# Novel test streamlines testing for Huntington Disease

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A new test may help to streamline genetic testing for Huntington Disease (HD) by generating accurate results, avoiding unnecessary additional testing, and improving turnaround time. The test, which uses chimeric or triplet repeat primed PCR (TP PCR) methodology, yielded results that were 100% concordant with standard genotyping methods in an analysis of 246 samples. The high sensitivity and specificity of the test could reduce the number of false negative results and facilitate both diagnosis and prognosis by correctly sizing the genetic abnormality characteristic of HD.

Huntington disease (also known as Huntington's disease or Huntington's chorea) is an inherited and progressive neurodegenerative disorder that typically becomes apparent during a person's thirties or forties. With time, HD patients develop diminished muscle coordination that is evident in walking, speaking, and swallowing and undergo changes in personality and thinking ability. A mutation in the Huntingtin gene leads to an abnormal number of repeats of a sequence of three nucleotides known as CAG. Based on the number of CAG repeats, a person may be deemed to be normal (10-35 repeats), at low risk (36-39 repeats), or at high risk (greater than 40 repeats) of having or developing HD symptoms. That is why accurately determining the number of CAG repeats is so important.

In this study, 246 samples that had been previously analyzed by other methods were tested with the new method (TP PCR). The samples included 14 DNA reference samples from the Coriell Cell Repositories,

three samples from the College of American Pathologists 2002 Survey, and 229 samples from individuals tested at ARUP Laboratories for clinical purposes by standard technologies, explained lead investigator Elaine Lyon, PhD, Medical Director of Molecular Genetics, ARUP Laboratories and its Institute for Clinical and Experimental Pathology, and Department of Pathology, University of Utah, Salt Lake City, UT. Normal samples were included as well as those with a wide range of CAG repeats. The samples were blinded and analyzed.

The results showed that TP PCR correctly sized 240 of the 246 samples. All of the 100 samples in the normal and low risk groups were correctly sized. In the 146 samples of those known to be affected by HD (those with > 39 CAG repeats), the results for 140 correctly matched that found with other methods whereas the number of CAG repeats differed by ±1 in 6 samples, a difference said by the authors to be within the precision of the method at higher repeat numbers. Up to 101 CAG repeats could be accurately sized with this test. Even samples that were found to be challenging to analyze with other methods could be assessed solely and accurately by TP PCR.

Another advantage of this new method is its ability to identify true homozygous normal samples, thus avoiding further testing. With other methodologies, if a sample appears homozygous for the normal allele, additional testing, often with Southern blot analysis, is still recommended because of the risk of false negatives. "Southern blotting is expensive, labor intensive, requires high concentrations of DNA, and can delay turnaround time," says Dr. Lyon. However, when HD is suspected in children, Dr. Lyon and colleagues recommend that even with TP PCR, apparently homozygous samples should undergo further testing.

TP PCR uses a forward and reverse chimeric primer to amplify from multiple priming sites within the trinucleotide repeat. TP PCR produces

a characteristic ladder on a fluorescence electropherogram that allows the rapid and inexpensive identification and quantification of expanded repeats. Major peaks and minor peaks (stutters) representing CAG repeats can be analyzed and sized automatically using commercially available software.

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