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What Data Can We Get? The Potential for Omics in DairyCare

Professor David Eckersall

Veterinary Gene & Protein Group

Institute of Biodiversity, Animal Health & Comparative Medicine

University of Glasgow

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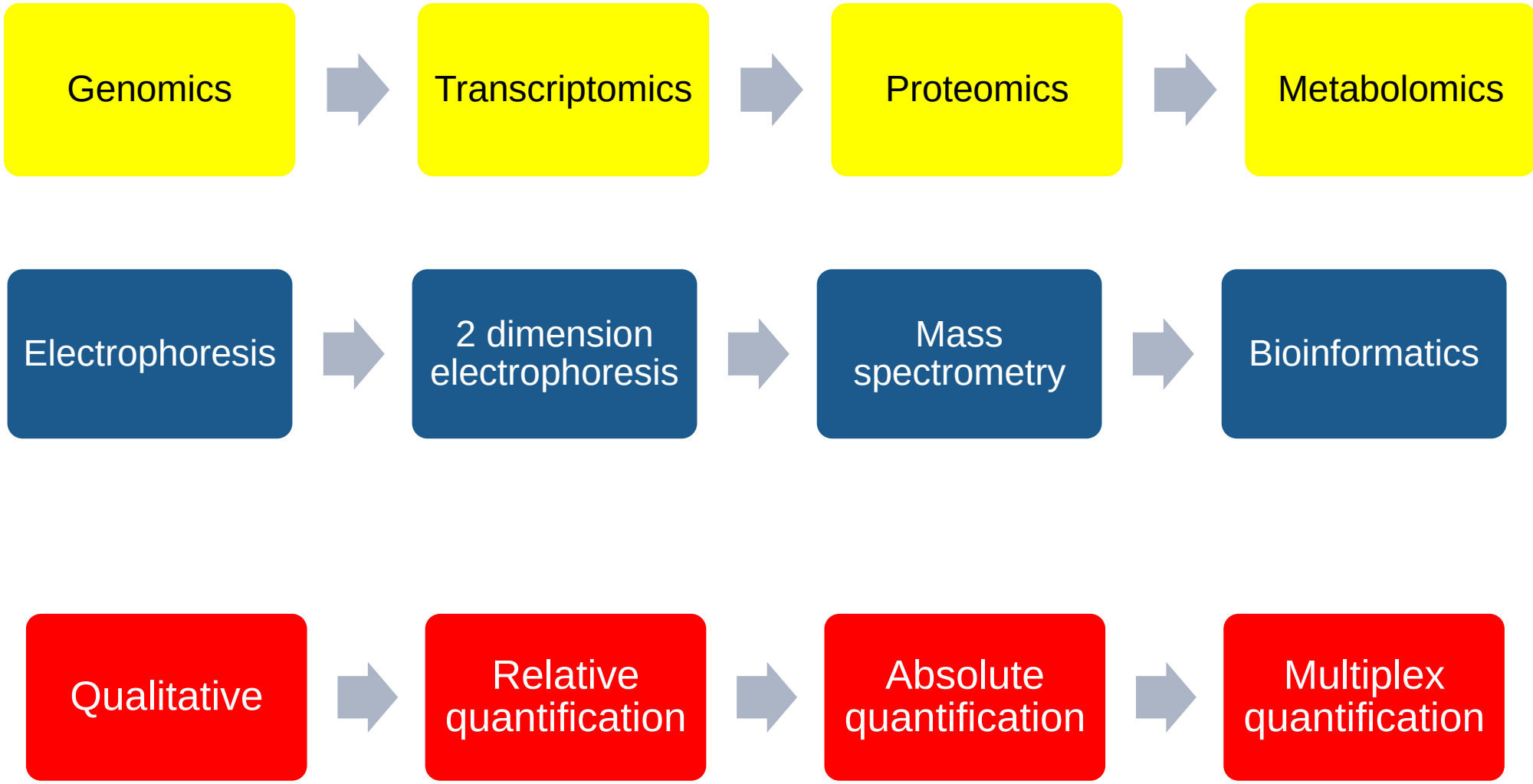
Omic progression



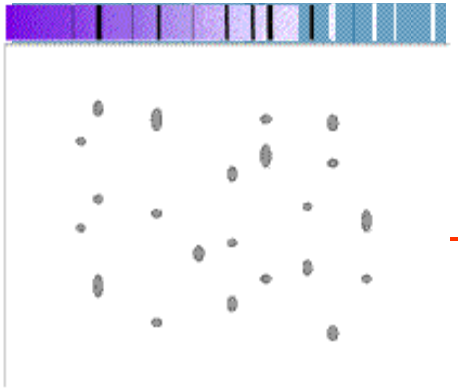
- **Proteomics and milk proteins**
  - Gel based proteomics
  - Non-gel based proteomics
- **Metabolomics and mastitis**

- **Non-invasive sampling**
  - Saliva
  - Sweat
  - Nasal secretion
  - Interstitial fluid

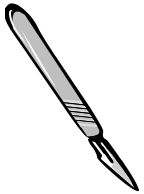
Classic Gel-Based Proteomics

2-DE and mass spectrometry

1st dimension
(isoelectric focusing)

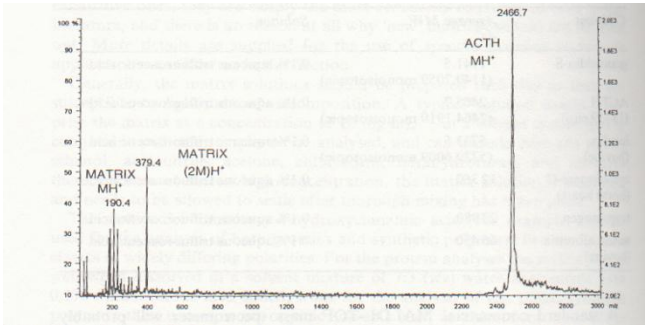


2nd dimension
SDS-PAGE



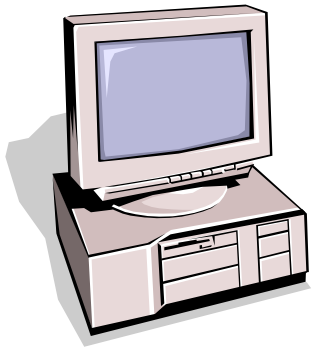
Excise spot of interest and digest with trypsin

MALDI-TOF mass spectrometry



Yields peptide mass fingerprint

Search protein database



Proteomics of milk : advances in methods

- **Galvani *et al* Rapid Comm in Mass Spec, 2000, 14:1889**
- **Spots:**
  - 9-11; β-lactoglobin
  - 13-15; α-lactalbumin
  - Others; caseins

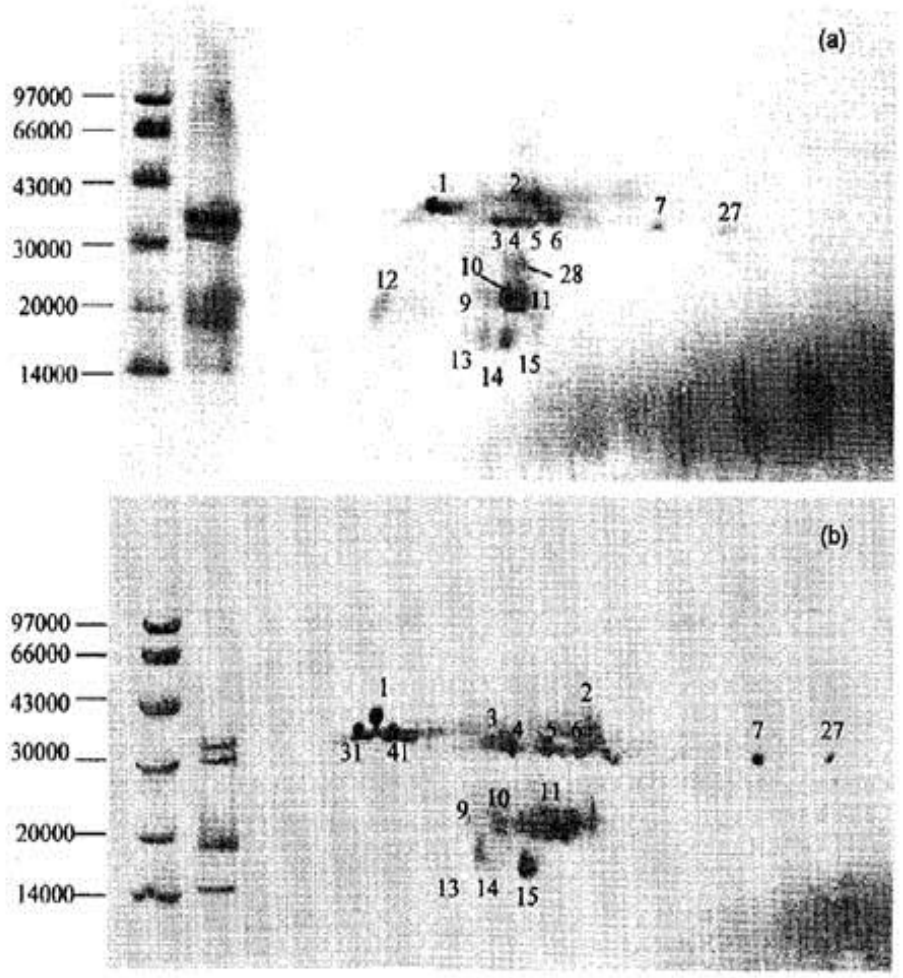


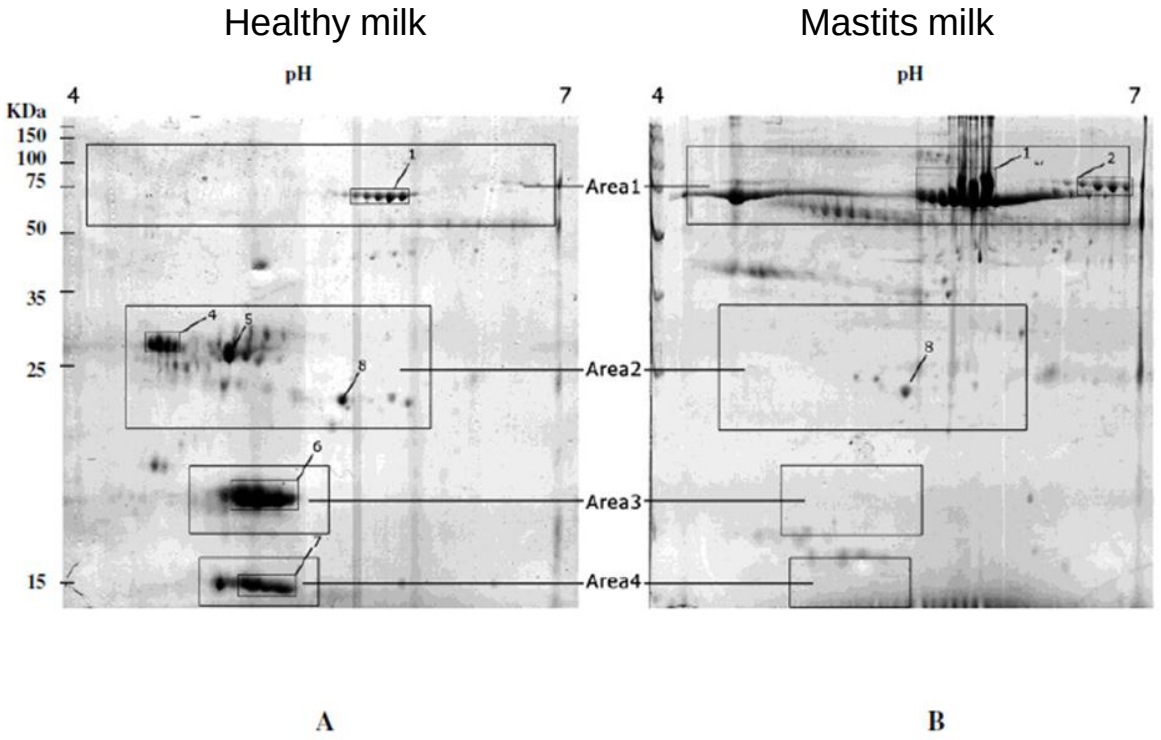
Figure 2. 2-D gel maps stained with Coomassie Brilliant Blue: (a) pH gradient 3–7 and (b) pH gradient 4–7.

Proteomics of Milk: Effect of Mastitis

Milk in clinical mastitis

Increased: albumin, lactoferrin (area 1), IgG

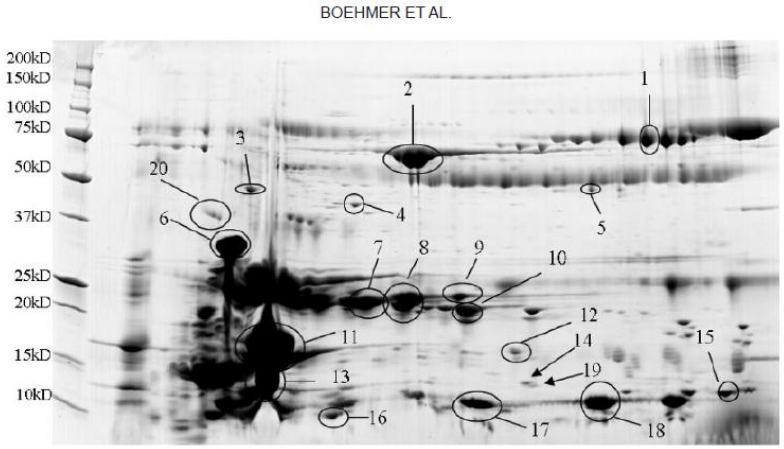
Decreased: casein (area 2), β-lactoglobin (area 3), α-lactalbumin (area 4)



Hogarth et al 2004

Proteomics of Milk: Advancing methods

- Boehmer et al 2008
- Ultracentrifugation to prepare samples
- High abundance of casein and beta-lactoglobulin,



- Healthy whey samples show low abundance proteins: transthyretin, lactadherin, beta-2-microglobulin, alpha-1-acid glycoprotein, and complement C3

•Mastitis whey samples increased serum albumin, transthyretin, complement C3 and alpha-1-acid glycoprotein and appearance of cathelicidin, indolicidin, and bactenecin 5 and 7, beta-fibrinogen, alpha-2-HS-glycoprotein, alpha-1-antiproteinase

Gel v. Non-Gel Proteomics

- **2 Dimension electrophoresis based proteomics**
  - Separate proteins on polyacrylamide gel
  - Stain and pick protein spots
  - Trypsin digest → peptides
  - Mass spectrometry → peptide mass
  - Protein identification by bioinformatic interrogation

Gel v. Non-Gel Proteomics

- **2 Dimension electrophoresis based proteomics**
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  - Stain and pick protein spots
  - Trypsin digest → peptides
  - Mass spectrometry → peptide mass
  - Protein identification by bioinformatic interrogation

- **Non-gel proteomics**
  - Trypsin digest → peptides OR ultrafiltration to remove large protein
  - Include labelled standards for quantification
  - Separate peptides on Liquid Chromatography or Capillary Electrophoresis
  - Mass spectrometry → peptide mass
  - Protein identification by bioinformatic interrogation
  - Calibrate with labelled standards

Proteomics of Milk: Non-gel methods

- **Danielson et al 2010 Proteomics 10:2240**
- **iTRAQ and LC-MS/MS**
- **Quantitative analysis of 80 milk proteins**
- **49 proteins changed in milk following experimental mastitis with *E. coli* infection of udder**
- **Increase compared to controls:**

– Apolipoprotein I, II & IV

– Bactenecins 5 & 7

– Complement C3 and C4

Proteomics of Milk: Non-gel methods

- **Reinhardt et al J Proteomics (2013) 82:141**
- **Proteomes determined in milk exosomes, milk fat globule membranes (MFGM) & whey**
- **iTRAQ & LC-MS/MS**
- **>300 milk proteins identified related to host defence**
- **94 significantly altered in *Staphylococcus aureus* infection of udder**
- **Identified neutrophil extracellular trap formation in MFGM**

