



Test development and perspectives for the future of lab diagnosis of angiostrongyliasis

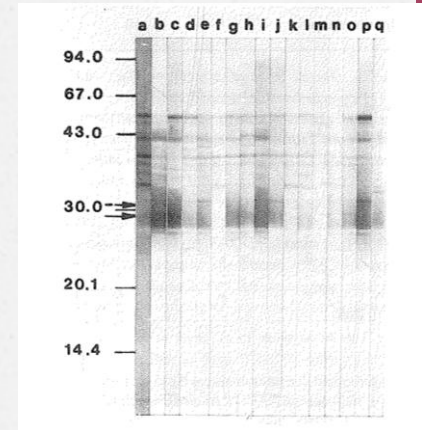
Alessandra L. Morassutti

PUC-RS – Brazil

The 31kDa antigen

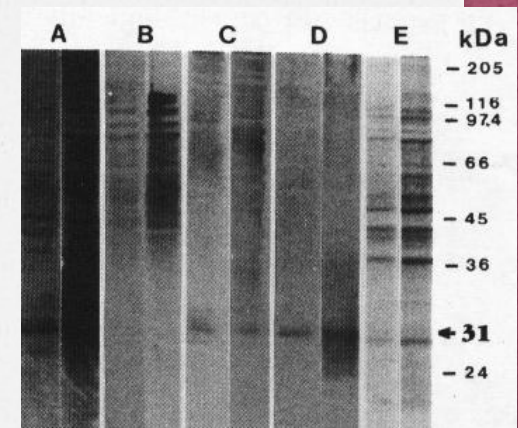
THE EVALUATION OF THE 29 AND 31 kDa ANTIGENS IN FEMALE *ANGIOSTRONGYLUS CANTONENSIS* FOR SERODIAGNOSIS OF HUMAN ANGIOSTRONGYLIASIS

Supaporn Nuamtanong



Characterization of a 31-kda specific antigen from *Parastrongylus cantonensis* (Nematoda: Metastrongylidae)

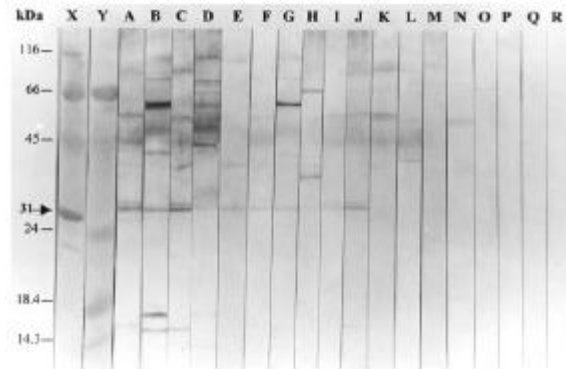
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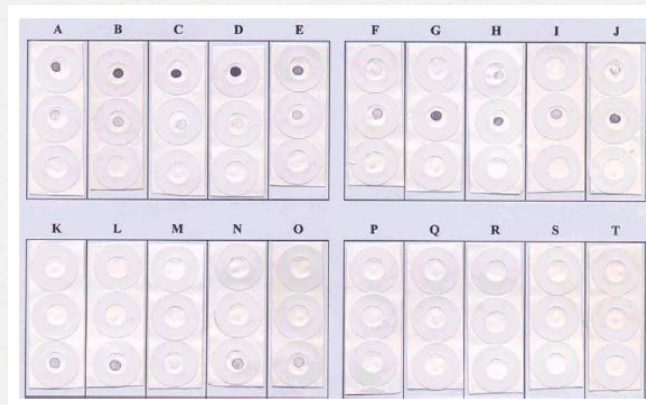
background

Immunological diagnosis of human angiostrongyliasis due to *Angiostrongylus cantonensis* (Nematoda: Angiostrongylidae)

Praphathip Eamsobhana^{a,*}, Hoi Sen Yong^b



o In house kit



Antigen preparation is dependent of:

- o Animals maint
- o Worm antigen
- o Time consumi
- o Low yield of pu
- o Laboratory inf

