Huntington disease (HD) is a neurodegenerative disease of middle onset that produces choreic movements and cognitive decline, often accompanied by psychiatric changes that affect approximately 1 in 10,000 individuals [1]. Inheritance is autosomal dominant with clinical manifestations associated with expansion of a polymorphic trinucleotide CAG repeat. Samples containing <27 CAG repeats are classified as normal samples spanning the range of CAG repeats useful in diagnosing HD. This SRM 2393 will be helpful to clinical diagnostic laboratories wanting to ensure the accuracy and comparability of their testing results to other testing laboratories and those wishing to validate their CAG repeat sizing methods.

DNA samples were obtained from Coriell Cell Repositories representing the following Huntington alleles: 15, 17, 19, 20, 35, 36, 41, 45, 50, and 74. These samples were amplified with a variety of PCR conditions and DNA polymerases to search for optimal conditions for reducing stutter product formation and trying to improve heterozygote peak height balance, particularly for alleles that exhibited extreme differences in size (e.g., 17, 74).

**Huntington Disease Classification**

From American College of Medical Genetics (ACMG) Standards and Guidelines for Clinical Genetics Laboratories [1]

<table>
<thead>
<tr>
<th>Language</th>
<th>Repeat Size</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt;27</td>
<td>Unaffected</td>
</tr>
<tr>
<td>Intermediate</td>
<td>27–35</td>
<td>Affected</td>
</tr>
<tr>
<td>Full Penetrance</td>
<td>36+</td>
<td>Affected</td>
</tr>
</tbody>
</table>

**SMR Certification Requirements**

NIST SRM 2393 will include 6 components which are Huntington Disease cell lines Coriell maintains, previously examined by the CDC's Gen-Res participants in an interlaboratory comparison.

To meet the NIST SMR certification requirements, materials obtained from Coriell have been and continue to be evaluated to be a certified value at NIST.

**Certification Value**

- Highest confidence in data accuracy and all known sources of bias have been investigated.
- To obtain a NIST certified value:
  - Certification using a single primary method with confirmation by other methods
  - Certificate Status using two independently-evaluated methods
  - DNA Sequencing and (2) Allele sizing using internal size standard compared to sizes of one or more sequenced alleles

**Certificate/Value Assignment Using one Method at NIST**

Coriell Samples

<table>
<thead>
<tr>
<th>Allele</th>
<th>Big Dye repeat</th>
<th>15/2029</th>
<th>15/2929</th>
<th>15/2030</th>
<th>15/4030</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>242.99 bp</td>
<td>70</td>
<td>71</td>
<td>72</td>
<td>73</td>
</tr>
<tr>
<td>30</td>
<td>254.38 bp</td>
<td>74</td>
<td>75</td>
<td>76</td>
<td>77</td>
</tr>
</tbody>
</table>

**Stutter products**

- ±1 repeat for alleles 27–35
- ±4 repeats for alleles >75

**Certification/Value Assignment Using one Method at NIST**

Coriell Samples

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**Accuracy genotyping (CAG) repeat number assignment** of the Huntington Disease SRM 2393 components involves more than base pair sizing of the PCR amplicons. While capillary electrophoresis base pair sizing has proven to be very reproducible when assay conditions are standardized, there is still the need to underpin these results with allele sequencing results. The graph of "Huntington's CAG repeat on set" shown on the right is from accumulated genotyping data from various steps in the sequencing process (see Materials and Methods section for conditions). Sequencing results, simply counting the number of (CAG) repeats present in a sample, can then be compared to the base pair sizing results.

**American College of Medical Genetics Guidelines for Huntington Disease Testing**

The ACMG Biochemical and Molecular Genetic Resource Committee recommends Huntington Disease alleles are sized with the following following CAG repeat ranges:

- ≥1 repeat for alleles 43
- ≥2 repeats for alleles between 44 and 50
- ≥3 repeats for alleles between 51 and 75
- ≥4 repeats for alleles >75

**Gel bands cut, TE-4**

**PCR: 20 cycles, 68°C anneal**

**Resolution**

- Nominal values for candidate materials are corroborated by interlaboratory comparison involving independent typing and sequencing of samples.

**Accurate genotyping (CAG) repeat number assignment** of the Huntington Disease SRM 2393 components involves more than base pair sizing of the PCR amplicons. While capillary electrophoresis base pair sizing has proven to be very reproducible when assay conditions are standardized, there is still the need to underpin these results with allele sequencing results. The graph of "Huntington's CAG repeat on set" shown on the right is from accumulated genotyping data from various steps in the sequencing process (see Materials and Methods section for conditions). Sequencing results, simply counting the number of (CAG) repeats present in a sample, can then be compared to the base pair sizing results.

**Genotyping Results from Genomic DNA**

**Genotyping Results from product used for sequencing “gel cut amp”**

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