

**Immunolocalization of target schistosomal  
antigens using anti-cysteine proteases  
antibodies in murine schistosomiasis**

**Presented by**

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

إِنَّمَا يَخْشَى اللَّهَ مِنْ عِبَادِهِ الْعُلَمَاءُ

إِنَّ اللَّهَ تَعَزَّزُ تَفَقُّرُ

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## **Approval Sheet**

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Immunolocalization of target schistosomal antigens using  
anti-cysteine proteases antibodies in murine schistosomiasis

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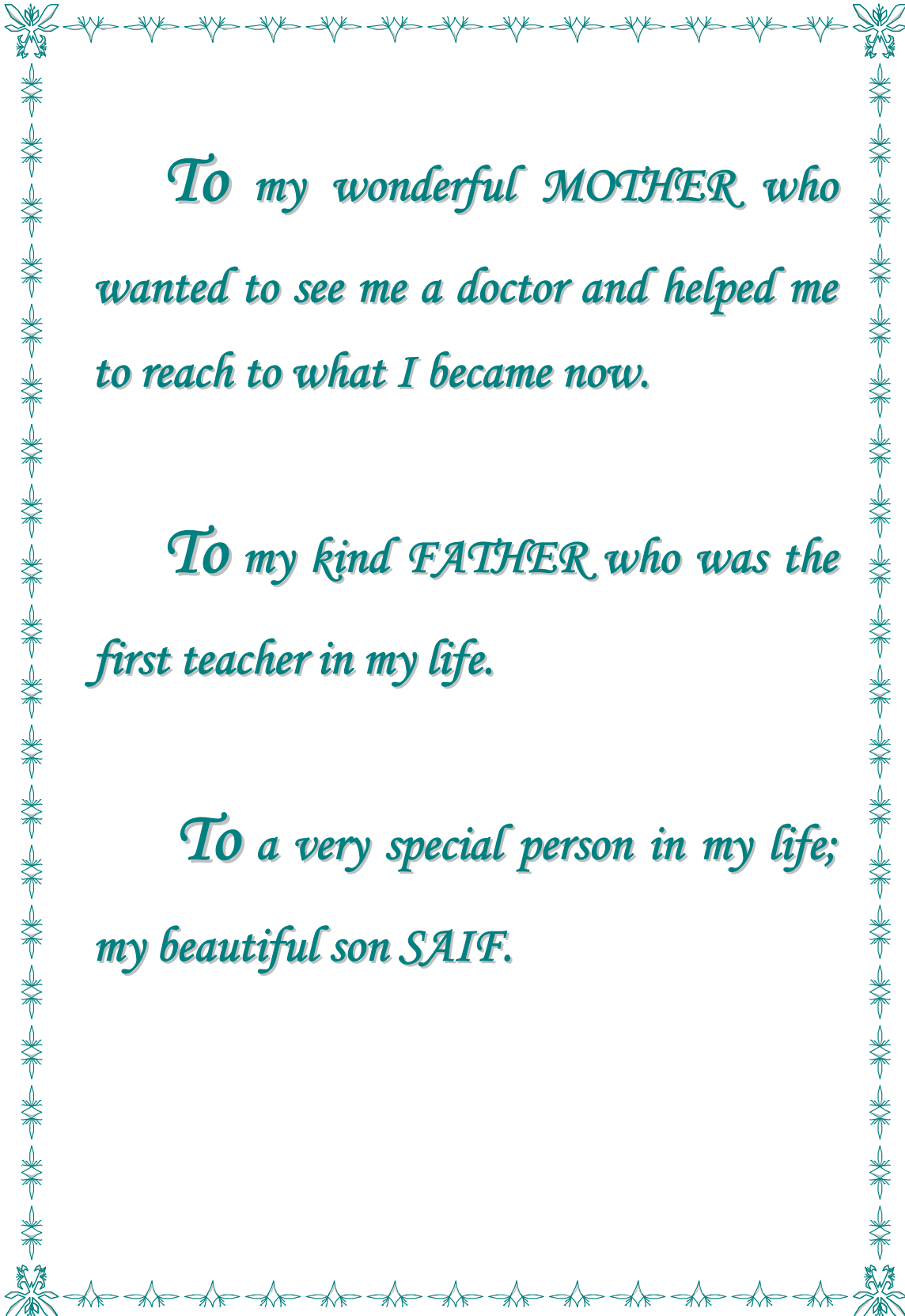
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wanted to see me a doctor and helped me  
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first teacher in my life.*

