Proteomics Course

LECTURE-18
Applications of two dimensional electrophoresis

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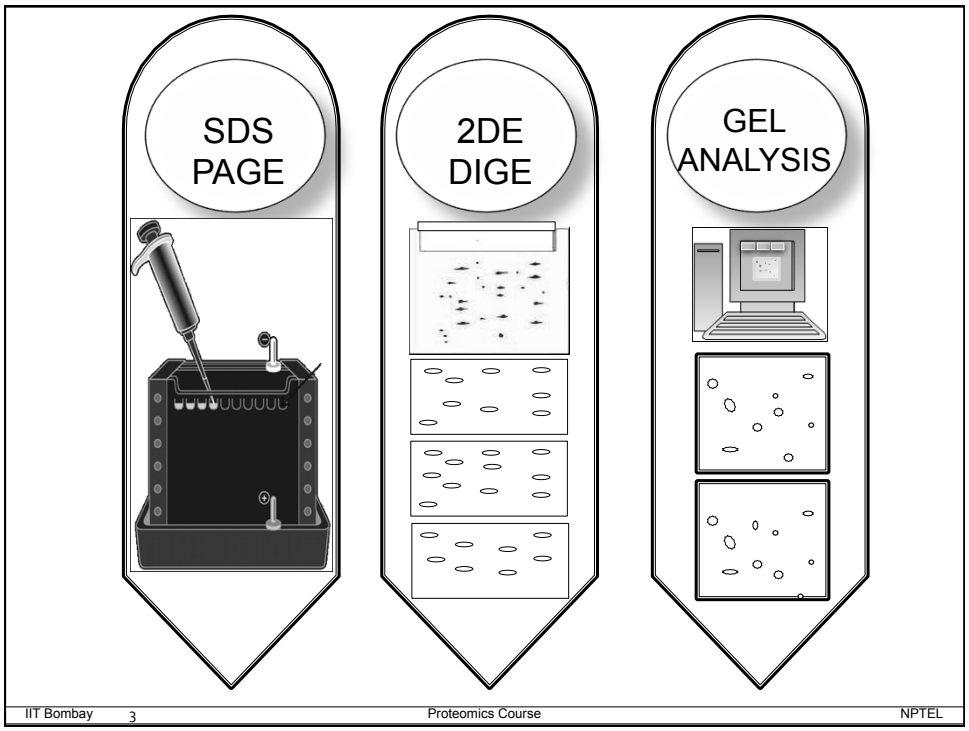
IIT Bombay

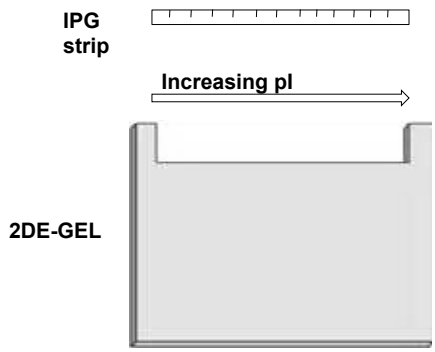
Lecture outline

- An overview of 2DE technique
- Applications
  - Case study – 1
  - Case study – 2



