DCU | Water Institute

From Separations to Sensors for Environmental Monitoring

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Outline

Emerging chemicals of concern

- Traditional separation methods
- Compliance monitoring - EQS

Polar and non-polar organics

- Longer-term monitoring
- Trend monitoring
- Passive sampling

Environmental pollutants (toxins, pharmaceuticals)

- Sensor platform development
- Single and multi-analyte systems
- Integration of technologies

Current monitoring approach

Compliance → Surveillance → Investigative

- Compliance requirement to "good chemical and ecological status of both surface and groundwater" based on chemical monitoring data.
- Collection of a spot/grab sample that is then analysed back in the laboratory.
- Disadvantages: cost, delay in obtaining results →time needed for laboratory analyses,
  - a snapshot of the measured variable at the time of sampling.
- Levels of pollutants can vary temporally and spatially→episodic events could be missed, or conclusions could be drawn on the basis of what may only be transitory high levels.

The use of relatively inexpensive *in-situ* sensors offers the potential to reduce costs considerably, making it possible to monitor an increasingly wider set of parameters in the field, as well as providing more useful, **continuous monitoring capabilities to give an accurate idea of changing water quality**.

Micropollutants and emerging watch list chemicals

- The levels of pollutants present in water bodies are most commonly judged against set **environmental quality standards (EQSs)**.
- These standards dictate the **maximum allowable concentrations (MAC EQS)** or **range of concentrations (Annual Average or AA EQS)** of specific pollutants allowed to ensure compliance.

Micropollutants and emerging watch list chemicals

- This list of priority and hazardous substances, which already includes PAHs, pesticides and more, ➔ a new daughter directive (Directive 2013/39/EU) ➔emerging chemicals of concern, including pesticides and biocides, industrial chemicals and endocrine disruptors.
- New watch list, in the EU ➔a number of new priority substances to be monitored under the WFD as well as suggested 'watch' compounds.
- New chemicals, termed **emerging chemicals**, include organohalogens, pharmaceutical compounds, endocrine disruptors and brominated flame-retardants.

General analytical approach

- A dual column GC-ECD multiresidue method for OCPs (including HCB HBCD, Heptachlor and heptachlor epoxide), PCBs and PBDEs and pesticides in biota →
- Adapted to a triple quadrupole GC-MS method and expanded to cover additional pesticides such as dicofol and to screen for the flame retardant HBCD.
- A further LCMSMS screening method was developed for other polar pesticides that are not amenable to GCMS method

Other method developments/ adaptations

PFOS method: an LCMSMS method was studied for PFOS in line with ICES PFOS guidelines for PFC monitoring in environmental compartments (Ahrens et al 2010)

