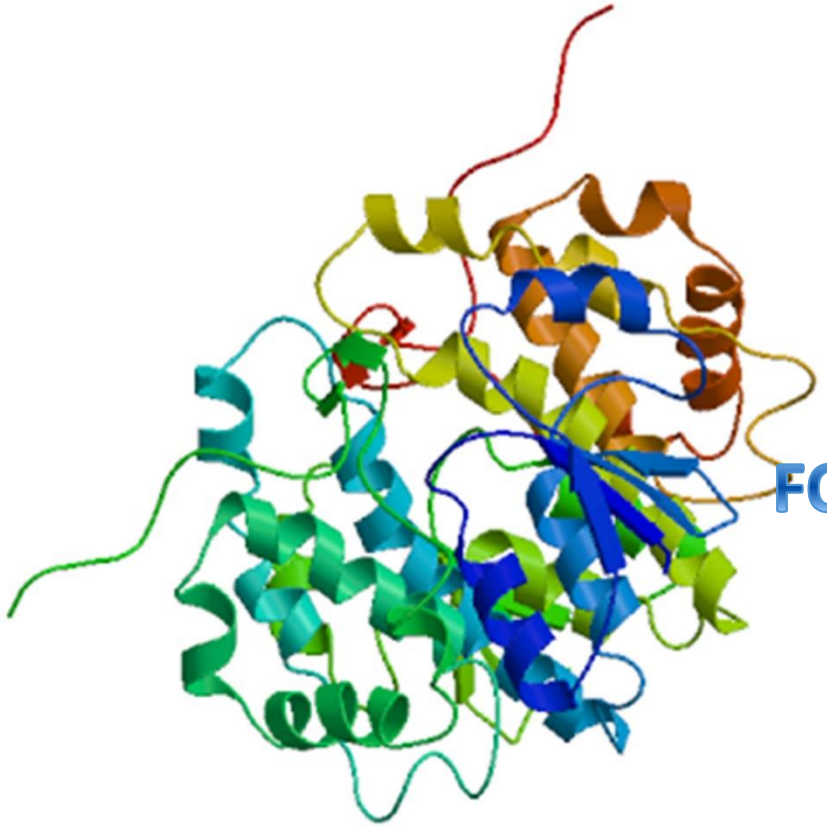

A NEW RECOMBINANT DIAGNOSTIC POLYPEPTIDE (RecDiPol) FOR THE SERODIAGNOSIS OF CYSTIC ECHINOCOCCOSIS

Predicted 3D structure of RecDiPol
https://swissmodel.expasy.org/interactive/qFVFL8/models/

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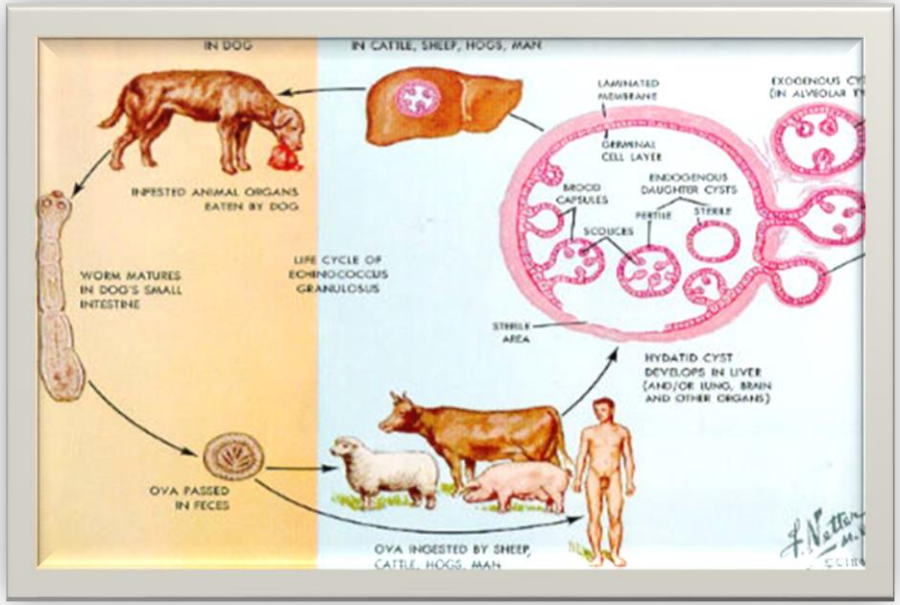
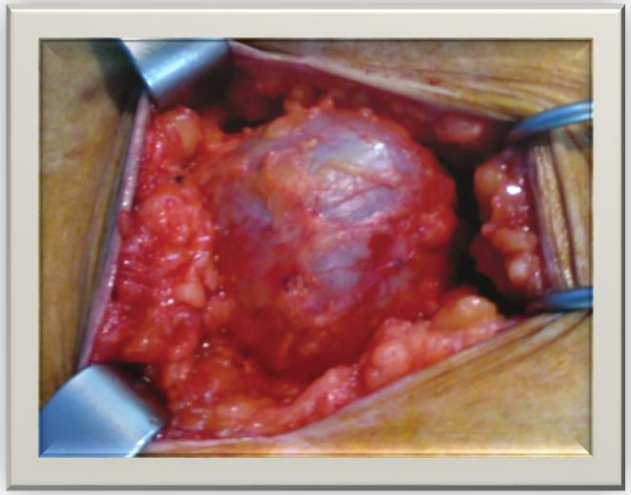
- **Human cystic echinococcus (CE) is a severe disease caused by the larval stage of the zoonotic *Echinococcus granulosus***
- **This infection is still one of the most important problems for issues relating to public health causing morbidity and mortality affecting 2-3 million people worlwide**