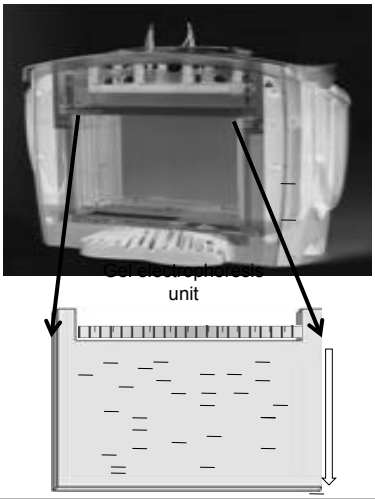
2DE overview: First dimension

Isoelectric focusing

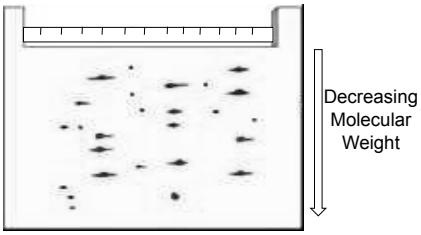


2DE overview: Second dimension

Molecular weight



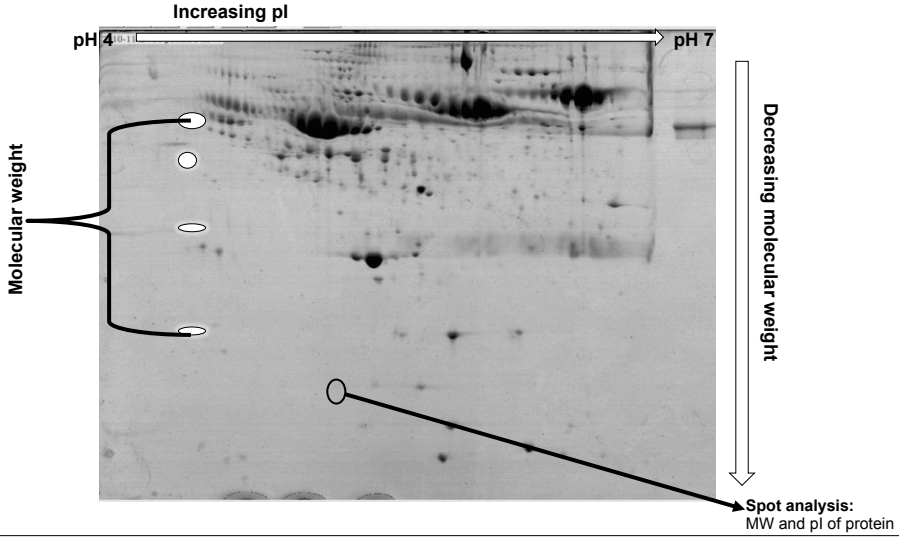
SDS-Polyacrylamide Gel



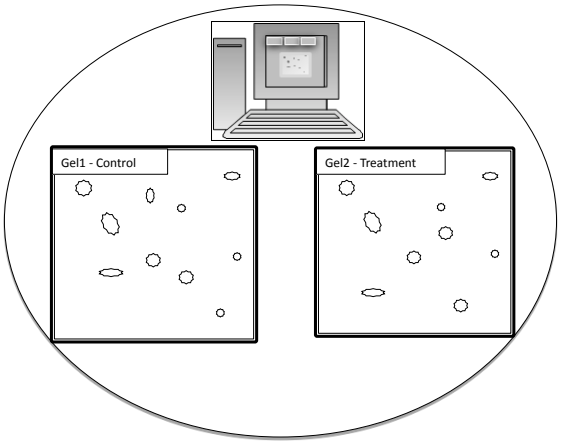
Second Dimension:
SDS-PAGE

2DE overview: Representative 2D Gel

A6: Staining



2DE overview: Data Analysis



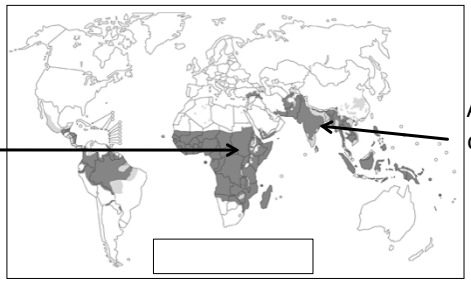
Case study-1

Serum proteome analysis of vivax malaria: An insight into disease pathogenesis and host immune response

Ray et al. Serum proteome analysis of vivax malaria: An insight into the disease pathogenesis and host immune response. J Proteomics. 2011. PMID: 22086083

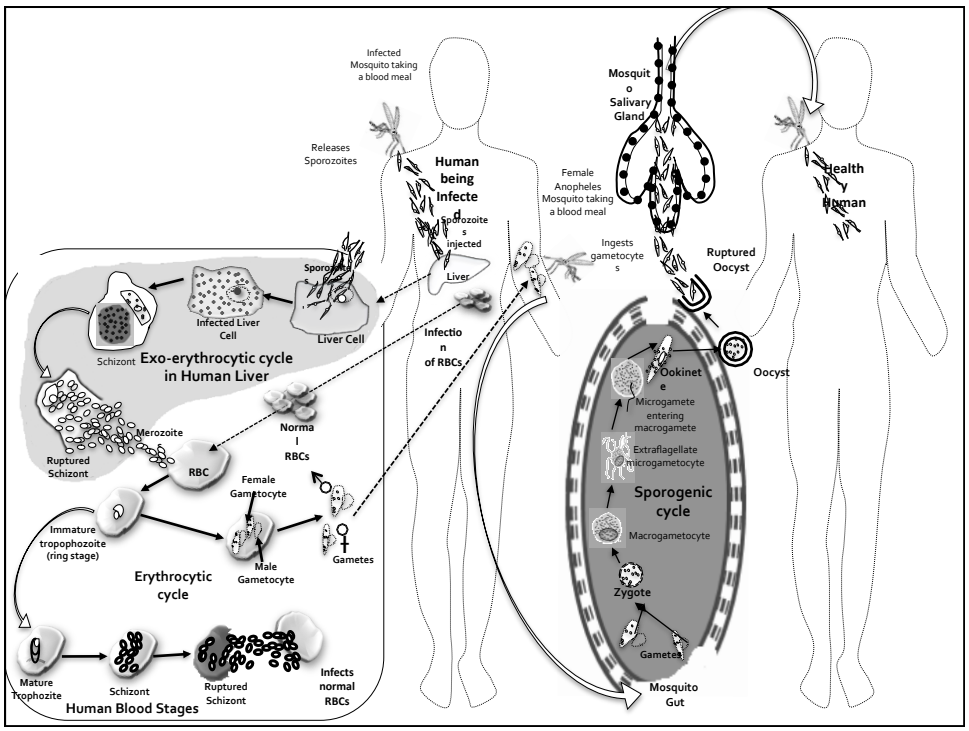
Malaria – a global view

AFRICA – single largest cause of death



ASIA – challenge of drug resistant strains

- Malaria - an epidemic in 103 countries around the globe
- Incidence of malaria worldwide ~300-500 million/ per year and death between 1.1-2.7 million people each year
- *P. vivax* & *P. falciparum* account for 95% of malaria worldwide