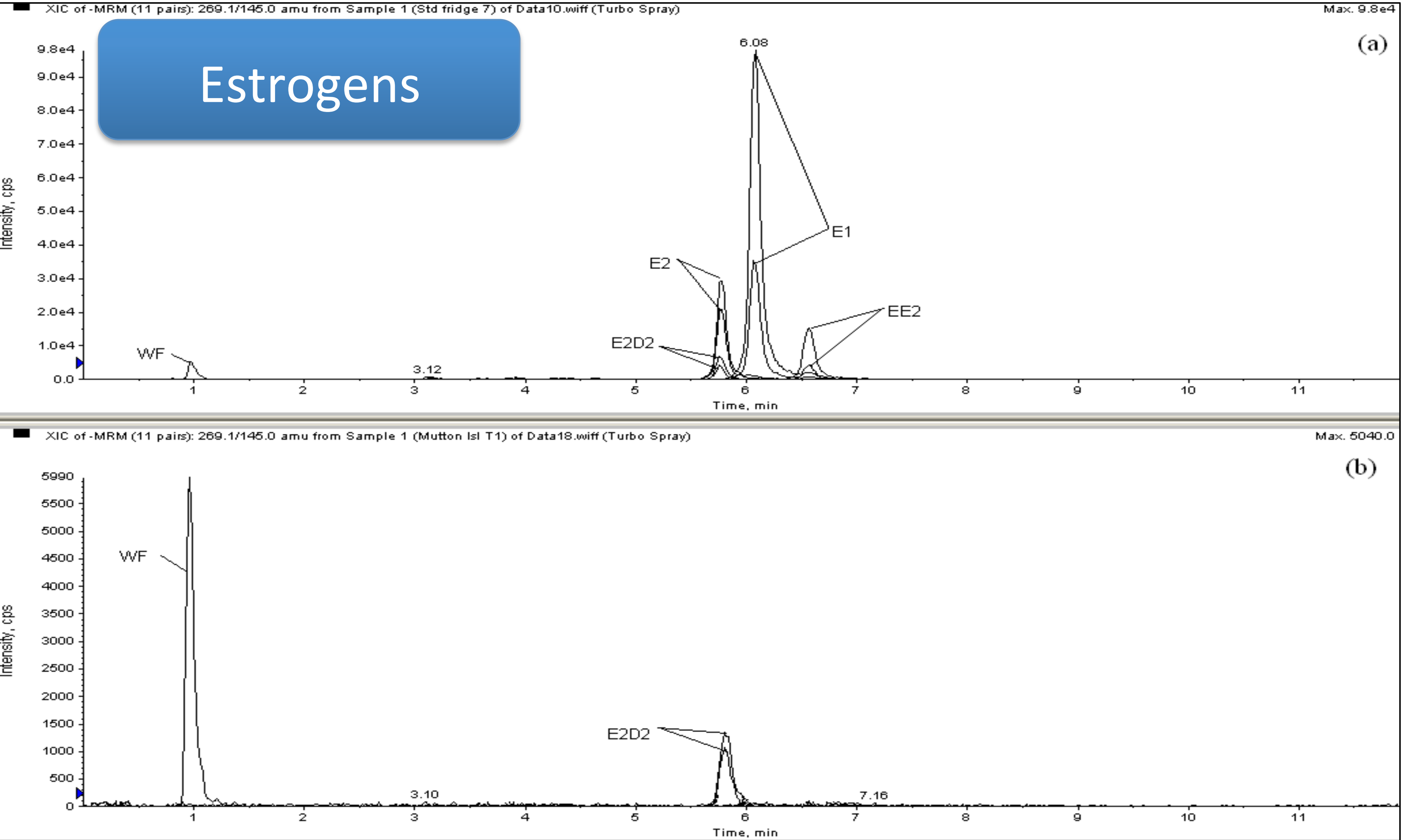
Endocrine Disrupting Substances: 17β estradiol (E2) and 17α ethynyl estradiol (EE2): LCMSMS for the analysis E2 and EE2 in water (including seawater) and biota.

Partners Marine Institute (MI) has taken part in the LGC Standards Proficiency Testing scheme (Aquacheck) for E2 and EE2 in effluent samples.

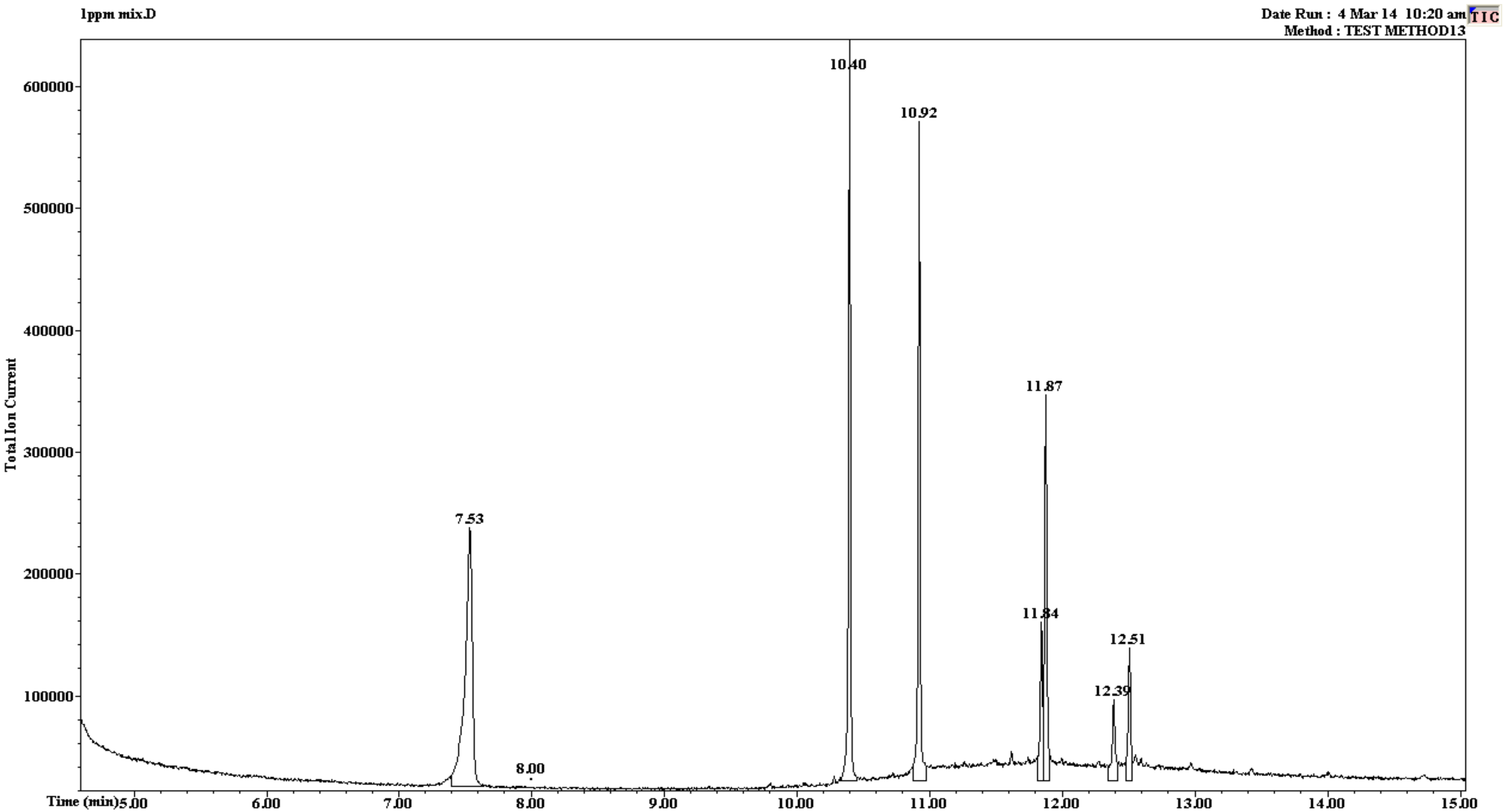
LCMS methods for Pharmaceutical Substances

