

NIST UPDATE

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Reference Materials for Genetic Testing**

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NIST participation in Huntington's Disease Validation Study

- **Sponsored by: CDC**
- **Purpose: To validate potential reference materials for use in HD testing**
- **Method: DNA sequencing**

Coriell Sample Number	10 Clinical Laboratories				NIST	Clinical Interpretation
	Mean	Mean	Mode	Mode	Mean	Allele 1/Allele 2
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1 Allele 2	
NA20245	15	15	15	15	15/15	normal/normal
NA20206	17	18	17	18	17/18	normal/normal
NA20207	19	21	19	21	19/21	normal/normal
NA20246	15	24	15	24	15/24	normal/normal
NA20247	15	29	15	29	15/29	normal/mutable normal
NA20248	17	36	17	36	17/36	normal/reduced penetrance
NA20249	22	39	22	39	22/39	normal/reduced penetrance
NA20250	15	40	15	40	15/40	normal/full penetrance
NA20208	35	45	35	45	35/45	mutable normal/full penetrance
NA20209	45	47	45	47	45/46	full penetrance/full penetrance
NA20251	39	50	39	50	39/50	reduced penetrance/full penetrance
NA20252	22	66	22	65 66	22/65	normal/full penetrance
NA20210	17	74	17	74	17/75	normal/full penetrance
NA20253	22	99	22	100	22/101	normal/full penetrance

Conclusion

- **Good agreement between the CAG repeat sizes obtained by the clinical laboratories and the NIST DNA sequence analysis.**

Participation in Fragile X Validation Study

- **Sponsored by: CDC/AMP**
- **Purpose: To achieve consensus validation on CGG repeat sizes in selected cell lines.**
- **SRM 2399 was used for assay validation of the laboratories in-house protocols and the common platform.**
- **Method: DNA sequencing**

Comparison of Male *FMR1* Allele Lengths Based on Consensus and Sequencing

Experimental	Cell Line	Allele Length Based on Consensus	Allele Length Based on Multiple Sequencing Assays	Quality score
	NA20244	41	41, 41, 41	56.4
	NA20232	46	46, 46, 46	56.6
	NA20230	53	54, 54, 54	52.9
	NA20231	76	78, 78, 78	45.9
	NA20233	117	>100, >100	50.1
Control				
	NA07174	30	30, 30, 30	51.7
	CD00014	56	56, 56, 56	45.6
	NA06892	86	88/89 88/89, 88/89	41.7

Conclusion

- **DNA sequencing confirms that there are available cell lines which are representative of clinically important diagnostic cutoffs.**
- **Benchmark sizes as determined by NIST sequencing were reproduced by in-house PCR-based methods in most of the clinical labs.**

Development of a SRM for Huntington's Disease

- 10 samples were selected which represent the clinically important diagnostic cutoffs.
- PCR amplification and DNA sequencing were used to confirm sizes.
- Final SRM will consist of PCR products representing a variety of CAG repeat lengths.

Future

■ Current SRM

- Fragile X Human DNA Triplet Standard (2399)

■ Future SRM's under development

- Huntington's Disease
- RNA controls for microarrays
- Pure viral DNA for use in CMV assays
- Corn DNA

<http://ts.nist.gov/MeasurementServices/ReferenceMaterials/232.cfm>



Questions?