Prenatal Molecular Guidelines

LHSC Molecular Diagnostic Laboratory should be notified in advance of arrival of a prenatal specimen. Referral for testing will be triaged by the Lab Director.

Prenatal Testing Eligibility:

- Gene must be licensed to be tested at LHSC
- This test request should be reserved for variants with early onset disease and high penetrance. In addition, testing should be reserved for those where a genetic diagnosis will guide management for the proband or family. It is the responsibility of the ordering provider to ensure the appropriateness of the requested testing.
- Testing request must include a copy of the familial report with a confirmed pathogenic/likely pathogenic variant tested by a CLIA or equivalent certified laboratory.
- Variants of Unknown Significance (VUS/ACMG 3) are not eligible for prenatal testing.
- mtDNA variants (m.) are not eligible for prenatal testing due to the biological and technical challenges related to low level heteroplasmy.
- In a situation where one parent is a known carrier for a recessive disorder, we require the other parent to be tested prior to fetal testing
- CF: only Southwestern Ontario catchment
- The lab must be notified to ensure we have reagents (primers) on hand to do the testing in advance of specimen collection.

Prenatal Methodology: Targeted mutation testing (Sanger sequencing / MLPA) for familial Pathogenic/Likely pathogenic variants or Agena MassArray for Cystic Fibrosis testing.

Turn-around time: 2 weeks (14 calendar days) from date received in the laboratory.

Specimen Requirements:

- Familial positive control specimens are required (2 x 4mL EDTA blood/extracted DNA).
- Prenatal sample (see table below for requirements)
- Maternal sample (2 x 4mL EDTA blood/extracted DNA if different from positive control): this is to be used for maternal cell contamination (MCC) studies.

Prenatal/Fetal Sample	Specimen Requirement
Cultured Cells (CVS/AF/Fetal tissue)	2 T25 flasks with confluent growth in RPMI or other culture media
Amniotic Fluid (AF)	**Internal referrals** - 20mL for direct DNA extraction if the pellet is sufficient and specimen is not bloody. A back-up culture is to be kept. If sample is bloody or insufficient, AF is cultured before DNA extraction **Note:** if culture is necessary results may be delayed. **External referrals** – **Referring center** may extract and ship direct AF DNA (see below for DNA requirements) but **is responsible for maintaining a backup culture.** Uncultured direct AF is not accepted
Chorionic Villi Sample (CVS)	10mg cleaned villi for direct DNA extraction (for external referrals, **cleaning and backup culture to be done by the referring center)**
Cord Blood	2x EDTA microtainer or 1x 4mL EDTA
Fetal tissue	5mm^3 (size of a pea)
Externally extracted DNA from any of the specimen types above	20ng/µl (A260 reading) or 10ng/µl if quantitated by fluorometry (Qubit) in a minimum volume of 50µl. (1µg total). **External assay for maternal cell contamination strongly preferred. Indicate "MCC completed" on the requisition and include results if possible.** **External center is responsible for maintaining a backup culture.**