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Development of Sensitive and Fast Immunoassay for Lyme Disease

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Development of Sensitive and Fast Immunoassay for Lyme Disease

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Introduction/ Background

Lyme disease is a common bacterial infection, generally caused by the bacterium *Borrelia burgdorferi,* transferred through blacklegged ticks. After infection, symptoms may not appear immediately, taking anywhere from 3-30 days to develop. When they do appear, symptoms include the well-known bull's-eye rash as well as others including fever, headache, and fatigue. If diagnosed quickly, Lyme can be treated with antibiotics. If not, symptoms can progress to include joint stiffness, irregular heartbeat, and dizziness, among others.[1]

Testing for Lyme disease has proven to be time consuming and inaccurate, especially in its early stages.[2] The goal of this project is to develop a new test to improve on its timeliness, sensitivity, and selectivity using an enzyme-linked immunosorbent assay, or ELISA. These tests utilize the selective nature of antibodies to create a method of producing a colorimetric signal to measure the presence of the specified target (See figure 1). In this scenario, the base protein is found on the bacterium responsible for Lyme disease. From here, the target human antibody response is added to bind to the protein. Another antibody, with an enzyme in tow, is added, which in the presence of a certain substrate can produce a color change. It is this color change that allows the quantification of the immune response through absorbance.

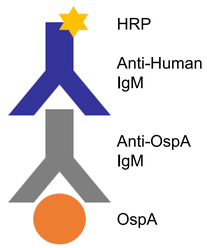

Figure 1: Visualization of Lyme ELISA.

To improve on traditional ELISAs, which are performed in 96-well plates, magnetic beads will be used.[3] Unlike a normal ELISA, these magnetic beads are the binding site of the target instead of the plate wall. With greatly increased surface area, the process can be much faster and detect far smaller concentrations of protein with greater accuracy. We expect this much faster magnetic bead ELISA to detect the disease in its earlier stages to minimize length of infection and chronic symptoms.

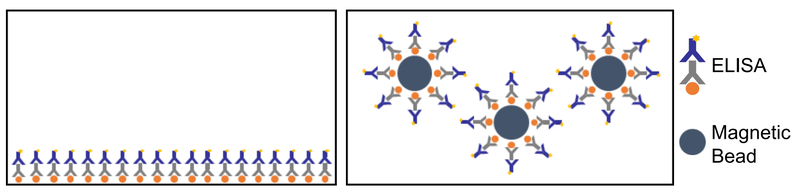

Figure 2: Regular ELISA vs. magnetic bead ELISA.

Rabbit IgG ELISA Procedure

- Purpose: Proof of Concept. Perform and optimize a magnetic bead ELISA with desirable results to then apply to the magnetic bead ELISA to Lyme Disease.
- Procedure:
  1) Add magnetic beads with conjugated capture antibody, blocking agent (Bovine Serum Albumin), and IgG to wells on 96 well plate
  2) Incubate for 15 minutes and wash 3 times with PBS with 0.1% Tween-20
  3) Add detection antibody to wells
  4) Incubate for 15 minutes under aluminum foil wash 3 times with PBS with 0.1% Tween-20
  5) Add TMB Substrate solution
  6) Incubate for 15 minutes under aluminum foil
  7) Add sulfuric acid to wells
  8) Measure absorbance at 450 nm

Rabbit IgG ELISA Optimization

- Figure 3 shows the experiments performed to select the ideal concentrations of both capture and detection antibody used in the magnetic bead ELISA.

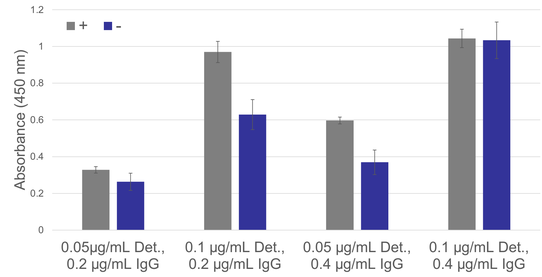

Figure 3: Absorbances at 450 nm for various concentrations of antibodies used. Each grouping of bars represents a variation of the procedure to test antibody concentrations. Error bars given by standard deviation.

