

Rapid Detection of Human Fecal Contamination Using sIgA as an Indicator

**Jessica Hamel
New Mexico State University
Biology Department**

Abstract

Water contaminated with human fecal matter is a significant public health concern especially in areas with limited water resources for consumption and irrigation. The objective of this study is to determine the feasibility of using human secretory immunoglobulin alpha (sIgA) as an indicator of human fecal contamination in water sources such as raw sewage and ground water to determine if concentrations of sIgA can be detected with a high degree of certainty and reliability. For ground water samples, 10L of water were collected and spiked with a ~200ng/mL concentration of human sIgA. The sample was then concentrated to 200mL using an ultrafiltration method. Eight 1:1 dilutions were placed on a 96-well plate for detection of sIgA by the Enzyme-linked Immunoabsorbant Assay (ELISA). The percent recoveries for the spike challenges performed on ground water were 33.74%. For raw sewage, a 1mL sample was collected, diluted (eight 1:1 dilutions), and plated to detect sIgA present before being processed at wastewater treatment facilities (71% tested positive for sIgA without concentration of the sample). The recoveries of sIgA was then determined from raw sewage following the concentration of the sample from 1L to 50 ml using an ultrafiltration method. The efficiency of recovery of ultrafiltration method for the concentrated raw sewage was 45.5%. A sIgA assay used for the detection of human fecal contamination proves to be an efficient, rapid, and cost effective procedure. These results indicate that it is feasible to detect human sIgA.

Introduction

In humans, there are five classes of immunoglobulins (or antibodies) including: IgE, IgD, IgG, IgM, and IgA (Kuby, 1997). Secretory immunoglobulin alpha (sIgA) is produced daily in copious quantities and is found in high concentrations in fecal matter. IgA makes up 10-15% of the total circulating immunoglobulin and its daily production is greater than that of any other immunoglobulin (Kuby, 1997). Studies have shown that IgA is the major immunoglobulin isotope found in human external secretions (Mestecky, 1983). Human sIgA may be a good indicator of fecal contamination, since it is produced and released with waste material from every individual and is also produced in large amounts (Kuby, 1997). Secretory IgA is secreted in the subepithelial tissue of the digestive, respiratory, and genitourinary tracts by plasma cells. The structure of sIgA makes it resistant to degradation and hydrolysis. It is a dimer held together by a “J chain” polypeptide and a polypeptide chain called secretory component. The secretory component consists of five domains and is synthesized by epithelial cells (Kuby, 1997). The secretory component of sIgA contributes to the stabilization of the dimer. Secretory IgA (sIgA) is an important part of the humoral immune response because it has the ability to cross-link large antigens with multiple epitopes to block infection of host epithelial cells due to its polymeric nature (Kuby, 1997). Ultrafiltration will be used to concentrate sIgA from water samples and has been shown to concentrate *Cryptosporidium parvum* oocysts and viruses very effectively. The filtration method has been found to be particularly effective with highly turbid and basic water found in the Rio Grande (Kuhn and Oshima, 2001; Oshima, 2001; Winona et al, 2001; Kuhn et al, 2001). Ultrafiltration is an efficient and effective method to concentrate pathogens from

water because organisms are concentrated by size exclusion. The hollow-fiber ultrafilters utilize a cross-flow circulation pattern which prevent the membranes of the filters from becoming obstructed with large particles and debris thus improving filter life (reusable) and efficiency (Winona et.al., 2001). The molecular weight of sIgA is 300,000 Daltons and the molecular weight cut-off of the filter pores is 50,000 Daltons, so the ultrafiltration method should effectively concentrate sIgA. This project explores the effectiveness of the human sIgA assay in water as an indicator of human fecal contamination. This approach has particular benefits like low cost, specificity, simplicity, and rapid detection of water contaminated with human fecal matter to regions with scarce water sources.

Methodology

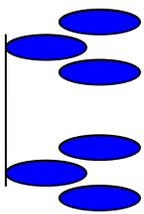
1. Optimization of ELISA System: Sandwich ELISA's are used to detect sIgA, using alkaline phosphatase conjugated anti-human IgA. A 96-well microtiter plate is used to bind the antibodies and antigens. The result of the ELISA is a color reaction that is read by a spectrophotometer at 450 nm. A standard dilution series with known amounts of sIgA is used to determine the sensitivity and linear range of an ELISA. The sandwich ELISA system works as shown in the figure below. From the optical density reading that's produced from the system (given from the spectrophotometer), a line can be plotted and the equation of a line, $y=mx+b$, can be found (using Graph Pad software) and used to determine the concentration (ng./mL.) of an unknown sample.

2. Optimization of Ultrafiltration System: Ultrafiltration is necessary to concentrate sIgA to detectable levels. A continuous-flow, hollow-fiber ultrafilter is a filter with a 50,000 MWCO was used due to its ability to retain sIgA. This system is used to concentrate

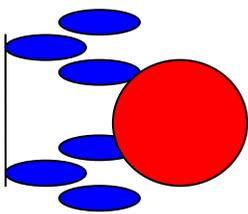
water samples from the Rio Grande and sewage treatment facilities to a much smaller volume (from 10L. to 250mL for Rio Grande water and from 1L to 50mL for raw sewage). Challenge experiments for Rio Grande water with human sIgA are done by adding 200 ng./mL. sIgA into 10 L. of water and concentrating to 250mL. Raw sewage experiments are preformed by concentrating 1L of sewage to 50mL. Recovery efficiency is determined by comparing the initial concentration of sIgA to the final concentration.