*To a very special person in my life;  
my beautiful son SAIF.*

## *ABSTRACT*

Schistosomiasis is an important parasitic disease in many parts of the tropics, second only to malaria as a source of morbidity and mortality. Diagnostic methods include microscopic examination of excreta such as the Kato-Katz method, radiography, and serological techniques.

Proteases have been under scrutiny as targets of immunological or chemotherapeutic anti-*Schistosoma* agents because of their vital role in many stages of the parasitic life cycle. Upon the basis of the mechanism of peptide hydrolysis, five major protease classes are known: serine, cysteine, aspartic, threonine, and metalloproteases. Proteases of all five catalytic classes have been identified from *S. mansoni* through proteomic or genetic analysis.

In this study, the produced polyclonal antibodies (pAb) against *S. mansoni* cysteine protease (CP) antigens were labelled with horseradish peroxidase (HRP) conjugate and used to demonstrate the presence of a highly reactive CP epitopes in the different organs of *S. mansoni* naturally infected mice and its different life cycle stages. Also, pAb successfully detects CP antigens in stool and serum samples of *S. mansoni* infected patients by sandwich ELISA with a sensitivity of 92.8% and 90.47% respectively. Moreover, a positive correlation was observed between *S. mansoni* egg counts and both stool and serum antigen concentrations. Thus, it can be concluded that purified 27.5 kDa CP obtained from E/S products of *S. mansoni* worms could be introduced as a suitable candidate antigen for early immunodiagnosis of *S. mansoni* infected human using sandwich ELISA for antigen detection and serodiagnosis.

### *Key words:*

*S. mansoni*  
Cysteine proteases  
Gel filtration chromatography  
SDS-PAGE

Immunolocalization  
ELISA  
Immunofluorescence  
Polyclonal IgG  
Immunostaining

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## **LIST OF ABBREVIATIONS**

<b>A</b>	<b>Central arteriole</b>
<b>Ab</b>	<b>Antibody</b>
<b>Ag</b>	<b>Antigen</b>
<b>AMC</b>	<b>7-amino-4-methylcomarin</b>
<b>AST</b>	<b>Antigenic sequence tags</b>
<b>AWA</b>	<b>Adult worm antigens</b>
<b>BC</b>	<b>Bowmen's capsule</b>
<b>BD</b>	<b>Bile duct</b>
<b>BS</b>	<b>Blood sinusoids</b>
<b>BS</b>	<b>Bowmen's space</b>
<b>BSA</b>	<b>Bovine serum albumin</b>
<b>bSmCL1</b>	<b>Bacterial SmCL1</b>
<b>CAA</b>	<b>Circulating anodic antigen</b>
<b>CB</b>	<b>Cathepsin B-like</b>
<b>CCA</b>	<b>Circulating cathodic antigen</b>
<b>CCL</b>	<b>Chemokine ligand</b>
<b>CL</b>	<b>Cathepsin L-like</b>
<b>Con A</b>	<b>Concanavalin A</b>
<b>COPT</b>	<b>Circumoval precipitin test</b>
<b>CP</b>	<b>Cysteine protease</b>
<b>CT</b>	<b>Computed tomography</b>
<b>CV</b>	<b>Central vein</b>
<b>DAB</b>	<b>Diaminobenzidine</b>
<b>DC</b>	<b>Dendritic cells</b>
<b>DDIA</b>	<b>Dipstick dye immunoassay</b>
<b>DEAE</b>	<b>Diethylaminoethyl</b>
<b>DNA</b>	<b>Deoxyribonucleic acid</b>
<b>DT</b>	<b>Distal convoluted tubule</b>
<b>DTH</b>	<b>Delayed-type hypersensitivity</b>



