中国疾病预防控制中心
CHINESE CENTER FOR DISEASE CONTROL AND PREVENTION



Development of clonorchiasis rapid test strip and its application in China

Principle Investigator : Prof. Xuenian Xu

xuxuenianqq@hotmail.com

Speaker: Na Cheng

National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention

16 June 2014 - Shanghai, China

Outline

- **Antigen candidates screening**
- **Molecular cloning, expression and the diagnostic potency of the antigen candidate**
- **Lab assessment and field application of clonorchiasis rapid test strip (Colloidal-Gold)**

Background

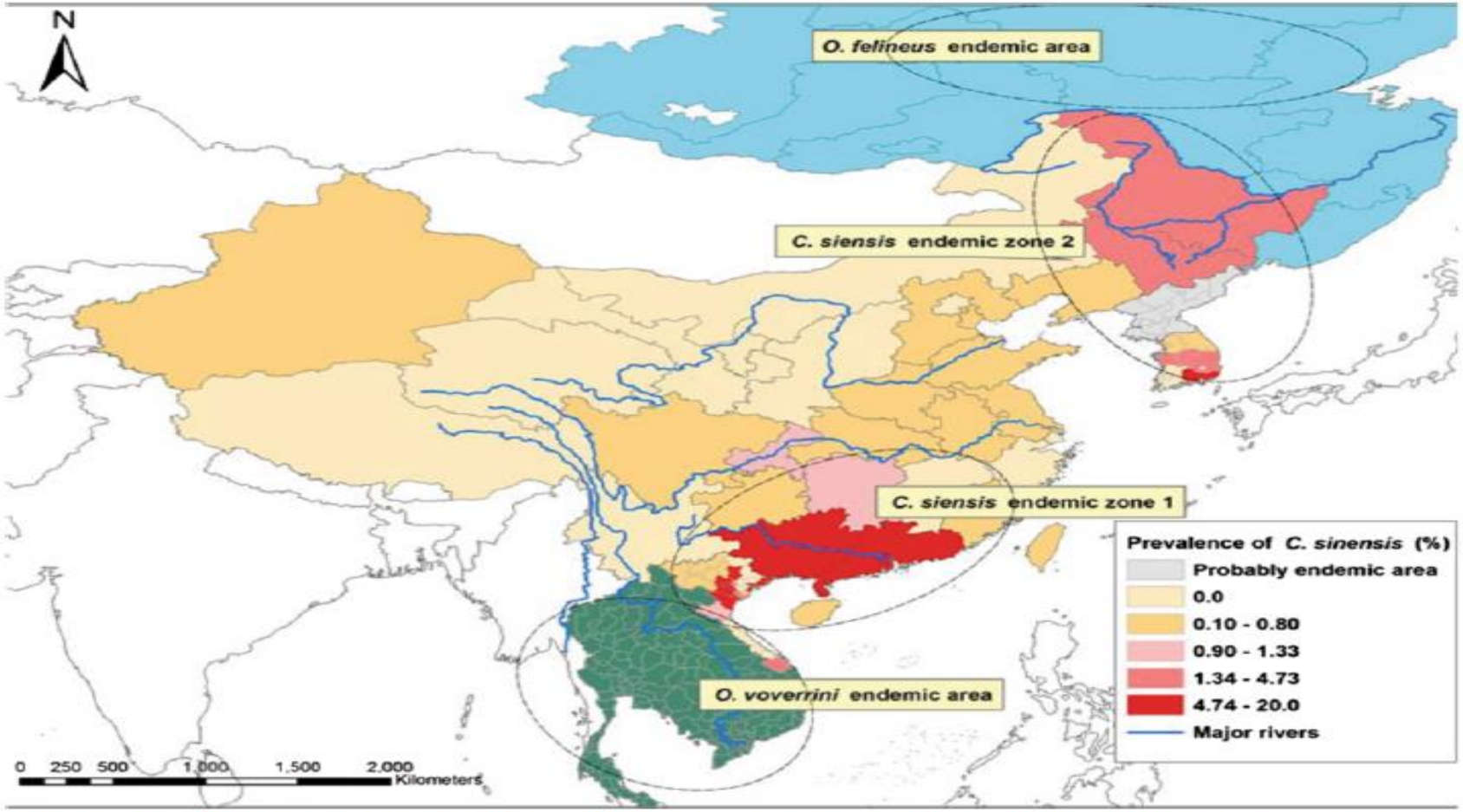


Figure 4 The global distribution of three major species of liver flukes, i.e. *C. sinensis*, *O. viverrini* and *O. felineus*. This map focuses on the endemicity of *C. sinensis* in China, the Republic of Korea and the northern part of Vietnam.

From "The global epidemiology of clonorchiasis and its relation with cholangiocarcinoma. Qian MB, etc. Inf Dis Poverty, 2012, 1:4"

Background

Obstacles- Lacking of simple, easy, sensitive and specific diagnostics

Gold standard: Stool examination

Limitations:

Time-consuming

Difficult in stool collection

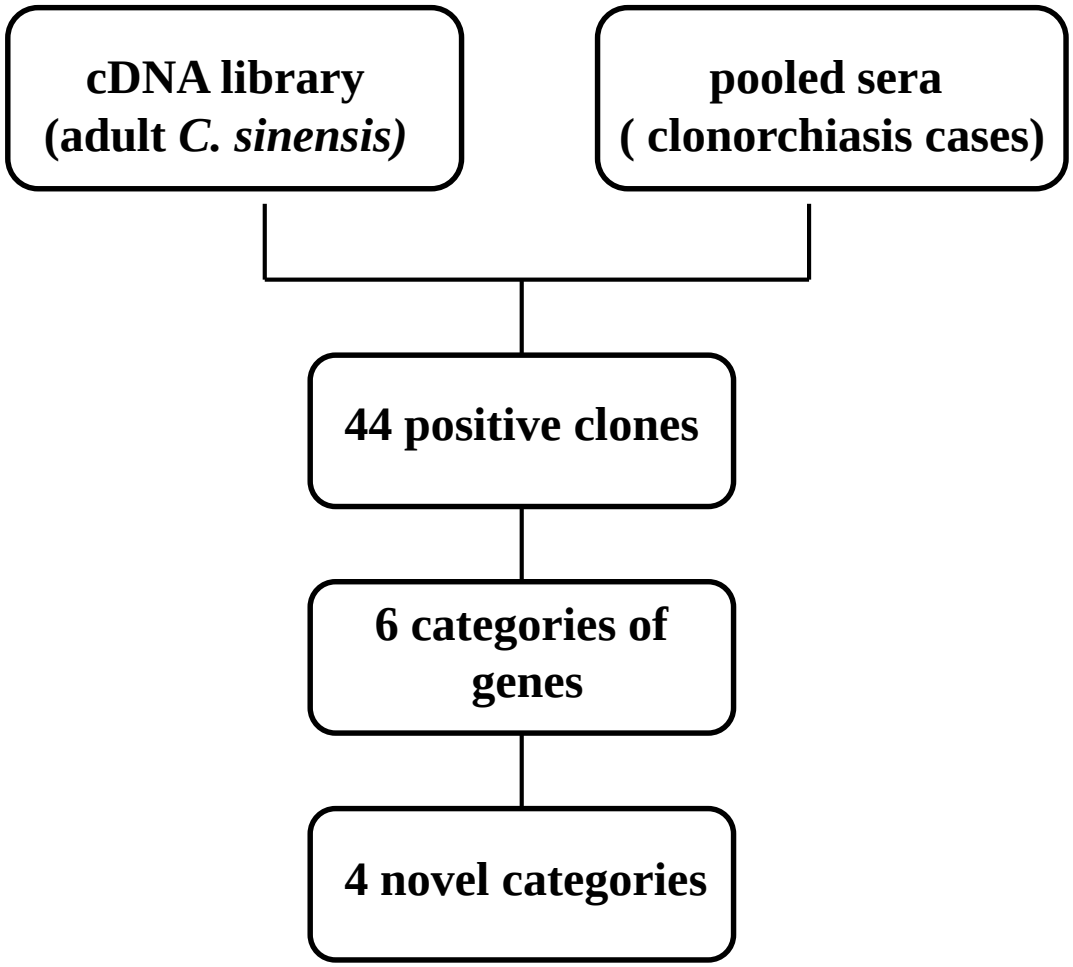
Missed diagnosis

Requirement of trained personnel

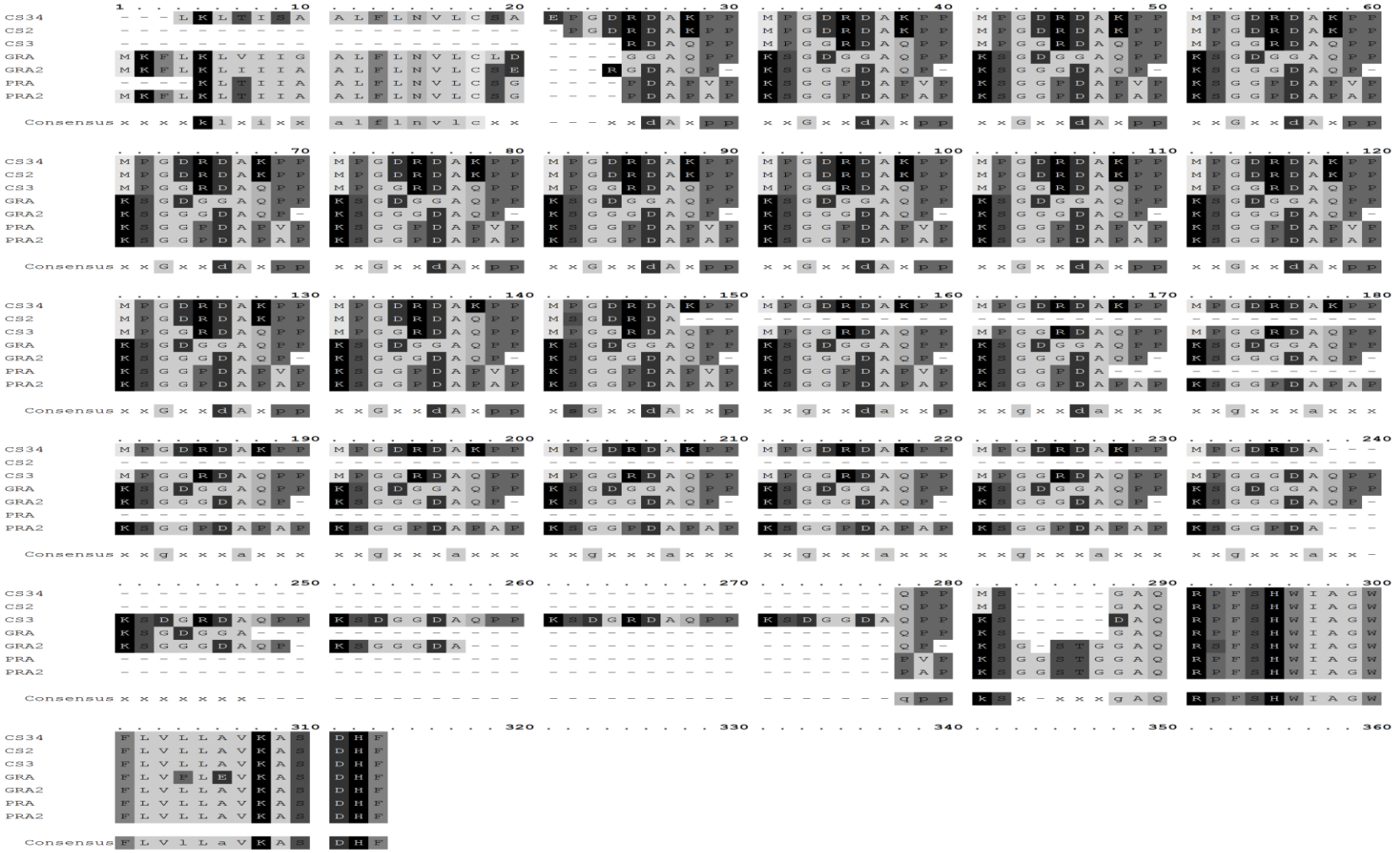


Antigen candidates screening

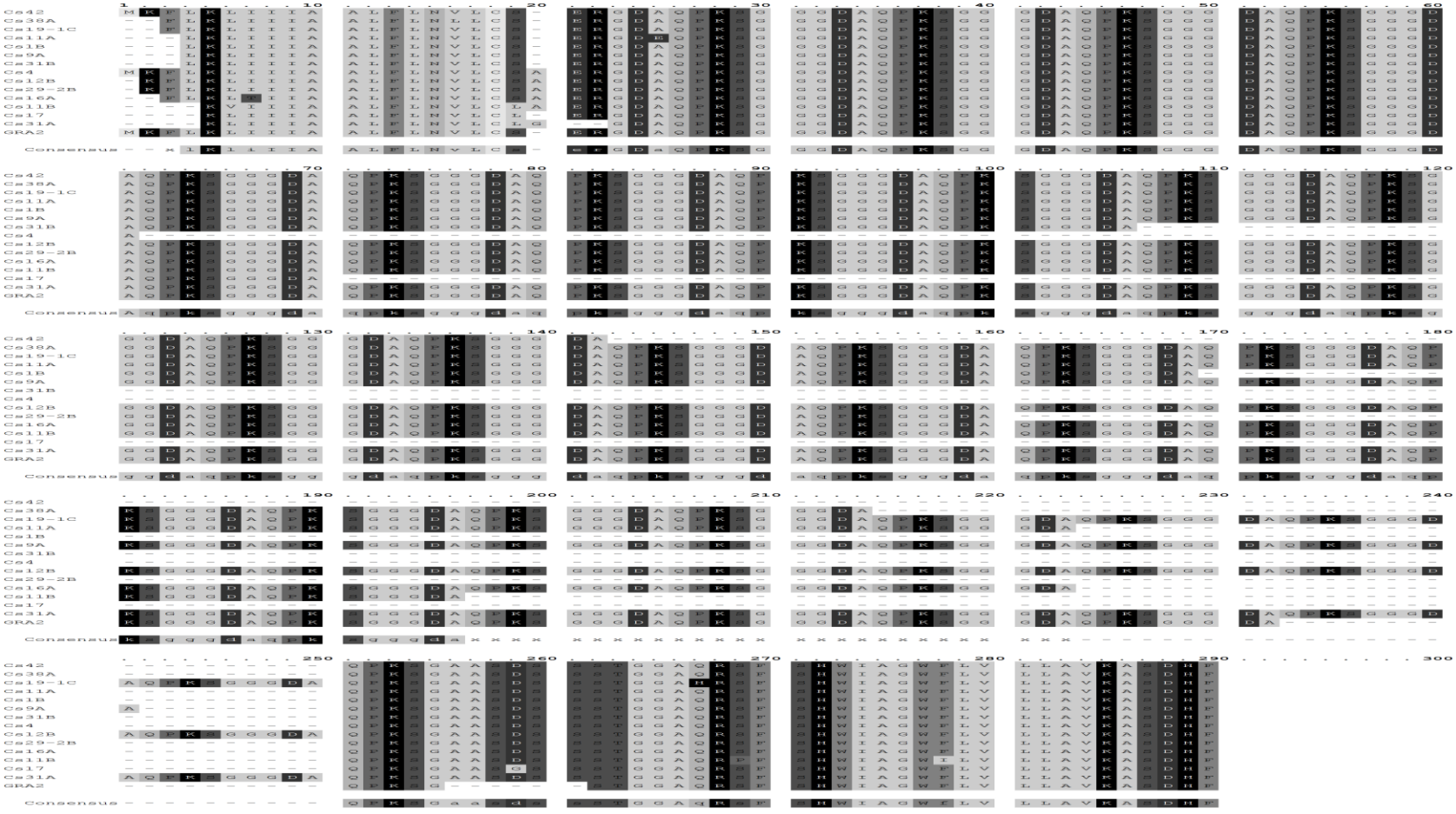
Immunoscreening of *C. sinensis* adult worm cDNA library



Multiple alignment of putative polypeptides of PPMP family *with other C. sinensis* proteins



Multiple alignment of putative polypeptides of GRA2a family *with C. sinensis* GRA2



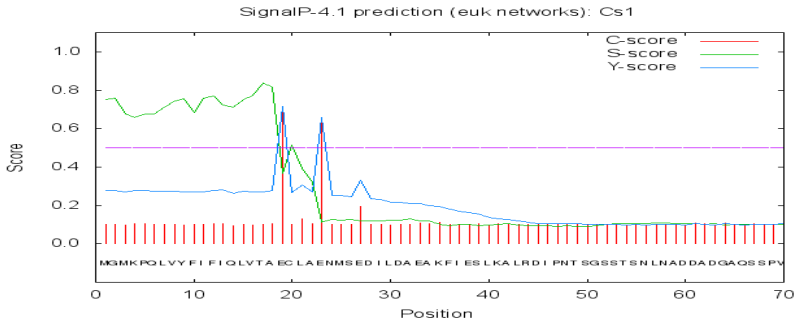
Screened antigen candidates

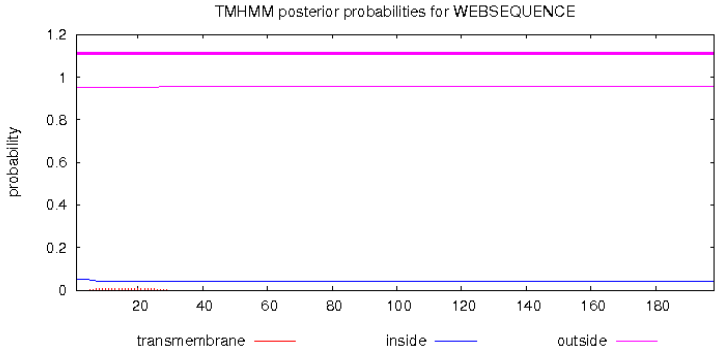
	No. Positive clones	Typical clone(s)		GenBank Accession No.
Glycine rich antigen 2a	30	Cs4	function unknown	GU124749.1
Glycine rich antigen 1	7	Cs7	reported	
PPMP antigen	3	Cs2, Cs3	unknown	HM347443.1, HM347444.1
cysteine proteinase	2	Cs12	reported	
Cs22	1	Cs22	unknown	KF004310.1
Cs1	1	Cs1	unknown	HM236312.1

Comparison of the diagnostic potency of four candidates

	Sensitivity (%)	Specificity (%)
Cs1	**94.3**	**97.2**
Cs2	74.3	94.4
Cs3	85.7	94.4
Cs4	80.0	97.2

Secretory protein prediction of Cs1





>Cs1

MGMKPQLVYFIFIQLVTAECLAENMSEDILDAEAKFIESLKALRDIPNTSGSSTS
NLNADDADGAQSSPVKGECDPESDGAVADDAPPSQVKPQGDPESDGAVADD
APPSQVKPQGDPESDGAVADDAPPSQVKPQGDPESDGAVADDAPPSQVKPQG
DPESDGAVAGDAPPSQVKPQGEIRMRKFRCQFIILRRIIS*