Diclofenac based on the method of (Zhang, Hibberd et al. 2008)

Estrogens



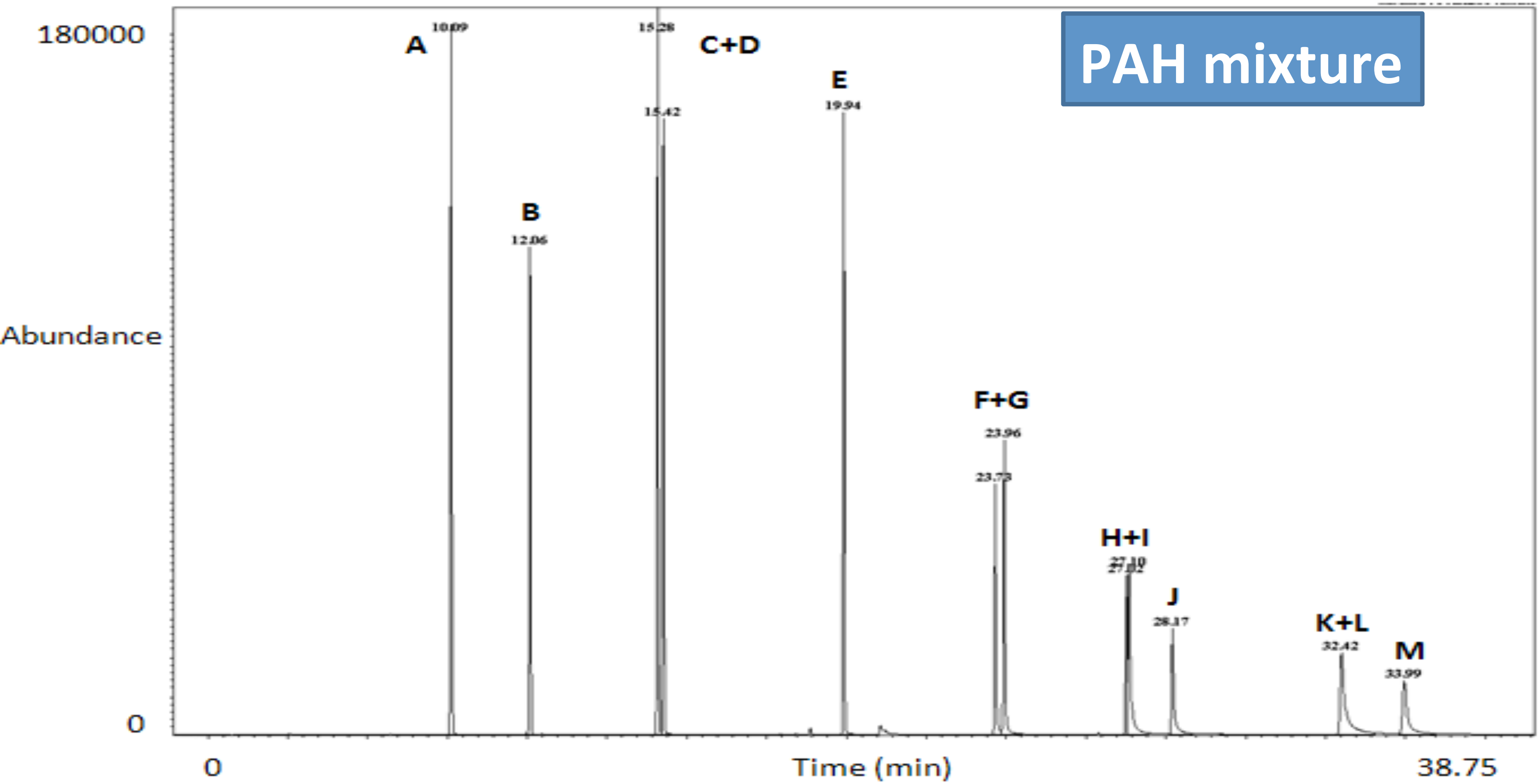
(a) Separation of external standard Warfarin (WF), followed by E2, E2d$_2$, E1 and EE2 by LC-MS/MS, (b) chromatogram of a formalin treated water sample.