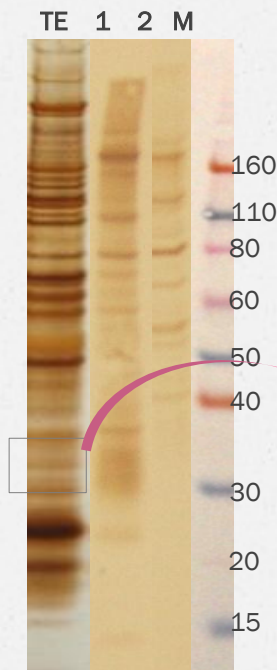
Goals :

- o Antigen available to all laboratories around the world;
- o Accuracy on the antigen preparation;
- o Reproducibility;

Goal

- o identify the 31kDa antigen for recombinant protein production
- o Distribute the antigen to all laboratories for independent analysis

1D SDS-PAGE



TE - silver stained
1- WB positive sera
2- WB negative sera

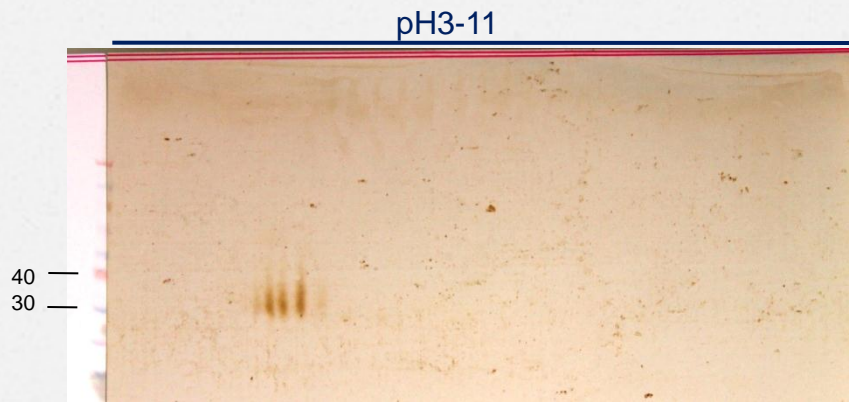
Mass spectrometry

2D eletrophoresis of female worm crude extract

pH 3-11



Identification of the 31 kDa - 2D analyses

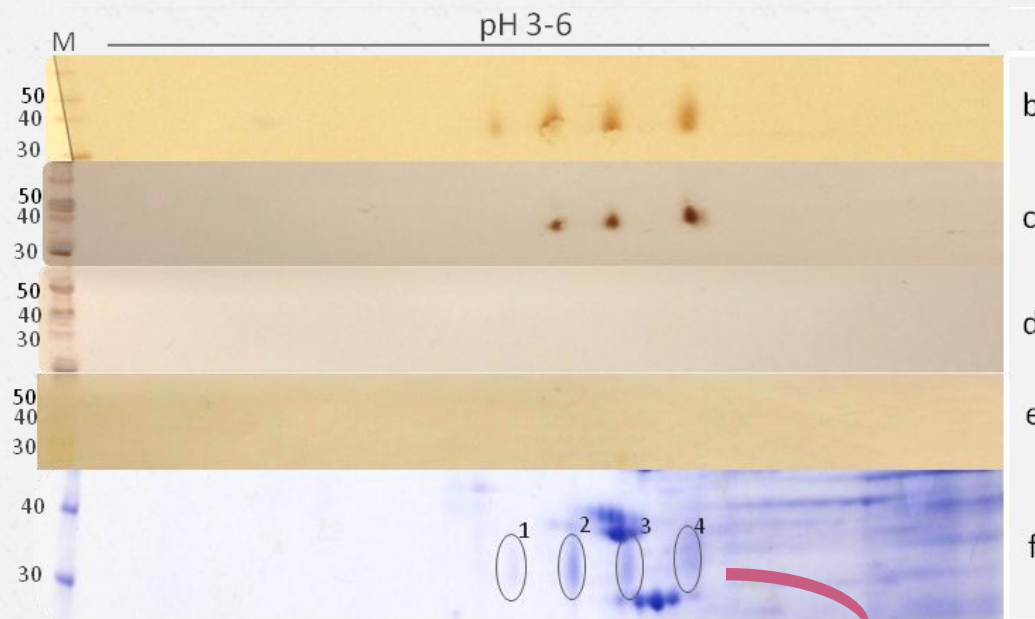


