Development of an interleukin-4-inducing principle from *Schistosoma mansoni* eggs (IPSE)-specific PCR assay as a quantitative predictor of schistosomiasis-associated morbidity

Dannah Farah³, Evaristus Mbanefo¹ Michael Hsieh¹²

¹Bladder Immunology Group, Biomedical Research Institute, Rockville, Maryland, USA  
²Division of Urology, Children’s National Medical Center, Washington, DC, USA  
³School of Medicine and Health Sciences, George Washington University, Washington, DC, USA  

**Abstract**

Schistosomiasis is a neglected tropical disease affecting between 200-500 million people worldwide. The two species causing most human cases of schistosomiasis are *Schistosoma mansoni* and *Schistosoma haematobium*. The gold standard for diagnosis is parasitological detection of parasite eggs in stool using the Kato-Katz method. Counting eggs shed in stool is labor-intensive and inaccurate. Interleukin-4-inducing principle from *Schistosoma mansoni* eggs (IPSE) is the most abundant secreted protein from schistosome eggs. We hypothesized that the mRNA transcripts of the IPSE protein may be found in the liver tissue and stool of experimentally infected animals, and that these transcripts can be specifically targeted as a molecular diagnostic for schistosomiasis in endemic areas. Liver tissue and stool samples were collected from *S. mansoni* infected mice. PCR amplification of IPSE mRNA from liver samples was correlated with positive controls from serial dilutions of a known concentration of pure *S. mansoni* egg RNA. Concentration of sample’s RNA was then compared to egg counts from stool samples. Results showed a positive correlation between increasing concentrations of IPSE RNA in infected liver tissue and increasing number of eggs found in stool. Our next steps are to repeat this experiment using *Schistosoma haematobium* infected hamsters, and to further develop the assay as a field diagnostic, correlate IPSE mRNA transcript levels in stool with stool egg counts.

**Introduction**

**Functions of IPSE**

IPSE: most abundant *Schistosoma* egg-secreted protein

1. IPSE binds Ig  
2. IPSE induces basophils and mast cells to release IL-4  
3. IPSE sequesters chemokines  
4. IPSE translocates into host cell nucleus to modulate gene expression

**Methods**

Week 7 - Post-Infection  
Sacrifice animal- collect liver and stool samples  
Samples in RNAzol for RNA extraction  
Digest liver samples in 4% KOH / Fix stool samples in 10% buffered formalin - wash in 1.2% NaCl for egg extraction  
Reverse transcription and qPCR  
Egg count  
Correlation analysis

**Goals**

1. Establish a standard curve using known concentrations of IPSE RNA  
2. Assess our sample concentrations of IPSE RNA  
3. Correlate our sample concentration of RNA with corresponding sample’s egg count

**Results**

**S. mansoni Stool Samples**

- m-IPSE qPCR  
- h-IPSE qPCR (10⁻µM)  
- h-IPSE qPCR (1µM)

**S. mansoni Liver Samples**

- MH-IPSE qPCR  
- MH-IPSE (Liver)

**Conclusion & Next Steps**

- For *S. mansoni*, we have established a strong positive correlation between the number of eggs and the amount of IPSE RNA in liver tissue. The same is true for stool, however this correlation is established with little to no eggs in our stool samples.  
- For *S. haematobium*, we have optimized our primer concentrations for qPCR amplification of IPSE RNA from pure egg mRNA  
- Our next steps are to  
  1. Infect mice with serial numbers of cercariae and sacrifice the at the same time point  
  2. Infect mice with the same number of cercariae and sacrifice at different time points  
  3. Optimize the egg extraction protocol for our stool samples  
- **Long-term goals**: We hope to develop IPSE-related molecular and immunological assays as more accurate indicators of schistosomiasis-related morbidity by characterizing  
  1. IPSE gene expression levels in stool and urine of schistosome-infected people  
  2. The immune responses to IPSE in infected individuals  
  3. Correlate these IPSE-related parameters to measures of host morbidity