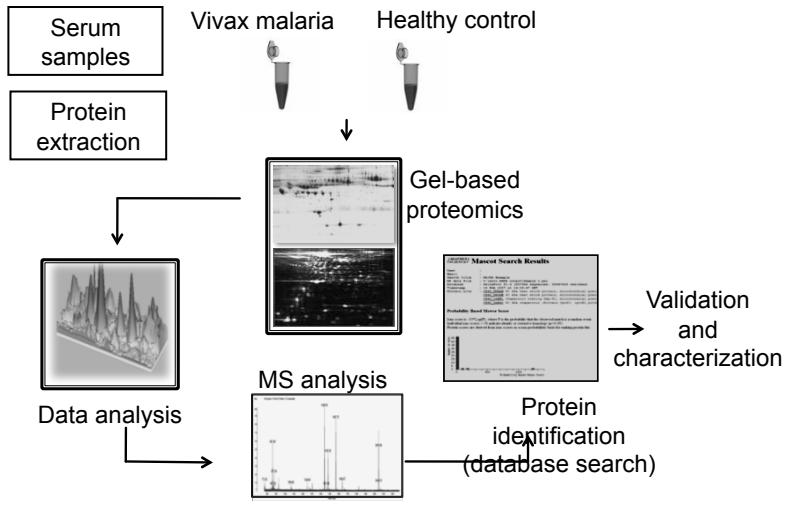


Plasmodium species which infect human

Species	Type of malaria
Plasmodium vivax	***Benign Tertian Malaria***
Plasmodium falciparum	***Malignant Tertian Malaria***
Plasmodium ovale	***Ovale Tertian Malaria***
Plasmodium malariae	***Quartan Malaria***

- *P. knowlesi* can also cause acute, severe illness but mortality rates are low

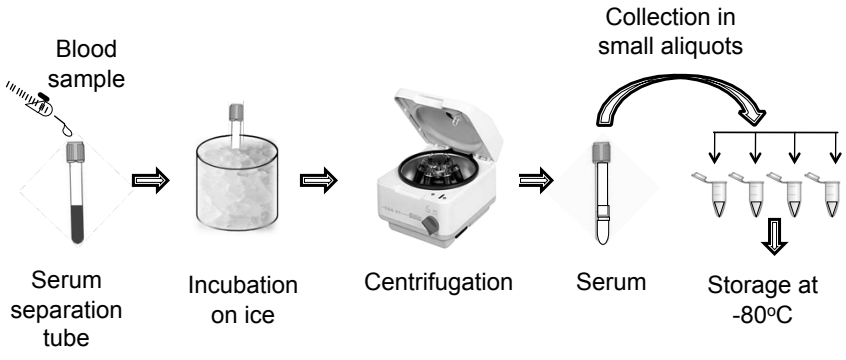
An overview of workflow



Sample collection

- Patient information
  - Age, Sex, Physiological status, Alcoholic patients
  - Previous history of diseases
  - Treatment [If already treated; treatment information]
- Selection of healthy control
- Pooled versus individual sample
- Process of sample collection
- Sample storage
- Reproducibility

Schematics of serum collection, handling and storage



Serum collection

5 ml blood collected into butter fly syringe
(kept on ice until the isolation of serum)

