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شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم





# جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

# قسم

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#### Evaluation of an Antigen Detection Nano Immunoassay for Assessing the Prevalence of Schistosoma haematobium Infection in Areas at Risk in Upper Egypt

#### Thesis

Submitted for Partial Fulfillment of Master degree in Medical Parasitology

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## Dedication

Dedicated to my caring and loving Mother, my supportive and understanding brothers, the most wonderful husband and the sunshine of my life Eyad.

Thank you for your support, really without you I would have never reached this point in my life

#### **Abstract**

**Background:** Urogenital schistosomiasis caused by *Schistosoma haematobium* is one of the major public health problems worldwide. It is thought that despite extensive efforts and integrated control programs implicated over the last few decades, the global disease burden of schistosomiasis remains unacceptably high. This persistence of the disease may be due to in part the lack of accurate diagnostic tools for case detection and community screening in endemic areas.

**Aim of the work** is to assess the prevalence of *Schistosoma haematobium* infection in certain villages at risk in Upper Egypt among children attending selected Primary and Preparatory schools. An antigen detection assay using nanoparticles conjugated with anti-schistosomal antibodies will be evaluated in comparison to direct microscopy, micro-haematuria and micro-proteinuria.

**Subjects and methods:** A cross sectional study was conducted on 290 students (192 male and 98 female) selected randomly from Primary and Preparatory schools in four villages in Beni-Suef governorate; the participating children were aged 8–15 years old. A simple questionnaire was designed based on the key indicators of urinary schistosomiasis then, terminal urine samples were collected between 10 am and 2 pm in clean container from each participant to be screened by chemical reagent strips (Combi 10) and examined by urine microscopy and sandwich ELISA techniques (traditional and IMB) for *S. haematobium* detection. Soluble egg antigen (SEA) was used to produce specific polyclonal antibodies (pAbs) which were then used as a primary capture in the sandwich ELISA techniques. The anti-SEA pAbs were labeled with horse-radish peroxidase (HRP) and used as a secondary capture.

**Results:** Out of the 290 participants, 39 children (13.4%) were positive by UM, 53 were positive by traditional sandwich ELISA, with diagnostic sensitivity (87.2%) and specificity (92.4%) and 50 were positive by IMB-sandwich ELISA with diagnostic sensitivity (94.9%) and specificity (95.2%) based on UM results. Micro-haematuria and proteinuria were assessed by chemical reagent strips which gave sensitivity of 29.5%, specificity of 90.8% for micro-haematuria alone, sensitivity of 18.4%, specificity of 92.4% for proteinuria alone, while sensitivity of 35.9%, specificity of 94.9% for combined micro-haematuria and proteinuria which indicated a highly significant association with *S. haematobium* infection (p value<0.001).

Conclusion: Combination of both clinical and epidemiological data in addition to sensitive diagnostic tools is essential for diagnosis. The present study as with other studies revealed that, IMB-ELISA based on gold nanoparticles provides more rapid and sensitive detection for SEA in urine samples of patient with active schistosomiasis. Simplicity and fast detection (10 min) are its main advantages. Moreover, its high sensitivity and specificity ensure its application with greater precision and rapid detection. Also, in addition, the prevalence of urinary schistosomiasis in these regions is considered relatively high requiring rapid implementation of control programs to decrease the prevalence and improve the community's health status.

**Keywords:** S. haematobium; chemical reagent strips; urine microscopy; ELISA; Immunomagnetic beaded ELISA technique; gold nanoparticles.

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### LIST OF ABBREVIATIONS

#### Abb. Full term

%Po	ercent
°CD	egree Celsius
μgΜ	Ticrogram
$\mu l$ $M$	
$\mu m$ $M$	licrometre
$\mu M$ $M$	Ticromole
Ag NPsS	ilver nanoparticles
AgA	ntigen
<i>AM</i>	nte meridiem (before noon)
<i>APS</i>	mmonium Per-Sulfate
AUCA	rea under curve
AuNPsG	old nanoparticles
AWAA	dult worm antigens
BCEB	efore Common Era
<i>BPB</i>	romo-phenol blue
BSAB	ovine serum albumin
C $C$	ross linking monomer concentration
Ca $C$	alifornia State
<i>CAA C</i>	irculating anodic antigen
CCAC	irculating cathodic antigen
CDC $C$	enters for Disease Control and Prevention
<i>CEFC</i>	ationic egg fraction
CEQC	atechol estrogen quinine
<i>CFAC</i>	omplete Freund's adjuvant
<i>CFPD C</i>	irculating cell-free parasite DNA
<i>CHRC</i>	ercarien Hüllen reaction
CI $C$	onfidence interval
Cm $C$	entimeter
CNS C	entral nervous system

# List of Abbreviations (cont...)

Abb.	Full term
CSA	. Circulating schistosome antigens
	. Computerized tomography
D	
	Droplet digital PCR
	Drug delivery system
	.Deoxyribonucleic Acid
	Delayed type hypersensitivity
DW	* **
e.g	
et al	_
	Fraction antigen
	Falcon assay screening test
	. Fet al Calf Serum
	. Gut associated soluble antigens
gm	
_	. Hydrogen peroxide
H2SO4	• •
HCl	. Hydrochloric acid
	. Human immunodeficiency virus
HR	. High resolution
HRP	. Horse-Radish Peroxidase
hrs	. Hours
<i>i.m</i>	.Intramuscular injection
<i>IARC</i>	.International Agency for Research on Cancer
<i>IFA</i>	.Incomplete Freund's adjuvant
<i>IFAT</i>	.Indirect immunofluorescence assay
<i>IFN</i>	.interferon
<i>Ig</i>	.Immunoglobulin