- Serodiagnosis is used as secondary confirmatory tests for CE infection

- So far, performance of currently available immunodiagnostic tests mainly based on HF, AgB and Ag5, yet their recombinant,are unsatisfactory !!!

- Mainly reasons that Cysts stage/location/number/dimension and different antigenic component of *E. granulosus*

Good way to improve the sensitivity and spesifity is
"more than one defined immunodominant antigen".
To this end, we desinged a new Recombinant Diagnostic
Polypeptide (RecDiPol)

DESINGING and CLONING

Choose a pGEX4T3

Prepare Insert DNA

-Diagnostic epitopes of AgB1 AgB2 and Ag5 were chosen by two tools

-PCR Amplified with the desired restriction sites

-Restriction digest-gel-purify

- the GenScript on line service;
  http://www.genscript.com/antigen-design.html
- the SVMTriP on line prediction tool;
  http://sysbio.unl.edu/SVMTriP/prediction.php

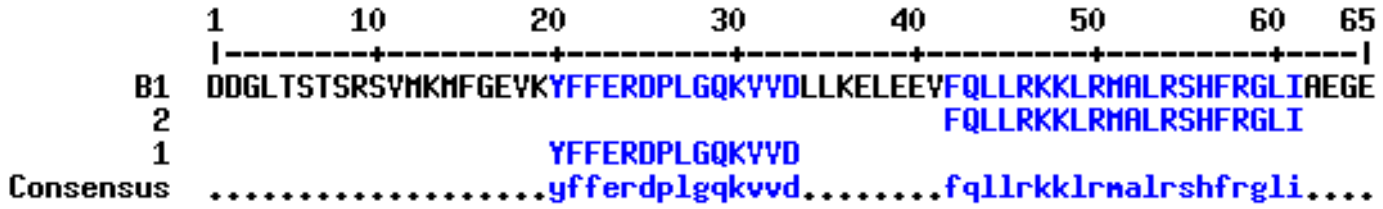
Sequential cloning

Ligation

- Transform into *E. coli* expressing strain (BL21 cells)

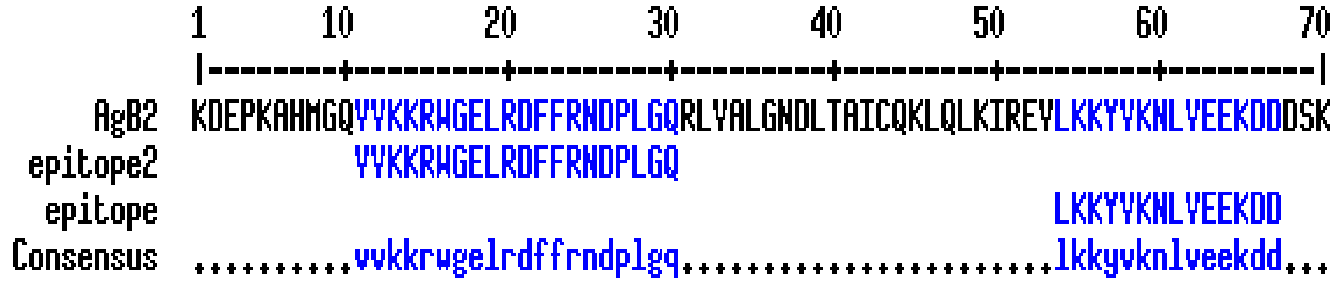
-The expression construct ;checked by PCR,restriction digestion,sequ.