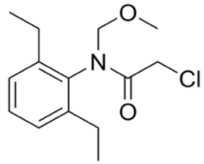
Method development for compounds by GCMS. Peaks: (1) octylphenol, (2) terbutryn, (3) cybutryne, (4) aclonifen, (5) quinoxyfen and (6) bifenox.

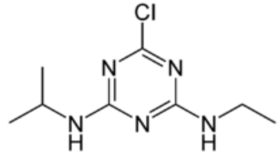
Properties of PAHs listed as Priority Pollutants in the WFD. Log K_{ow} is the octanol/water partition coefficient value which indicates the solubility of the chemical.

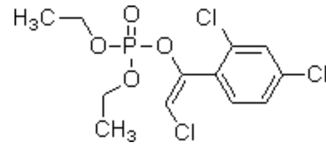
Compound	Molecular Weight (g)	Log K_{ow}	Compound	Molecular Weight (g)	Log K_{ow}
Anthracene	178.23	4.55	Fluorene	166.22	4.18
Acenaphthylene	152.19	3.94	Pyrene	202.25	4.88
Phenanthrene	178.23	4.46	Benzo(a)anthracene	228.29	5.70
Benzo(a)pyrene	252.31	6.11	Chrysene	228.29	5.63
Benzo(b)fluoranthene	252.31	6.04	Dibenzo(a,h)anthracene	278.34	6.86
Benzo(g,h,i)perylene	276.33	6.78	Benzo(k)fluoranthene	252.31	6.21
Indeno(1,2,3-cd) pyrene	276.33	6.58			

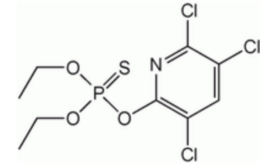


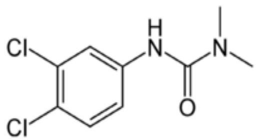
Separation of PAH mixture by GCMS; (A) Acenaphthylene, (B) Fluorene, (C) Phenanthrene, (D) Anthracene, (E) Pyrene, (F) Benzo(a)anthracene, (G) Chrysene, (H) Benzo(b)fluoranthene, (I) Benzo(k)fluoranthene, (J) Benzo(a)pyrene, (K) Indeno(1,2,3-cd)pyrene, (L) Dibenzo(a,h)anthracene, (M) Benzo(ghi)perylene