⇩

Allowed to clot for 1 h

⇩

Centrifuged at 2500 rpm at 20°C, 10 min

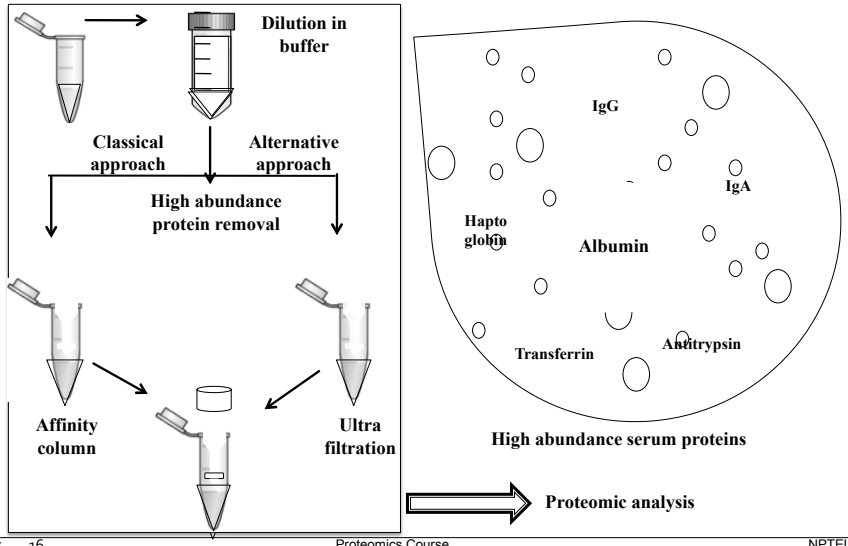
⇩

Serum was collected immediately

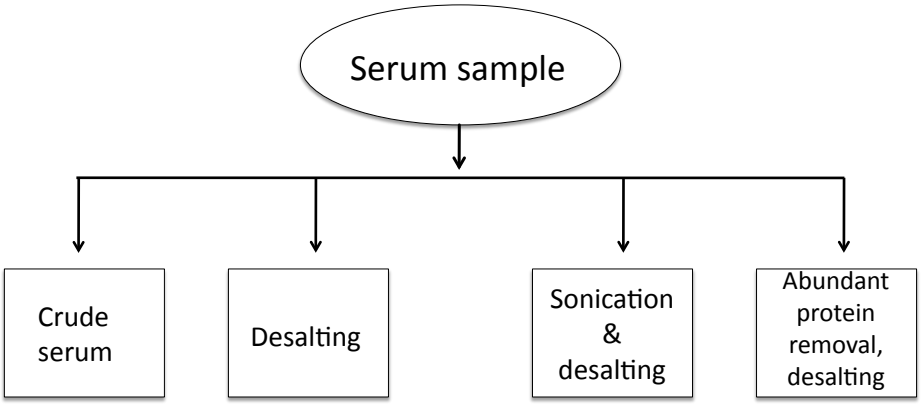
⇩

Collected serum was divided into aliquots and stored at -20°C

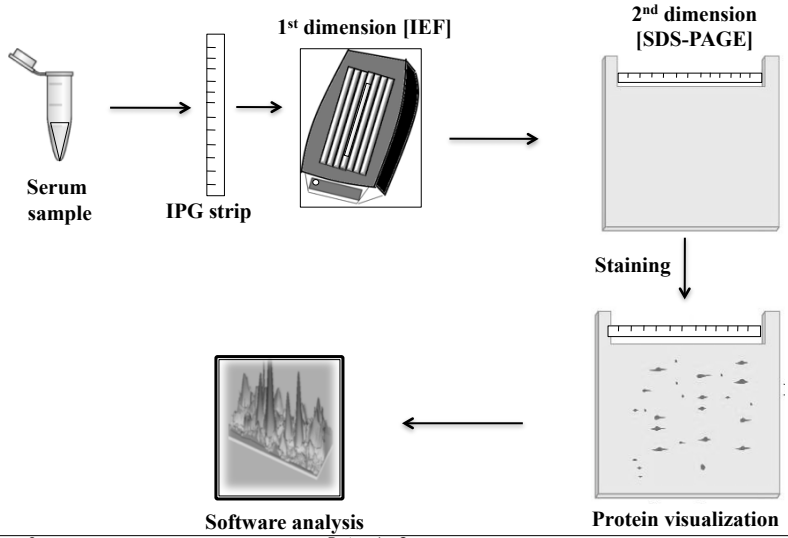
Serum sample processing for proteomic analysis



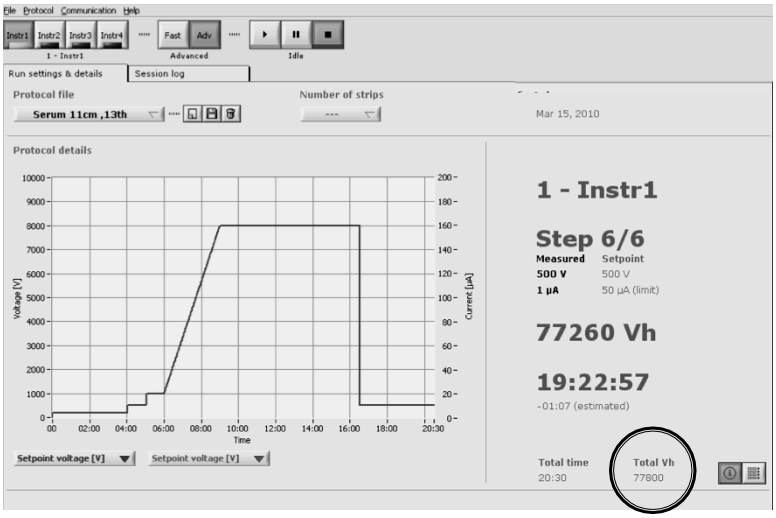
Sample preparation optimization



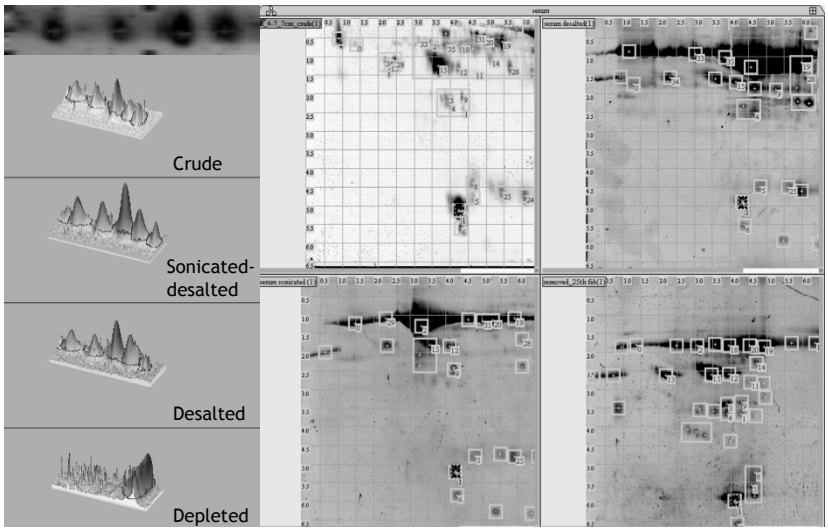
Two dimensional electrophoresis



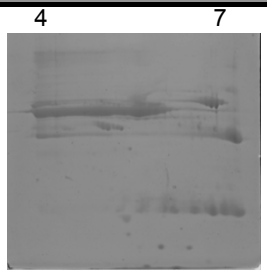
IEF Settings



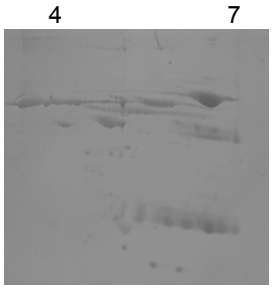
2-DE analysis



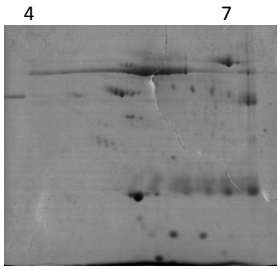
2-DE optimization (7 cm)



