymorphism (RFLP) and for Polymerase Chain Reaction (PCR) based methods.

CHANGES IN THE NDIS STANDARDS FOR ACCEPTANCE OF DNA DATA

From time to time, changes to the NDIS STANDARDS FOR ACCEPTANCE OF DNA DATA (NDIS STANDARDS), may be issued. Changes to the NDIS STANDARDS are to be posted on the FBI Web page (fbi.gov). These changes shall be promptly instituted by NDIS participating laboratories upon notification of the changes. Any laboratory recommending a change to the NDIS STANDARDS shall contact the NDIS Custodian, in writing. This communication should include the name of a contact person and telephone number, as well as a description of the proposed change and the reasons supporting the need for such a change. After review of such request, the NDIS Custodian shall notify the NDIS participating laboratory of his/her determination.

NDIS shall accept a DNA profile after it is determined to be compliant with the NDIS STANDARDS in effect at the time the DNA profile was derived or compliant with the standards that are in place at the time the DNA profile is offered. For example, a "new" molecular weight size marker may be added to the list of acceptable molecular weight size markers. Any DNA profiles offered but previously rejected solely as a result of the use of the previously unrecognized molecular weight size marker shall be accepted after the NDIS STANDARDS are revised to include the "new" molecular weight size marker.

¹The Combined DNA Index System (CODIS): A Theoretical Model, Appendix II, from DNA Fingerprinting, An Introduction, Kirby, L. T., 1990, Stockton Press, New York.

Laboratory Procedures and Practices

All DNA profiles offered to NDIS by NDIS participating laboratories shall be produced in accordance with the Quality Assurance Standards, as required in the DNA Identification Act of 1994. The Quality Assurance Standards for Forensic DNA Testing Laboratories were approved by the Director of the FBI and became effective October 1, 1998. The Quality Assurance Standards for Convicted Offender DNA Databasing Laboratories, also approved by the Director of the FBI, became effective April 1, 1999. These Quality Assurance Standards supersede the quality assurance guide lines adopted by TWGDAM, entitled "Guidelines for a Quality Assurance Program for DNA Analysis"² (TWGDAM Guidelines).

Restriction Fragment Length Polymorphism Section

Protocol for RFLP

1. The laboratory shall demonstrate that it continues to use a protocol that produces NDIS-compatible DNA results by analysis of the K562 human DNA control (American Type Culture Center, [ATCC], registered cell line). The K562 human DNA control shall be run on every RFLP electrophoretic analytical gel that exhibits a DNA profile offered to NDIS. The protocol is acceptable as long as the K562 human DNA control measurements are routinely within NDIS tolerances.
2. The restriction enzyme shall be *Hae III*.
3. Only DNA profiles derived by applying DNA probes to loci listed on the "List of NDIS Accepted Loci" shall be accepted by NDIS.
4. Derivation of base pair values shall be obtained using computer software approved by the Federal Bureau of Investigation.

Changes to the RFLP Protocols

1. A laboratory that changes its protocol shall not use the modified protocol in the analysis of specimens that are intended for submission to NDIS until the laboratory demonstrates that the modified protocol produces NDIS-compatible results.
2. The use of a protocol that does not achieve K562 human DNA control measurements within NDIS established tolerances shall be discontinued.
3. At the request of NDIS, a laboratory shall demonstrate the reliability of data generated by the proposed protocol.

²Guidelines for a Quality Assurance Program for DNA RFLP Analysis, Crime Laboratory Digest, April-July 1989, Vol. 16 (2), pp. 40-59; Guidelines for a Quality Assurance Program for DNA Analysis, Crime Laboratory Digest, April 1991, Vol 18 (2), pp. 44-75; and Guidelines for a Quality Assurance Program for DNA Analysis, Crime Laboratory Digest, April 1995, Vol. 22 (2), pp. 21-43.