Angiostrongyliasis sera



Normal human

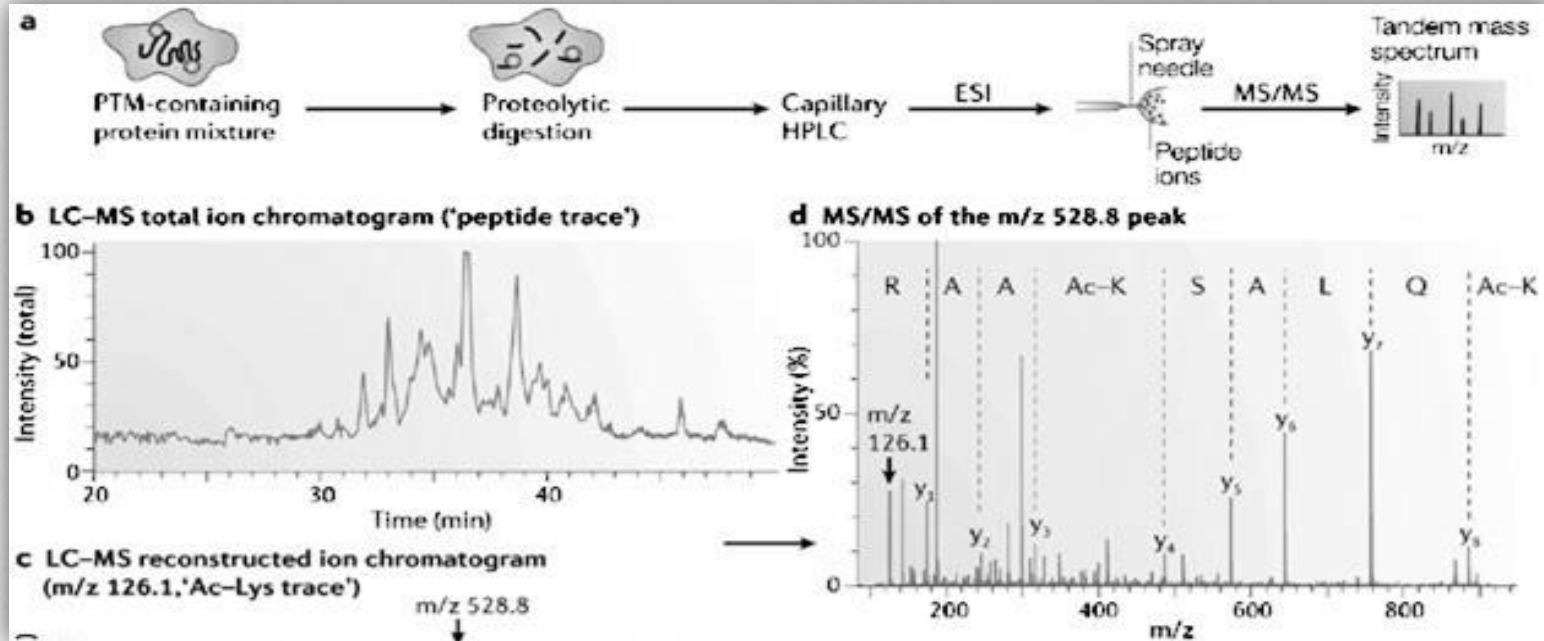
2DE pH 3-6



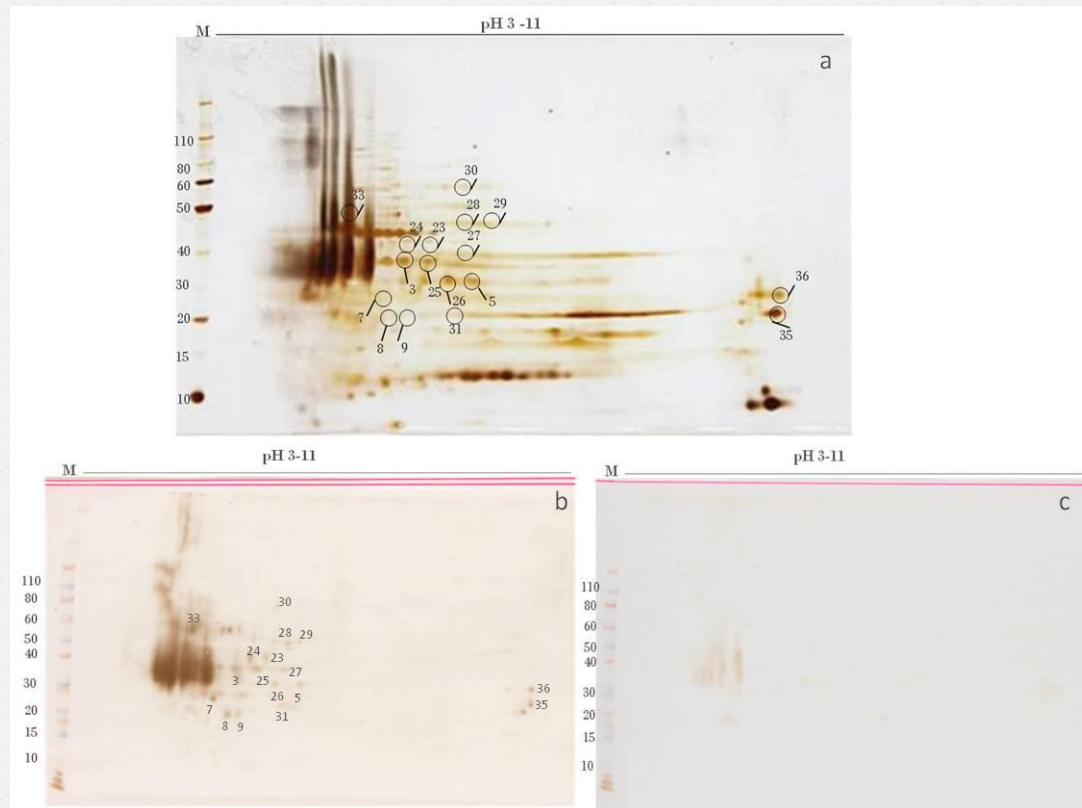
Mass spectrometry

Legend: sera: b- *A.costaricensis*; c- *A. cantonensis*; d normal human; e- *m*-periodate treatment; f- 2DE 3-6 pH Comassie blue stained.