Molecular cloning, expression and the diagnostic potency of Cs1

Expression and purfication of recombinant Cs1 (rCs1) protein

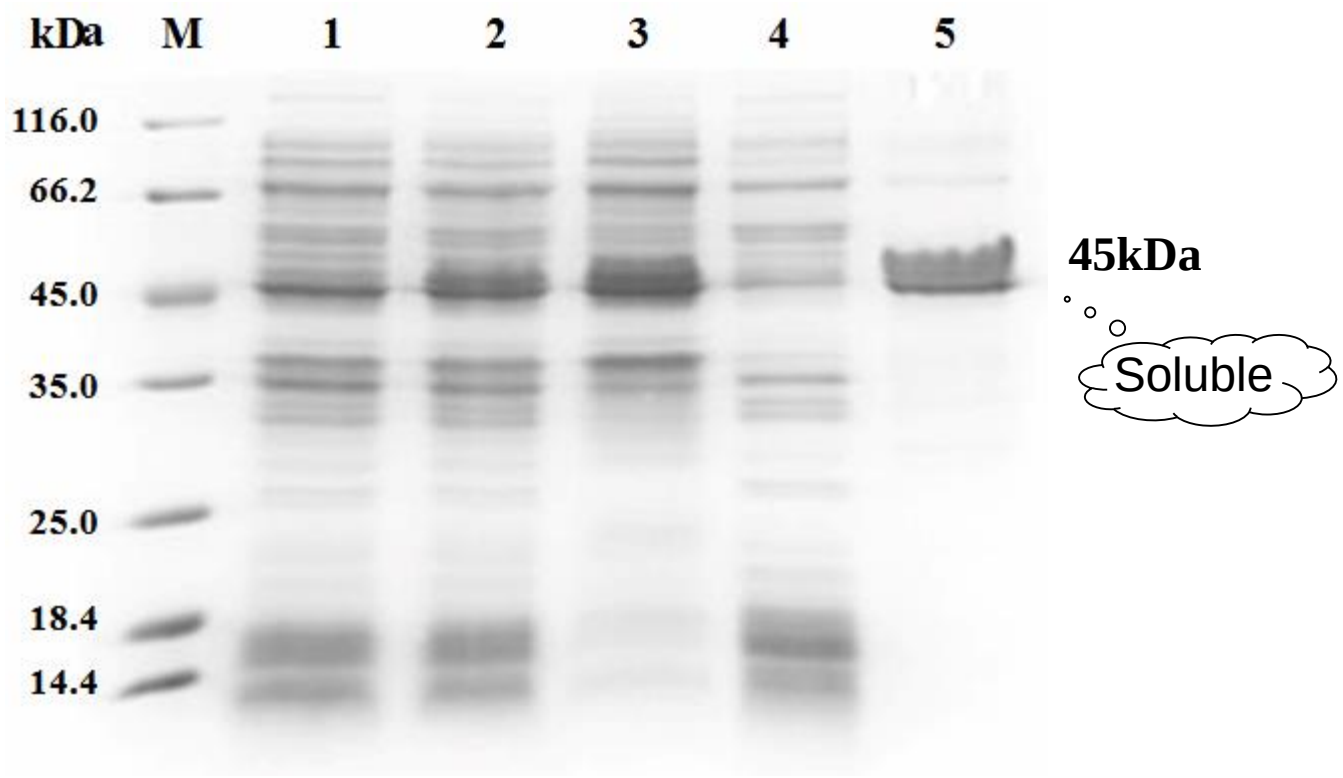


Fig . Lane identification: M: Protein marker (1) IPTG-uninduced E.coli lysate; (2) IPTG-induced E.coli lysate ; (3) supernatant portion of IPTG-induced E.coli lysate; (4) precipitate portion of IPTG-induced E.coli lysate ; (5) purified rCs1.

Comparison of the sensitivity and specificity of rCs1-ELISA and ESA-ELISA

Table 1. Comparison of sensitivity and specificity of rCs1-ELISA and ESA-ELISA

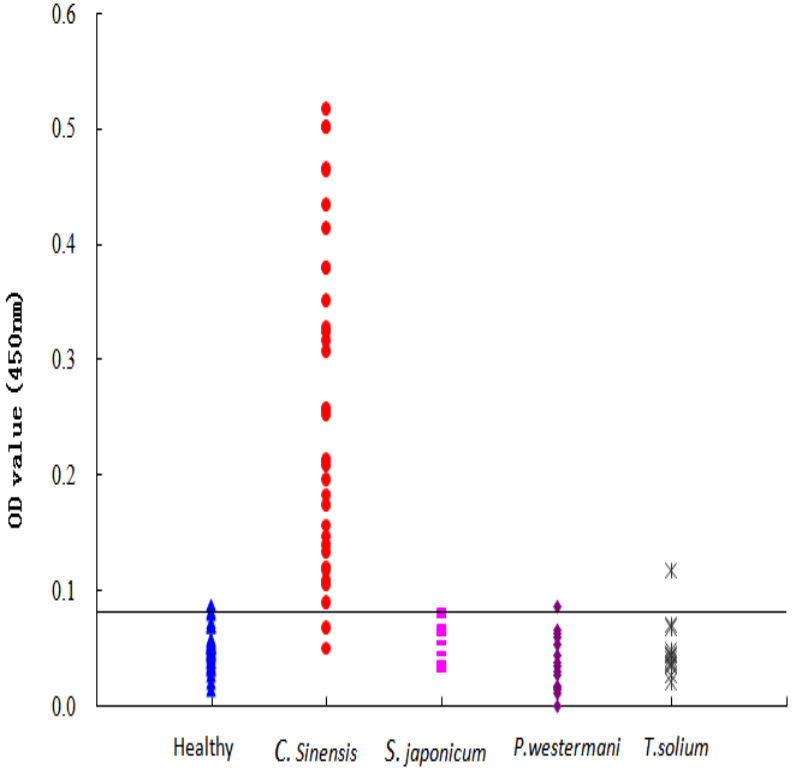
	Sensitivity (%)	Specificity (%)	Total coincidence rate (%)
rCs1-ELISA	94.3	94.9	94.7
ESA-ELISA	88.6	86.1	86.8