ANTIGEN B1:



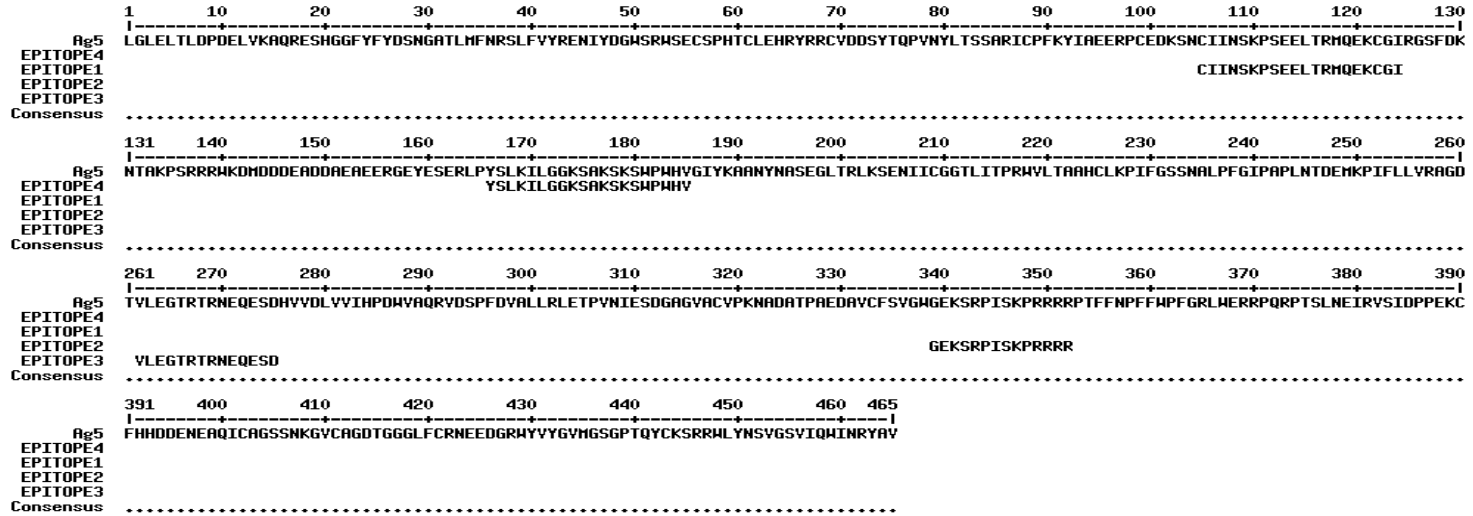
YFFERDPLGQKVVDLLKELEEVFQLLRKKLRMALRSHFRGLI

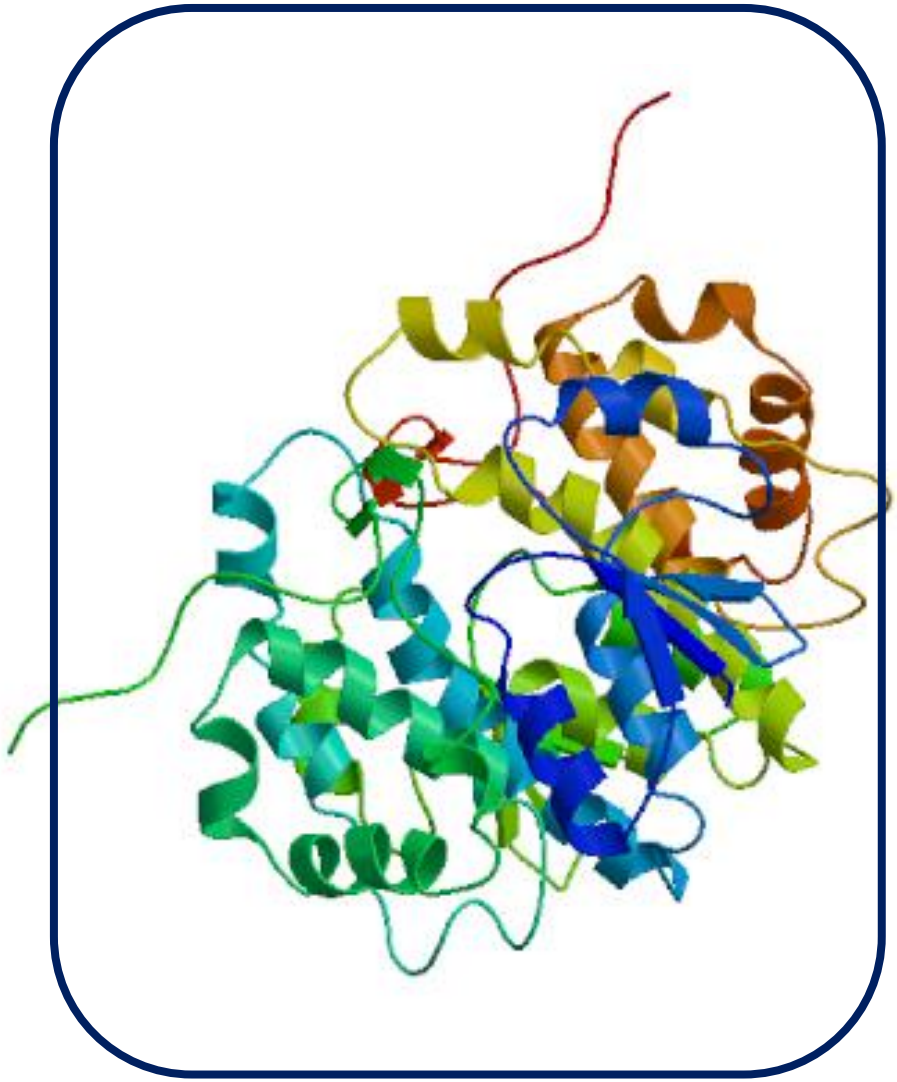
ANTIGEN B2:



VVKKRWGELRDFFRNDPLGQRLVALGNDLTAICQKLQLKIREVLKKYVKNLVEEKDD

ANTIGEN Ag5:



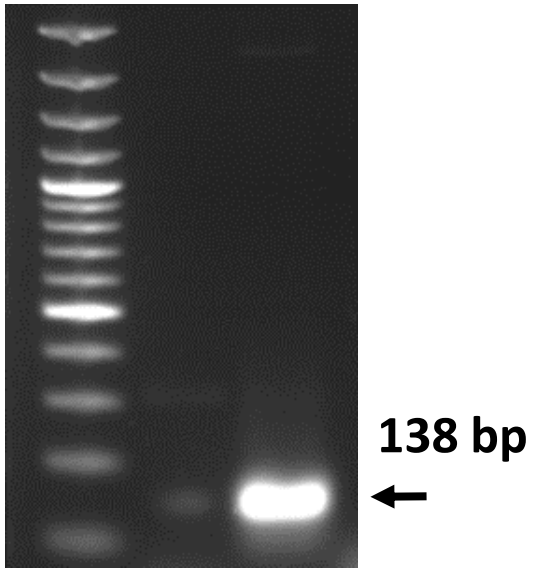


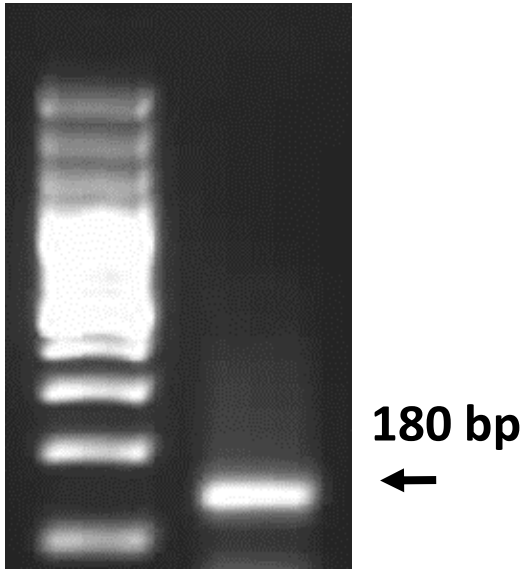
Predicted 3D structure of RecDiPol
https://swissmodel.expasy.org/interactive/qFVFL8/models/

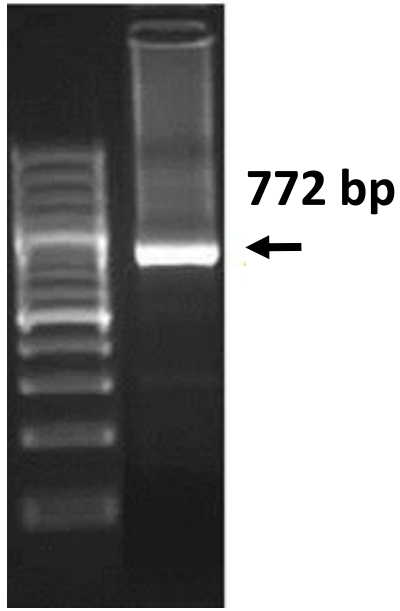
Length of combine selected epitopes with desired enzymes side;

- AgB1(*Bam*HI- *Eco*RI) ;138 bp
- AgB2(EcoRI- SalI); 180 bp
- Ag5(*Not*I- *Not*I) ;772 bp

Diagnostic epitopes of AgB1, AgB2 and Ag5 were PCR Amplified with the desired restriction sites