Molecular Weight Size Marker (MWSM)

1. An MWSM from the list of acceptable MWSMs shall be run on each gel that exhibits a DNA profile that is submitted to NDIS.
2. All MWSMs, specimens and K562 human DNA control(s) shall be of sufficient clarity and intensity within the relevant measurement area of the gel so that meaningful measurements can be made.
3. No more than five (5) lanes shall be between any two MWSMs.
4. The MWSM lanes shall contain only MWSM.
5. The addition of a "new MWSM" to the list of acceptable MWSMs shall be made by NDIS only after data presented to NDIS demonstrates that the "new MWSM" shall generate NDIS-compatible results.

K562 Human DNA Control

1. The K562 human DNA control shall be on each analytical electrophoretic gel that exhibits a DNA profile submitted to NDIS.
2. Each NDIS subscribing laboratory shall request approval, in writing, from the NDIS Custodian, for established K562 human DNA control tolerances to be used by the NDIS subscribing laboratory. Once approved by the NDIS Custodian, such K562 human DNA control tolerances shall be accepted by NDIS.
3. K562 human DNA control measurements submitted to NDIS shall be within each subscribing laboratory's approved K562 human DNA control tolerances. K562 human DNA control measurements outside acceptable tolerances shall result in the rejection of all associated DNA profiles submitted from that analytical electrophoretic gel, at that locus.
4. Any NDIS subscribing laboratories seeking to change established tolerances shall request of the NDIS Custodian, in writing, approval of the new tolerances for associated DNA profiles to be accepted by NDIS, and the reason(s) for seeking to change established tolerances.
5. Any human DNA controls other than K562 included in a DNA analysis shall not be evaluated by NDIS (except as may be described elsewhere in this document). All sized K562 human DNA control measurements shall be evaluated before DNA results from any specimens are accepted by NDIS (for either use or inclusion in NDIS files).

6. As per Table 1, the NDIS Custodian shall calculate and record K562 human DNA controls for quality assurance as defined according to the following function:

$$\frac{\left(\frac{X-\bar{X}}{SD_x}\right)^2 + \left(\frac{Y-\bar{Y}}{SD_y}\right)^2 - 2R\left(\frac{X-\bar{X}}{SD_x}\right)\left(\frac{Y-\bar{Y}}{SD_y}\right)}{(1-R^2)} \leq K_{1-\alpha}$$

where: X, Y

Measured

band size of alleles 1 and 2.

\bar{X} , \bar{Y}

Expected interlaboratory band size of alleles 1 and 2

SD_x , SD_y

Expected interlaboratory reproducibility SD of alleles 1 and 2

R

Expected intralaboratory correlation between allele 1 and 2 measurements

$K_{1-\alpha}$

Constant for coverage of 100(1- α)% of a bivariate normal distribution

Table 1						
Locus	\bar{X}	SD_x^c	\bar{Y}	SD_y^c	R ^d	$K_{0.99}^e$
D1S7	4571 ^a	34	4231 ^a	31	0.62	9.21
D2S44	2907 ^a	21	1791 ^a	14	0.62	9.21
D4S139	6474 ^a	58	3438 ^a	24	0.62	9.21
D5S110	3714 ^b	26	2942 ^b	21	0.62	9.21
D10S28	1757 ^a	14	1182 ^a	12	0.62	9.21
D17S79	1982 ^a	15	1520 ^a	13	0.62	9.21

^aCertified allele band size as stated in the National Institute of Standards and Technology Certificate of Analysis for Standard Reference Material 2390 "DNA Profiling Standard", available from Standard Reference Materials Program, NIST, Gaithersburg, MD 20899 (1992). NIST will update this periodically.

^bMedian of data from 10 laboratories, compiled by Brian Hoey of the Missouri State Highway Patrol.