Table 2. Comparison of cross-reactivity of rCs1-ELISA and ESA-ELISA

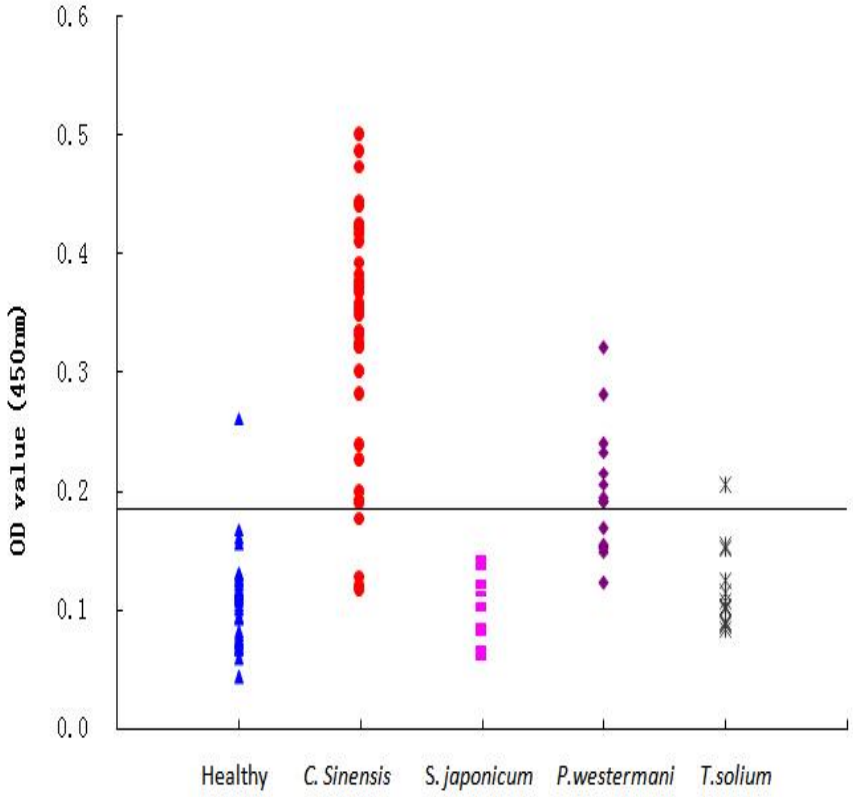
	False-positive rate for S.j sera (%)	False-positive rate for P.w sera (%)	False-positive rate for T.s sera (%)
rCs1-ELISA	6.67	6.67	7.69
ESA-ELISA	0.00	60.00	7.69

*ESA=Excretory-secretory antigen

Comparison of the sensitivity and specificity of rCs1-ELISA and ESA-ELISA



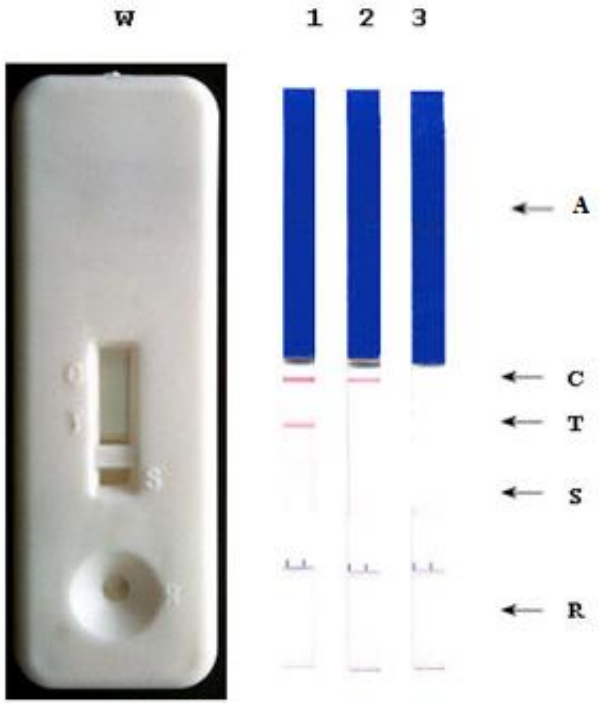
rCs1-ELISA



ESA-ELISA

Lab assessment and field application of the rapid test strip

Structure diagram of the rapid test strip



1: Positive Result; 2: Negative Result; 3. Invalid Result

A: Absorbent Pater; C: Control Line; T: Test Line;