- Results: 0.1 µg/mL detection antibody and 0.2 µg/mL IgG provided the highest positive while maintaining a low negative signal.
- Figure 4 shows three different washing procedures to determine which would result in the lowest negative absorbance.

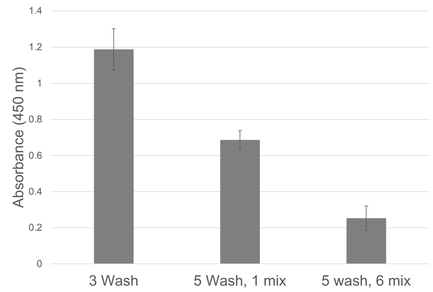

Figure 4: Average negative well absorbance over three modified procedures. Error bars given by standard deviation.

- Results: 5 washes with mixing using a micropipette tip after each, including after addition of detection antibody provides the lowest background noise with this ELISA.

Time Testing

- Figure 5 shows the results of the optimized two-step ELISA and results from a one-step ELISA, which skips steps 2 and 3 as seen on the original procedure.

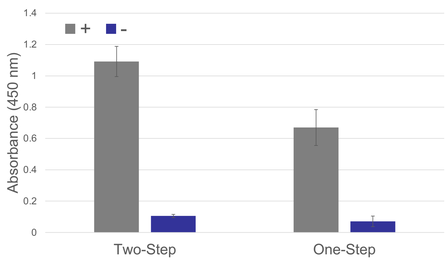

Figure 5: Average absorbances of positive and negative samples comparing original and simplified procedure. Error bars given by standard deviation.

- Results: The two-step provides a better difference between positive and negative signal, while the one-step provides lower overall signal as well as a shorter assay time. Time decreased from 120 minutes to 90.

Application to Lyme Disease

- Purpose: Use optimized magnetic bead procedure to measure the human response to Lyme disease.
- Figure 6 shows the results of an ELISA using the same one-step procedure developed through the optimization process.

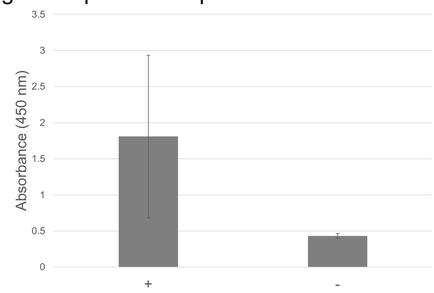

Figure 6: Average absorbances of positive and negative samples with the Anti-OpsA IgM ELISA. Error bars given by standard deviation.

- Results: When using the same procedure as with Rabbit IgG this one-step procedure gave a greater difference between positive and negative absorbances with a significant increase in the positive well error.

Conclusions

- The use of magnetic beads in an ELISA decreases the time needed to perform test to around 90 minutes from around 420 minutes with a plate ELISA
- 0.1 µg/mL detection antibody and 0.2 µg/mL IgG provided the most optimal absorbance results.
- 5 washes with mixing using a micropipette tip after each, including after addition of detection antibody provides the lowest background noise with this ELISA.
- The two-step procedure provides a better difference between positive and negative absorbances, while the one-step provides lower overall absorbances as well as a lower overall time from around 120 minutes to 90
- The optimization of the Rabbit IgG ELISA applies to the Anti-OpsA IgM ELISA.

Future Work

- Decrease overall test time to goal of 30 minutes.
- Incorporate a parallel test aimed at measuring bacterial proteins itself instead of the human immune response to the bacterium.

References

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3. Markwalter, C. F., Ricks, K. M., Bitting, A. L., Mudenda, L., & Wright, D. W. (2016). Simultaneous capture and sequential detection of two malarial biomarkers on magnetic microparticles. Talanta, 161, 443-449. https://doi.org/10.1016/j.talanta.2016.08.078

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