Quantitative Proteomics of Milk

Journal of proteome research

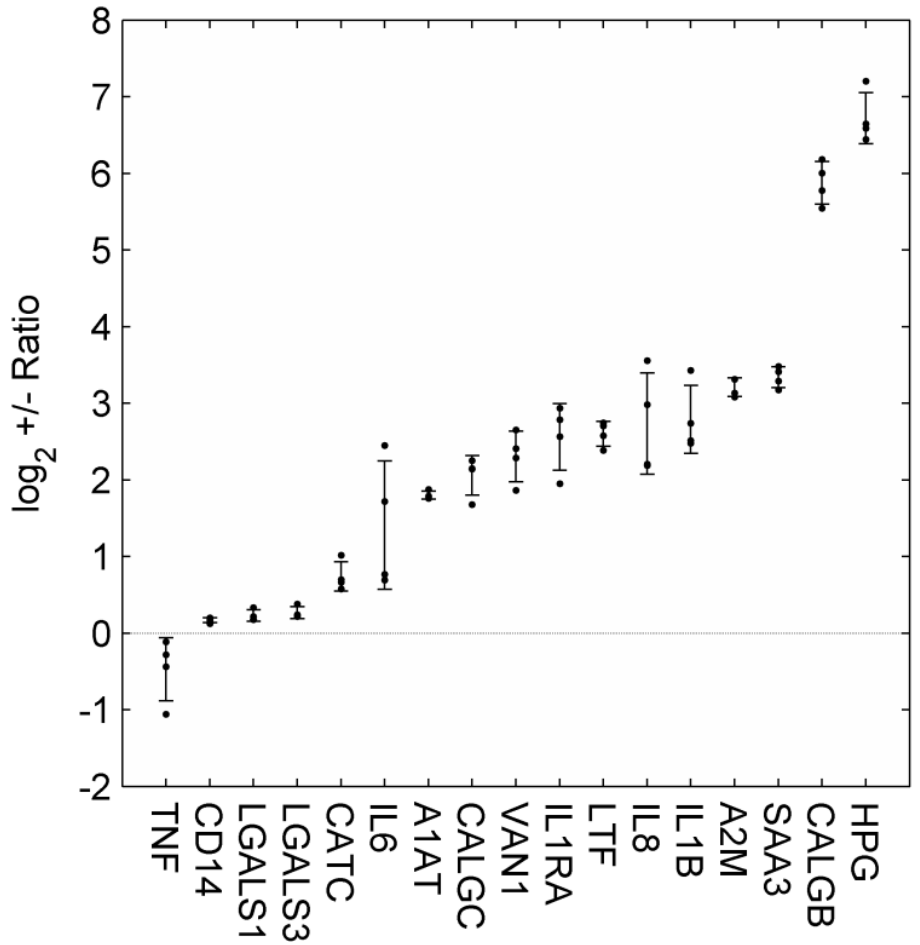
Article

pubs.acs.org/jpr

Quantotypic Properties of QconCAT Peptides Targeting Bovine Host Response to *Streptococcus ube*

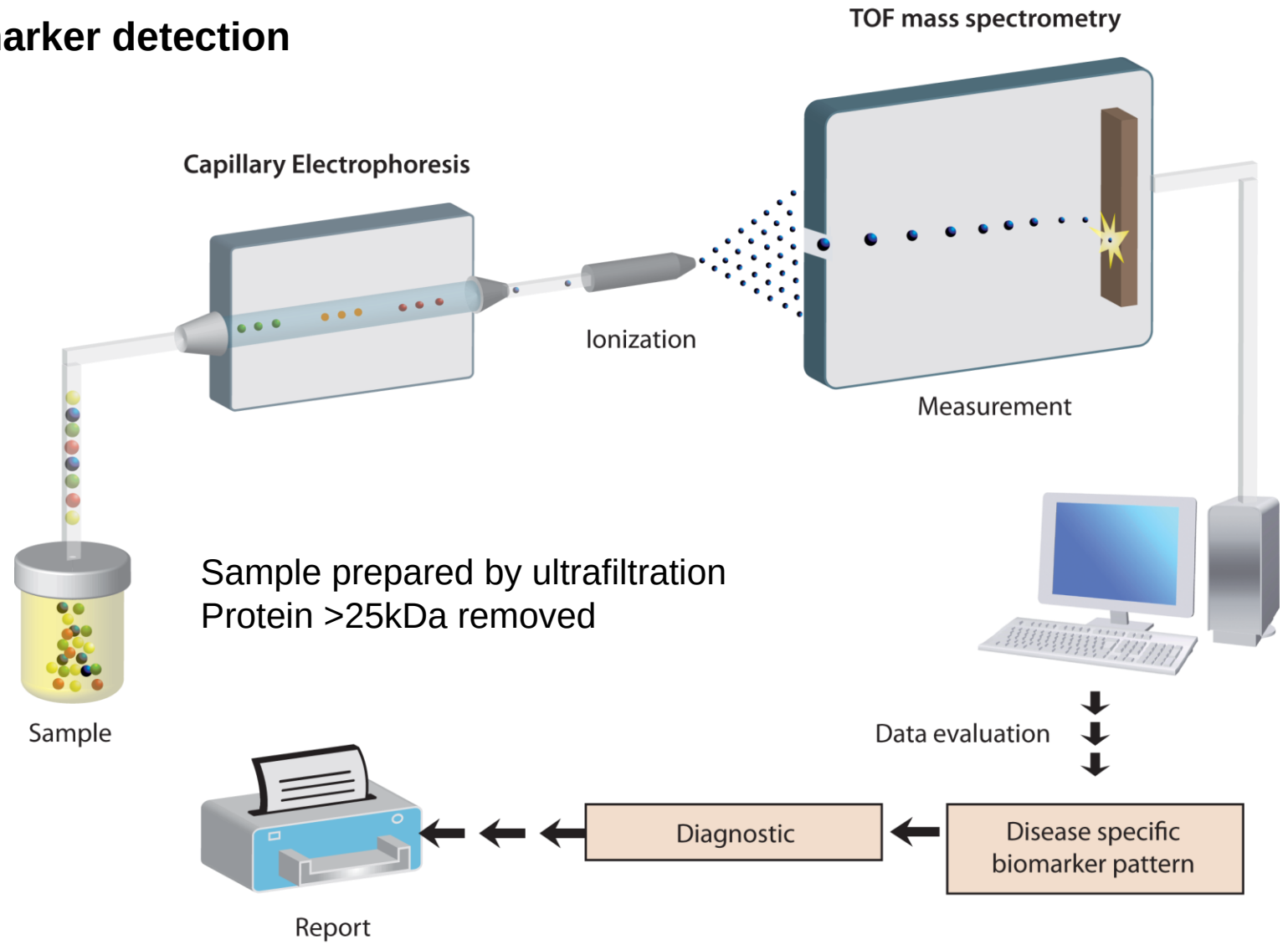
Stine L. Bislev,[†,‡] Ulrike Kusebauch,[‡,§] Marius
Christine M. Røntved,[†] Ruedi Aebersold,[#,¶,△]

J Prot Res, 2012, 11:1832



Milk Peptidomics by CE-MS

Biomarker detection



Combined Peptidomic of Milk

Control (n=12) and clinical mastitis milk (n=25)

Clinical mastitis detected by swelling, heat, pain, clots in milk

Ultrafiltered to remove protein >25kDa

Run on CE-MS

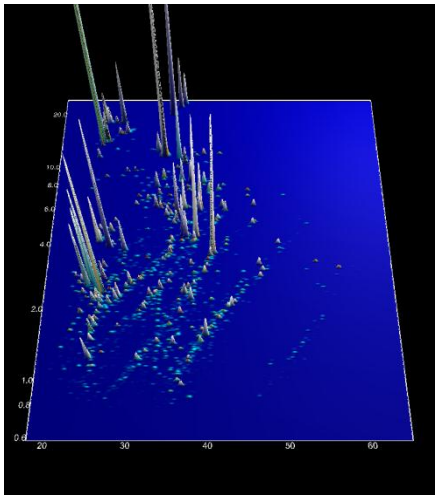
Peaks combined by statistical analysis to develop algorithm

>1000 peptide peaks detected

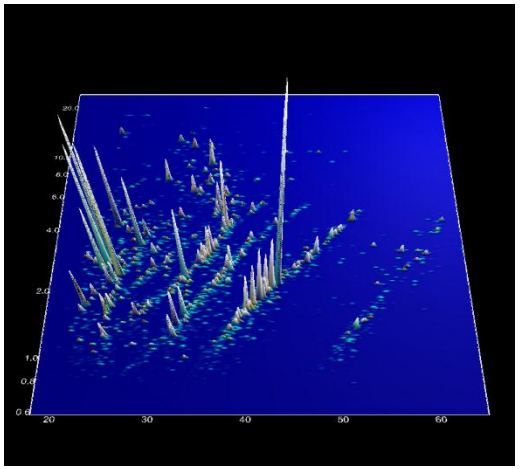
508 peptides differ between healthy & mastitic milk

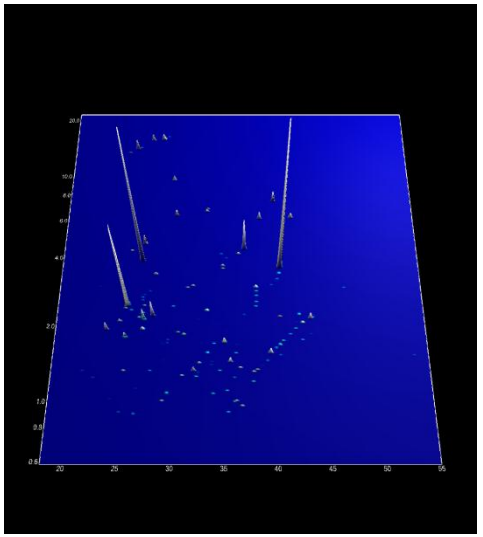
Mansor et al Journal of Proteomics 2013 85:89

Healthy

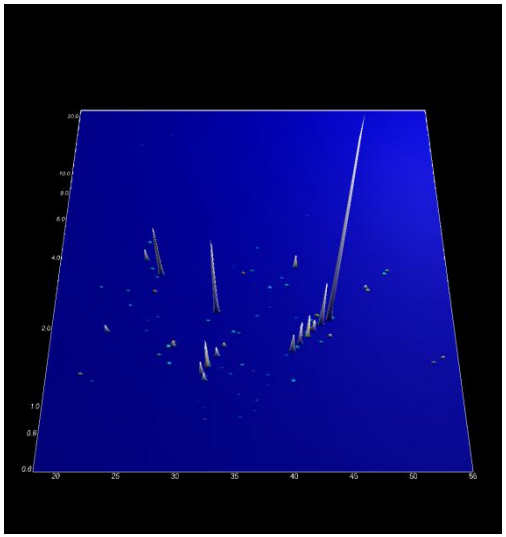


Mastitis





Greater in healthy



Greater in mastitis

Biomarkers: Healthy v Mastitis

Peaks combined by statistical analysis

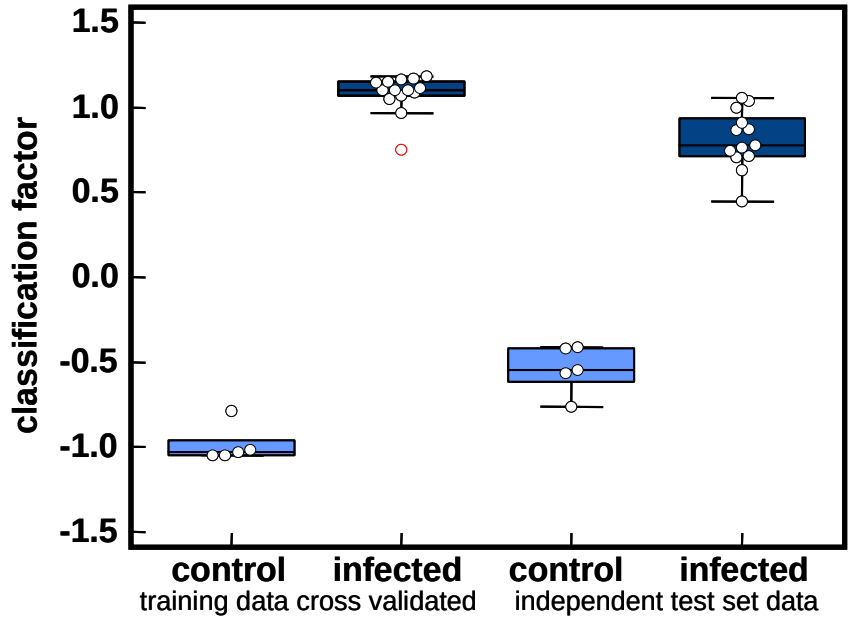
154 peptide multiplex biomarker panel

Training sets: 5 healthy samples v 14 clinical mastitis samples

Test sets: 5 healthy samples v 13 clinical mastitis samples

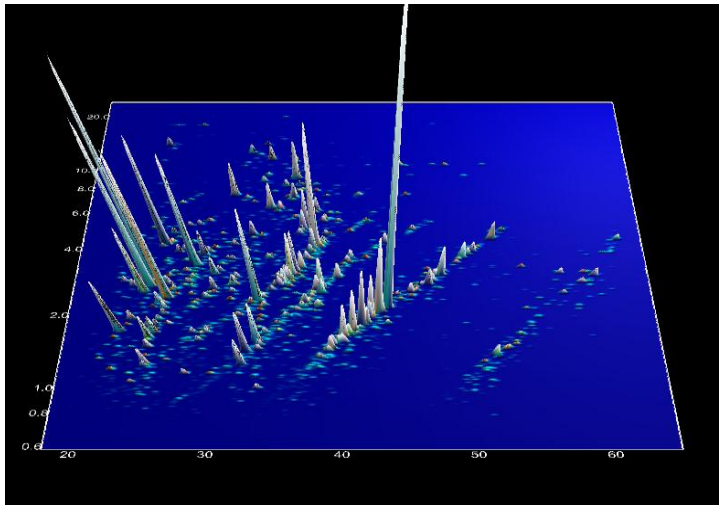
100% sensitive & 100% specific

Peptides identified mostly casein breakdown products

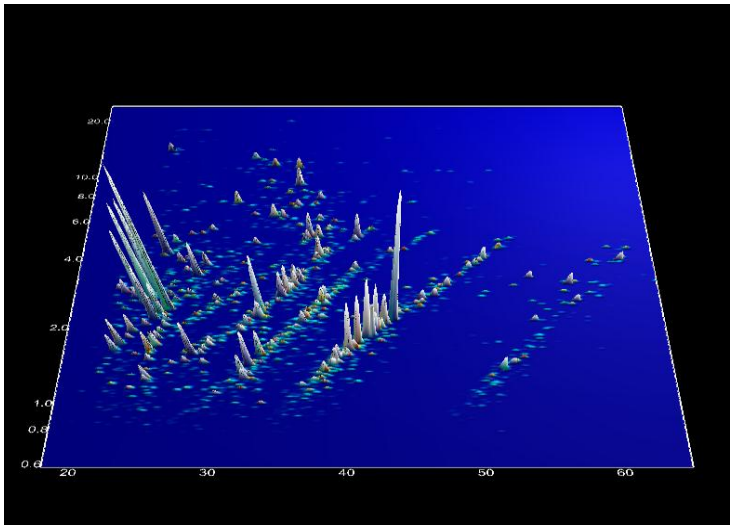


Milk Peptidome of *E. coli* v *S. aureus*

Differential diagnosis of bacterial cause of mastitis



E. Coli



S. aureus

Bacterial infection determined by Glasgow Veterinary Diagnostic Service

Milk peptidome in differential diagnosis

Peaks combined by statistical analysis (Mosaique Diagnostics)

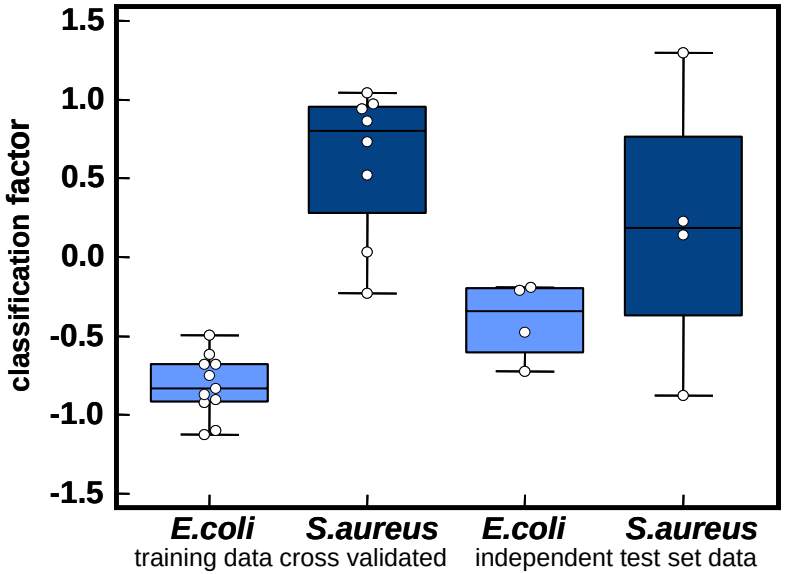
47 peptide multiplex biomarker panel

Training sets: 11 *E. Coli* v 8 *S. aureus* samples

Test sets: 4 *E. Coli* v 4 *S. aureus* samples

75% sensitive & 100% specific

Peptides identified mostly casein breakdown products



Metabolomics

Metabolomics

•Characterization and quantification of the whole collection of both intra and extra-cellular metabolites.