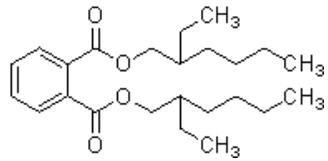

Alachlor

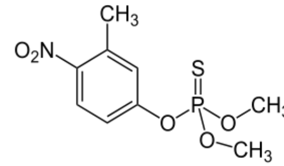

Atrazine

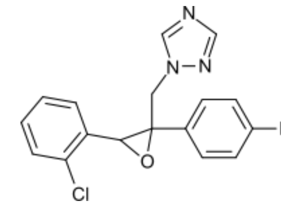

Chlorfenvinphos

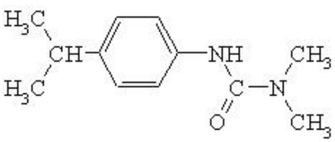

Chlorpyrifos

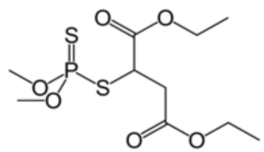

Diuron

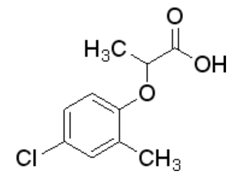

DEHP

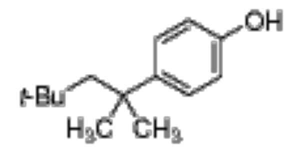

Fenitrothion

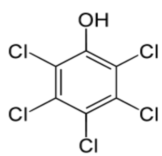

Epoxiconazole

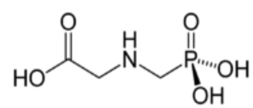

Isoproturon

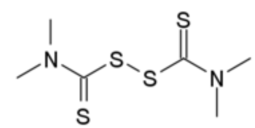

Malathion

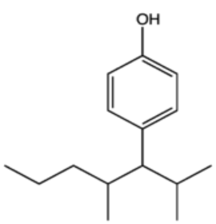

Mecoprop

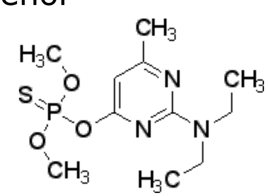

Octylphenol

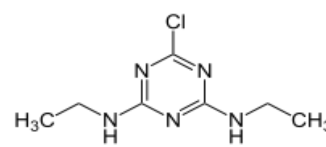

Pentachlorophenol

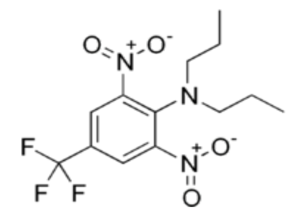

Glyphosphate


Thiram


Nonylphenol


Pirimiphos methyl


Simazine


Trifluralin

Pesticides Included in Analysis

Pesticide	Internal standard	Priority pollutant
Alachlor	Alachlor-D13	✓
Atrazine	Atrazine-D5	✓
Chlorfenvinphos	Chlorfenvinphos-D10	✓
Chlorpyrifos	Chlorpyrifos-D10	✓
Di-2-ethylhexyl-phthalate	DEHP-D4	✓
Diuron	Diuron-D6	✓
Epoxiconazole	-	
Isoproturon	-	✓
Fenitrothion	Fenitrothion-D6	
Malathion	Malathion-D6	
Mecoprop	Mecoprop-D3	
Octylphenol	-	✓
Nonylphenol	Nonylphenol-D8	✓
Pentachlorphenol	Pentachlorphenol-13C6	✓
Pirimiphos-methyl	Pirimiphos-methyl-D6	
Simazine	Simazine-D10	
Thiram	Thiram-D12	
Trifluralin	Trifluralin-D14	✓
Glyphosate	Glyphosate -13C2 15N	

CIT

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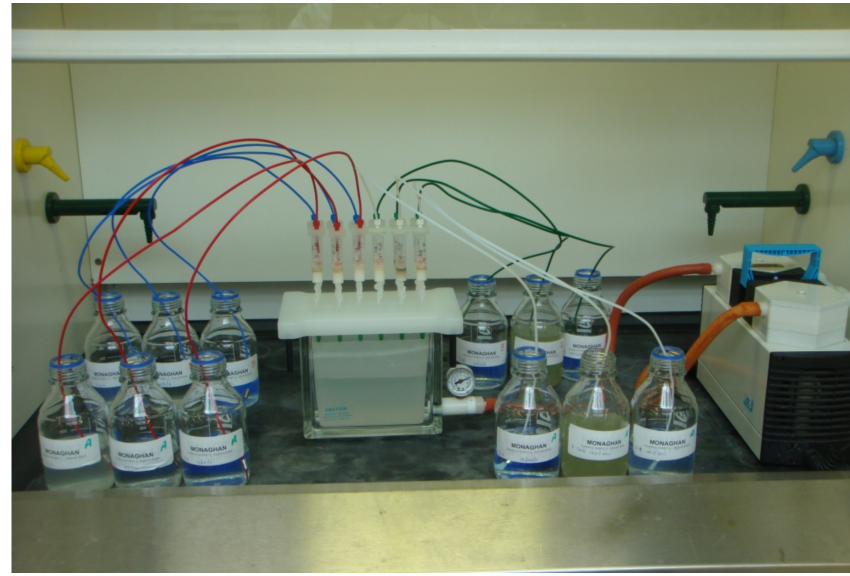
Sample Preparation

