Frequently Asked Question: How does the replacement of Southern blot analysis with tp-PCR in testing for *FMR1*-related disorders, Myotonic dystrophy type I (DM1) and Friedreich ataxia impact my practice?

Summary:

In March of 2016, triplet primed (tp) PCR replaced Southern blot analysis in the testing algorithm for *FMR1*-related disorders, myotonic dystrophy type 1 (DM1), and Friedreich ataxia. This change improves patient care by decreasing turn-around-times (TATs) and reducing blood collection volumes. However, in rare cases, Southern blot analysis performed by an external laboratory will be required in order to provide additional information for patient counseling. Out-of-province MSP approval of funding is not required; but, sample recollection and completion of related out-of-province documents will be necessary, including completion of the consent to release information outside of Canada.

Detailed discussion:

***FMR1*-related disorders:**

Validation studies have been completed using the Amplidex PCR/CE *FMR1* reagents (Asuragen, Inc). The use of this tp-PCR kit replaces Southern blot analysis in the testing algorithm (see figure 1), improving patient care by decreasing turn-around-times and reducing blood collection volumes (see table 1). The Amplidex PCR/CE *FMR1* reagents do not allow assessment of the methylation status of the *FMR1* gene; however, in most cases the repeat is sized well into the full mutation range and, thus, hypermethylation can be assumed. In rare cases, a repeat collection and testing by Southern blot analysis will be recommended. Southern blot analysis will be referred out to an external laboratory located in the USA. Out-of-province MSP approval of funding is not required; but, sample recollection and completion of related out-of-province documents will be necessary, including completion of the consent to release information outside of Canada.

Figure 1: *FMR1*-related disorders test algorithm.

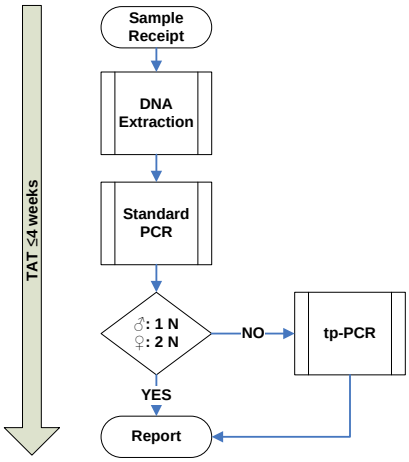

Table 1: Comparison of tp-PCR and Southern blot analysis TAT and sample requirements.

		tp-PCR	Southern blot
TAT	Routine	≤4 weeks	≤8 weeks
	Prenatal	≤3 weeks	≤3 weeks
Sample requirements	Routine	1 ml EDTA blood	4 ml EDTA blood
	Prenatal	25 ml Amniotic fluid 20 mg CVS	25 ml Amniotic fluid 20 mg CVS

Myotonic dystrophy type I (DM1):

Validation studies have been completed using a lab-developed tp-PCR test. tp-PCR replaces Southern blot analysis in the testing algorithm (see figure 2), improving patient care by decreasing turn-around-times and reducing blood collection volumes (see table 2). This new test algorithm accurately sizes up to ~100 repeats; beyond ~100 repeats, the assay is only able to determine presence of an expansion. For prenatal diagnosis, or where the size of the expanded allele is necessary for reproductive counselling or patient management, Southern blot analysis will be required; Southern blot analysis will be referred out to an external laboratory located in the USA. Out-of-province MSP approval of funding is not required. Sample collection and completion of related out-of-province documents will be necessary, including completion of the consent to release information outside of Canada. Prenatal diagnosis must be ordered through the provincial Medical Genetics program, to ensure appropriate case management. For ordering physicians who wish to request Southern blot analysis for patient management, please contact the laboratory to discuss further.

Figure 2: DM1 test algorithm

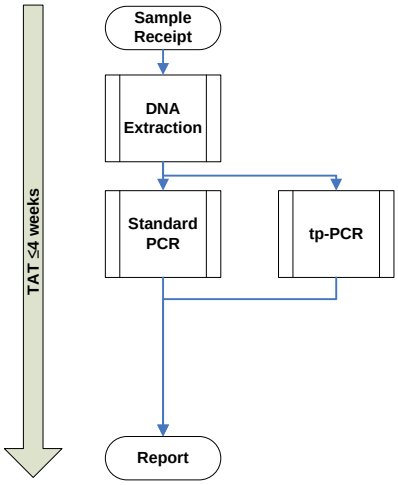

Table 2: Comparison of tp-PCR and Southern blot analysis TAT and sample requirements.

		tp-PCR	Southern blot
TAT	Routine	≤4 weeks	≤6 weeks
	Prenatal	≤3 weeks	≤3 weeks
Sample requirements	Routine	1 ml EDTA blood	4 ml EDTA blood
	Prenatal	25 ml Amniotic fluid 20 mg CVS	25 ml Amniotic fluid 20 mg CVS

Friedreich Ataxia (FRDA):

Validation studies have been completed using a lab-developed tp-PCR test. tp-PCR replaces Southern blot analysis in the testing algorithm (see figure 3), improving patient care by decreasing turn-around-times and reducing blood collection volumes (see table 3). This new test algorithm allows detection individuals heterozygous and homozygous for expanded repeats. Southern blot analysis will not be required for further assessment.

Figure 3: FRDA test algorithm.

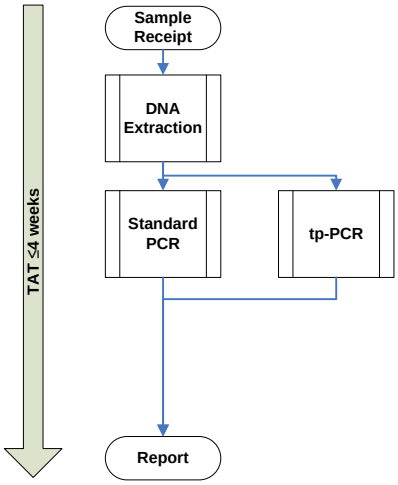

Table 2: Comparison of tp-PCR and Southern blot analysis TAT and sample requirements.

		tp-PCR	Southern blot
TAT	Routine	≤4 weeks	≤6 weeks
	Prenatal	≤3 weeks	≤3 weeks
Sample requirements	Routine	1 ml EDTA blood	4 ml EDTA blood
	Prenatal	25 ml Amniotic fluid 20 mg CVS	25 ml Amniotic fluid 20 mg CVS