CRUDE SERUM

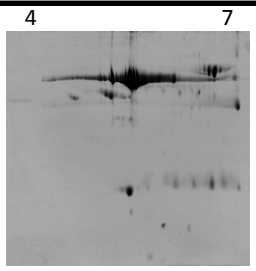


DESALTED SERUM

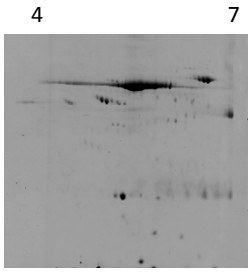


SONICATED-DESALTED SERUM

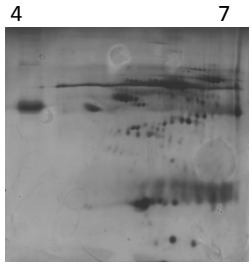
2-DE optimization (11 cm)



Desalted serum



Sonicated-desalted serum
Coomassie



Sonicated-desalted_silver

Protein extraction

40 µL serum was precipitated -4 volumes of ice-cold acetone containing 10% w/v TCA

⇩

incubated at -20^{0}C for 90 min

⇩

Centrifuged at 15 000 X g, 4^{0}C, for 20 min.

⇩

1 mL of ice-cold acetone was added to wash the precipitate

⇩

incubated on ice for 15 min and centrifuged as above

⇩

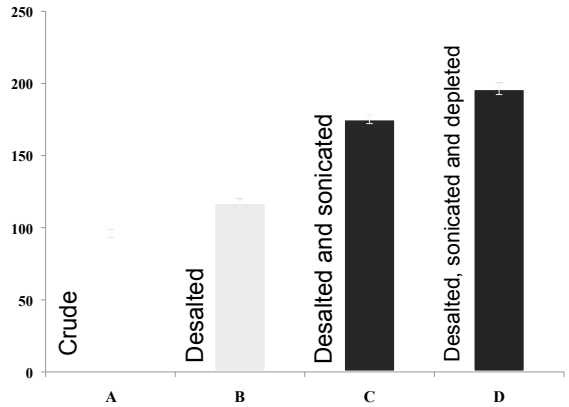
Acetone-containing supernatant was removed

⇩

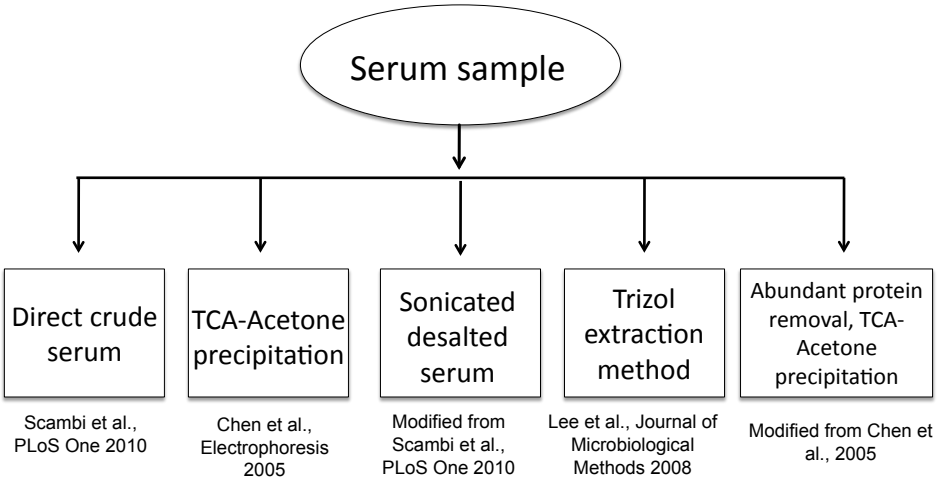
Pellet dissolved in lysis buffer [Urea 8M, CHAPS; 4% ; 2% IPG buffer; DTT 40mM; 1 % BPB]

Effect of different sample processing

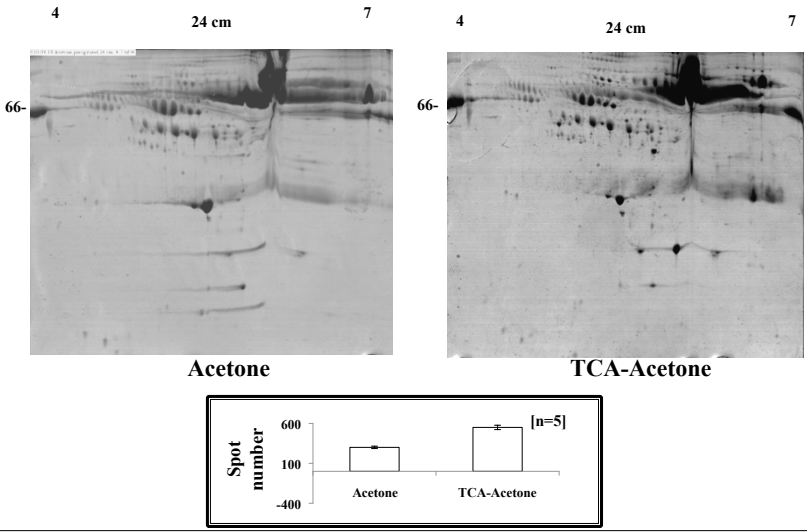
Number of spots [n=3]