Metabolome

•the qualitative and quantitative collection of all low molecular weight of molecules

• in biological fluid, organism or cell

• can be peptides, amino acids, nucleic acids, carbohydrates, organic acids, vitamins, polyphenols, alkaloids

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Metabolomics, Milk & Mastitis

- Does the milk metabolome change with mastitis?

- Are there differences in the milk metabolome with different pathogens?

- Can change in specific metabolites be used as biomarkers for mastitis?

Metabolomic method

Mass spectrometry analysis

- Milk extracted with methanol:chloroform:water (3:1:1)
- LC system coupled with MS (Dionex UltiMate 1)

-MS Acquisition (Orbitrap Exactive)

-Performed in both –ve and +ve ionisation modes and full scan modes

Metabolomic results

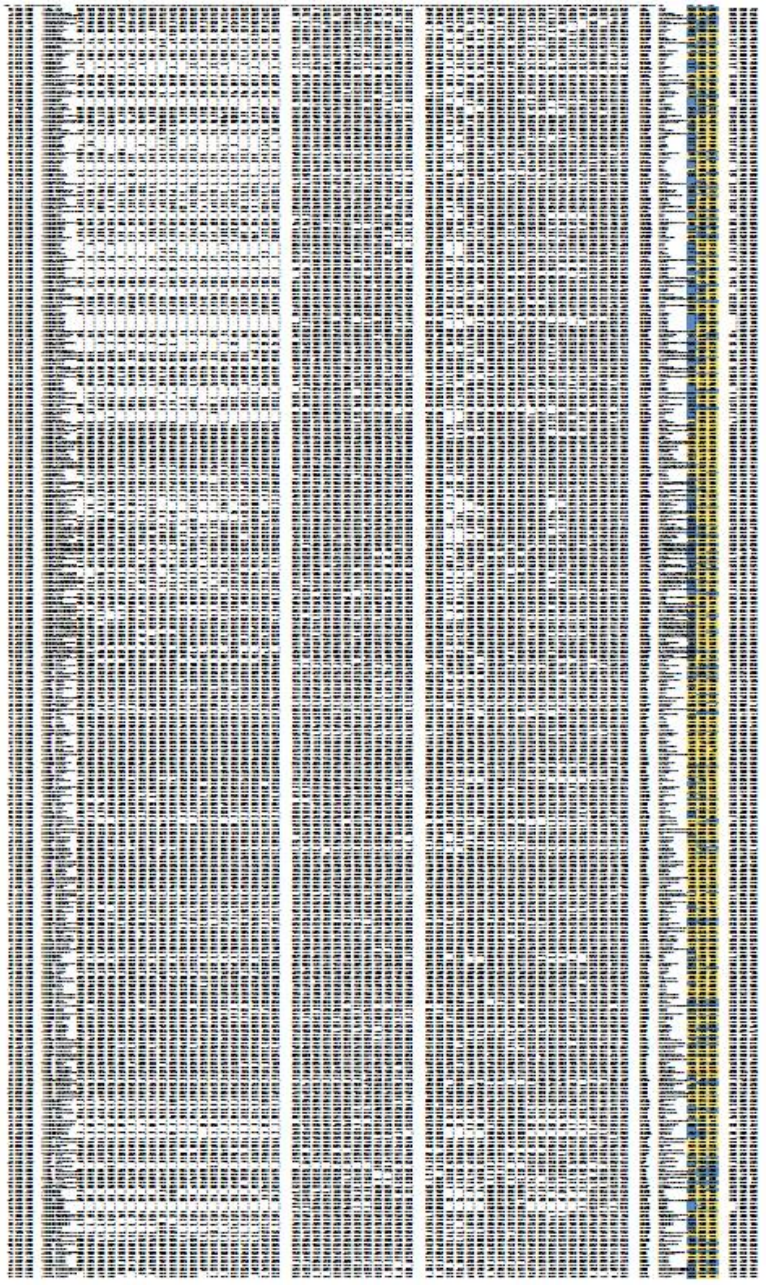
- 10 Healthy milk samples
- 10 *E.Coli* milk samples
- 6 *Staph. aureus* milk samples

- 5502 MS negative ion peaks
- 573 identified metabolites

- 9908 MS positive ion peaks
- 814 identified metabolites

- 1 of 12 pages of MS data for negative ion data set

- Challenge for bioinformatics



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The first 50 of 573 peaks (Negative Ion Mode)

Metabolites	Control	Name	E.Coli	Name	saureus
5-Dehydro-4-deoxy-D-glucarate	1.75E+09	5-Dehydro-4-deoxy-D-glucarate	1.38E+09	5-Dehydro-4-deoxy-D-glucarate	1.71E+09
1-alpha-D-Galactosyl-myo-inositol	1.51E+09	1-alpha-D-Galactosyl-myo-inositol	1.16E+09	1-alpha-D-Galactosyl-myo-inositol	1.25E+09
N-Acetyl-D-glucosamine 6-phosphate	2.62E+08	(R)-Lactate	8.12E+08	(R)-Lactate	9.22E+08
cis-Aconitate	1.67E+08	myo-Inositol	1.83E+08	L-Glutamate	1.56E+08
myo-Inositol	1.06E+08	Succinate	1.8E+08	cis-Aconitate	1.24E+08
Hippurate	98680533	cis-Aconitate	1.05E+08	myo-Inositol	1.16E+08
Urate	89863389	2-C-Methyl-D-erythritol 4-phosphate	91564904	(S)-Malate	1E+08
2_5-Dioxopentanoate	84341727	2-Dehydro-3-deoxy-L-arabinonate	86395991	Urate	94020844
Miserotoxin	78910119	Pyruvate	81948401	2-C-Methyl-D-erythritol 4-phosphate	85004997
2-C-Methyl-D-erythritol 4-phosphate	78482788	Creatine	73380634	L-Erythrulose	74942006
L-Erythrulose	75351299	Hippurate	72131854	N-Acetyl-D-glucosamine	73840064
N-Acetyl-D-glucosamine	74489145	(S)-Malate	71188741	Miserotoxin	70788430
Creatine	74407132	2-C-Methyl-D-erythritol 4-phosphate	63327089	2_5-Dioxopentanoate	69476176
(S)-Malate	73918031	Urate	60860379	Taurine	62376397
L-Glutamate	73661846	L-Glutamate	60367636	(R)-3-Hydroxybutanoate	62310337
sn-glycero-3-Phospho-1-inositol	66333040	2_5-Dioxopentanoate	59941285	Pyruvate	58442163
D-Glucose 6-phosphate	52176193	N-Acetyl-D-glucosamine 6-phosphate	59306858	Creatine	56614851
2-Dehydro-3-deoxy-L-arabinonate	44090003	Glycerol 2-phosphate	51930002	Hippurate	53708833
2-Butyne-1_4-diol	43558519	2-Oxoglutarate	47839083	2-Dehydro-3-deoxy-L-arabinonate	45261272
Pantothenate	43488200	(2S)-2-Isopropylmalate	46199319	Mesaconate	42718964
2-Oxoglutarate	41739044	Taurine	43331718	2-Butyne-1_4-diol	38833822
Glycerophosphoglycerol	35372625	N-Acetyl-D-glucosamine	37618824	N-Acetyl-D-glucosamine 6-phosphate	35648796
L-Alanine	32778314	Pantothenate	36966410	Succinate	29519867
2-Oxooctadecanoic acid	31016415	L-Erythrulose	32796067	L-Alanine	25713006
(R)-Lactate	30676971	Mesaconate	32519002	Pantothenate	25649695
2-C-Methyl-D-erythritol 4-phosphate	17129722	Miserotoxin	32238333	L-Arginine	24661840
Phenylacetylglycine	16394154	2-Butyne-1_4-diol	31756211	Acetoacetate	23241854
Glycerol 2-phosphate	15741286	L-Alanine	30500954	Leucyl-leucine	23052455
Uridine	15169547	D-Glycerate	21083181	(R)-2-Hydroxystearate	21361935
D-Glycerate	13282239	(R)-3-Hydroxybutanoate	20058371	L-1-Pyrroline-3-hydroxy-5-carboxylate	21140417
2-Hydroxy-2_4-pentadienoate	11126302	Glycerophosphoglycerol	17011504	L-Aspartate	20924711
Fumarate	10123233	alpha-D-Mannoheptulopyranose	14571959	Glycerophosphoglycerol	20292854
1-Aminocyclopropane-1-carboxylate	8933048	Glycerophosphoglycerol	14066463	Leu-Gln-Ser	18858881
Acetoacetate	8114826	Diacetyl	13189653	Lys-Val-Ser	18012351
Pyruvate	7995273	L-Arginine	12673627	2-Oxoglutarate	17828323
Succinate	5936534	(S)-3-Methyl-2-oxopentanoic acid	12014074	Diacetyl	17016063
L-2-Aminoadipate	5451488	D-Glucose 6-phosphate	10716111	2-Oxooctadecanoic acid	16754393
(S)-Dihydroorotate	5164245	Fumarate	9768704	Leu-Tyr	15228717
D-Glucuronolactone	5130855	sn-glycero-3-Phospho-1-inositol	9457991	D-Glycerate	15214056
Taurine	5106576	Leu-Tyr	8427266	Leu-Thr	14873123
Met-Asn-Asp	5096341	2-Oxooctadecanoic acid	8162199	Fumarate	14530951
Xylitol	5046532	Met-Asn-Asp	8105485	Leu-Phe	13157998
Fumarate	4844560	(R)-2-Hydroxystearate	7952443	Leu-Leu-Val	12690777
1-4-beta-D-Glucan	4819930	Phenylacetylglycine	7817072	2-C-Methyl-D-erythritol 4-phosphate	12648423
(R)-3-Hydroxybutanoate	4219629	L-1-Pyrroline-3-hydroxy-5-carboxylate	7631977	9_10-DHOME	11837381
alpha-D-Mannoheptulopyranose	3797943	Acetoacetate	7415715	Uridine	11031207
Bicine	3590543	1-4-beta-D-Glucan	6210871	Leu-Trp	10963377
4-Oxoproline	3581575	Fumarate	6149763	Val-Arg	10020592
4-Pyridoxate	3464541	5-Hydroxypentanoate	5744097	1-Aminocyclopropane-1-carboxylate	9663711

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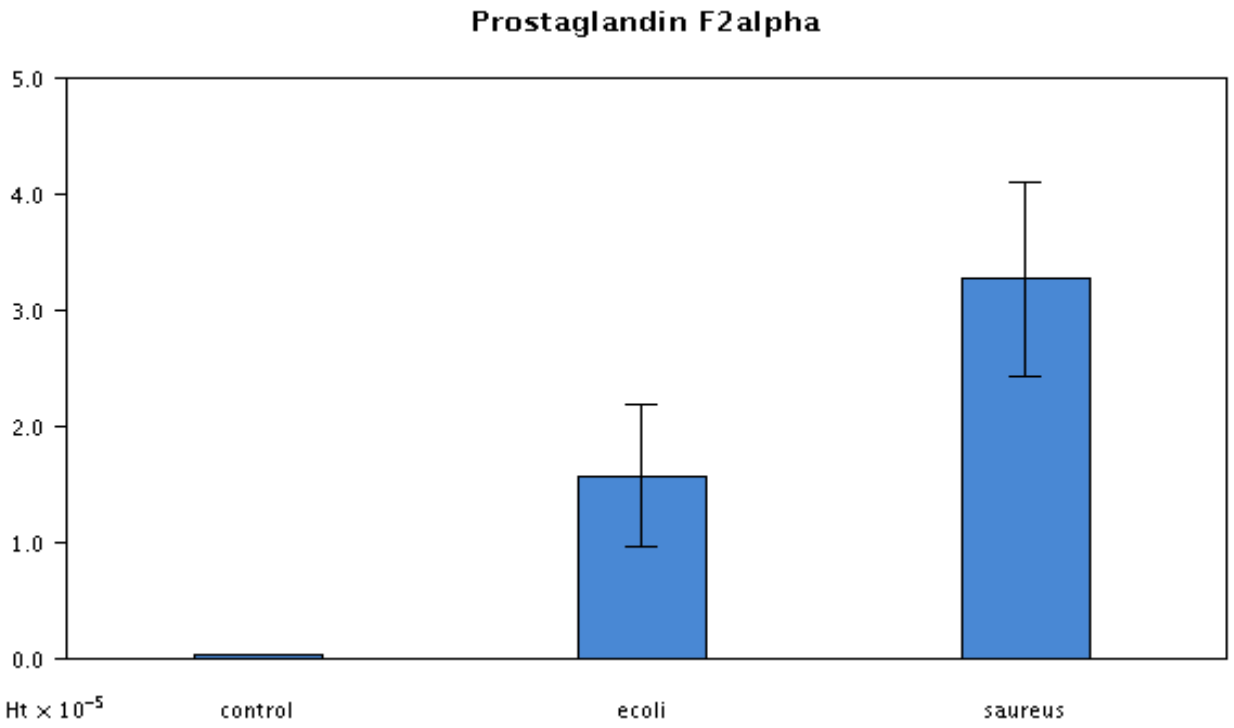
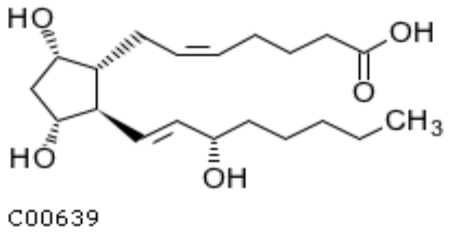
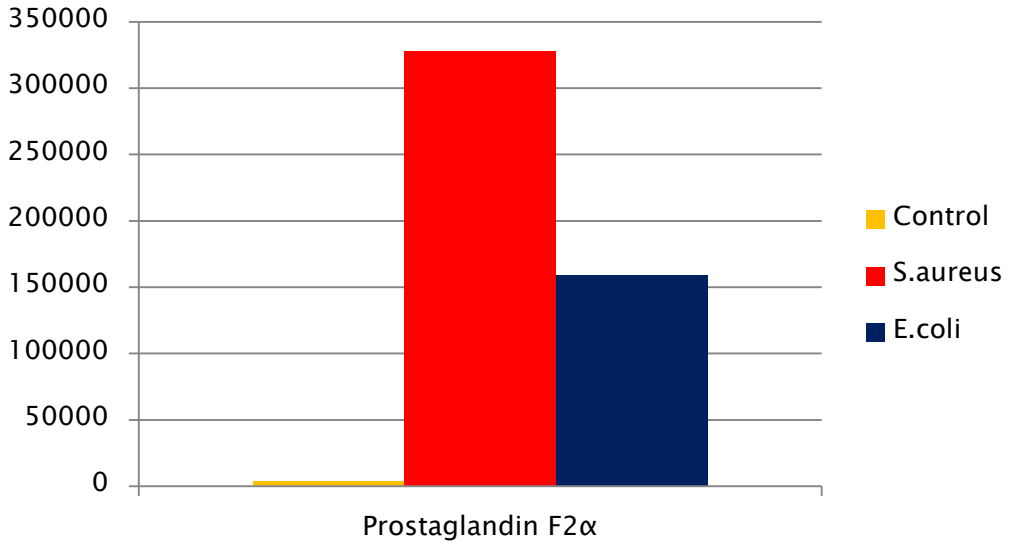
Change in individual metabolites





C00639

Kegg map metabolites with formula $C_{20}H_{34}O_5$



Prostglandin F2α are significantly **higher** in **both bacterial groups** compared with control group

