Introduction

- Tuberculosis (TB) is caused by the bacterium *Mycobacterium tuberculosis* which is primarily a pulmonary pathogen (1).
- TB is one of the top 10 causes of death worldwide and the leading cause of death from a single infectious agent (2).
- In 2018 there were 10 million people who fell ill with TB and 1.5 million TB-related deaths (2).
- Most infected individuals live in resource-limited countries (3).
- Due to the high mortality rate and resource limitations, an efficient, consistent and low-cost TB diagnostic solution is of critical importance.
- Staining slides for microscopy is cost-effective.
- With the use of the ELITechGroup Aerospray® TB Series 2 Stainer, staining can be efficient and provide consistent results.

Goals

- Investigate and determine the dye uptake kinetics of *Mycobacterium tuberculosis*.
- Improve the formulation of reagents used for staining *Mycobacterium tuberculosis*.
- Optimize the staining program for the ELITechGroup Aerospray® TB Series 2 Stainer.

Proposed Methods

**Phase 1: Dye Uptake Kinetics**
- Development of a sputum mimetic containing *M. tuberculosis* and non-acid-fast bacteria.
- Measure *M. tuberculosis* kinetic absorption of primary stain using Cytation™ 1.
- Measure dye retention after decolorization using acid alcohol wash.

**Phase 2: Improved Reagent Formulation**
- Create multiple compositions of primary stain by varying parameters such as dye concentration, pH, lipophilic agent, and buffer concentration.
- Repeat process of measuring *M. tuberculosis* kinetic absorption of primary stain using Cytation™ 1.

**Phase 3: Optimized Staining Programming**
- Use the results of the dye uptake kinetics of the most effective primary stain formulation to optimize programming for the Aerospray® TB Series 2 stainer.
- Programming parameters that will be modified include output volume as well as staining duration.

Results

- Tested various sputum mimetic matrices and found that a methycellulose based matrix most closely represented a typical sputum sample.
- Due to the high mortality rate and resource limitations, an efficient, consistent and low-cost TB diagnostic solution is of critical importance.

Conclusion

- The dye uptake analysis will provide critical information regarding the properties of the cell wall of acid-fast bacteria, specifically *Mycobacterium tuberculosis*.
- The dye uptake analysis will be important for creating improved stain reagents and optimizing the programming of the ELITechGroup Aerospray® TB Series 2 stainer.
- The outcome of improved staining would be highly beneficial, especially in resource-limited countries and could lead to millions of lives saved, aiding in the goal to eradicate TB.

References