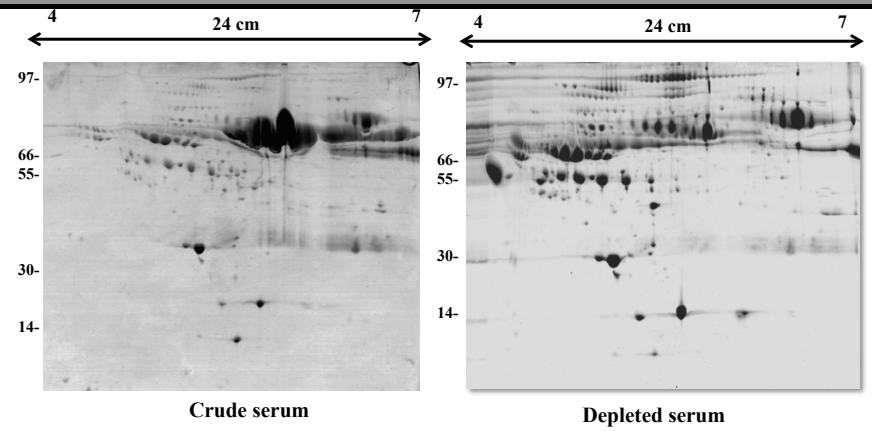
Optimization of protein extraction protocols



Protein extraction: Acetone vs TCA-Acetone precipitation

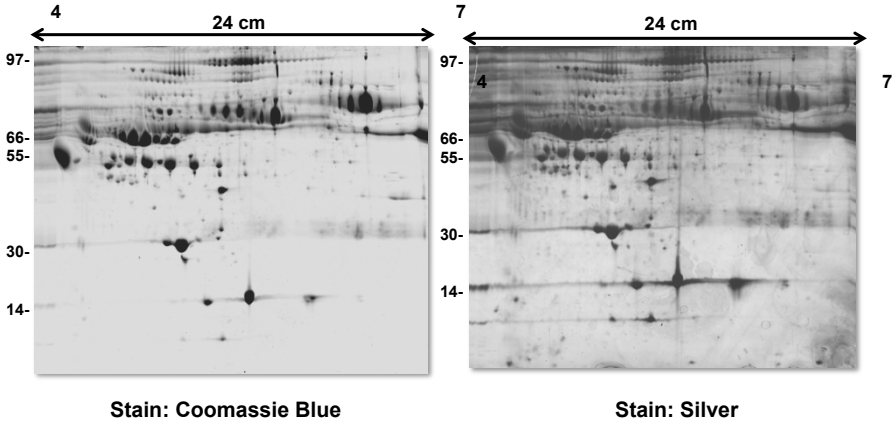


Protein extraction: Crude vs Depleted

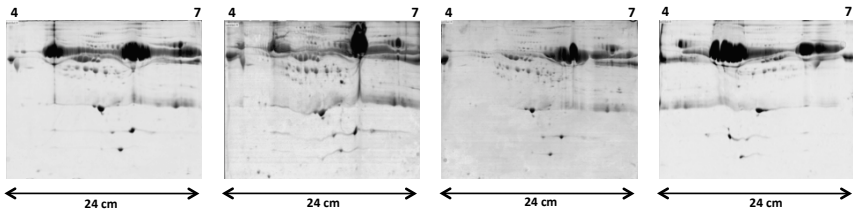


Spot number	Crude serum	Depleted serum
Software analysis	533	719

Staining agent: Coomassie vs Silver

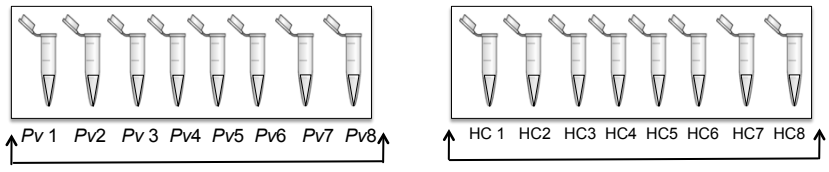


Spot number	CBB	Silver
Software analysis	719	995



Parameters	Crude	Sonicated-desalted	TCA-Acetone	Trizol
1. Sonication	-	+	+	+
2. Desalting	-	+	+	-
3. Rehydration	Active	Active	Passive	Active
4. Amount of protein loaded	1200 µg	1200 µg	600 µg	1200 µg
5. Strip	24 cm	24 cm	24 cm	24 cm
6. Staining Soln	Coomassie	Coomassie	Coomassie	Coomassie
7. Spot Number [Software detected]	513	503	509	359
8. Spot Number [After refinement]	351	363	308	208