^cPredicted standard deviation for the band sizes, using equation:

$$SD = 7.5(1+bp/19500)^{7.1}$$

of: A.M. Stolorow; D.L. Duewer; Dennis J. Reeder; E. Buel; G. Herrin, Jr.; Interlaboratory Comparison of Autoradiographic DNA Profiling Measurements. 3. Repeatability and Reproducibility of Restriction Fragment Length Polymorphism Band Sizing, Particularly Bands of Molecular Size >10k Base Pairs. Analytical Chemistry 1996: 68(11), 1941-1947.

^dEmpirically determined for each locus using data supplied by numerous city, county, state, or Federal forensic laboratories. Correlations were determined for each laboratory supplying data (between 16 and 26 unique data sets, depending on locus). The median correlation at each locus was found to be 0.62±0.04.

^e $K_{0.99} = \chi^{-1}(.01,2) = 9.21$. The inverse one-tailed (1-0.99) probability of the chi-squared distribution with two degrees of freedom is the limiting (infinite data) critical K for 99% coverage of a bivariate normal distribution.

Monomorphic Human DNA Controls

Monomorphic probes shall not be used concurrently with a probe for any locus in the table of RFLP Loci Accepted at NDIS.

Interpretation of DNA Profiles

1. DNA profiles submitted to NDIS shall be interpretable (interpretable - any DNA data that could be used to make an exclusion).
2. A laboratory submitting a DNA profile to NDIS that is derived from forensic evidence, shall only offer those bands that are attributed to the putative perpetrator(s). Alleles derived from forensic profiles that are unambiguously attributed to a victim or individuals other than the perpetrator(s), such as, but not limited to a husband or boyfriend, shall not be offered to NDIS.
3. The DNA results from any locus in which an ambiguity exists in the assignment of one or more alleles to the putative perpetrator(s) may be offered to NDIS. The mere observation of alleles that may be attributed to individuals other than the putative perpetrator, does not in itself, preclude offering DNA profiles to NDIS at that locus.
4. After image analysis, no "correction factors" that alter or adjust the readings derived directly from an image analysis workstation shall be applied to the DNA profile offered to NDIS.

RFLP Loci Accepted and Minimum RFLP Loci for a DNA Profile to be Accepted at NDIS

The inclusion of DNA profiles in NDIS derived from convicted offender, forensic samples, unidentified human remains, and population samples requires conclusive fragment size determinations from certain specific loci. There is a minimum number of loci from which conclusive results are required for profiles submitted to the forensic, unidentified human remains and convicted offender indexes. Additional loci on these samples shall then be accepted. DNA profiles which fail to include these loci (number and name) shall not be accepted by NDIS.

Table 2 constitutes all RFLP loci from which results shall be accepted by NDIS. The absence of any particular locus from this table does not suggest the unsuitability of the locus for forensic application. The addition of new RFLP loci shall be accepted by NDIS, upon approval by the NDIS Custodian.

Table 2 RFLP Loci Accepted at NDIS					
Locus	Probe	Convicted Offender ¹	Forensic ²	Unidentified Human Remains ²	Population
D1S7	MS1	Accepted	Accepted	Accepted	Accepted
D2S44	YNH24	Required	Required	Required	Accepted
D4S139	PH30	Required	Required	Required	Accepted
D5S110	LH1	Required	Required	Required	Accepted
D10S28	TBQ7	Required	Required	Required	Accepted
D14S13	CMM10 1	Accepted	Accepted	Accepted	Accepted
D16S85	3'HVR	Accepted	Accepted	Accepted	Accepted
D17S26	EFD52	Accepted	Accepted	Accepted	Accepted
D17S79	V1	Accepted	Accepted	Accepted	Accepted

Any of these RFLP loci so indicated shall be accepted at NDIS.

¹The number required to be a complete profile for Convicted Offender is the required 4.

²An analysis of all 4 required loci must be attempted for both Forensic and Unidentified Human Remains. The minimum number of RFLP loci required for search purposes is 3 for Forensic and Unidentified Human Remains.