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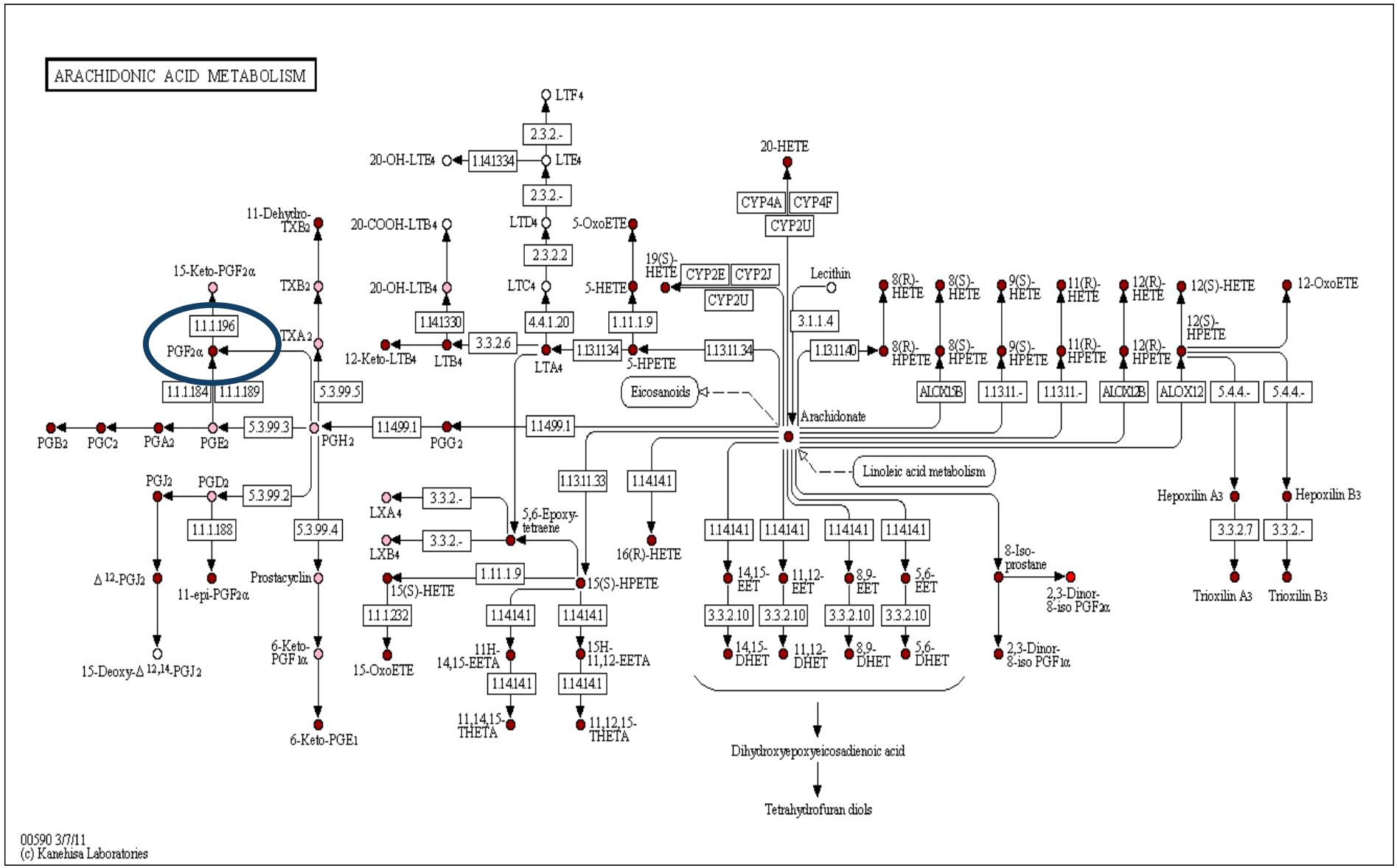
Kegg Diagram of pathway changes: red dot increased in mastitis



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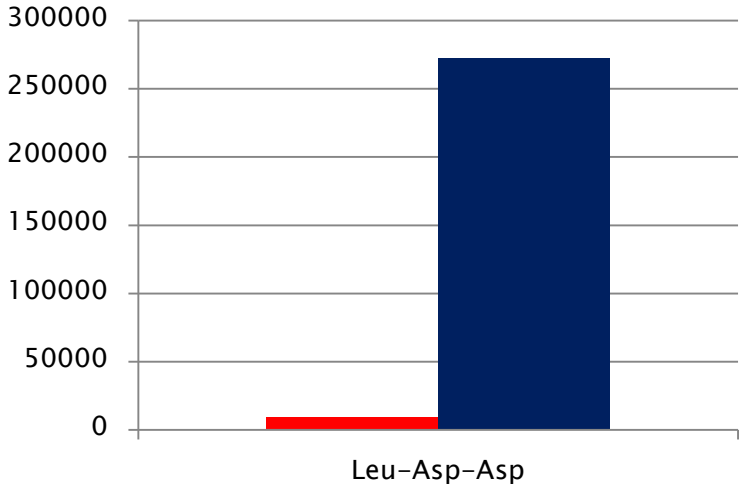
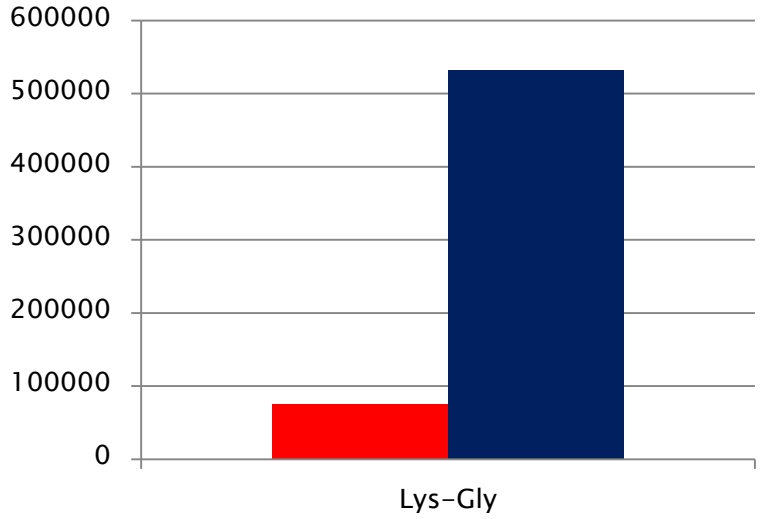
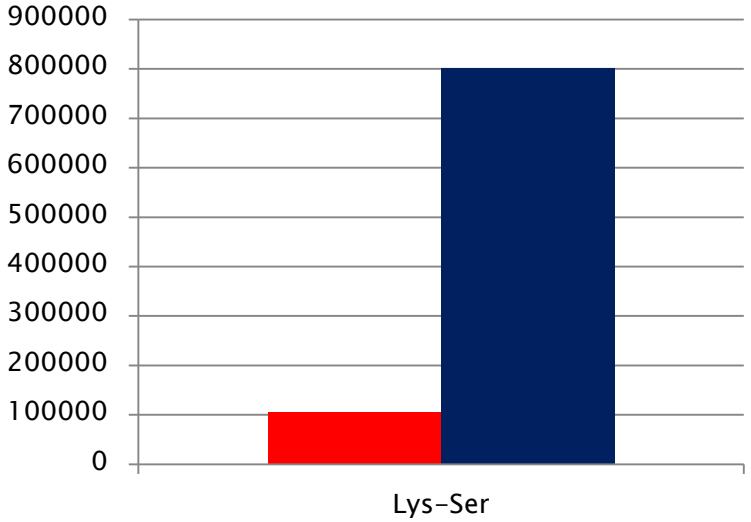
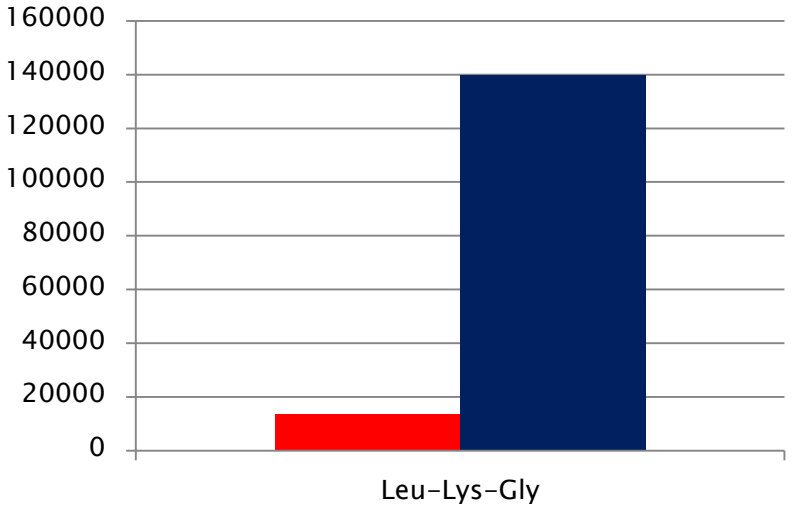
Metabolites found highest in *S. aureus* group



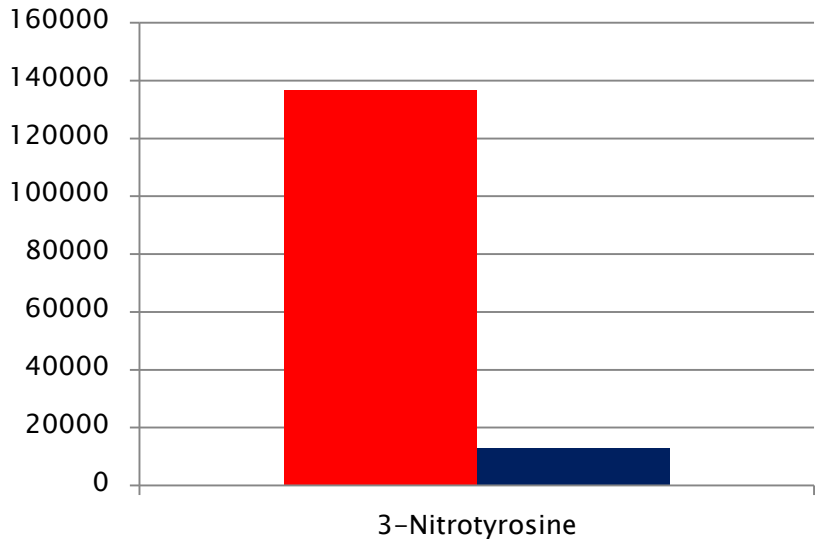


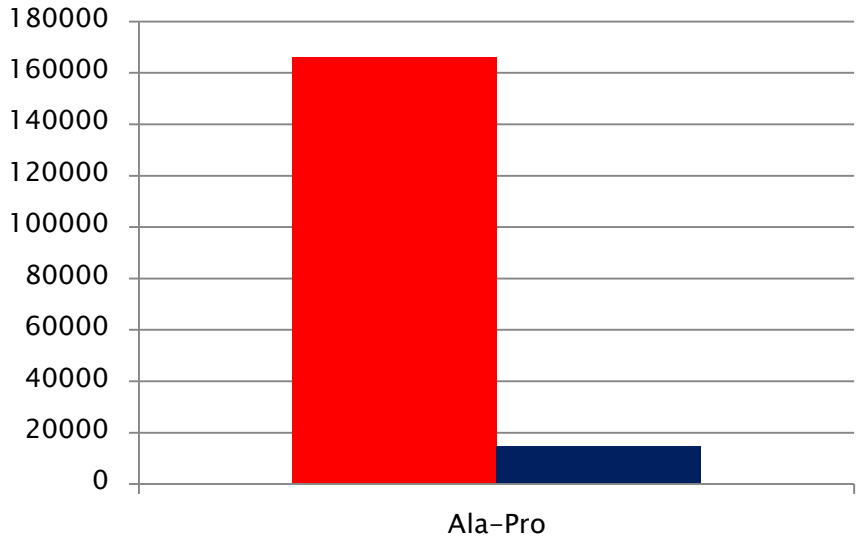


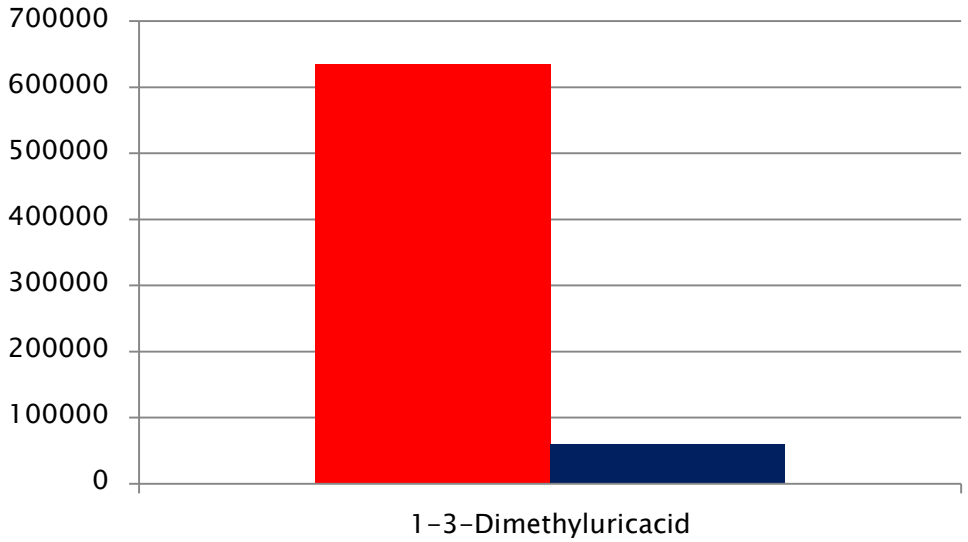


S.aureus
E.coli

Metabolites found highest in *E.coli* group







S.aureus
E.coli

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Metabolomics

- **Lipid, protein and carbohydrate metabolism are all affected by mastitis**
- **Arachidonic acid metabolites are increased in mastitis**
- **Amino acids, di & tri peptides increase in mastitis**
- **Significant metabolite differences found in healthy versus mastitis milk**
- **Significant metabolite differences found in *E. coli* versus *S. aureus* milk**
- **Many other causes of change in milk metabolome**

- **Proteomics and milk proteins**
  - Gel based proteomics
  - Non-gel based proteomics
- **Metabolomics and mastitis**

- **Non-invasive sampling**
  - Saliva
  - Sweat
  - Nasal secretion
  - Interstitial fluid

Bovine Salivary Proteome & Glycoproteome

- Bovine saliva collected on gauze swabs
- 4 different pre-fractionation methods
- 402 salivary protein
- 45 N-linked glycoprotein
- Immunoglobulin, prolactin-inducible protein, cystatins, carbonic anhydrase, lactoferrin, lysozyme
- No amylase
- Ang et al J Prot Res 2011 10 5059

Bovine Salivary Proteome & Glycoproteome

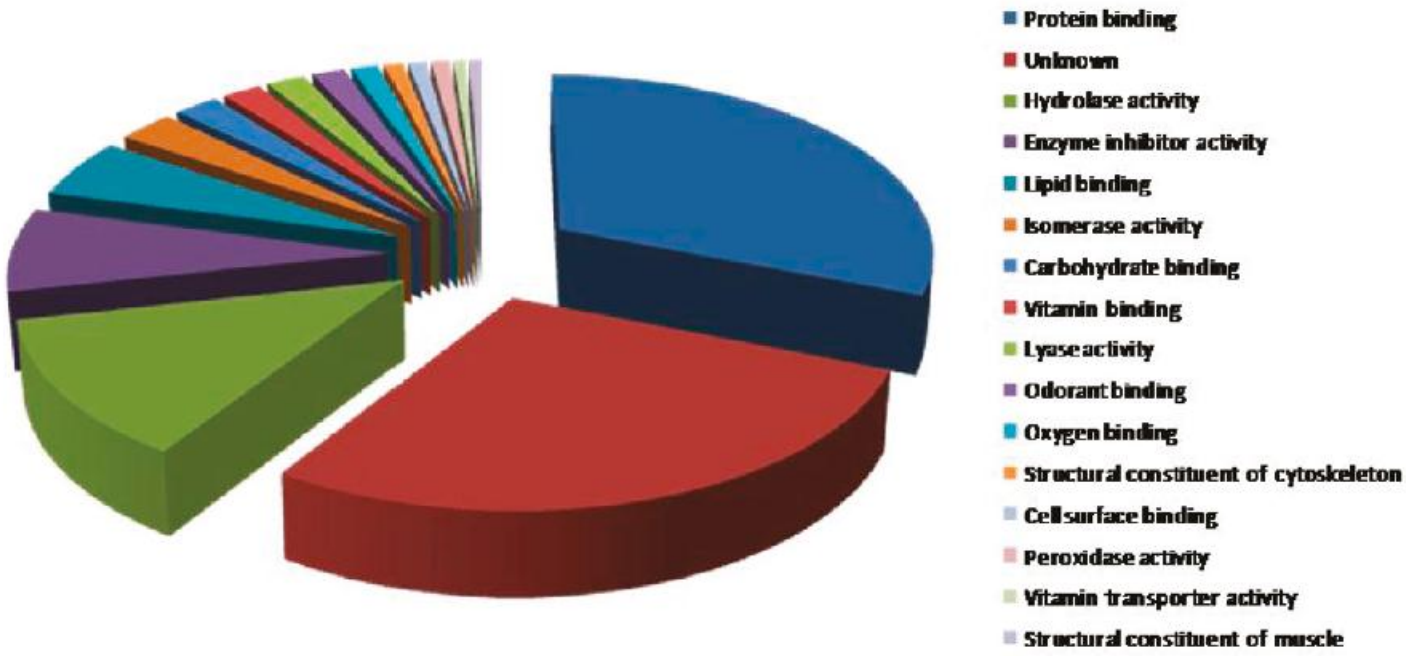


Figure 4. Functional classification of the 396 proteins identified from the nontargeted approach (SDS-PAGE, Off-gel, RP-HPLC and SCX-HPLC prefractionation). Classification is based on the GeneOntology molecular function annotation using the DAVID Functional Annotation Clustering tool (http://david.abcc.ncifcrf.gov/).