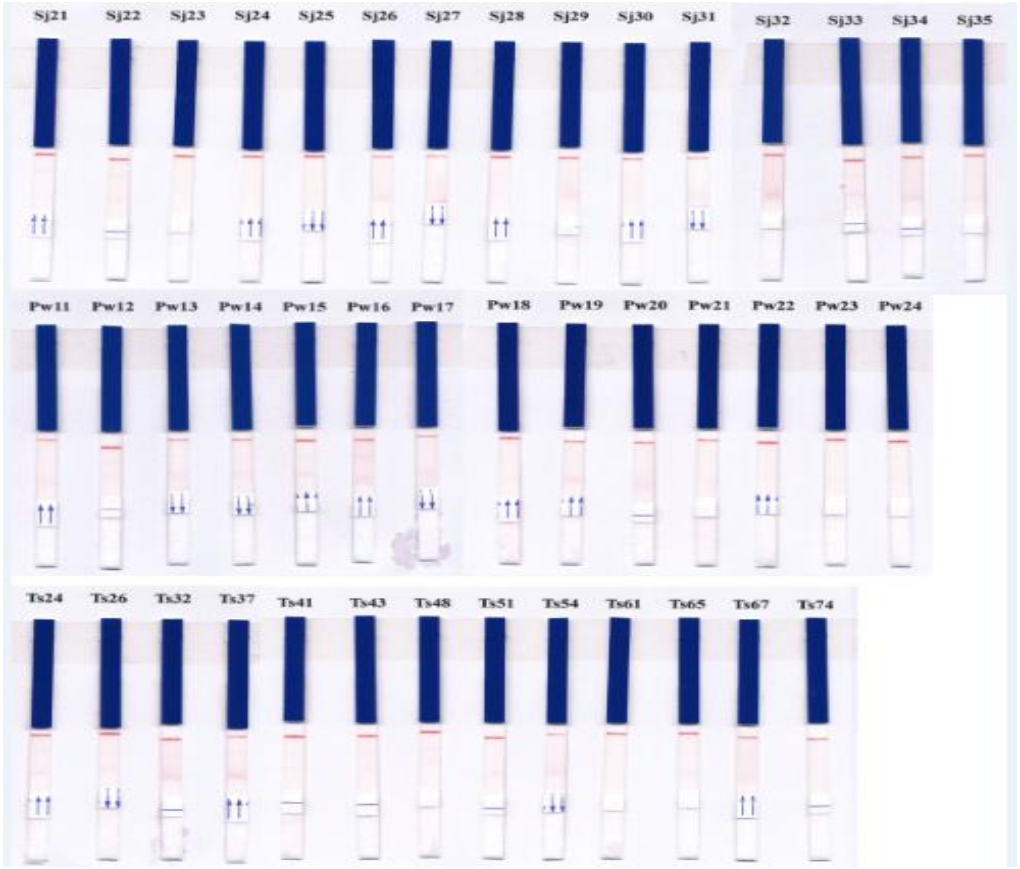
S: Sample Pad; R: Reagent Pad

Sensitivity and specificity of rCs1-GICA (Colloidal-Gold Immunochromatographic Assay)



Upper: *C.sinensis*; Bottom: Healthy control

Cross reactivity of GICA



Upper：*S. japonicum*
Middle：*P. westermani*
Bottom：*T.solium*

Comparison of the sensitivity and specificity of rCs1-GICA and rCs1-ELISA

Table 1. Comparison of sensitivity and specificity of rCs1-GICA and rCs1-ELISA

	Sensitivity (%)	Specificity (%)	Total coincidence rate (%)
rCs1-GICA	91.4	90.6	90.9
rCs1-ELISA	88.6	96.0	93.6

Table 2. Comparison of cross-reactivity of rCs1-GICA and rCs1-ELISA

	False-positive rate for S.j sera (%)	False-positive rate for P.w sera (%)	False-positive rate for T.s sera (%)
rCs1-GICA	13.3	7.1	7.7
rCs1-ELISA	0.00	7.1	7.7

Field Application-1

	Sensitivity (%)	Specificity (%)	Total coincidence rate (%)
Initial	93.5	73.8	82.2
Corrected	96.1	82.1	88.8

Information:

1. **rCs1-GICA:** Minimum Detection Limit of EPG<10

2. **Kato-Katz:** Missed Diagnosis

Field Application-2

	Sera samples	Coincidence rate（%）
Sensitivity	High-infection intensity	100.0
	Moderate-infection intensity	97.5
	Mild-infection intensity	87.6
	total	95.0
Specificity	Healthy control (endemic area)	80.83
	Healthy control (non-endemic area)	96.00
	Other parasites-infected	85.29
	total	85.23

*Infection intensity: Mild, EPG <1000 ; Moderate,1000≤ EPG ＜10000; High, EPG≥10000

SUMMARY

Performance：

Total: **1213** samples

Areas: **Guangdong, Guangxi, Heilongjiang**

Results:

Sensitivity: **94.1%-96.7%**

Specificity: **82.1%-87.78%**

Minimum detection limit: **EPG<10**

CONCLUSION

Clonorchiasis Rapid Test Strip

- **Specimen collection**: serum (10ul）
- **High sensitivity**: 94.1%-96.7%
- **High specificity**: 82.1%-87.78%
- **Minimum detection limit:** EPG<10
- **Rapid:** reaction time<10 minutes
- **Easy-to-perform:** no requirement of specific equipments or professional personnel

ACKNOWLEDGEMENTS

Guangxi CDC & Guangxi Binyang CDC

Guangdong CDC & Guangdong Shunde CDC

Heilongjiang CDC

Department of Soil- and food-borne parasitic diseases, NIPD, China CDC

Consultation and Detection Center for Health Education on Parasitic Disease, NIPD, China CDC

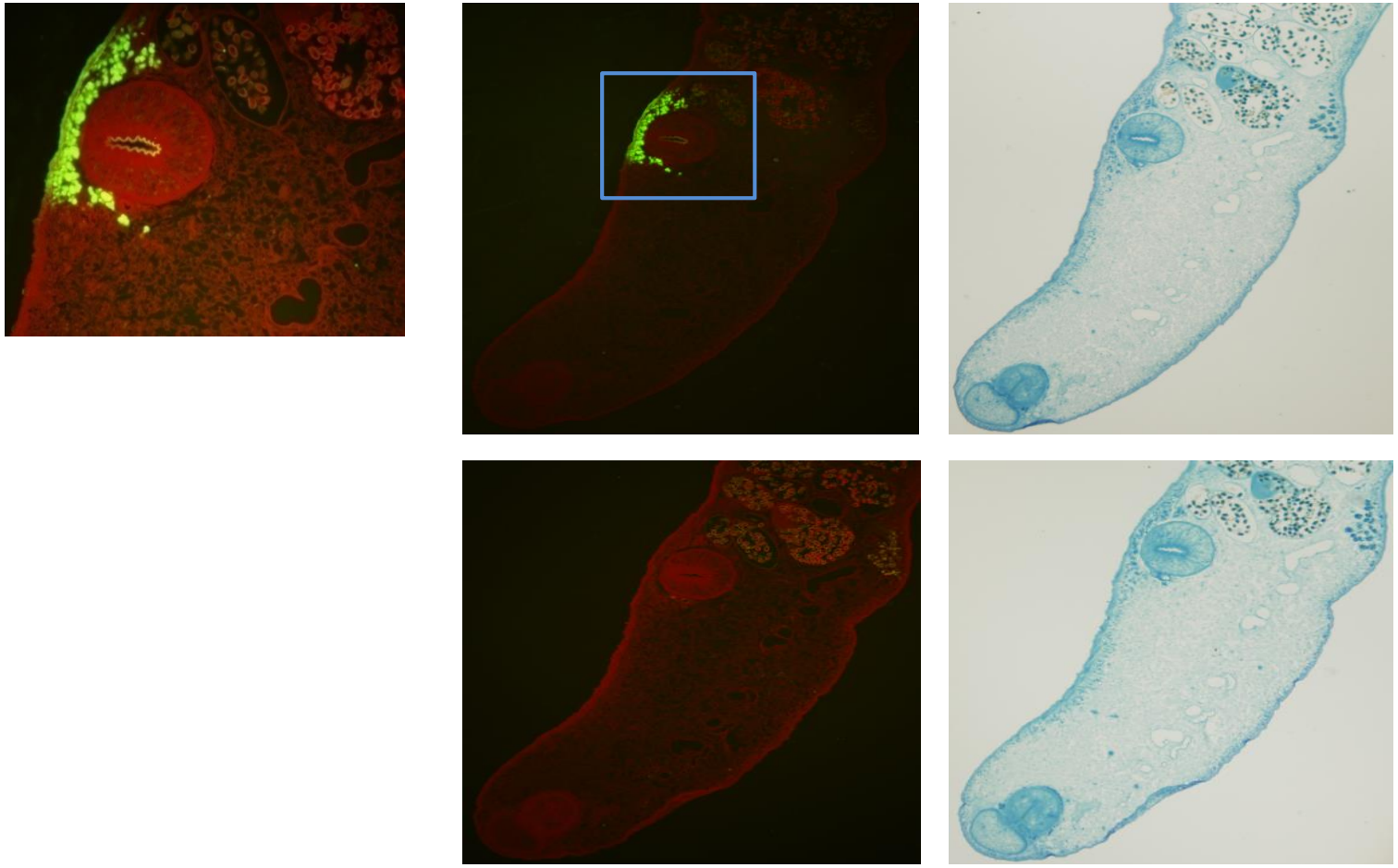
This work was supported by grants from National Science and Technology Major Project of the Ministry of Science and Technology of China (Grant no. No.2008ZX10004-011, No.2009ZX10004-302, 2012ZX10004220).