- **Solid-Phase Extraction**
- Phenomenex Strata-X
  - Extracts wide range of compounds

Protocol:

- Filter water sample if necessary
- Condition and equilibrate SPE cartridge
- Apply water samples onto SPE cartridge
- Rinse with deionised water
- Dry SPE cartridge
- Store SPE cartridge at -20°C if necessary
- Elute pesticides with ACN and IPA
- Evaporate under N_2 in Turbovap
- Add 1 mL ACN
- Filter and analyse



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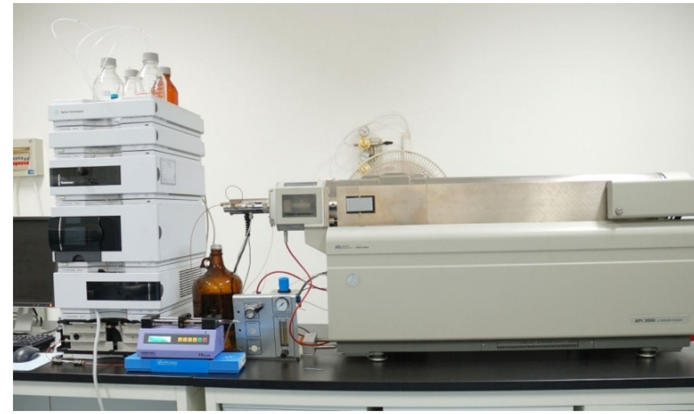
LC-MS/MS Conditions - Triple Quadrupole



MS: Applied Biosystems API 3000

- Source temperature: 450°C
- Nebulizer/desolvation gas: Zero air
- Curtain and collision gas: Nitrogen
- Ion spray voltage: 5500V ESI^+ / 4500V ESI^-

HPLC: Agilent 1100 series

- Column: Waters XBridge C18 (150 x 4.6 mm, 3.5µm) + guard column (20 x 4.6 mm, 3.5µm)
- Column temperature: 40°C
- Mobile phase A: H_2O:ACN (90:10 v/v) + 1mM ammonium acetate + 0.01% acetic acid
- Mobile phase B: ACN + 1mM ammonium acetate + 0.01% acetic aci
- Flow rate: 300 µL/min
- Injection volume: 20µL
- 35 min gradient ESI^+, 30 min gradient ESI^-

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EQS Limits & LOQs

Pesticide	EQS (µg/L)	Target LOQ < 30% EQS	Conc in 1 mL sample extract (µg/L)*	LOQ (µg/L)
Alachlor	0.3	0.09	45	1
Atrazine	0.6	0.18	90	1
Chlorfenvinphos	0.1	0.03	15	1
Chlorpyrifos	0.03	0.009	4.5	5
DEHP	1.3	0.39	195	1
Diuron	0.2	0.06	30	1
Isoproturon	0.3	0.09	45	1
Simazine	1.0	0.3	150	1
Epoxiconazole	-	-	-	1
Fenitrothion	-	-	-	1
Malathion	-	-	-	1
Pirimiphos-methyl	-	-	-	1
Mecoprop	-	-	-	1
Pentachlorophenol	-	-	-	1
Thiram	-	-	-	5
Glyphosate	-	-	-	>1000

*500mL water samples were concentrated down to 1 mL

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Analyte	ESI mode	RT (min)	Q1 (Da)	Q3 (Da)	Dwell time (msec)	DP (V)	FP (V)	CE (V)	CXP (V)
Simazine D_{10}	+	8.86	212.71	137.02	100	56	190	29	6
Simazine C	+	8.95	202.00	103.89	100				
Simazine Q	+	8.95	202.00	131.94	100				
Isoproturon Q	+	9.76	207.08	71.92	100				
Isoproturon C	+	9.76	207.08	164.97	100				
Diuron D6	+	9.77	239.03	77.95	100				
Diuron Cl^{37}	+	9.84	234.92	71.90	100				
Diuron Q	+	9.85	232.89	71.90	100				
Atrazine D_5	+	9.96	221.19	178.83	100				
Atrazine Q	+	10.03	216.20	173.79	100				
Atrazine C	+	10.03	216.20	67.92	100				
Epoxiconazole Q	+	11.02	330.13	120.96	100				
Epoxiconazole C	+	11.02	330.13	100.97	100				
Malathion D6	+	11.44	355.09	99.98	100				
Malathion Q	+	11.49	348.02	126.93	100				
Malathion C	+	11.49	348.02	98.92	100				
Alachlor D_3	+	11.87	283.03	251.11	100				
Alachlor Q	+	11.97	270.16	237.89	100				
Alachlor C	+	11.97	270.16	161.99	100				
Chlorfenvinphos D_{10}	+	11.97	368.88	100.93	100				
Chlorfenvinphos Q	+	12.05	358.88	154.90	100	46	150	19	8
Chlorfenvinphos C	+	12.05	358.88	98.92	100	46	150	47	6
Pirimiphos D_6	+	13.00	311.99	163.94	150	51	150	31	8
Fenitrothion D_6	+	13.02	283.94	130.91	150	51	100	33	8
Pirimiphos C	+	13.04	306.03	66.98	150	56	190	61	4
Pirimiphos Q	+	13.04	306.03	164.05	150	56	190	31	10