Human Sweat: non-invasive sampling

- **Human sweat proteome**
- **Armpit sweat compared in controls v Schizophrenia patients**
- **LC-MS/MS analysis**
- **17 protein had 2x or higher difference in patient group**

- **Raiszadeh et al J Prot Res 2012, 11:2127**

Protein	Peptide	Patient Pool: Control Pool
Alpha-2-glycoprotein	YSLTYIYTGLSK	1.9 : 1
	AYLEEECPATLR	1.7 : 1
Annexin 5	VLTEIIASR	4.6 : 1
	GTVTDFPGFDER	5.1 : 1
Arginase	GGVEEGPTVLR	2.9 : 1
Bleomycin Hydrolase	DGEAVWFGCDVGK	inf.
Calmodulin-like Skin Protein	VNYEEFAR	2.1 : 1
	AGLEDLQVAFR	2.0 : 1
Caspase 14	FQQAIDSR	2.7 : 1
	LENLFEALNNK	5.4 : 1
Corneodesmosin	IILQPCGSK	2.5 : 1
	GSPGVPSFAAGPPISEGK	3.0 : 1
Cystatin A	TQVVAGTNYYIK	2.8 : 1
Dermcidin	ENAGEDPGLAR	1.0 : 1
Desmoglein	YQGTILSIDDNLQR	3.6 : 1
DJ-1	DGLILTSR	4.0 : 1
G3PDH	VPTANVSVVDLTCR	6.7 : 1
Kallikrein 11	LLCGATLIAPR	2.6 : 1
Keratin 10	YENEVALR	1.0 : 1
	ALEESNYELEGK	0.97 : 1
Peroxiredoxin 1	GLFIIDDK	6.2 : 1
	LVQAFQFTDK	9.3 : 1
Prolactin-induced Protein	TVQIAAVVDVIR	0.57 : 1
Prostatic-binding Protein	LYTLVLTDPDAPSR	7.2 : 1
S100A7	GTNYLADVFEK	9.2 : 1
Thioredoxin	VGEFSGANK	8.9 : 1
	TAFQEALDAAGDK	10.6 : 1

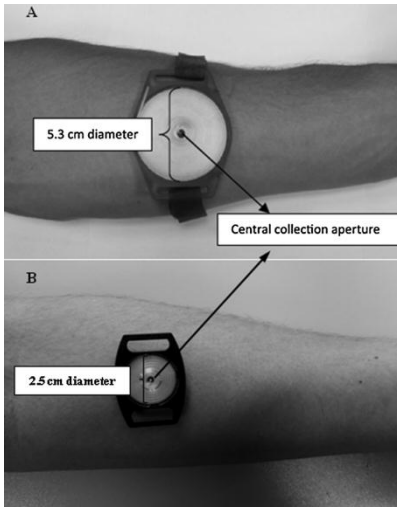
Sweat metabolomics for cancer biomarker

- **Sweat collecting device required 10μl for metabolomics**

- **Compared sweat from control and lung cancer patient**

- **Possible biomarkers:**

citrulline, maltotriose, maltotetraose, suberic, urocanic, muconic acid and nonanedioic acids, trisaccaride phosphate, phenylalanine, histidine, taurine, citruline, tryptophan, sulfonic lipid, and l-octen-3yl-glucoside



Mena-Bravo et al J Pharma Biomed Anal 2014 ,90 :139

Lactoferrin & antimicrobial peptides in sweat

- **Human sweat examined by LC-MS/MS**
- **Antimicrobial found in sweat**
- **Lactoferrin confirmed by western blot**

Park et al Exper Derm 2011 20 367

Table 1. AMP identified by Mass spectrometry from human sweat sample

Accession number	Mass [Da]	Protein score	Description
gi|34412	78 029	828	Lactoferrin
gi|16751921	11 277	299	Dermcidin precursor
gi|12053626	10 410	238	Psoriasin
gi|299033	14 679	128	Lysozyme = amyloid fibril protein [human, Peptide Mutant, 130 aa]
gi|31979	13 899	107	Histone H2A.2
gi|7245541	37 076	75	Chain a, r210k n-terminal lobe human lactoferrin
gi|13399625	14 678	71	Chain A, Mutant Human Lysozyme (q86d)
gi|307141	16 544	70	Lysozyme precursor (EC 3.2.1.17)
gi|386772	15 238	48	Histone H3
gi|32097	11 269	47	Histone cluster 2, h4a

AMP, antimicrobial peptides or proteins.

Proteome list from sweat by LC-MS/MS

	Accession number	Mass [Da]	Protein score	Description
1	gi|1346343	65978	989	Cytokeratin 1
2	gi|34412	78029	828	Lactoferrin
3	gi|29667	53117	781	Carboxypeptidase E
4	gi|38026	34714	779	Zn-alpha2-glycoprotein
5	gi|28317	59492	668	Cytokeratin 10
6	gi|619383	27975	578	Apolipoprotein D
7	gi|435476	62092	484	Cytokeratin 9
8	gi|56554584	17914	473	Chain A, Crystal Structure Of Human Tear lipocalinvon EBNERS GLAND Protein
9	gi|42716297	57796	400	Clusterin isoform 1
10	gi|229532	8446	388	Ubiquitin
11	gi|178855	48772	379	Apolipoprotein J precursor
12	gi|55859601	15883	345	Calmodulin-like 5
13	gi|51094526	16562	335	Prolactin-induced protein
14	gi|6912286	27662	333	Caspase 14 precursor
15	gi|5902072	44537	319	Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 3
16	gi|4557014	59719	299	Catalase
17	gi|16751921	11277	299	Dermcidin precursor
18	gi|57997569	52459	266	Hypothetical protein
19	gi|483474	65289	264	90K , galectin 3 binding protein
20	gi|28590	69250	246	Albumin
21	gi|4504963	19238	238	Lipocalin 1 precursor
22	gi|12053626	10410	238	Psoriasin
23	gi|238236	83262	232	Transmembrane secretory component; poly-Ig receptor; SC
24	gi|189308	53698	229	Nucleobindin
25	gi|5729909	9918	224	Lipophilin B precursor

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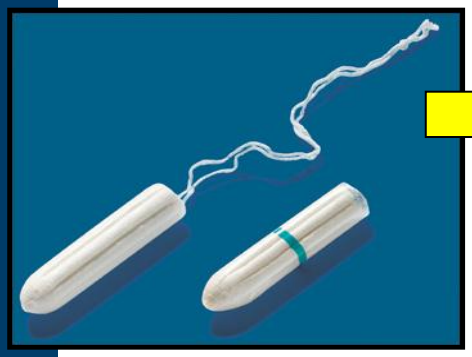
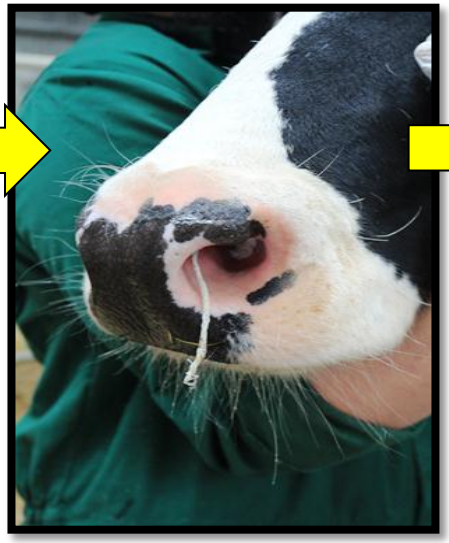
Nasal Secretion

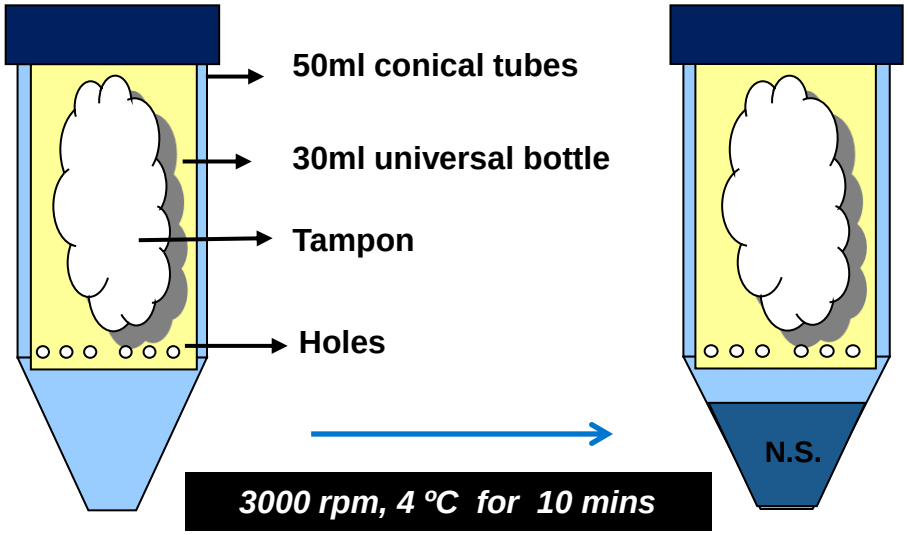
- Nasal secretion for non-invasive sampling
- Nasal secretion collection – up to 12ml in 10 min
- Biochemical analysis revealed high activity of alkaline phosphatase
- Protein content examined by 2DE
- Nasal secretion proteome characterised
- Change in proteins in response to malignant catarrhal fever determined

- Mohd Faizal Ghazali, University of Glasgow

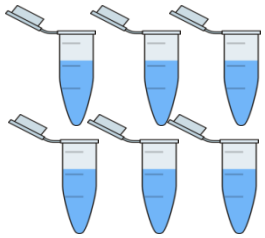
Nasal secretion - Collection





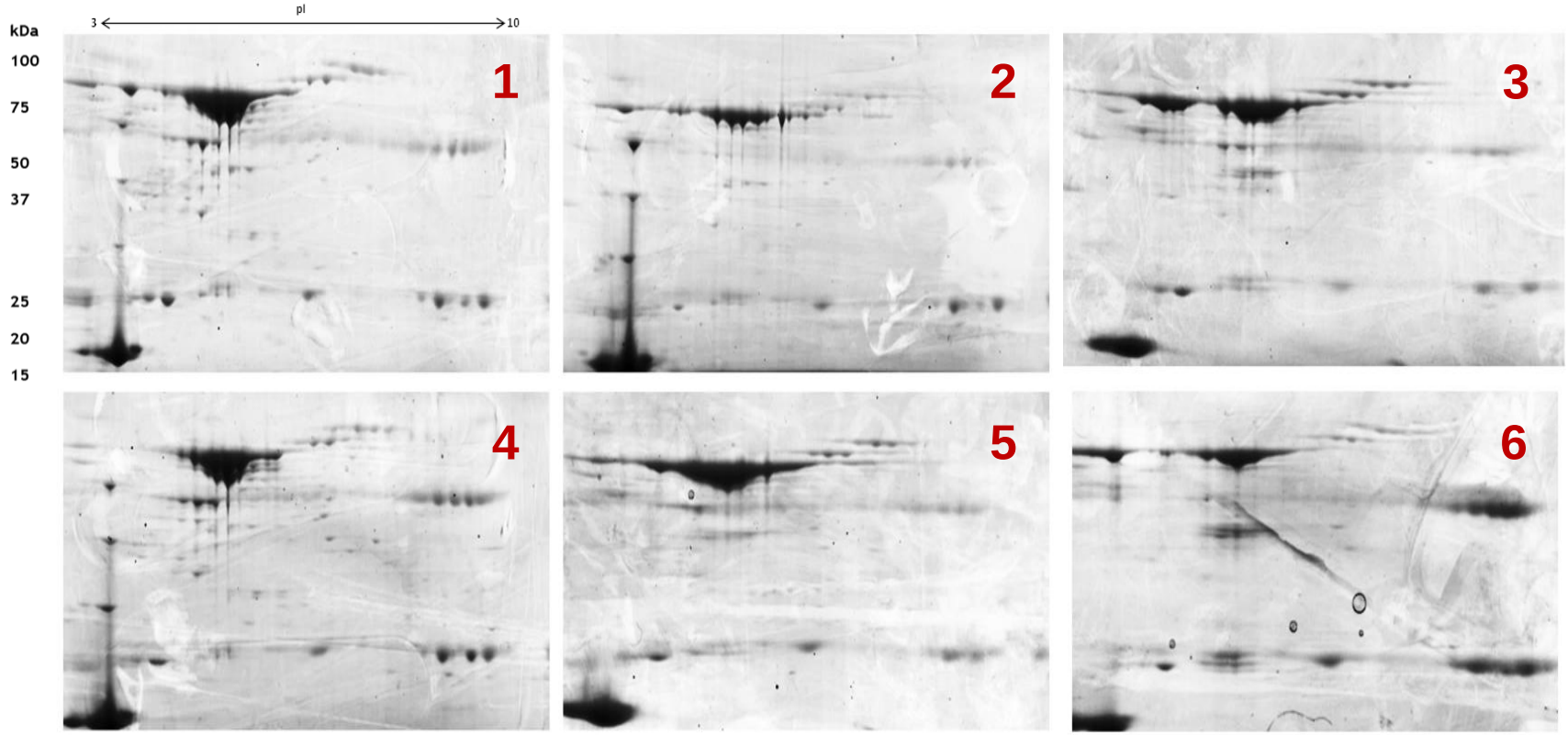


3000 rpm, 4 ºC for 10 mins



5 – 12 ml / tampon

Individual 2-D electrophoresis of nasal secretion from six different cattle



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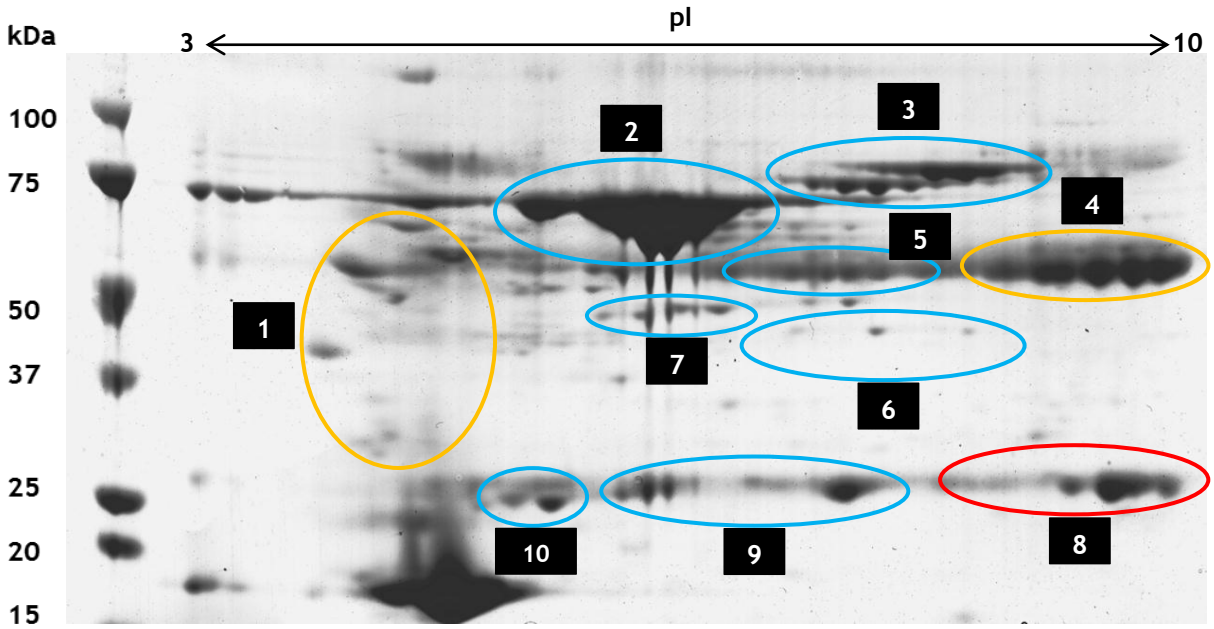
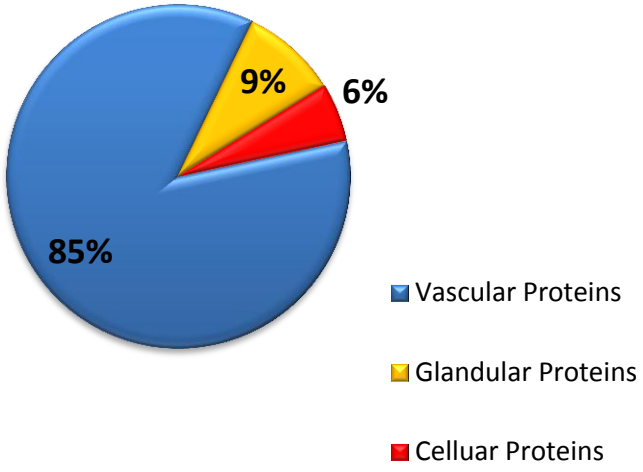
Nasal Secretion Protein