Application of probes

Alleles detected following the hybridization of a membrane shall be unambiguously ascribed to a single locus. Therefore, only one locus may be probed during the hybridization of a membrane. The mixing of probes to more than one locus for concurrent application to a single membrane is prohibited.

Molecular Weight Size Markers (MWSMs) Accepted by NDIS for RFLP Loci

The following MWSMs shall be accepted at NDIS:

1. Life Technologies BRL, DNA Analysis Marker System
2. Lifecodes, 23 kb sizing standard
3. Promega Genetic Analysis Marker Ladder

Polymerase Chain Reaction (PCR) Section

Protocol for PCR

PCR DNA Controls, allelic ladders and primer sets that were validated together shall be used together.

1. The laboratory shall demonstrate that it continues to use a protocol that produces NDIS compatible DNA results by its application of a positive PCR DNA Control that has been appropriately validated.
2. All DNA profiles offered to NDIS must be associated with an accurate result for PCR DNA Controls.
3. Only DNA profiles derived from analysis of NDIS Accepted PCR Kits (Table 3) shall be accepted at NDIS.

Changes to PCR Based Protocols (Per the FBI Quality Assurance Standards, page 2)

1. Any significant changes made to a protocol must be demonstrated to be non-detrimental to the PCR results, as indicated by appropriate PCR DNA Control results.
2. The use of a protocol that does not achieve the correct results for the PCR DNA Controls shall be discontinued.
3. At the request of NDIS, a laboratory shall demonstrate the reliability of data generated by the proposed protocol.

Allelic Ladders

1. The allelic ladders used must be from the list of NDIS Accepted PCR Kits (Table 3).
2. The allelic ladders used for each locus must give NDIS compatible results, as demonstrated by the PCR DNA Controls.
3. At each locus, the allelic ladder should have the commonly occurring alleles of the repeat element.
4. An NDIS Accepted Allelic ladder must be associated with each sample set.

Interpretation of DNA Profiles

1. DNA profiles submitted to NDIS shall be interpretable (interpretable - any DNA data that could be used to make an exclusion).
2. A laboratory submitting a DNA profile to NDIS that is derived from forensic evidence, shall only offer those alleles that are attributed to the putative perpetrator(s). Alleles derived from forensic profiles that are unambiguously attributed to a victim or individuals other than the perpetrator(s), such as, but not limited to a husband or boyfriend, shall not be offered to NDIS.
3. The DNA results from any locus in which an ambiguity exists in the assignment of one or more alleles to the putative perpetrator(s) may be offered to NDIS. The mere observation of alleles that may be attributed to individuals other than the putative perpetrator, does not in itself, preclude offering DNA profiles to NDIS at that locus.

NDIS Accepted PCR Kits

1. The following table (Table 3) provides the PCR Kits accepted by NDIS.
2. The absence of a PCR Kit from Table 3 does not suggest the unsuitability of that particular PCR Kit for forensic application.
3. The addition of a PCR Kit to Table 3 (NDIS Accepted PCR Kits) or modification of an existing PCR Kit, shall be made only after data are presented to NDIS, that demonstrates that the new PCR Kit generates NDIS compatible results, or the modification is justified.

Table 3 - NDIS Accepted PCR Kits	
Manufacturer	Kit Name
Promega	GenePrint PowerPlex 1.1(Catalog numbers DC6091/6090)
Promega	GenePrint PowerPlex 1.2 (Catalog numbers DC 6101/6100)
Promega	GenePrint PowerPlex 2.1 (Catalog numbers DC 6471/6470)
PE Applied Systems	AmpF/STR Profiler Plus (PIN 4303326)
PE Applied Systems	AmpF/STR Cofiler (PIN 4305246)
PE Applied Systems	AmpF/STR Profiler Plus and AmpF/STR Cofiler (PIN 4305979)
Promega Monoplex*	Monoplex D5S818 (Catalog number DC6161)
Promega Monoplex*	Monoplex D7S820 (Catalog number DC6141)
Promega Monoplex*	Monoplex D13S317 (Catalog number DC6151)
Promega Monoplex*	Monoplex D16S539 (Catalog number DC6131)
Promega Monoplex*	Monoplex TH01 (Catalog number DC5081)
Promega Monoplex*	Monoplex TPOX (Catalog number DC5111)
Promega Monoplex*	Monoplex CSF1PO (Catalog number DC5091)
Promega Monoplex*	Monoplex vWA (Catalog number DC5141)

