**GLOSSARY**

**Language and Landscape of Molecular Diagnostics for TB Drug Resistance**

**Codon** - a combination of three consecutive bases, within a specific gene, that specifies or encodes for a particular amino acid. Specific codons also signal the beginning (i.e., start codon) or end (i.e., stop codon) of protein synthesis.

**Cross-resistance** – bacterial resistance to different antibiotics that share a similar mechanism of action. Cross-resistance may be intrinsic (e.g., impermeability of cell wall) or caused by the presence of a mutation associated with resistance. For example, an *M. tuberculosis* isolate with an A1401G mutation in the *rrs* gene will likely exhibit cross-resistance to amikacin, capreomycin, and kanamycin. All three of these second-line injectables affect protein synthesis in the bacterial cell and the A1401G mutation modifies the drug target such that these antibiotics are rendered ineffective in that particular isolate.

**Deletion** - a mutation that can occur when a single nucleotide or set of nucleotides is removed from a DNA sequence. A deletion can be small (i.e., one to few nucleotides) or large (i.e., a whole segment of the chromosome) and the effect of the deletion on viability of the organism or antibiotic resistance will depend on the location of the deletion and how the deletion affects protein synthesis.

**DNA** – deoxyribonucleic acid. DNA is a double stranded molecule that carries genetic information. Strands are held together through complementary pairing of nucleotide bases: Adenine to Thymine (A to T) and Guanine to Cytosine (G to C).

**DNA replication** - the process through which the genetic information contained within DNA is copied during bacterial replication to ensure an exact copy of the genetic material is transferred to progeny cells. However, replication is error prone and mutations can be introduced during this process.

**Ethambutol (EMB)** – a drug often used as part of the standard first-line antituberculosis regimen. EMB is bacteriostatic but is thought to minimize the risk of drug resistance development to companion first-line drugs, particularly isoniazid. Monoresistance to EMB is rare.

**Fluoroquinolones** – a member of this class of drug (e.g. moxifloxacin, levofloxacin, ofloxacin, and ciprofloxacin) is typically included in second-line treatment regimens for multidrug resistant TB disease. These drugs target DNA gyrase which is important for DNA replication. Heteroresistance may be observed with fluoroquinolones.

**Gene** - a distinct sequence of bases along a segment of DNA that provide the coded instructions for synthesizing a protein or RNA molecule.
**Drug susceptibility testing (DST)** – a laboratory test performed to characterize the susceptibility of *M. tuberculosis* to a panel of antituberculosis drugs for the purposes of informing clinical treatment of patients. DST generally involves exposing an isolate to a single concentration of drug (known as the critical concentration) to see if the organism can grow in the presence of the drug (i.e. resistant). The use of the term DST is generally used to refer to growth based testing.

**Heteroresistance** – The result of varying levels of resistance within a population of *M. tuberculosis* due to the presence of sub-populations with differing nucleotides at a loci associated with drug resistance, resulting in both drug-resistant and drug-susceptible organisms. This phenomenon is not limited to, but is commonly noted with, fluoroquinolones.

**Isolate** – an organism isolated (i.e., grown) from culture of a clinical specimen (i.e., sputum).

**Isoniazid (INH)** - used as a first-line agent in the treatment of both TB disease and TB infection. INH inhibits cell wall synthesis by interfering with production of mycolic acid, a major component of the *M. tuberculosis* cell wall.

**Laboratory developed test (LDT)** - new tests developed, evaluated, and validated within a particular laboratory that are not approved by the FDA. Often, a laboratory will choose to develop and use an LDT because a commercial test is not currently available or is not available for the specific purpose required by the laboratory.

**Limit of detection (LOD)** - the lowest amount of a substance or genetic target that can reliably be detected by an analytic method.

**Locus** – a region of interest in a gene.

**Macromolecules** – large, complex molecules that consists of multiple, linked, smaller molecules. Proteins are an example of a macromolecule made up of amino acids.

**Messenger RNA (mRNA)** – form of ribonucleic acid (i.e., RNA) produced through the process of transcription from specific segments of DNA that contain genetic information for protein synthesis. The message contained within mRNA is read in sets of three bases called codons that are used as the blueprint for incorporation of amino acids during translation in the production of proteins.

**Missense (or non-synonymous)** - mutation that occurs when the nucleotide change in the DNA results in a subsequent change in the codon such that a different amino acid is incorporated during protein synthesis.