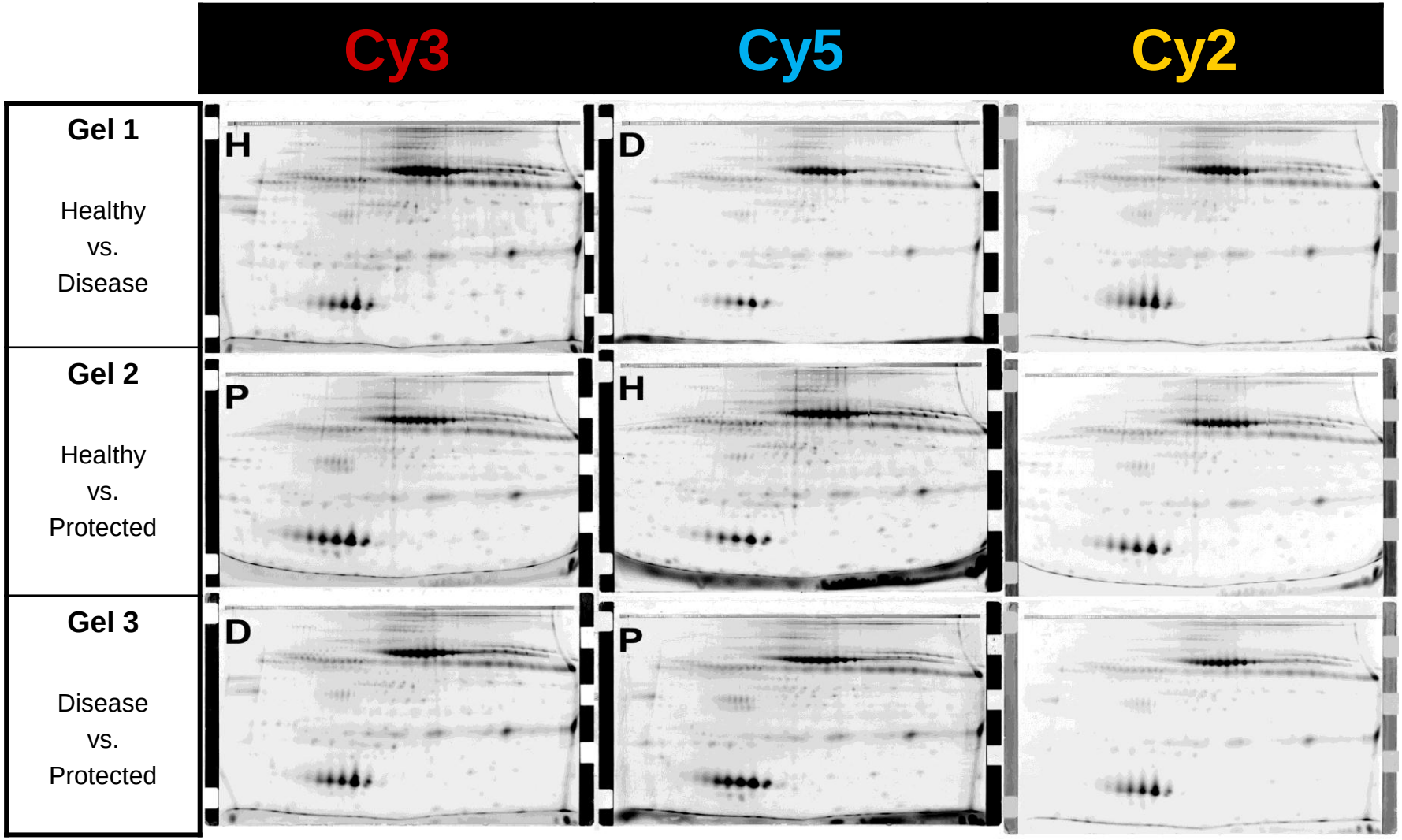
Protein spot no.	Protein name	Accession	Theoretical mass (Da)	Highest MS score	Highest Matches
1	Odorant-binding protein	OBP_BOVIN	18492	498	32
2	Albumin	ALBU_BOVIN	71244	2568	163
3	Serotransferrin	TRFE_BOVIN	79870	1394	102
4	Lactotransferrin	TRFL_BOVIN	80002	129	12
5	Ig heavy chain	O46780_BOVIN	51391	621	38
6	Fibrinogen beta chain	FIBB_BOVIN	53933	474	40
7	Complement C3	CO3_BOVIN	188652	501	51
8	Glutathione S-transferase	GSTP1_BOVIN	23826	525	26
9	Ig light chain	Q1RMN8_BOVIN	25032	189	23
10	Apolipoprotein A1	APOA1_BOVIN	30258	1296	77

Malignant Catarrhal fever

- **Nasal secretion from experimental model of Malignant Catarrhal Fever**
- **Calves immunised with attenuated C500 strain AlHV-1**
- **Challenge with virulent C500 strain AlHV-1**
- **Healthy samples: Pre-challenge NS sample**
- **Disease: not immunised, NS taken at 2 weeks, no survivors at week 12**
- **Protected: immunised, NS taken at 2 weeks, all survived at week 12**
- **Pools of NS from each group prepared (n=7) and run on DiGE in duplicate**

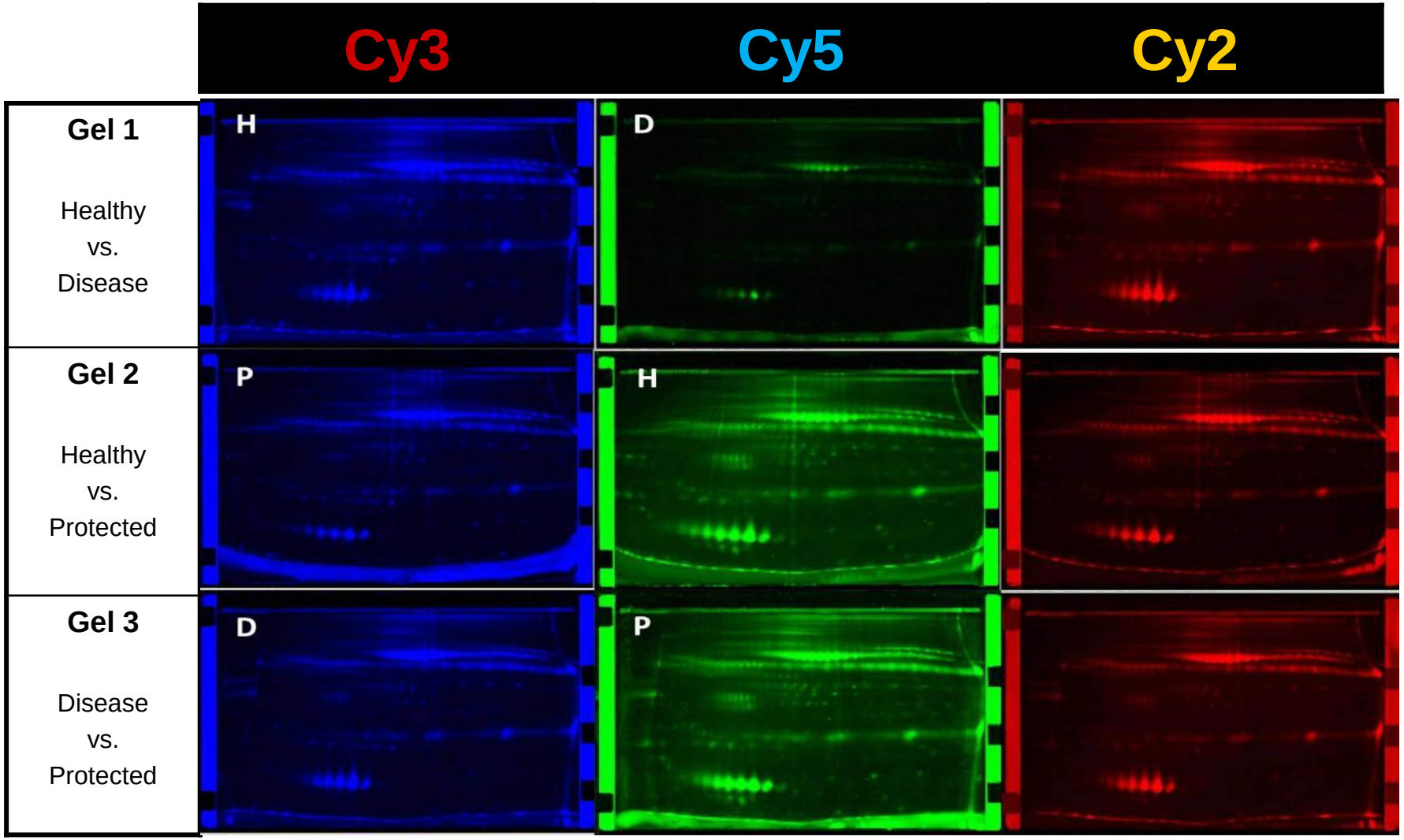
Collaboration with Moredun Research Institute; Haig, D.M. et al., 2008. *Vaccine*, 26(35)

Relative Quantification by Difference Gel Electrophoresis (DiGE) of Nasal secretion from calves with MCF



Malignant Catarrhal fever

Relative Quantification by Difference Gel Electrophoresis (DiGE) of Nasal secretion from calves with MCF



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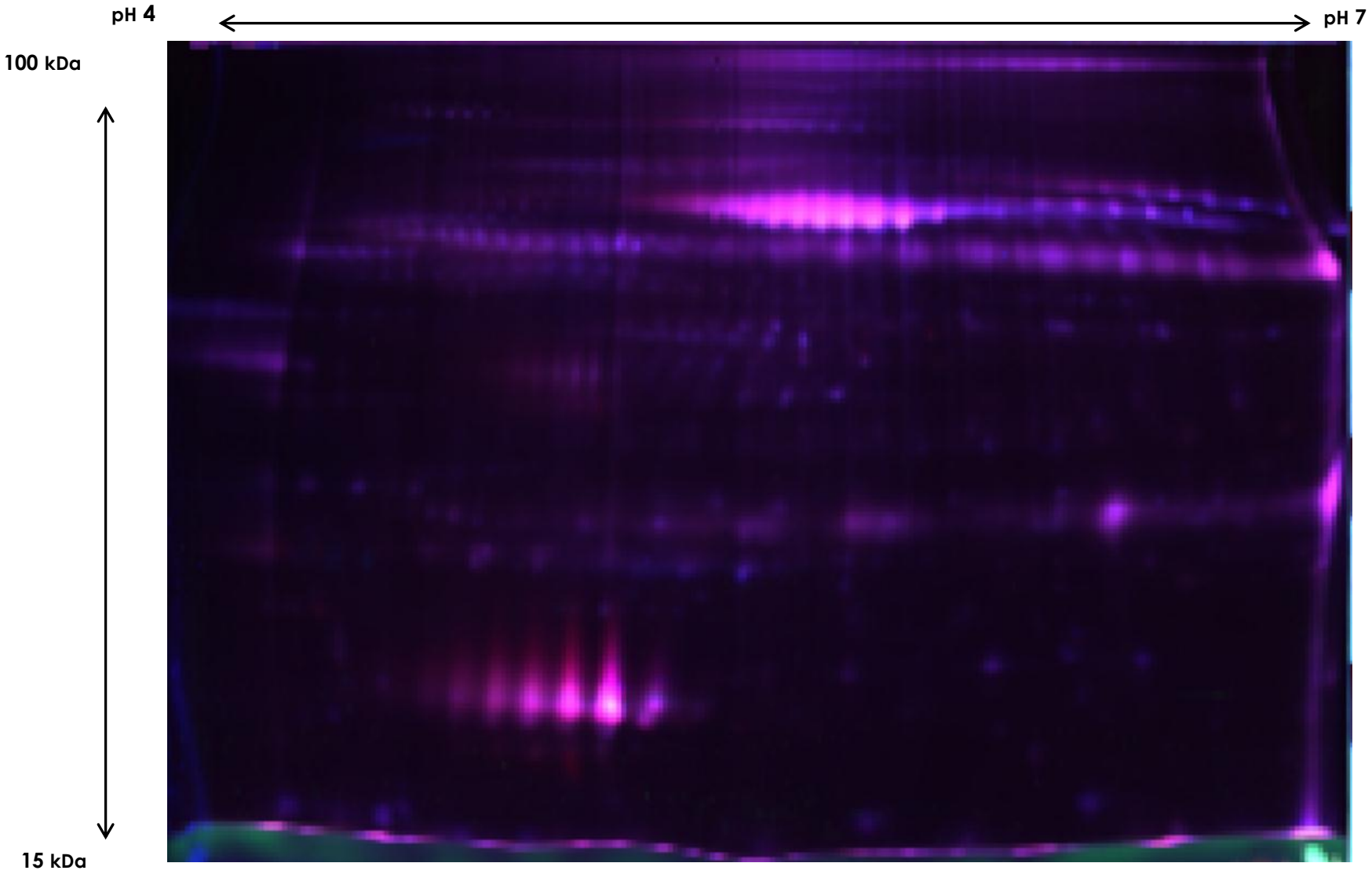
DiGE of Nasal Secretion in MCF