* Monoplexes are all fluorescence-labeled and have same chemistry as when in multiplex kits

PCR Profiles Offered to NDIS

1. The DNA result from each locus will be in the form p,q for heterozygotes (in ascending order) and p,p for homozygotes.
2. Alleles below or above the allelic ladder are entered as < (lowest allele) or > (highest allele), respectively.

PCR Loci Accepted and Minimum PCR Loci for a DNA Profile to be Accepted at NDIS

The inclusion of DNA PCR profiles in NDIS derived from convicted offender, forensic samples, unidentified human remains and population samples require conclusive results from a minimum number of specific loci/systems. DNA profiles which fail to include these loci, at a minimum, shall not be accepted by NDIS. There is a minimum number of loci from which conclusive results are required for profiles submitted to the forensic, unidentified human remains and convicted offender indexes. Additional loci on these samples shall then be accepted. DNA profiles which fail to include these loci (number and name) shall not be accepted by NDIS.

Table 4 constitutes all PCR loci from which results shall be accepted by NDIS. The absence of any particular locus from this table does not suggest the unsuitability of the locus for forensic application. The addition of new PCR loci shall be accepted by NDIS, upon approval by the NDIS Custodian.

Table 4 PCR Loci Accepted at NDIS					
Locus	Chromosome Location	Convicted Offender ¹	Forensic ²	Unidentified Human Remains ²	Population
CSF1PO	5q33.3-34	Required	Required	Required	Accepted
FGA	4q28	Required	Required	Required	Accepted
THO1	11p15.5	Required	Required	Required	Accepted
TPOX	2p23-2pter	Required	Required	Required	Accepted
VWA	12p12-pter	Required	Required	Required	Accepted
D3S1358	3p	Required	Required	Required	Accepted
D5S818	5q21-31	Required	Required	Required	Accepted
D7S820	7q	Required	Required	Required	Accepted
D8S1179	8	Required	Required	Required	Accepted
D13S317	13q22-31	Required	Required	Required	Accepted
D16S539	16q24-qter	Required	Required	Required	Accepted
D18S51	18q1.3	Required	Required	Required	Accepted
D21S11	21	Required	Required	Required	Accepted
Amlogenin	X:p22.1-22.3 Y:p11.2	Accepted	Accepted	Accepted	Accepted

Any of these PCR loci so indicated shall be accepted at NDIS.

¹The number required to be a complete profile for Convicted Offender is the required 13.

²An analysis of all 13 required loci must be attempted for both Forensic and Unidentified Human Remains. The minimum number of PCR loci required for search purposes is 10 for Forensic and Unidentified Human Remains.

Appendix

Waivers - General Information

NDIS shall conditionally accept DNA results obtained prior to the “Guidelines for a Quality Assurance Program for DNA Analysis” (TWGDAM Guidelines first published in 1989, footnote page 2), the effective date of the NDIS STANDARDS. The Quality Assurance Standards for Forensic DNA Testing Laboratories (effective October 1, 1998), and The Quality Assurance Standards for Convicted Offender DNA Databasing Laboratories (effective April 1, 1999), supersede the quality assurance guide lines adopted by TWGDAM. Waivers shall not be granted to DNA records derived after the issuance of NDIS STANDARDS, except as noted herein.

A waiver granted shall remain in effect until NDIS Custodian issues superseding NDIS STANDARDS, at which time previously granted waivers may be renewed upon approval by the NDIS Custodian.