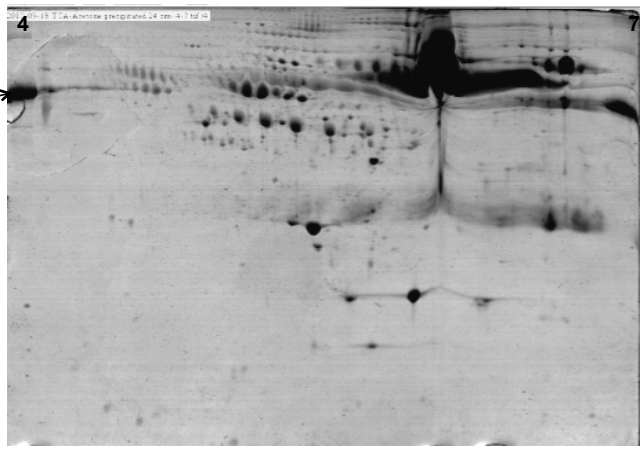
Comparative analysis of serum samples



Vivax malaria samples

Healthy control samples

Representative gel



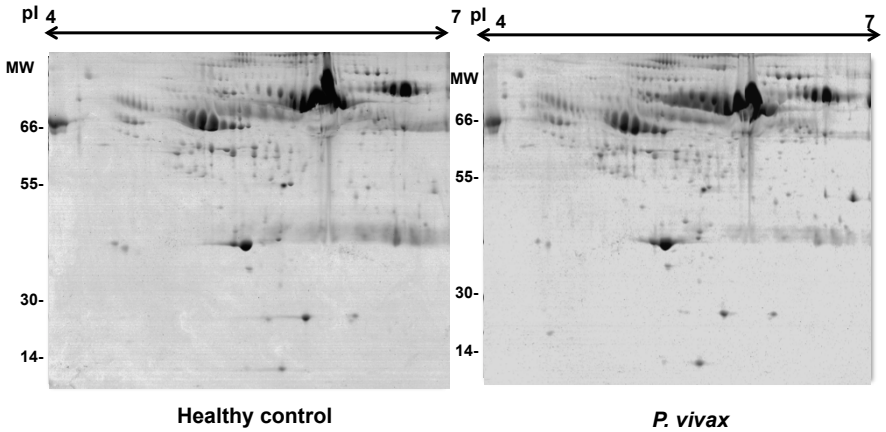
600 µg protein

24 cm

Software detected: 539

After refinement: 392 ±10

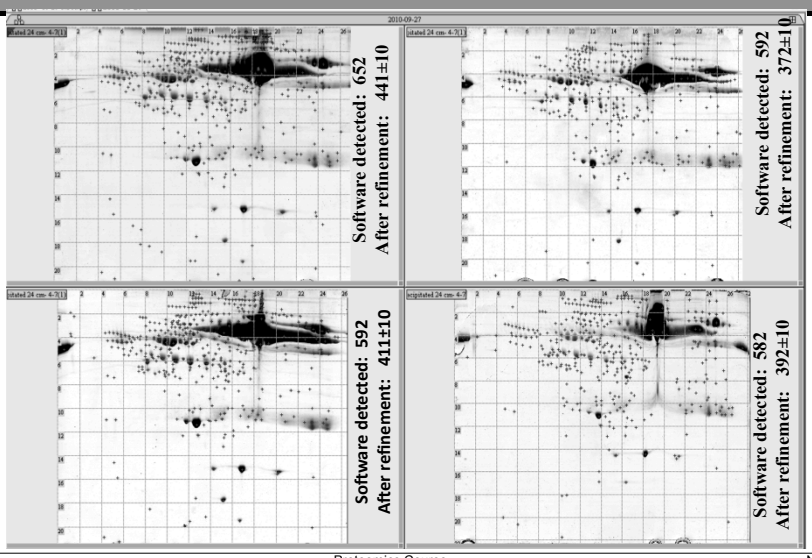
P. vivax vs healthy controls: representative gels



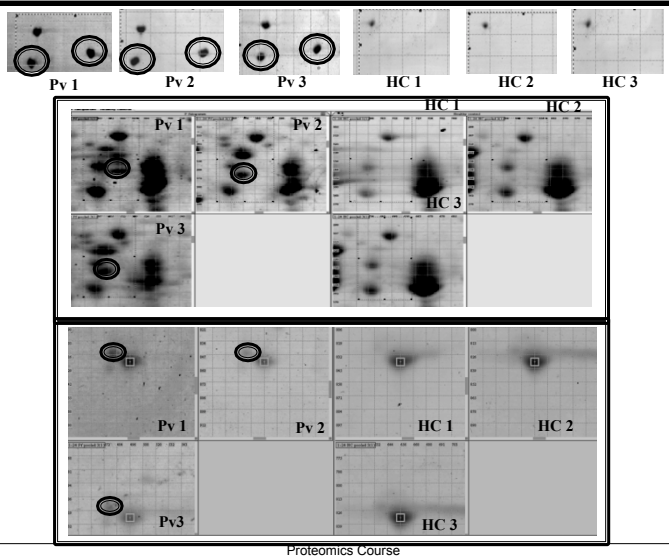
Healthy control

P. vivax

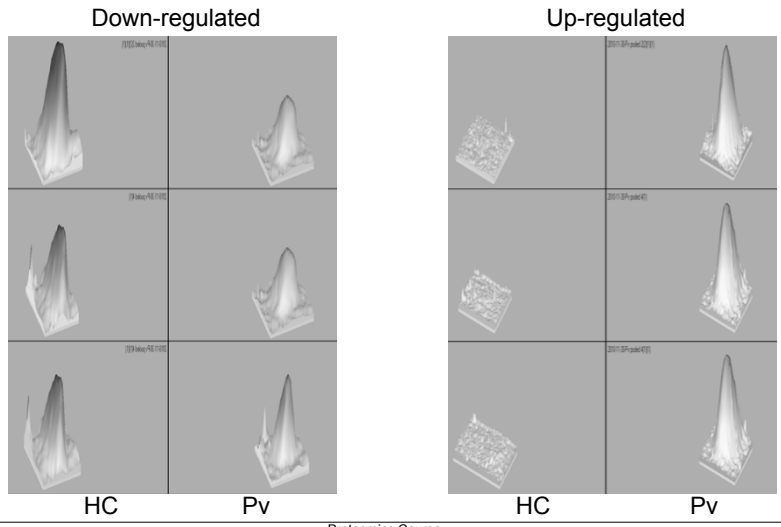
2D gel analysis: automated matching and refinement