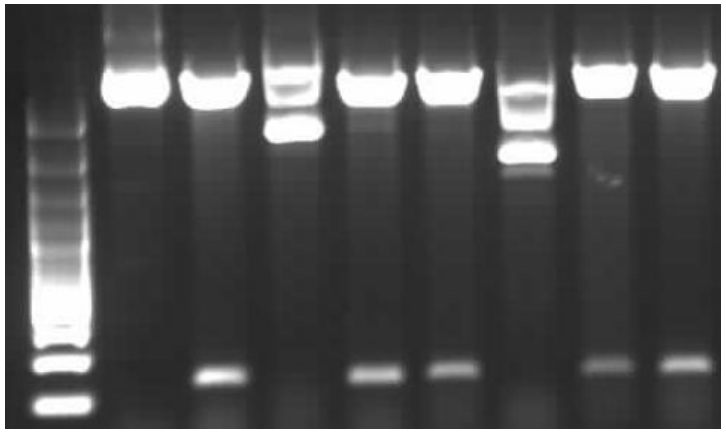






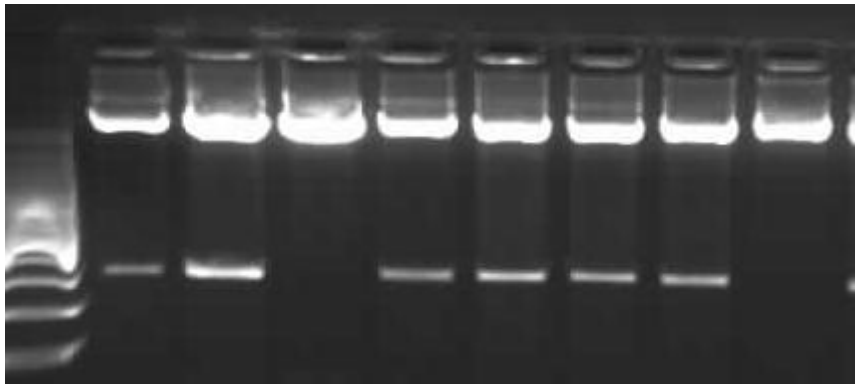
These inserts were inserted to pGEX4T3 one by one then checked by restriction digestion

1



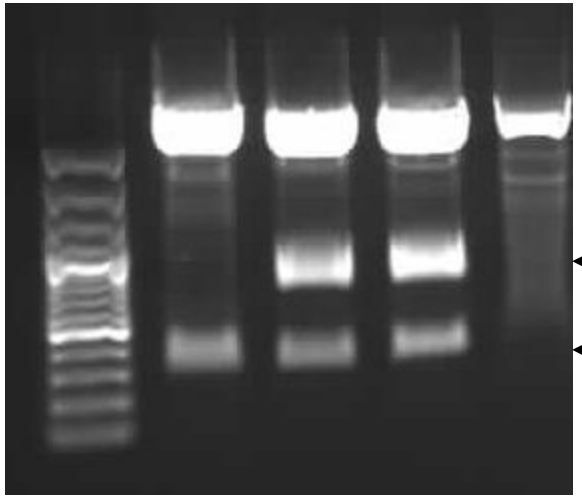
AgB1 after digestion with *Bam*HI and *EcoR*I

2



AgB1+AgB2 after digestion with *Bam*HI and *Sal*I

3



AgB1+AgB2 and Ag5 after digestion with *Bam*HI and *Not*I

EKSPRESSİON

Transform into Expression Host(BL21)

-Transform host carrying T7 RNA polymerase gene

-İncubation at 37 ºC(untill DO= 0.7-0.8),then cooled down at 15ºC

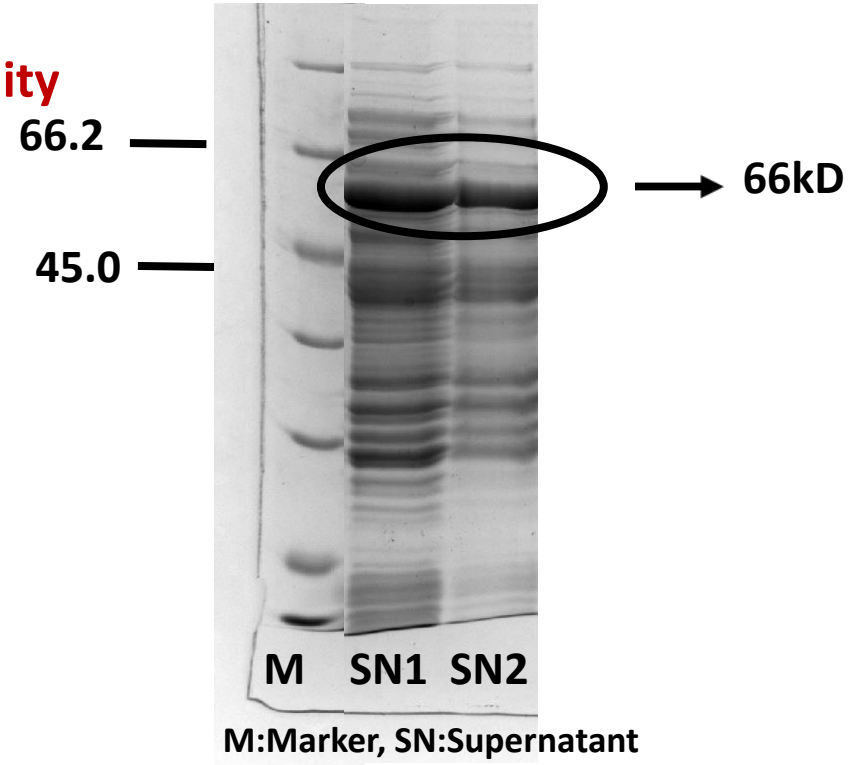
⬇

Induce and Optimize Expression of Target Protein

-induction with IPTG (1mM)(incubate for 3 hour at 37ºC)