**Molecular detection of drug resistance** – laboratory method used for examining DNA for the presence of mutations associated with drug resistance. DNA sequencing involves the identification of mutations by comparing the resulting sequence from the sample of interest to a reference (i.e., wild-type) sequence. However, methods other than DNA sequencing (e.g., real-time PCR for GeneXpert MTB/RIF) may also be used to detect the presence of mutations.

**Mutant** - an organism that contains a mutation that makes it different from its parent cell.
**Mutation** - a change in the DNA sequence that results in variation from previous generations that can be transmitted to subsequent generations. Mutations are rare and occur spontaneously. In *M. tuberculosis* mutations occur primarily through the accumulation of point mutations and not through acquisition of antibiotic resistance genes as seen with other types of bacteria.

**Non-synonymous** – see Missense

**Nucleotide bases** – also called nucleotides or simply bases, the basic building blocks of DNA and RNA. There are 4 bases in DNA adenine (A), thymine (T), cytosine (C), and guanine (G) and 4 bases in RNA, with a substitution of uracil (U) for thymine (T).

**Nonsense mutation** - a base change that results in a STOP codon and ends the process of translation prematurely during protein synthesis.

**Point mutation** - a change in a single nucleotide in the DNA sequence, an A, T, C, or G from what is commonly observed (i.e., wild type). Multiple point mutations can occur within the same locus.

**Prodrug** - drug that must be converted into an active form for effectiveness. Pyrazinamide is an example of a prodrug that must be converted by the *M. tuberculosis* pyrazinamidase for activity. Mutations in the gene coding for pyrazinamidase (*pncA*) can inhibit enzyme activity and prevent conversion of the prodrug thereby resulting in PZA resistance.

**Promoter region** - a nucleotide sequence at the beginning of a gene to which RNA polymerase must bind before the process of transcription (i.e., synthesis of mRNA) can start. Promoter regions contain important elements that control the level of gene expression. This becomes important for *M. tuberculosis* with the example of the promoter for *inhA*. Mutations in the promoter region of *inhA* can result in upregulation of the expression of InhA thereby resulting in low-level resistance to isoniazid.

**Pyrazinamide (PZA)** – drug used for TB treatment that has important sterilizing activity that shortens the duration of TB treatment when used in combination with RIF. PZA is a prodrug that is converted to its active form, pyrazinoic acid, by a pyrazinamidase encoded by *pncA* in *M. tuberculosis*.

**Resistant** – categorical result for drug susceptibility testing defined by the ability of a microorganism to grow in the presence of an antimicrobial agent to a defined level when compared to growth in the absence of drug.

**Rifampin (RIF)** - cornerstone of first-line anti-TB treatment. Rifampin acts by inhibiting transcription of mRNA and ultimately leads to cell death. Resistance to RIF is a surrogate marker for multidrug resistant TB.

**RNA** – ribonucleic acid. Exists as a single stranded molecule. There are different types of RNA including ribosomal RNA, transfer RNA, and messenger RNA (mRNA). The mRNA serves as a message to provide instructions from the DNA for protein synthesis during translation.

**Second-line injectable drugs** – typically included in second-line treatment regimens for multidrug resistant TB disease (i.e. amikacin, capreomycin, and kanamycin). These three drugs affect protein
synthesis in the bacterial cell. Mutations associated with resistance to these drugs can result in cross-resistance.

**Sequencing** - the process of determining the specific order of nucleotides within a DNA molecule.

**Silent (or synonymous)** - mutation in which the DNA sequence is changed from what is normally observed (i.e., wild-type) but the resulting amino acid sequence remains the same.

**Single nucleotide polymorphism (SNP)** - a variation in a base pair in a DNA sequence.

**Susceptible** - categorical result for drug susceptibility testing defined by the lack of growth of a microorganism in the presence of an antimicrobial agent at a given concentration.

**Synonymous** – see Silent

**Translation** - biological process whereby messenger RNA is translated for incorporation of amino acids during protein synthesis.

**Transcription** - biological process of converting the genetic information from a DNA sequence into a messenger RNA using a specific enzyme called RNA polymerase.

**Wild type** - a typical or common version of a gene.

**Xpert® MTB/RIF (Cepheid)** - a commercial real-time PCR test performed in an automated system for detecting *M. tuberculosis* complex and mutations associated with resistance to the first-line drug rifampin. The assay evaluates the rifampin resistance-determining region (RRDR) of the *rpoB* gene.