Mass spectrometry scheme



Excretion and Secretion (ES) Proteins as immunodiagnostic targets

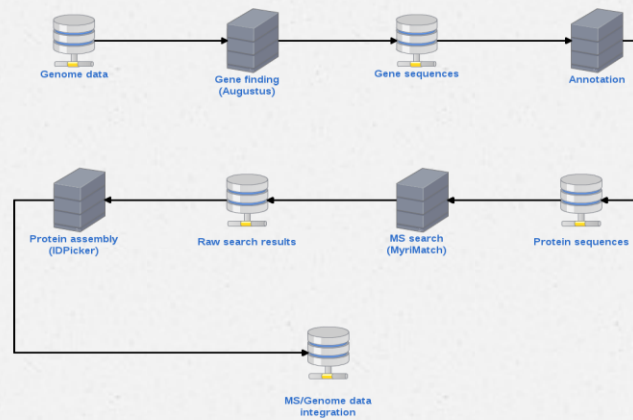


Protein identification

Spot #	Peptide sequence	Protein name (Uniprot)	Organism	Score*
1	K.GDNDFDYEAGEK.V	CBR-PVP-1 protein (28219)	<i>Coccinellidae braggae</i>	86
	As-27			147
5	K.CVPSYK.E K.NDVAAGQK.E R.ICIADSEK.I R.HDLVYFFK.C	cathepsin B-like cysteine proteinase 1 (66715)	<i>Angiostrongylus cantonensis</i>	215
	HSP70			
7	K.IFFGLDK.G K.LYSGTLFVTK.K R.AFVQLPQIEK.L K.GFVDMRPGYVLDQIEK.G	hypothetical protein (21073)	<i>Angiostrongylus cantonensis</i>	171
	R.WLDEWYTAISK.D	heat shock protein 70a (71252)	<i>Foramsia albobrunnea</i>	132
	K.NKTYLQVGGPFAK.I		verrea	116
	R.DLTPSEIEEK.V	Agmatyl protease 190301er (28921)	<i>Paratyphlocyba ornata</i>	68
8	K.LYSGTLFVTK.K R.AFVQLPQIEK.L	hypothetical protein (21073)	<i>Angiostrongylus cantonensis</i>	62
	R.MGQFKNILTR.D K.GAIVSYDQVQIEK.L	Proteasome subunit beta type-1 (28655)	<i>Acanthamoeba castellanii</i>	125
9	K.DDEGQAYR.G	geronitricedonin (21946)	<i>Haemaphysalis concinna</i>	65
22	K.IATEPVE.W K.ALQEMIEK.K K.NFLSVLQGEK.S R.WQADLAAAYVLMR.N	hemoglobin α -type cysteine proteinase (69849)	<i>Angiostrongylus cantonensis</i>	99
	R.HDLVYFFK.C R.GVDECGESQVVGQPK.S	cathepsin B-like cysteine proteinase 1 (66715)	<i>Angiostrongylus cantonensis</i>	79
24	K.AGFAGDQAF.A R.VAFRHPVLLTRAPLNK.A	actin A3 (61845)	<i>Drosophila melanogaster</i>	85
	K.IATEPVE.W K.NFLSVLQGEK.S R.WQADLAAAYVLMR.N	hemoglobin α -type cysteine proteinase (69849)	<i>Angiostrongylus cantonensis</i>	79
	K.CVPSYK.E K.NDVAAGQK.E R.HDLVYFFK.C	cathepsin B-like cysteine proteinase 1 (66715)	<i>Angiostrongylus cantonensis</i>	215

25	K.DLDDIPETFDAR.Q R.GVDECGESQVVGQPK.S K.VQVTLSDQLLSCRR.T			
	R.VAVGHWDEK.T	Serine carboxypeptidase (25652)	<i>Acanthamoeba castellanii</i>	78
26	K.IATEPVE.W K.ALQEMIEK.K K.NFLSVLQGEK.S R.WQADLAAAYVLMR.N	hemoglobin α -type cysteine proteinase (69849)	<i>Angiostrongylus cantonensis</i>	79
	Hemoglobinase			89
27	K.KDAGQVICTAK.N K.APAPPPQGVAR.Q R.DDQVIVVIEK.A K.E K.E K.E	AdT (18922)	<i>Acanthamoeba castellanii</i>	120
	Peroxiredoxin			129
28	K.NFLVSEYVQAGEK.T K.FFIEDDFPVELE.A K.MDATANDVPLFEVLR.G			129
29	K R K			160
	Aldolase			
30	R.ALQASYLKA K.VTRQVLAFTYK.A K.GRLAAGESTGTTGK.R	Fructose-bisphosphate aldolase 2 (28122)	<i>Coccinellidae elegans</i>	129
	K.IETVELSYQVLA K.DADLPLPSEK.F R.SRPPK.A K.PQVFAEK.V R.LFVYQGEK.I R.VQDILVLR.E	Aldolase (29673)	<i>Haemaphysalis concinna</i>	88
31	R.SRPPK.A K.PQVFAEK.V R.LFVYQGEK.I R.VQDILVLR.E	Galactin (CBR-LEC-5) (26555)	<i>Coccinellidae braggae</i>	108
32	R.VGPGGVYFQK.E R.ASAANDPHEISDFSEK.F	putative Formin protein 2 (6892)	<i>Angiostrongylus cantonensis</i>	90
33	K.VTRQVLAFTYK.A	hypothetical protein P01F1.12 (28822)	<i>Coccinellidae elegans</i>	96

Sequence achievement



perspectives

- o Large scale recombinant protein production;
- o Preliminary tests of specificity and sensitivity;
- o Distribution of the recombinants;
 - o Standardization of the antigens by independent evaluations;



Pontifícia
Universidade
Católica do RS



Bioinformatics' team

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APHL
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