-Analyze solubility and activity of target protein by SDS-PAGE

SDS_PAGE image for analyzing solubility and activity



M:Marker, SN:Supernatant

PURİFİCATİON

Scale-up culture size and Extract Target Protein

Mechanical methods

⬇

Purify Target Protein

Affinity purification of (GST) fusion

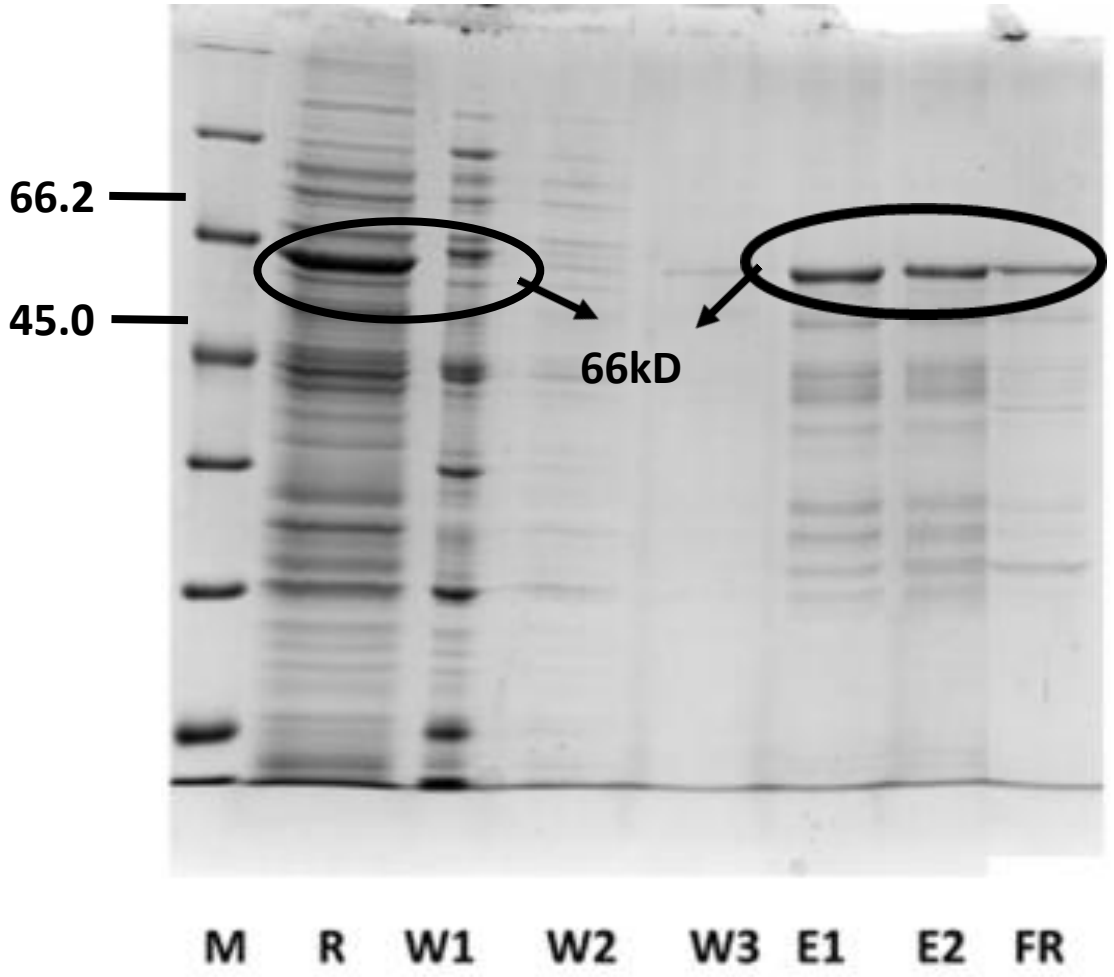
Protein using Glutathione Sepharose B resin

⬇

The purity of the recombinant;
checked by SDS-PAGE
The concentration was estimated by comparison with a BSA gradient and ChemiDoc™ MP analysis

recDipol : 400 ug/L

SDS-PAGE image of reDiPol with GST



M:Marker, R:Resin , W1:Wash1, W2: Wash 2
E1:Elution1 , E2:Elution2 , FR:Final resin