Summary of the retention times, diagnostic ions, and the MS/MS operating conditions for the 13 pesticides and 11 internal standards included in the study.

Q is quantitative ion and C is confirmatory ion.

Results of the analysis of real waste water samples from seven WWTPs (n = 3) and limits of quantitation of the LC-MS/MS method and the corresponding surface EQS limits.

						Month 2		Month 3		Month 4	
WWTP	**Analyte**	**(ng/mL)**	**EQS (ng/mL)**	**Mean (ng/mL)**	**RSD**	**Mean (ng/mL)**	**RSD**	**Mean (ng/mL)**	**RSD**	**Mean (ng/mL)**	**RSD**
Bandon	Atrazine	2	600	9.0	5.6	406.7	2.3	62.0	6.3	41.1	1.7
	Diuron	2	200	82.6	21.8	374.7	0.7	65.2	2.9	977.0	5.3
	Simazine	2	-	29.5	7.8	84.9	6.2	36.5	15.3	38.4	8.6
	Mecoprop	2	-	-	-	446	8.1	-	-	-	-
Ballincollig	Atrazine	2	600	13.9	3.6	4.1	7.3	14.9	4.7	2.8	3.6
	Diuron	2	200	87.2	6.8	80.9	5.1	81.3	5.5	163.6	4.1
	Simazine	2	-	43.1	10.7	45.3	7.9	16.1	3.7	53.4	9.0
	Mecoprop	2	-	-	-	-	-	56.1	51.3	311.3	5.0
Clonakilty	Atrazine	2	600	7.3	4.1	30.8	3.2	9.3	7.5	4.8	6.3
	Diuron	2	200	23.7	9.7	30.6	9.2	51.0	6.9	41.9	1.4
	Simazine	2	-	5.0	20.0	-	-	-	-	3.9	-
Charleville	Atrazine	2	600	11.7	3.4	11.1	3.6	9.2	4.3	7.0	2.9

Outline

Emerging chemicals of concern

- Traditional separation methods
- Compliance monitoring - EQS

Polar and non-polar organics

- Longer-term monitoring
- Trend monitoring
- Passive sampling

Environmental pollutants (toxins, pharmaceuticals)

- Sensor platform development
- Single and multi-analyte systems
- Integration of technologies

A single sample

Spot sampling

4-6 weeks

Longer-term monitoring of trends

Up-to a year autonomously

Continuous real-time monitoring of certain parameters

Passive Sampling

- Determination of pollutants in aquatic environment
- Free flow of analyte molecules from sampled medium to collecting medium – only dissolved analytes, no energy source

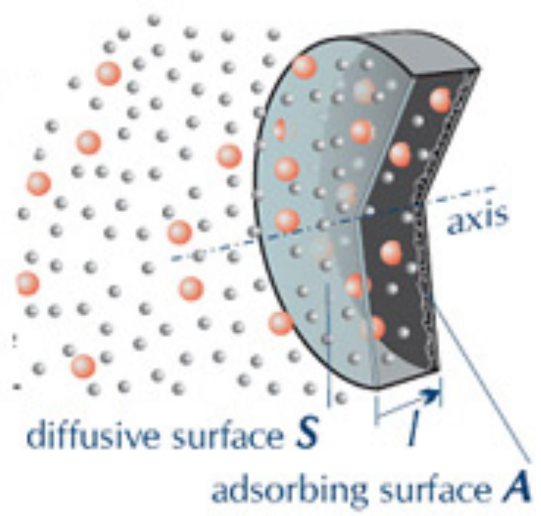


Fig. 1 – Passive sampling mechanism

Fig. 2 – Passive sampling device (interior)

