Detecting Leptospirosis in Water: Preliminary Results from a Regional Collaboration
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ABSTRACT
The United States Centers for Disease Control has re-added Leptospirosis to the list of emerging infectious diseases. Leptospirosis is caused by one of many strains of infectious Leptospira bacteria, and can be fatal if left untreated. The increased frequency of this disease is due to a complex transmission cycle with many site-specific regulatory factors. This makes localized environmental detection a necessary step in the development of adequate control measures. Hospital data and seroprevalence surveys indicate more than 70% of human infections result from physical contact with contaminated freshwater supplies. The objective of this project is to develop and evaluate a freshwater test for Leptospirosis that could be useful for routine monitoring of freshwater streams. Preliminary results indicate a 0.2 µm nitrocellulose filter would be the optimal choice for Leptospira collection. They also indicate Leptospira DNA can be extracted from Leptospirosis-trapped on a nitrocellulose filter, and that this DNA can be identified using a polymerase chain reaction (PCR) and gel electrophoresis. Research is ongoing and is currently evaluating the sensitivity of this methodology in three distinct Hawaiian streams.

INTRODUCTION

The Disease: Leptospirosis
• Common in the tropics, also found in temperate regions. 1,2
• The U.S. reports approximately 40 to 120 cases/year.3
• Most cases result from physical contact with contaminated freshwater.2,4,5
• Often runs a biphasic course with highly variable symptoms:
  1)  250µL of Leptospira culture was mixed in 10mL of 0.01M Phosphate Buffered Saline.
  2)  Cell density was assessed with a Petroff Hausser counting chamber in darkfield microscopy with 10 repetitions.
  3)  The concentration of cells in the filtered solution was assessed using the procedure from step 2.
  4)  Each filter was evaluated three times, and average cell counts were determined for each filter type.
  5)  Averages were used to calculate the percent flow-through of each filter using the following formula:

\[ \text{Percent Flow-Through} = \frac{\text{Flow-Through}}{\text{Total Volume}} \times 100 \]

• Several factors which impact DNA recovery include: filter material, the extraction kit and DNA Detection
method’s sensitivity.

RESULTS

Selecting an Appropriate Filter
The following filters were evaluated using pure suspensions of Leptospirosis icterohaemorrhagiae M20: 0.8 µm glass, 0.45 µm Durapore (polyvinylidene fluoride), 0.4-µm nitrocellulose, 0.22 µm Durapore, 0.2 µm nitrocellulose, 40-µm nylon mesh. 1) 250µL of Leptospira culture was mixed in 10mL of 0.01M Phosphate Buffered Saline. 2) Cell density was assessed with a Petroff Hausser counting chamber in darkfield microscopy with 10 repetitions. 3) The suspension was vacuum filtered at 10 to 25 kPa using each of the filters listed above. 4) The concentration of cells in the filtered solution was assessed using the procedure from step 2. 5) Each filter was evaluated three times, and average cell counts were determined for each filter type. 6) Averages were used to calculate the percent flow-through of each filter using the following formula:

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• Results confirm the visual quantification technique works for leptospires in pure suspension.

DISCUSSION

Filtration
• Results confirm the visual quantification technique works for leptospires in pure suspension.
• Filters made of Nitrocellulose or possessing 0.2µm pore sizes retain the most leptospires.
• Bacterial surfaces stick to negatively charged materials like nitrocellulose, glass, and nylon.
• Conclusions: Bacterial recovery is most enhanced by using a nitrocellulose or nylon filter with 0.2µm pores.

DNA Detection
• We have been successful in recovering bacterial DNA from less selective filters, indicating the procedure is sensitive.
• Several factors which impact DNA recovery include: filter material, the extraction kit and protocol, and factors related to the PCR reaction.
• Using a filter with smaller pore sizes will likely enhance DNA recovery and improve the method’s sensitivity.

METHODS

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Obtaining and Identifying Leptospira DNA
To establish whether DNA can be collected and properly identified from used filters, two filter types were evaluated: 0.45 µm nitrocellulose & 40 µm nylon mesh. These pore sizes are less selective and less likely to give a positive result.

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