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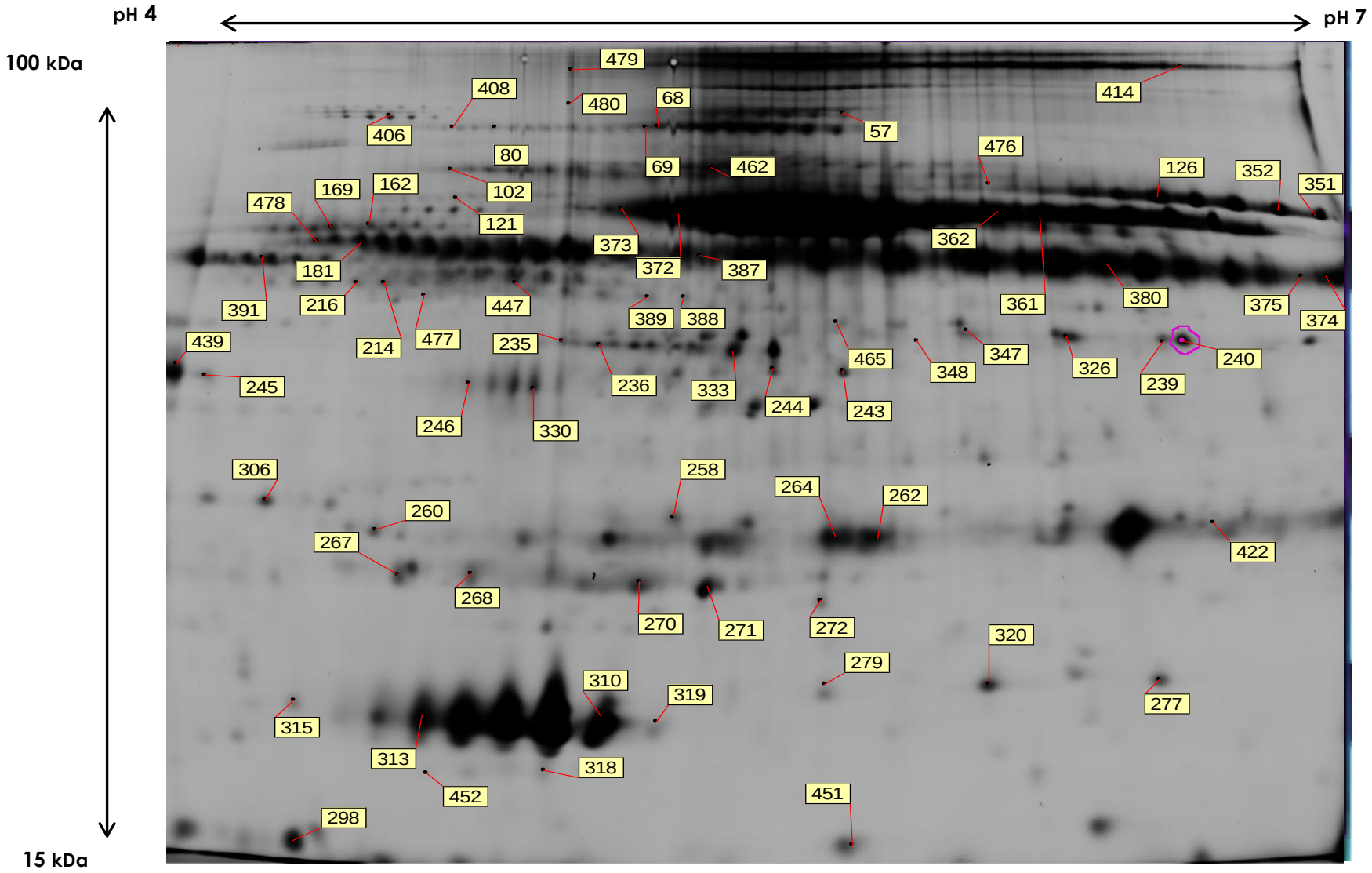
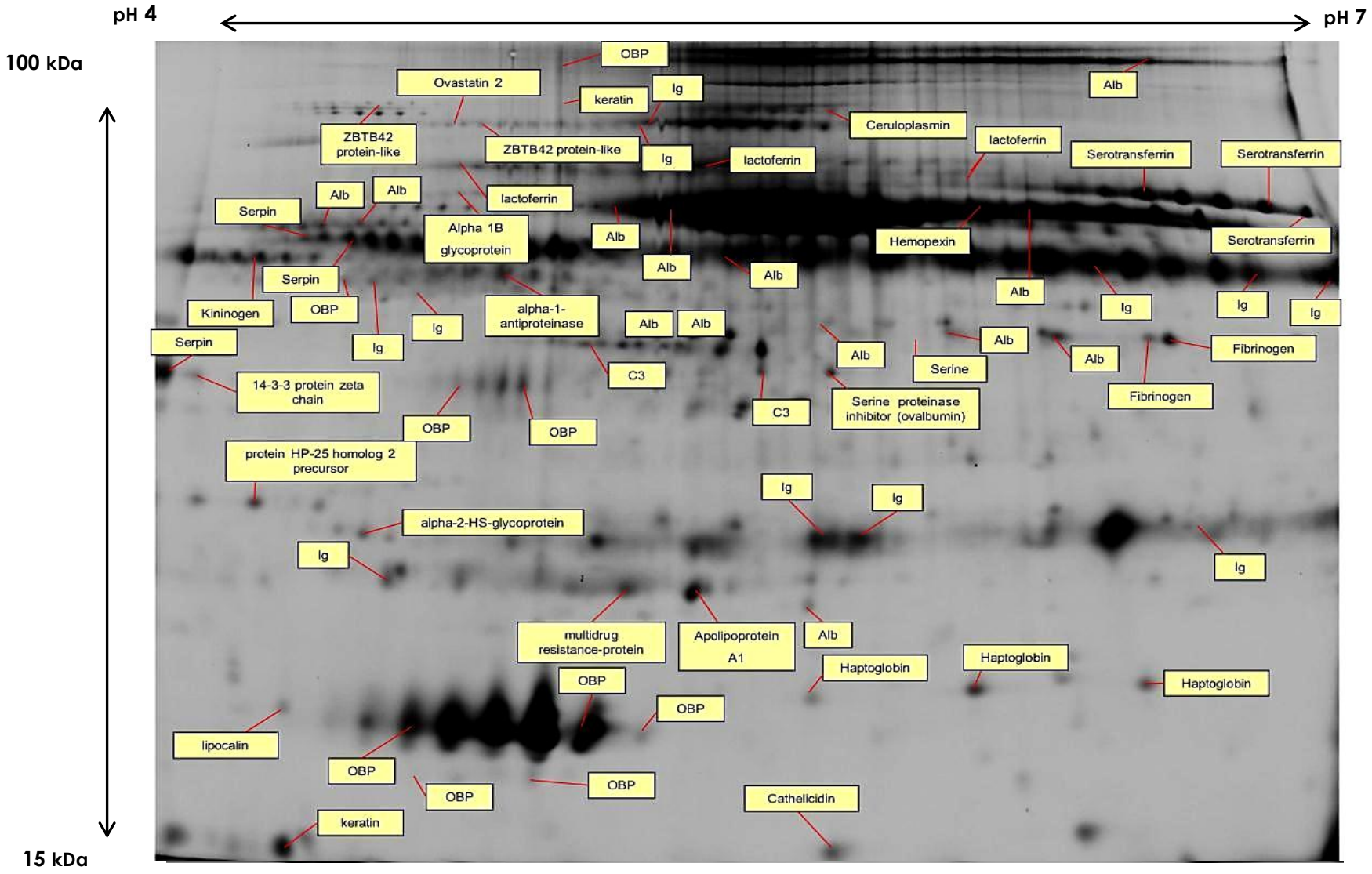
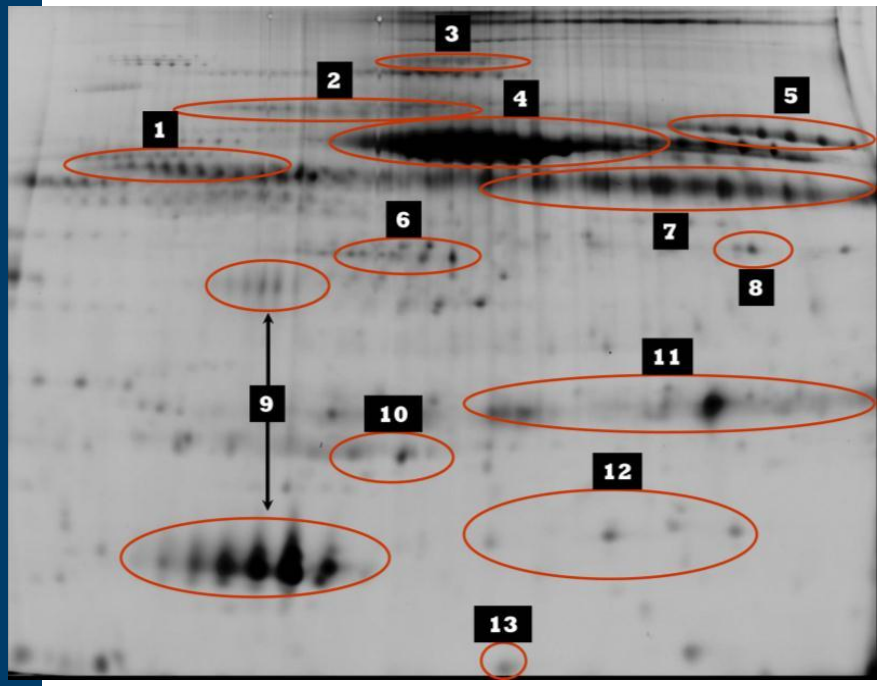
DiGE of Nasal Secretion in MCF



DiGE of Nasal Secretion in MCF



DiGE of Nasal Secretion in MCF



Protein spot no.	Protein name	Accession	Theoretical mass (Da)	Highest MS score	Highest matches
1	Serpin	SPA35_BOVIN	46482	578	78
2	Lactoftransferrin	TRFL_BOVIN	80002	1107	149
3	Ceruloplasmin	NP_001243485	121901	434	40
4	Albumin	ALBU_BOVIN	71244	1681	228
5	Serotransferrin	TRFE_BOVIN	79870	1404	147
6	Complement C3 (fragments)	CO3_BOVIN	188652 (~ 68000)	554	110
7	Ig heavy chain	O46780_BOVIN	51391	749	58
8	Fibrinogen beta chain	FIBB_BOVIN	57146	722	112
9	Odorant-binding protein	OBP_BOVIN	18492	579	97
10	Apolipoprotein A1	APOA1_BOVIN	30258	368	74
11	Ig light chain	Q1RMN8_BOVIN	25032	343	45
12	Haptoglobin	HPT_BOVIN	45629	383	60
13	Cathelicidin	CTHL1_BOVIN	17931	412	59

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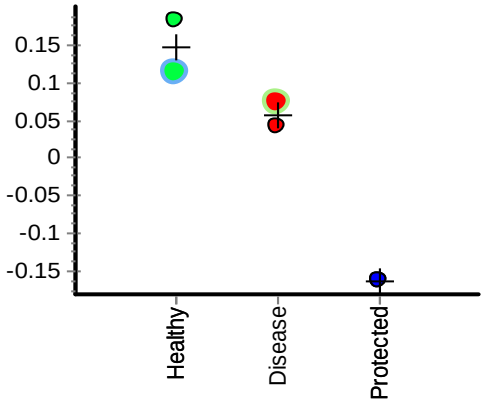
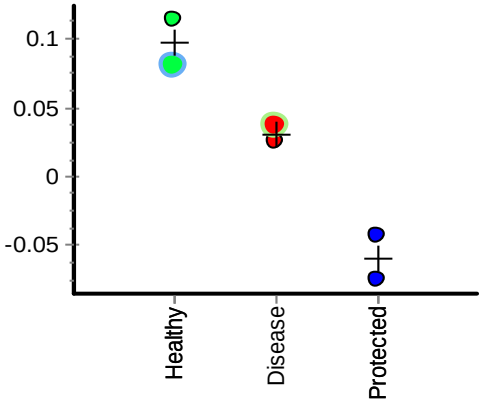
DiGE of Nasal Secretion in MCF

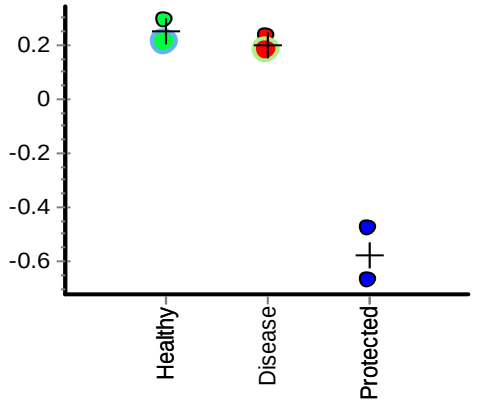
Fibrinogen beta chain



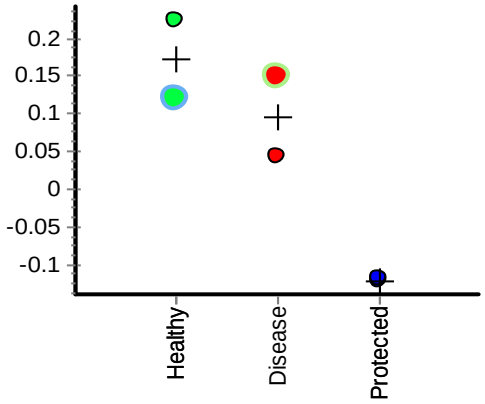
Albumin



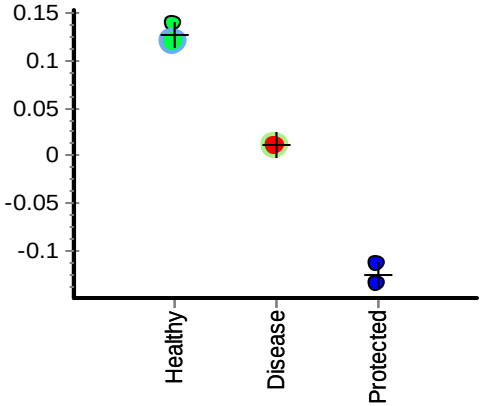
Apolipoprotein A1



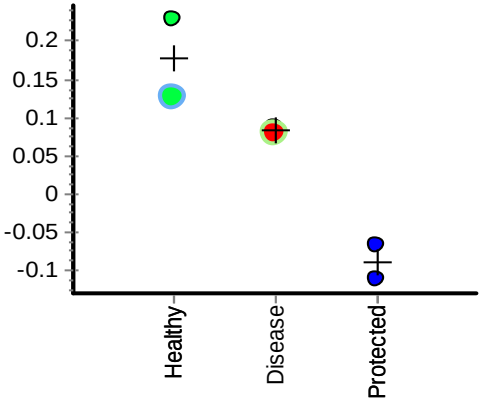
Ceruloplasmin



Kininogen



Hemopexin



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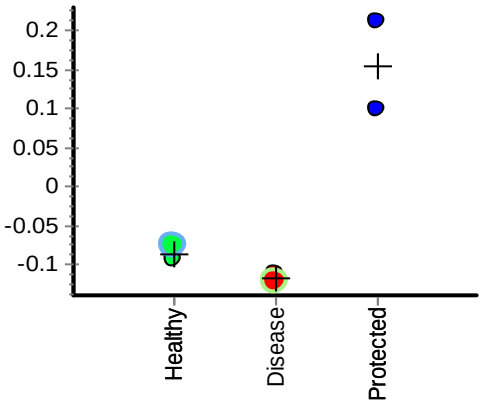
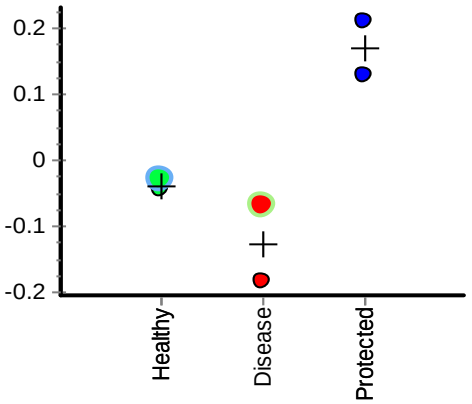
DiGE of Nasal Secretion in MCF