Thank You!

Part Ⅱ

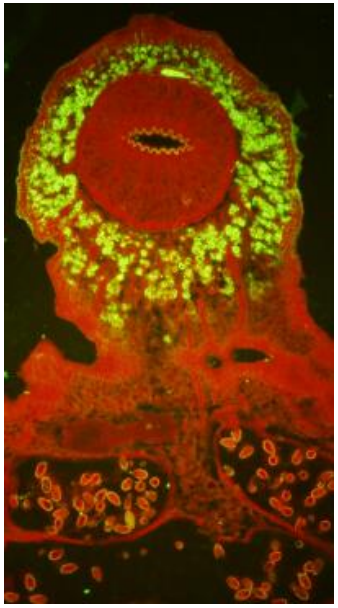
Characterization of Cs1 protein

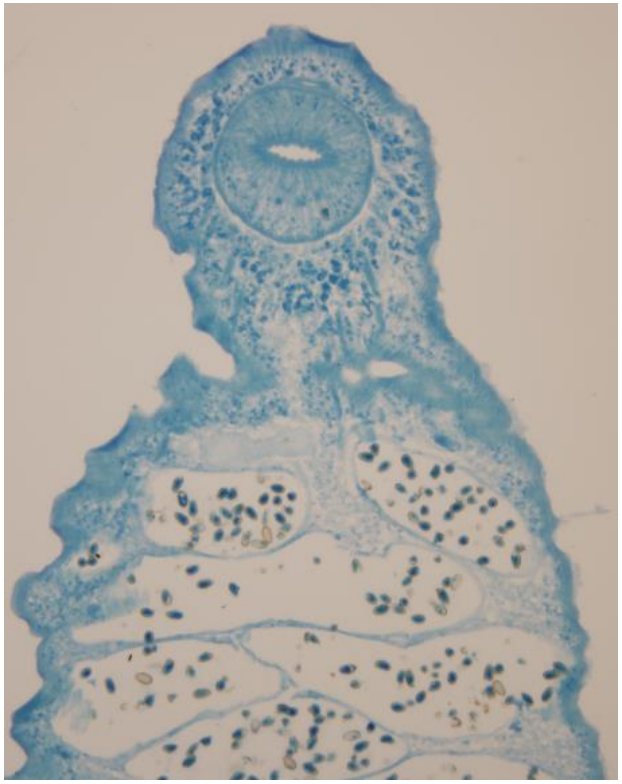
Immunolocalization



Section of *C. sinensis adult worms probed with anti-rCs1 (left panel) or normal mouse serum (right panel). The magnification is* × *40.*