Provisions Subject to NDIS RFLP Waivers

Applications for waivers to the sections from the NDIS STANDARDS relative to RFLP data listed previously may be submitted to the NDIS Custodian. The application shall specify the DNA results that are to be covered by the waiver. No other waivers shall be granted. Granting of a waiver is at the sole discretion of the NDIS Custodian.

Waiver - DNA Records Derived Prior to April, 1989

Waivers may be granted for those DNA records that were derived prior to the issuance of the TWGDAM Guidelines in April, 1989, at the discretion of the NDIS Custodian. The laboratory must demonstrate that the qualified DNA records were derived in a manner largely consistent with the TWGDAM Guidelines. The certification shall be signed and dated (date signed) by a DNA Supervisor, an individual who is administratively responsible for the DNA analysis work of laboratory personnel.

Waiver - Alternative Image Analysis Workstation (IAW) System

Data demonstrating that an IAW system other than that developed by the FBI (alternative IAW) produces reliable and NDIS compatible DNA records is required. Also, a test plan and data demonstrating the conversion of the electronic format of the DNA records to a NDIS compatible format are required. The electronic conversion of DNA records to a NDIS data compatible format must be demonstrated to retain the integrity of the DNA record through the conversion process.

DNA profiles derived using an alternative IAW software/work station shall only be accepted by NDIS after the alternative IAW has been demonstrated to meet all NDIS performance standards, including reliability, compatibility, and data integrity.

Waiver - RFLP Human DNA control

All analytical electrophoretic gels exhibiting DNA profiles for use by or inclusion in NDIS shall also exhibit a human DNA control. Human DNA controls other than K562 (alternative human DNA control) shall only be accepted when sufficient data are presented to determine acceptable values for the alternate human DNA control. The waiver shall only apply to analyses conducted prior to 90 days after the effective date of the NDIS STANDARDS.

Waiver for Minimum Loci Constituting a DNA Profile Accepted by NDIS

NDIS shall accept any locus listed as “NDIS Accepted Loci” for “convicted offender,” “forensic,” “unidentified human remains,” and “population” classes of specimens, where results are available for the specified minimum number of loci. Thus, NDIS shall accept any combination of loci for the “population” class of specimen and any combination of accepted loci beyond the required loci for the “convicted offender,” “forensic” or “unidentified human remains” classes of specimens; where these locus combinations are defined from among the “Loci Accepted at NDIS”: Pages 6 (Table 2) and 9 (Table 4).

Application for a Waiver

States intending to make application for a waiver of NDIS STANDARDS should write the NDIS Custodian for details.

Application for Acceptance of New Loci by NDIS

Applications for new loci to the NDIS STANDARDS may be submitted to the NDIS Custodian by a criminal justice agency. The addition of new loci to NDIS STANDARDS shall be made by the NDIS Custodian only after data presented to NDIS demonstrates that the new loci have been appropriately validated including forensic and population studies, and provide NDIS comparable results. The NDIS Custodian may request further validation by additional criminal justice agencies.

Correspondence

Any correspondence regarding NDIS STANDARDS FOR ACCEPTANCE OF DNA DATA should be sent to:

Attention: NDIS Custodian
Forensic Science Systems Unit
FBI Laboratory
935 Pennsylvania Avenue, Northwest, Room GRB-3R
Washington, DC 20535-0001

Revision History		
Date	Author	Comments
11 November 1996	Barry Brown	Revised per TWGDAM*
12 June 1998	Barry Brown	Revised per TWGDAM*
4 January 1999	Barry Brown	Revised per SWGDAM*
12 July 1999	Barry Brown	Reviewed at SWGDAM*
11 January 2000	Barry Brown	Revised per SWGDAM*

*The TWGDAM CODIS Subcommittee, later SWGDAM CODIS Subcommittee, reviews and makes revision suggestions on a regular basis.