<b>E/S</b>	<b>Excretory/secretory</b>
<b>EF-hand motifs</b>	<b>Calcium-binding motifs composed of two helices, E and F, joined by a loop</b>
<b>EITB</b>	<b>Electroimmunotransfer blotting</b>
<b>ELISA</b>	<b>Enzyme- linked immunosorbent assay</b>
<b>epg</b>	<b>Eggs per gram stool</b>
<b>EST</b>	<b>Expressed sequence tag</b>
<b>FABP</b>	<b>Fatty acid-binding protein</b>
<b>FCS</b>	<b>Fetal calf serum</b>
<b>FDA</b>	<b>Fast Dot ELISA</b>
<b>FITC</b>	<b>Fluorescein isothiocyanate conjugate</b>
<b>G</b>	<b>Glomerulus</b>
<b>GAPDH</b>	<b>Glyceraldehyde-3-phosphate dehydrogenase</b>
<b>GC</b>	<b>Gynaecophoric canal</b>
<b>GL</b>	<b>Gut lining</b>
<b>Glc</b>	<b>Glucose</b>
<b>GST</b>	<b>Glutathione S-transferase</b>
<b>H<sub>2</sub>O<sub>2</sub></b>	<b>Hydrogen peroxide</b>
<b>HA</b>	<b>Hepatic artery</b>
<b>HBV</b>	<b>Hepatitis B virus</b>
<b>HCV</b>	<b>Hepatitis C virus</b>
<b>HE</b>	<b>Hematoxylin and eosin</b>
<b>HEPES</b>	<b>N-2-hydroxyethyl piperazine-N-2-ethanesulfonic acid</b>
<b>HL</b>	<b>Hepatic lobule</b>
<b>hr</b>	<b>Hour</b>
<b>HRP</b>	<b>Horseradish peroxidase</b>
<b>HS</b>	<b>Hepatic strand</b>
<b>HSC</b>	<b>Hepatic stellate cell</b>
<b>HSP</b>	<b>Heat shock protein</b>
<b>HSS</b>	<b>Hepatosplenic schistosomiasis</b>
<b>i.m</b>	<b>Intramuscular injection</b>
<b>ID</b>	<b>Intradermal test</b>

<b>IFN-<math>\gamma</math></b>	<b>Interferon gamma</b>
<b>IgG</b>	<b>Immunoglobulin G</b>
<b>IgG pAb</b>	<b>IgG polyclonal antibody</b>
<b>IHA</b>	<b>Indirect hemagglutination assay</b>
<b>IIF</b>	<b>Indirect immunofluorescence</b>
<b>IL</b>	<b>Interleukin</b>
<b>kDa</b>	<b>Kilo Dalton</b>
<b>LC</b>	<b>Langerhans cell</b>
<b>LDNF</b>	<b>Fucosylated LacdiNAc</b>
<b>LeX</b>	<b>Lewis X</b>
<b>LP</b>	<b>Lamina propria</b>
<b>LPS</b>	<b>Lipopolysaccharide</b>
<b>LR</b>	<b>London resin</b>
<b>Lutz/HPJ</b>	<b>Lutz/Hoffman, Pons &amp; Janer</b>
<b>M</b>	<b>Mucosa</b>
<b>m</b>	<b>Miracidium</b>
<b>mAb</b>	<b>Monoclonal antibody</b>
<b>MEG</b>	<b>Major egg glycoprotein</b>
<b>MIFC method</b>	<b>Merthiolate iodine formaldehyde concentration method</b>
<b>MIP</b>	<b>Mitochondrial intermediate protease</b>
<b>ML</b>	<b>Muscle layer</b>
<b>mol. wt.</b>	<b>Molecular weight</b>
<b>MPP</b>	<b>Mitochondrial processing protease</b>
<b>MSA</b>	<b>Major serological antigens</b>
<b>MZ</b>	<b>Marginal zone</b>
<b>NO</b>	<b>Nitric oxide</b>
<b>NPV</b>	<b>Negative predictive value</b>
<b>OD</b>	<b>Optical density</b>
<b>OPD</b>	<b>O-phenylenediamine</b>
<b>Os</b>	<b>Oral sucker</b>
<b>pAb</b>	<b>Polyclonal antibody</b>
<b>PALS</b>	<b>Periarterial lymphatic sheath</b>