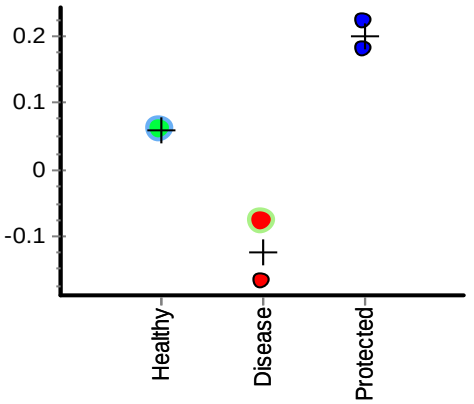
Lactoferrin



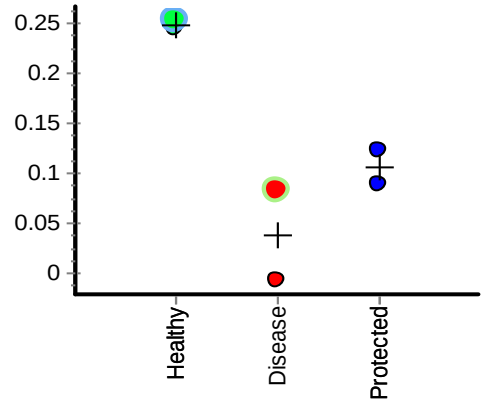
Complement C3



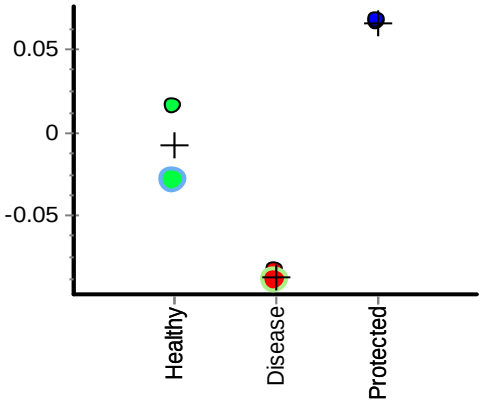
Haptoglobin



Cathelicidin



Serotransferrin



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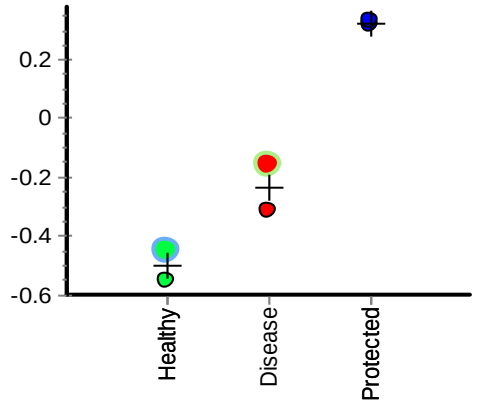
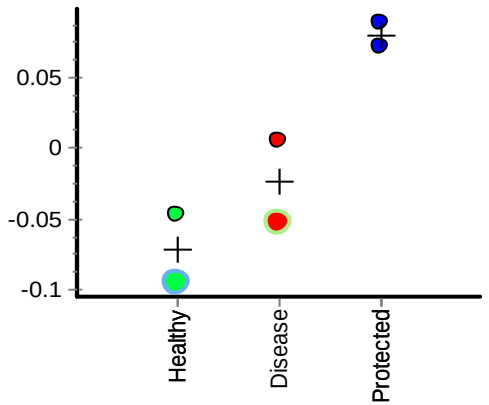
DiGE of Nasal Secretion in MCF

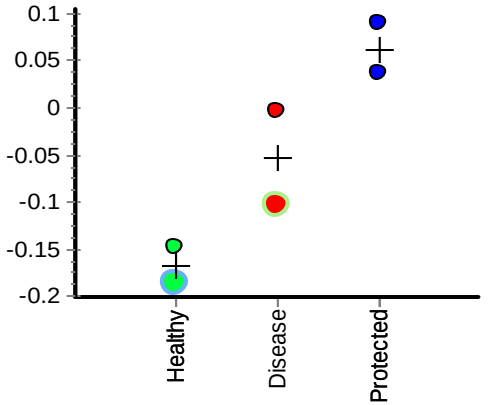
Odorant Binding Protein



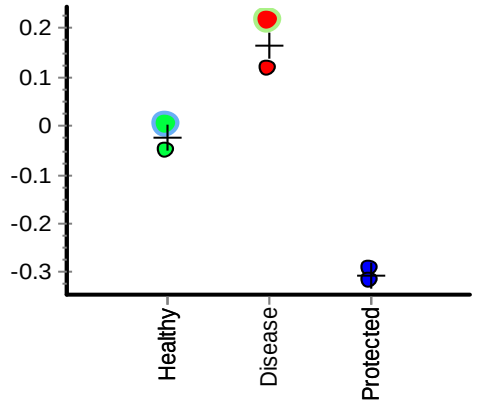
Ig A



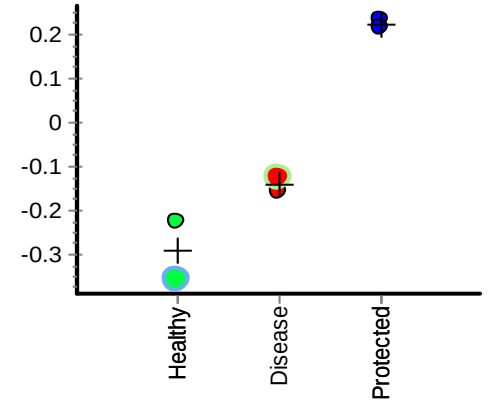
Ig G



Serpin



Alpha-2-macroglobulin



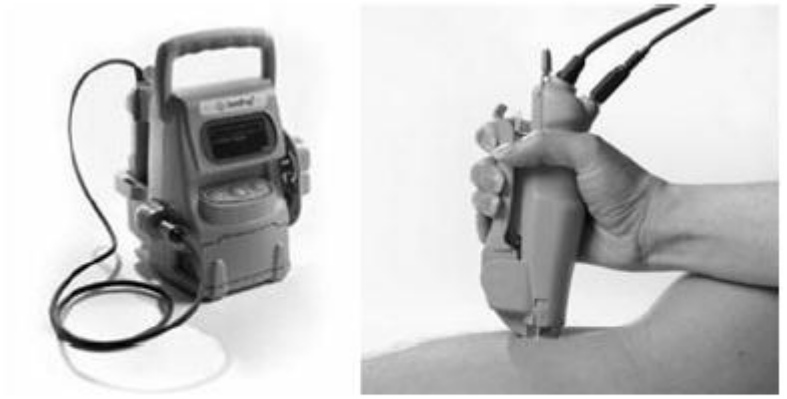
DiGE of Nasal Secretion in MCF

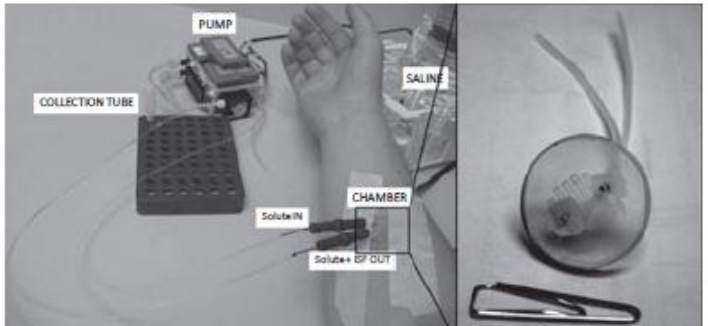
- **23 protein spots were differentially expressed as determined by DiGE**
- **Most plasma proteins ↓ in challenge cattle**
- **Immunoglobulins, OBP and Serpin ↑ disease and protected cattle**
- **Proteins involve in innate immunity and antimicrobial ↑ in protected ↓ in disease**

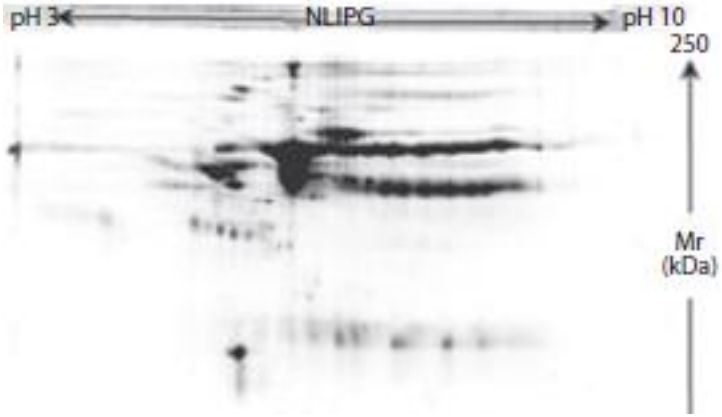
Proteomics of Interstitial fluid

- **Device for collecting interstitial fluid 0.5ml of ~1mg/ml protein**
- **15min collections up to 90 min**
- **Protein analysed by 2DE**
- **Identification underway**
- **Not suitable for Dairy Care but ISF is a substrate for proteomics**

Lecombe et al Skin Res & Tech, 2013 19:27

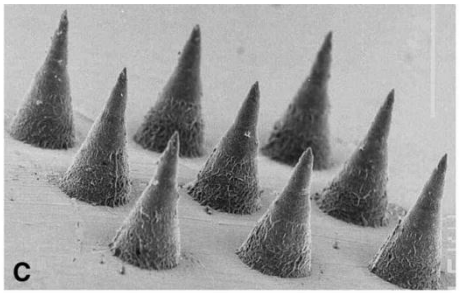


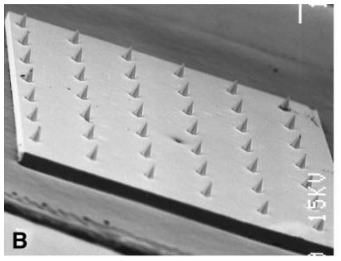




ISF collection by Microneedle arrays

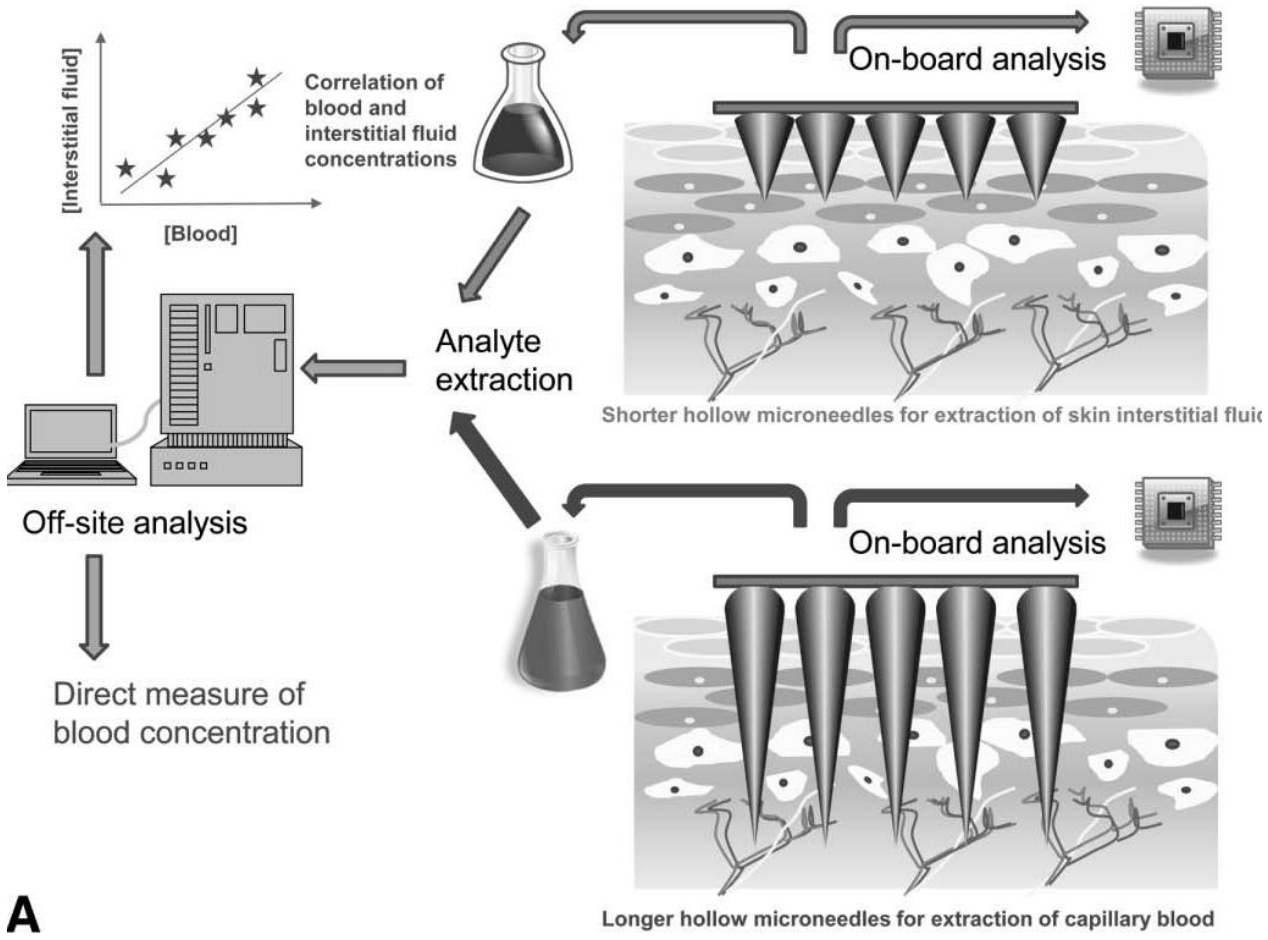
- **Microneedle arrays ~100 per cm^2**
- **Made of polymer (b) poly(methylvinylether-co-maleic acid) and poly(ethyleneglycol) (c) silicone or metal**
- **For therapeutic drug delivery – also sampling of ISF**
- **Painless penetration of skin**
- **Collect interstitial fluid: 50-150 nm**
- **Collect capillary blood: 1000-1500nm**
- **Sample collected for analysis**
- **Potential to link to point of care biosensors**





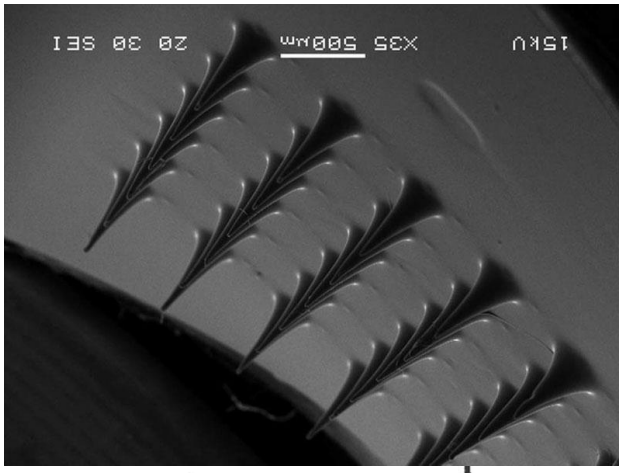
Donnelly et al Ther Drug Monit 2014, 36:10

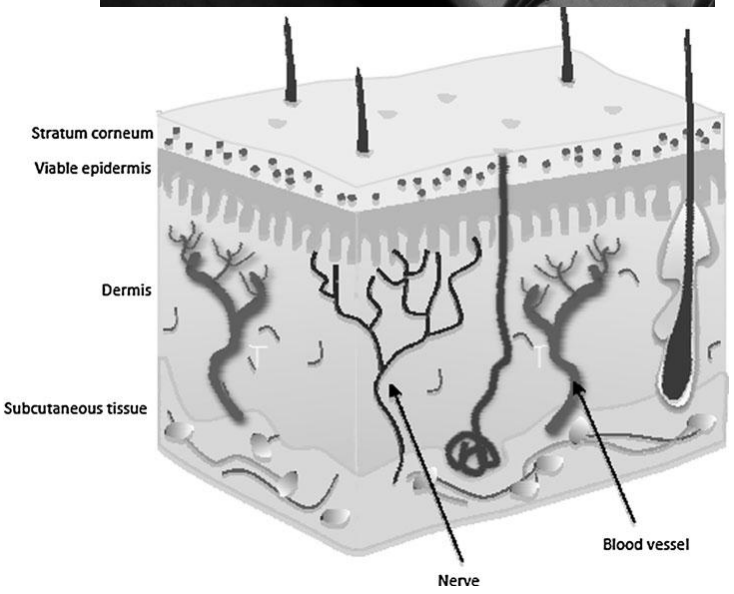
Microneedle sampling



ISF and Microneedle Arrays

- **Review of microneedle array for glucose monitoring**
- **ISF collected for continuous glucose monitoring**
- **Glucose in ISF and serum**
- **Pain assessed on visual analog scale**
  - **Control 0.42 mm**
  - **26 gauge needle 23.9mm**
  - **Microneedle array 0.67 mm**





- **El-Aboudi et al Diabetes Tech Therap 2013 15:101**

University of Glasgow

Summary

- **Proteomics can detect changes in the milk protein composition during mastitis**
- **Quantitative proteomics can provide sensitive & multiplexed assays of low abundance milk protein**
- **Peptidomics can provide a panel of peptides which could be used as biomarkers of the cause of mastitis**
- **Metabolomics detects many metabolites in milk, potential as biomarkers of pathogens**
- **Bovine saliva and nasal secretion can be substrate for proteomics**
- **Sweat and interstitial fluid could be collected for omic analysis**

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Metabolomic method

Mass spectrometry analysis

•LC system coupled with MS (Dionex UltiMate 1)

-separation using a 4.6 x 150mm ZIC-HILIC column at 300µl/min

-gradient ran from 80% ACN to 20% ACN in 30 min, step to 5% ACN, wash for 9 min, re-equilibrate for 6 min at 80%

•MS Acquisition (Orbitrap Exactive)

-Performed in both –ve and +ve ionisation modes and full scan modes

-50 000 resolution, scan range 70- 1400, noise threshold of 5000, m/z tolerance of 0.0005Da, alignment with a mass tolerance of 0.0005 and rt tolerance of 15 sec.