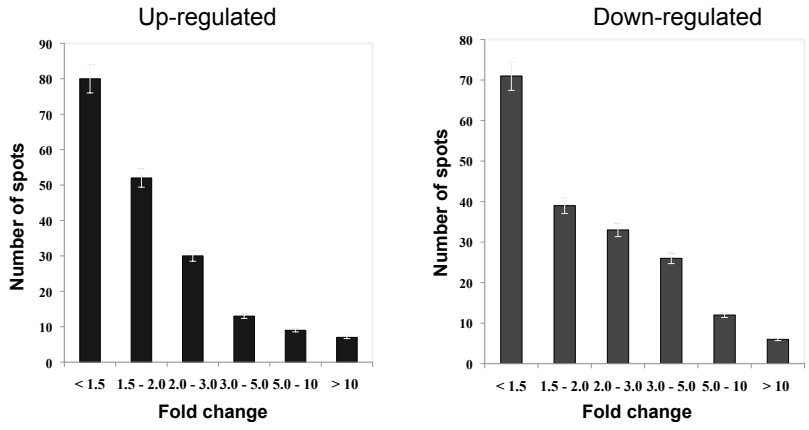
2D gel analysis: differentially expressed spots



2D gel analysis: differentially expressed spots

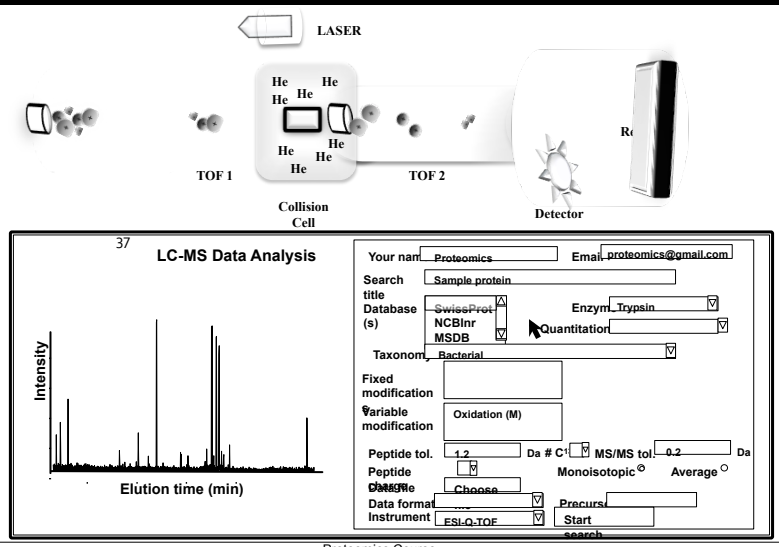


Altered protein expression levels in vivax malaria patients

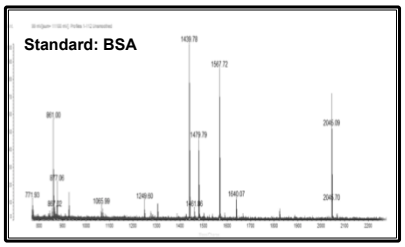


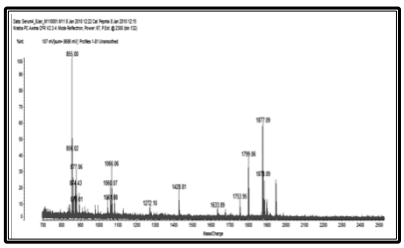
Data are represented as mean ± SEM where (*n*=3)

Mass spectrometry analysis

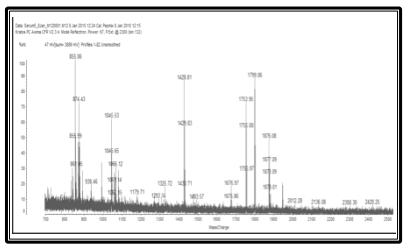


Mass spectrometry analysis





Serum Proteins: Spot 1



Serum Proteins: Spot 2

Significant differentially expressed proteins identified using 2DE

Down-regulated proteins	Up-regulated proteins
* Haptoglobin precursor (HP)	* Serum amyloid A (SAA)
* Apolipoprotein A-1 (APO A-1)	* Ceruloplasmin precursor (CP)
* Serum albumin precursor (ALB)	* Leucine-rich α-2-glycoprotein precursor (LRG)
* Clusterin precursor (CLU)	* Alpha-1-antitrypsin precursor (Alpha-1 protease inhibitor)

Conclusions

- Few differentially regulated serum proteins identified in this study have not been reported earlier in vivax malaria pathogenesis
- An important role of serum amyloid A and P, haptoglobin, apolipoprotein A-1 and E proteins elucidated in vivax malaria

Summary

- Two dimensional electrophoresis can be applied for various applications
- Case studies –
  - Host response to malaria infection
  - Drug treatment to malaria parasite

REFERENCES

- Ray et al. Serum proteome analysis of vivax malaria: An insight into the disease pathogenesis and host immune response. J Proteomics. 2011. PMID: 22086083
- Ray et al. Proteomic Investigation of Falciparum and Vivax Malaria for Identification of Surrogate Protein Markers. PLoS ONE 7(8): e41751. doi:10.1371/journal.pone.0041751
- Herbert BR, Harry JL, Packer NH, Gooley AA, Pedersen SK and Williams KL. What place for polyacrylamide in proteomics? Trends Biotechnol. 2001, 19 (10 Suppl), S3-9.
- Hanash S. Disease proteomics. Nature 2003, 422, 226-232.