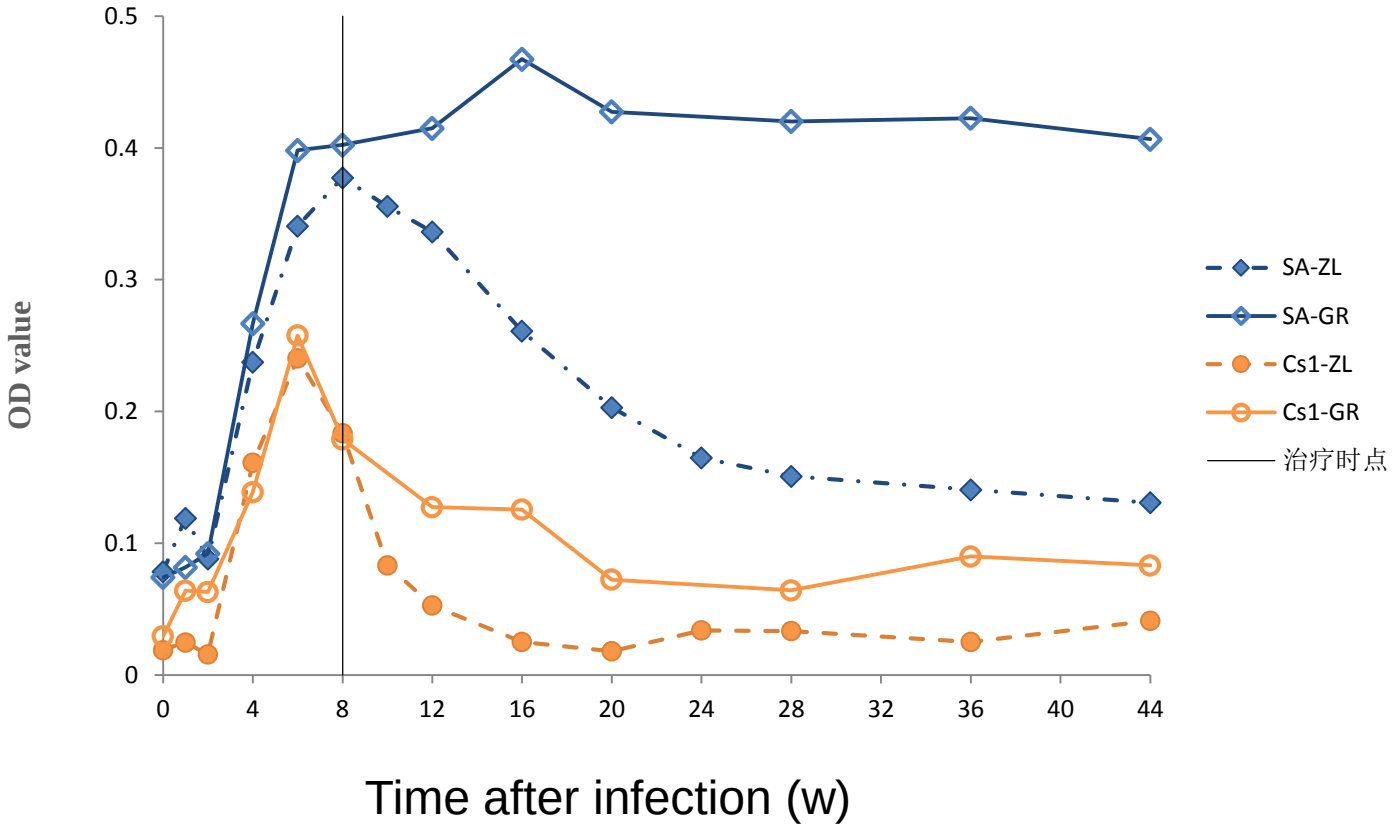
Immunolocalization





Section of *C. sinensis adult worms probed with anti-rCs1 (left) or normal mouse serum (right). The magnification is* × *100.*

Dynamic changes of specific antibody in infected rabbits sera



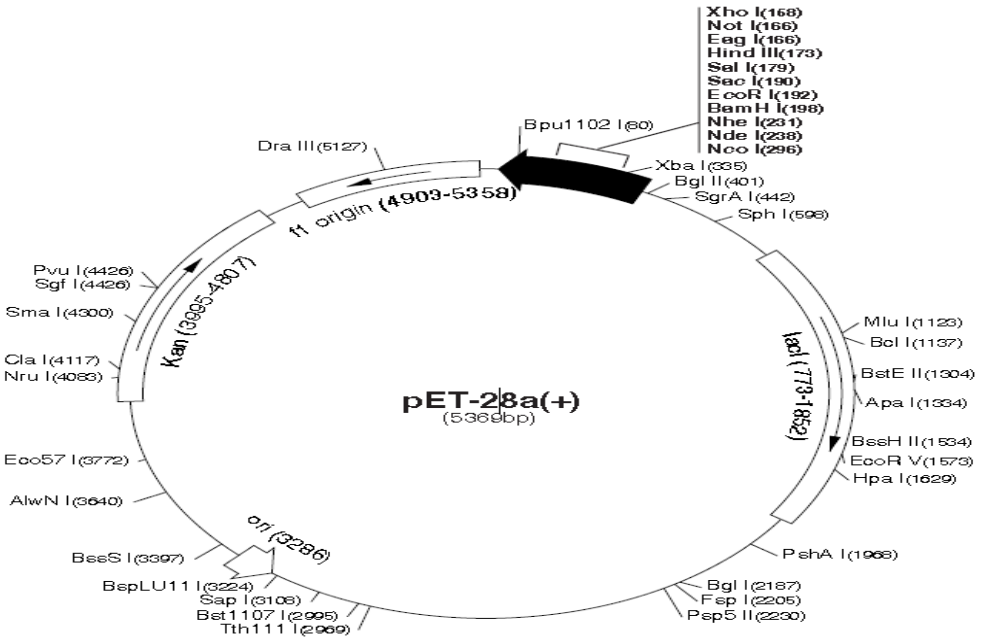
SA-ZL：treatment of soluble antigens immunized group --治疗组；SA-GR：粗抗原-感染组；

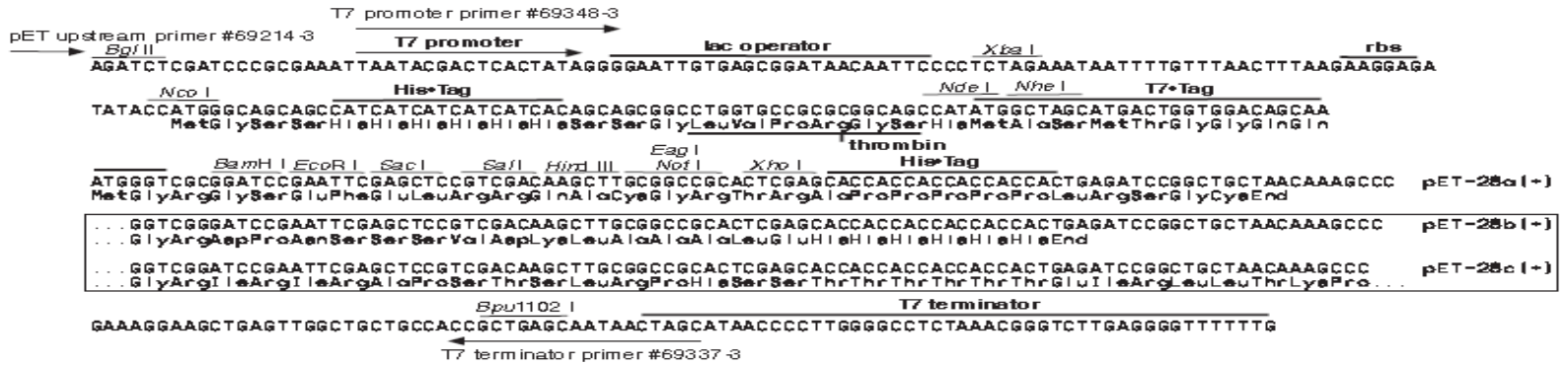
Cs1-ZL：Cs1抗原-治疗组；Cs1-GR：Cs1抗原-感染组

Prokaryotic Expressing System

pET-28a(+) sequence landmarks	
T7 promoter	370-386
T7 transcription start	369
His•Tag coding sequence	270-287
T7•Tag coding sequence	207-239
Multiple cloning sites	
(*Bam*H I - *Xho* I)	158-203
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	773-1852
pBR322 origin	3286
Kan coding sequence	3995-4807
f1 origin	4903-5358

The maps for pET-28b(+) and pET-28c(+) are the same as pET-28a(+) (shown) with the following exceptions: pET-28b(+) is a 5368bp plasmid; subtract 1bp from each site beyond *Bam*H I at 198. pET-28c(+) is a 5367bp plasmid; subtract 2bp from each site beyond *Bam*H I at 198.





pET-28a-c(+) cloning/expression region