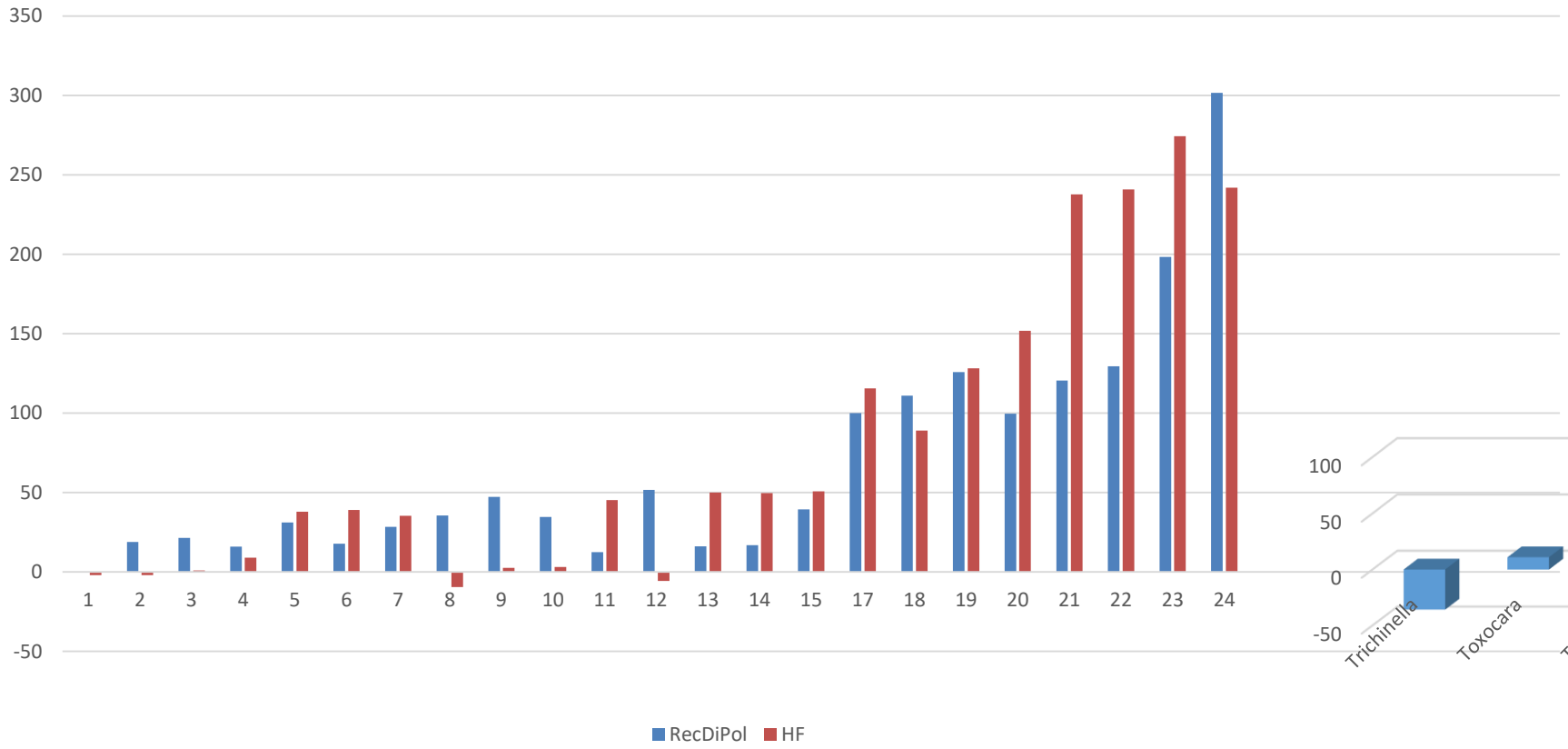
The preliminary integrity of the purified protein as a diagnostic marker has been evaluated by ELISA assay

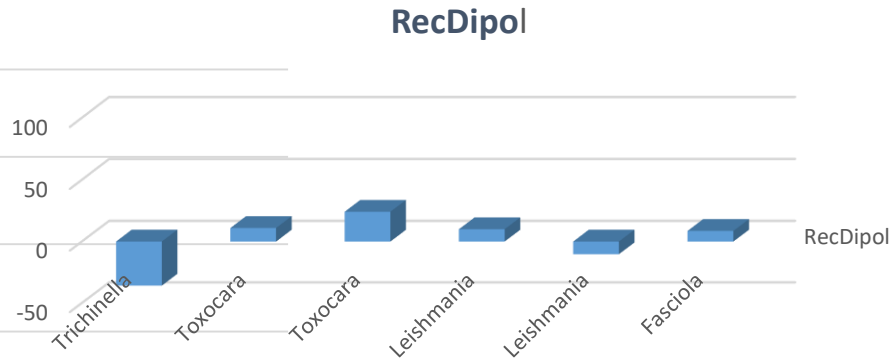
- **8 cases of cystic echinococcosis (operated and previosly checked with HF)**
- **8 healthy individuals (previosly checked with HF and IHA),8 Doubtful sera**
- **6 cases of other parasitic diseases (previosly diagnosed)**

GENERAL ELISA PROTOCOL

- **Plates were coated with recDiPol at 0.4 ug/ml in PBS overnight**
- **blocked with 5% casein buffer at 37 C for 1 hr.**
- **Sera were diluted 1:100 in 5% casein buffer**
- **Plates with diluted sera were incubated in duplicate wells at 37 C for 1 hr and then washed three times with PBST.**
- **Anti-human IgG konjuge(ALP) goat was diluted at 1:5000 in PBS buffer and incubated at 37 C for 1 hour. Subsrat ρ-Nitrophenyl phosphate (pNPP)**
- **The optical density at 405 nm was evaluated with an ELISA reader** The cut-off point was determined as the mean optical density plus 3 times standard deviation for a panel of serum samples obtained from healthy donors