<b>PAS</b>	<b>Periodic Acid Schiff reagent</b>
<b>PBMCs</b>	<b>peripheral-blood mononuclear cells</b>
<b>PBS</b>	<b>Phosphate-buffered saline</b>
<b>PBS/T</b>	<b>PBS/tween</b>
<b>PCR</b>	<b>Polymerase chain reaction</b>
<b>PEPCK</b>	<b>Phosphoenolpyruvate carboxykinase</b>
<b>PI</b>	<b>Isoelectric point</b>
<b>PNA</b>	<b>Peanut agglutinin</b>
<b>PPV</b>	<b>Positive predictive value</b>
<b>PT</b>	<b>Proximal convoluted tubule</b>
<b>PTGS</b>	<b>Posttranscriptional gene silencing</b>
<b>PTK</b>	<b>Protein tyrosine kinase</b>
<b>PV</b>	<b>Portal vein</b>
<b>PZQ</b>	<b>Praziquantel</b>
<b>R</b>	<b>Correlation coefficient</b>
<b>RC</b>	<b>Renal corpuscles</b>
<b>RP</b>	<b>Red pulp</b>
<b>RPMI</b>	<b>Roswell Park Memorial Institute</b>
<b>RT-PCR</b>	<b>Reverse transcriptase-PCR</b>
<i>S. bovis</i>	<i>Schistosoma bovis</i>
<i>S. haematobium</i>	<i>Schistosoma haematobium</i>
<i>S. intercalatum</i>	<i>Schistosoma intercalatum</i>
<i>S. mansoni</i>	<i>Schistosoma mansoni</i>
<i>S. mekongi</i>	<i>Schistosoma mekongi</i>
<b>SBSP</b>	<b>Schistosome Biological Supply Program</b>
<b>SD</b>	<b>Standard deviation</b>
<b>SDS-PAGE</b>	<b>Sodium dodecyl-sulfate polyacrylamide gel electrophoresis</b>
<b>SE</b>	<b>Saline extract</b>
<b>SE2</b>	<b>Reextraction of adult worms</b>
<b>SEA</b>	<b>Soluble egg antigen</b>
<b>SEi</b>	<b>Initial fraction of SE</b>
<b>Ser</b>	<b>Serine</b>

<b>Sj22.6</b>	<i>S. japonicum</i> 22.6
<b>SjCL1</b>	<i>S. japonicum</i> CL1
<b>Sm</b>	<i>S. mansoni</i>
<b>Sm20.8</b>	<i>S. mansoni</i> 20.8
<b>Sm21.7</b>	<i>S. mansoni</i> 21.7
<b>Sm22.6</b>	<i>S. mansoni</i> 22.6
<b>SmCB</b>	<i>S. mansoni</i> Cathepsin B-like endopeptidase 1
<b>SmCE</b>	<i>S. mansoni</i> cercarial elastase
<b>SmCL1</b>	<i>S. mansoni</i> Cathepsin L-like
<b>SmDPP IV</b>	<i>S. mansoni</i> dipeptidyl peptidase IV
<b>SmFes</b>	PTK in <i>S. mansoni</i>
<b>SmPepM8</b>	<i>S. mansoni</i> polypeptide M8
<b>TBRI</b>	Theodor Bilharz Research Institute
<b>TCA</b>	Trichloroanisole
<b>Teg</b>	Tegument
<b>TEM</b>	Transmission electron microscope
<b>Th1</b>	T helper cell 1
<b>Th2</b>	T helper cell 2
<b>Thr</b>	Threonine
<b>TLRs</b>	Toll like receptors
<b>TNF-<math>\alpha</math></b>	Tumor necrosis factor- $\alpha$
<b>USG</b>	Ultrasonography
<b>Vs</b>	Ventral sucker
<b>WB</b>	Western blot
<b>wk</b>	week
<b>WHO</b>	World health organization
<b>WP</b>	White pulp
<b>0–3-h RP</b>	Released proteins by larvae 0-3 hr after transformation

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