3. Presence of sIgA in Raw Sewage: Conducted to determine if human sIgA is present in raw sewage samples.

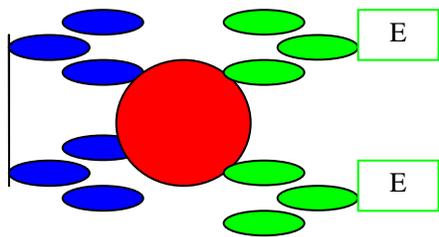
4. Detection of sIgA in ground water: Conducted to determine if the proposed approach to concentrate and detect sIgA from the Rio Grande/ground water is feasible. Positive samples verify that the approach using ultrafiltration and ELISA assays will detect sIgA in water.



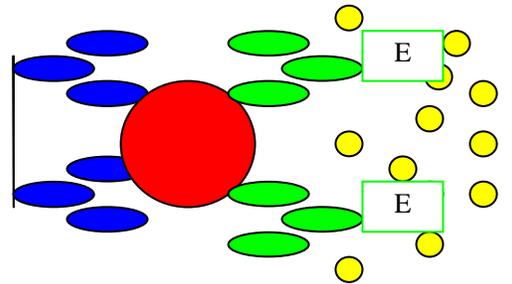
1. Passive Absorption of Antibody to Plate (Aby. to sIgA).



2. Addition of Antigen (sIgA).



3. Addition of Enzyme-labeled Antibody Against Antigen (sIgA).



4. Addition of Color Development System.

Results

Figure 1: Percent Recovery of Spiked (with sIgA) Surface Water via the Ultrafiltration System

| Date | Water | Goal Input ng./mL. | Actual Input ng./mL. | Ret. prime % Recovery |
|-----------|-----------------------|--------------------|----------------------|-----------------------|
| 1/8/04 | Spiked R.G.(Mesilla) | 200 | 72.3 (36.2%) | 66.3% |
| 1/29/04 | Spiked R.G. (Mesilla) | 200 | 39.9(20.0%) | 52.4% |
| 1/30/04 | Spiked R.G.(Mesilla) | 200 | 26.2(13..1%) | 31.9% |
| 7/9/2005 | Spiked R.G. (Mesilla) | 200 | 55.965 (27.985%) | 12.50% |
| 7/15/2005 | Spiked R.G. (Mesilla) | 200 | 56.890(28.445%) | 5.58% |
| 7/29/2006 | Spiked R.G. (Mesilla) | 200 | 238.217 | 24% |
| 8/2/2006 | Spiked R.G. (Mesilla) | 200 | 163.157 | 16.36% |

Figure 2: Detection of human sIgA in Raw Sewage (unconcentrated sample)

| Raw Sewage Concentrations of sIgA | | |
|-----------------------------------|----------|---------------------|
| Source | Results | Concentration ng/mL |
| | Positive | |
| | Positive | |
| | Positive | |
| | Negative | |
| Juarez | Positive | 42.6 |
| Las Cruces | Positive | 2.27 |
| Las Cruces | Positive | 44.235 |
| Las Cruces | Positive | 7.529 |

Figure 3: Recovery Efficiency Human sIgA from Raw Sewage through a Ultrafiltration-based Concentration System

| Raw Sewage Concentrations of sIgA after Ultrafiltration | | | | | | | |
|---|----------|---------|--------------------------|----------|----------------|-----------------|-------------------------|
| ELISA# | Date | Source | Methods | Results | Input RS ng/mL | Final Con ng/mL | % Filtration Efficiency |
| 87 | 06/17/05 | El Paso | 1L concentrated to 50 mL | Positive | 12.9 | 38.6 | 33.40% |
| 92 | 07/19/05 | El Paso | 1L concentrated to 50 mL | Positive | 66.7 | 90.8 | 73.40% |
| 96 | 09/06/05 | | 1L concentrated to 50 mL | Positive | 21.1 | 20.2 | 4.80% |
| 101 | 12/13/05 | El Paso | 1L concentrated to 50 mL | Positive | 113.8 | 20.2 | 70.30% |

Discussion and Significance

- Optimization of Ultrafiltration System: The filtration results show that the ultrafiltration system can effectively concentrate human sIgA from water and sewage samples. The filtration experiments that have been conducted so far have given an average percent recovery of 29.9% however more replicates should be conducted. Variations in efficiency of recovery may be in part due to water temperature and turbidity.
- Detection of sIgA in Raw Sewage: Of the 8 raw sewage samples tested directly with no concentration, 71.4% were positive. When ultrafiltration was used to concentrate one liter of raw sewage to ~50 ml the average recovery efficiency was 45.5%.

Results indicate that human sIgA has promise as an indicator of human fecal contamination. Previous results have shown little to no cross-reactivity of the human sIgA assay with bovine, porcine, horse or chicken sIgA. A human indicator for fecal contamination is beneficial to the region and elsewhere because it will provide a new method for identifying water that has been impaired by human fecal material. This method will be inexpensive, specific to humans, and the assay is capable of high throughput and is more rapid than bacterial culture methods.

References

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