Preliminary Comparison of RecDipol and HF Serological Index



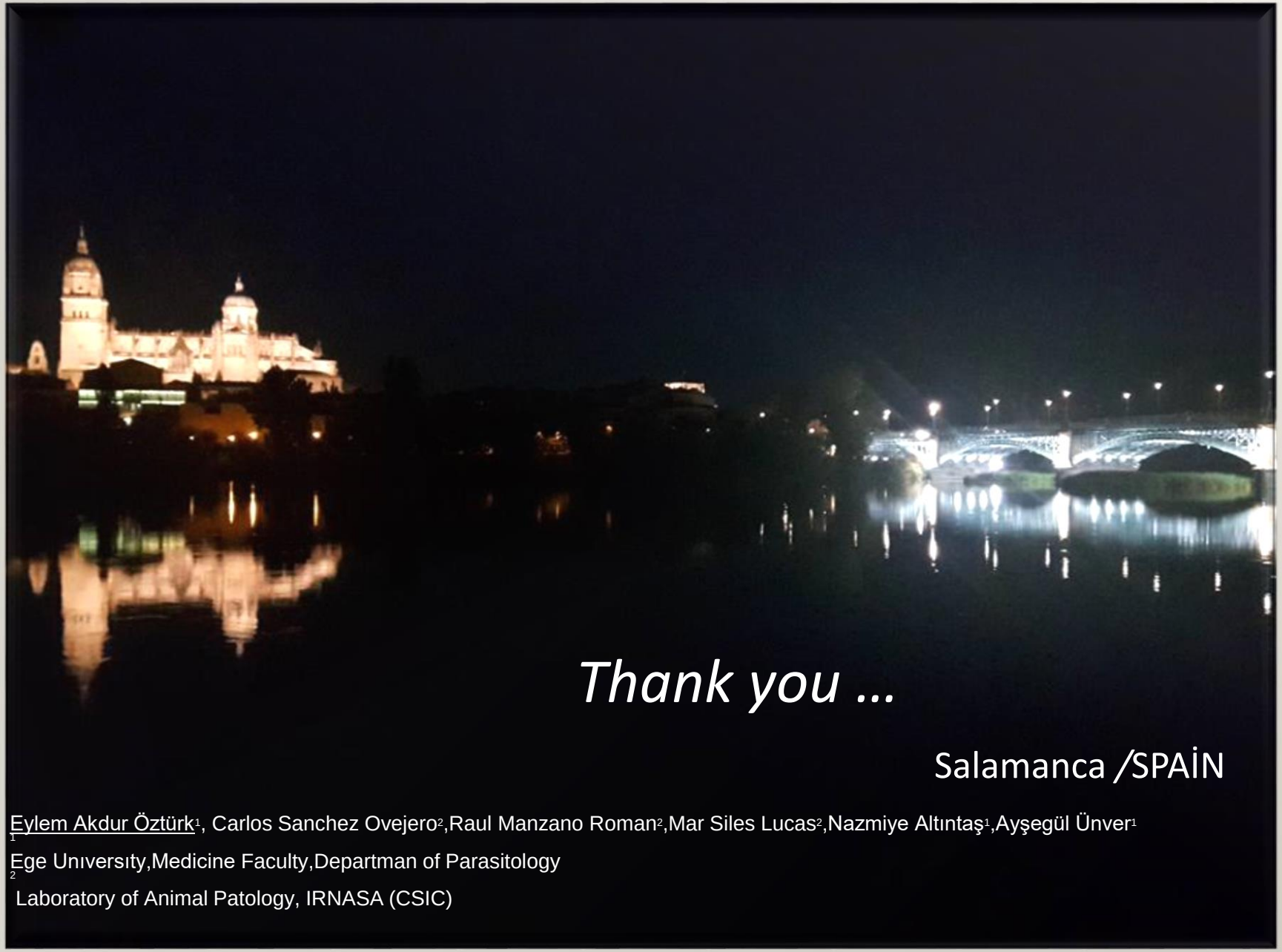


Therefore for a more successful evaluation; need to more well characterized sera ,clinical / radiological data of the CE patients due to the reasons that I mentioned in the introduction(cyst stage,cyst number,cyst location etc)

- In conclusion; according to the results of conventional serological methods, one antigen is unable to detect the antiserum belonging to different serovars and not incorporate high degrees of sensitivity and specificity

- In this content, we have designed new multiepitope recombinant peptide antigen (named recDiPol) to improve CE serodiagnosis

- Preliminary results of Elisa suggested that the performance of the generated RecDiPol has promising potential in serodiagnosis of CE



Thank you …

Salamanca /SPAİN

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