Molecular Diagnostics - Infectious Disease Detection
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Methods
1. Pre-treat chips to improve performance
2. Mix mastermix solution in biosafety cabinet to prevent contamination of samples
3. Incubate samples at 65°C in a dry bath or thermocycler to facilitate the LAMP process
4. Dispense sample on chip for impedance testing
   • DNA vs. Non-Template Control (NTC)
   • 20°C (Not amplified) vs. 65°C (Post amplification)
5. Graph data using Matlab to analyze resistance separation of amplified DNA from all controls

Results

![Graph showing resistance signal for amplified DNA with clear separation from the DNA 20°C, NTC 20°C, and NTC 65°C and good triplicate precision.]

Figure 1: Large resistance signal for amplified DNA with clear separation from the DNA 20°C, NTC 20°C, and NTC 65°C and good triplicate precision.

![Resistance signal for amplified DNA with clear separation from the room temperature control. Good triplicate precision is observed for the high concentration of methylene blue. Lack of signal between amplified DNA and room temperature controls for the low concentration of methylene blue.]

Figure 2: Resistance signal for amplified DNA with clear separation from the room temperature control. Good triplicate precision is observed for the high concentration of methylene blue. Lack of signal between amplified DNA and room temperature controls for the low concentration of methylene blue.

Conclusions
- Manipulation of mastermix components decreased DNA amplification time to results by ~50%
- Addition of methylene blue to mastermix was found to produce repeatable resistance signals in the electrical system
- A methylene blue concentration ~40% higher than suggested by literature showed improved separation between incubated and room temperature DNA
- Current pre-treatment process was suspected to be a key variable affecting resistance signal results; addition of chemicals to solution is a potential replacement option

References

Future Work
Methylene blue
- Optimize concentration in mastermix to improve separation
- Test limits of DNA detection for optimal methylene blue concentration

Remove Pre-treatment
- Introduce additional chemicals to mastermix as pre-treatment replacement
- Removing current pre-treatment process will decrease production time and manufacturing cost

Real-time electrical detection
- Implement on-chip heating to facilitate LAMP
- Determine optimal impedance testing frequency

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Methylene Blue
Methylene blue is an intercalating, redox-active molecule. When methylene blue binds to DNA the resistance of the solution increases (conductivity drops). This boosts the signal between incubated samples with high concentrations of DNA and their respective controls.

Electrical Detection
Fluid flows over two electrodes present on-chip. A current is passed through the fluid to measure change in resistance caused by DNA amplification.