Fig. 3 – Passive sampling devices

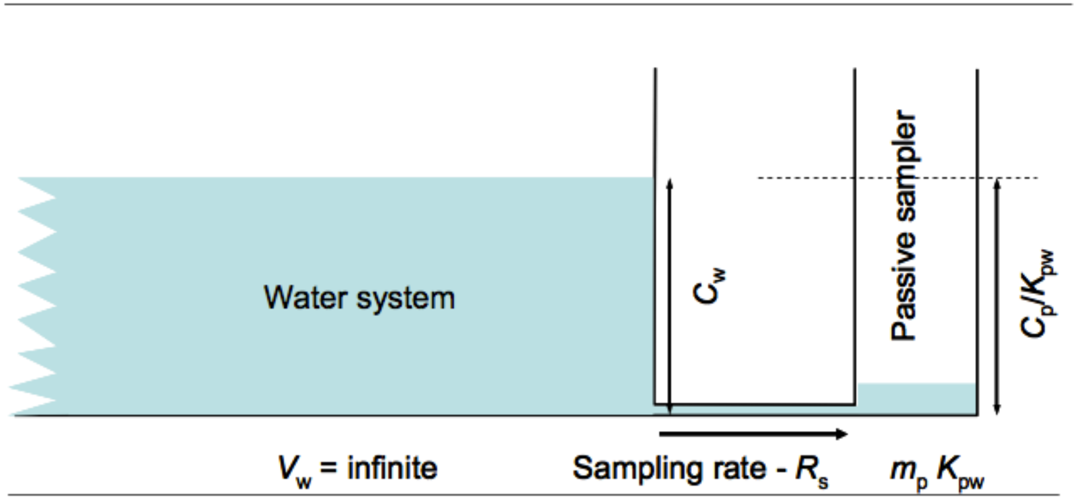
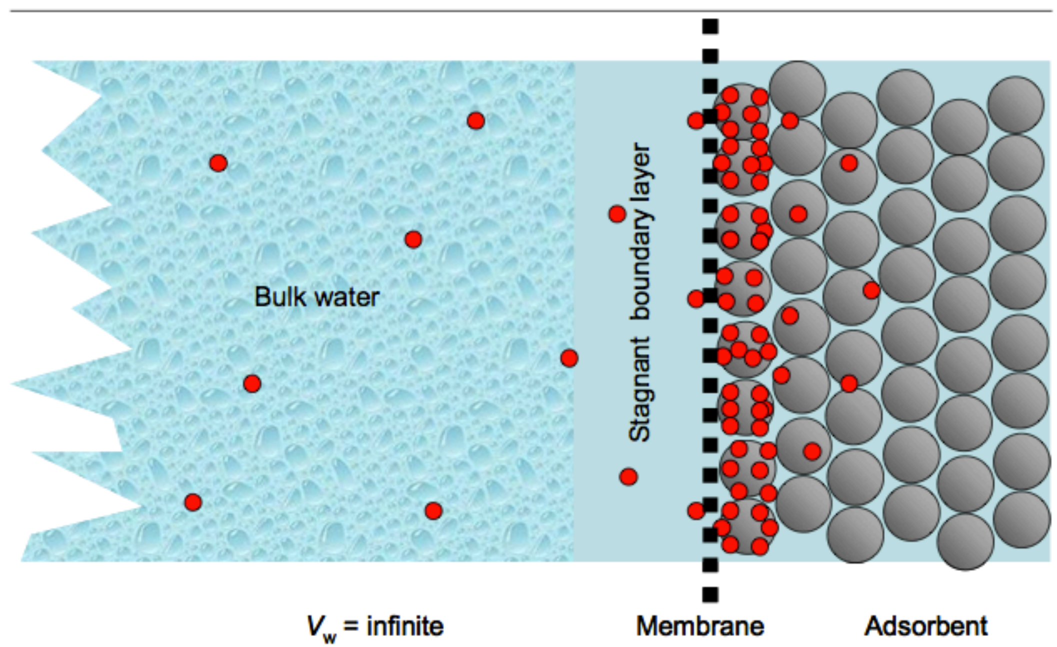


Fig. 4 – Absorption passive sampling mechanism

Equilibrium is reached and time-weighted average is determined. Mainly for non-polar compounds.

Fig. 5 – Adsorption passive sampling mechanism

Kinetic regime is maintained and calculations are based on time-integrated measurements Mainly for polar analytes.



Ref: Smedes *et al.*, 2010

Protocol for Passive Sampler Deployment

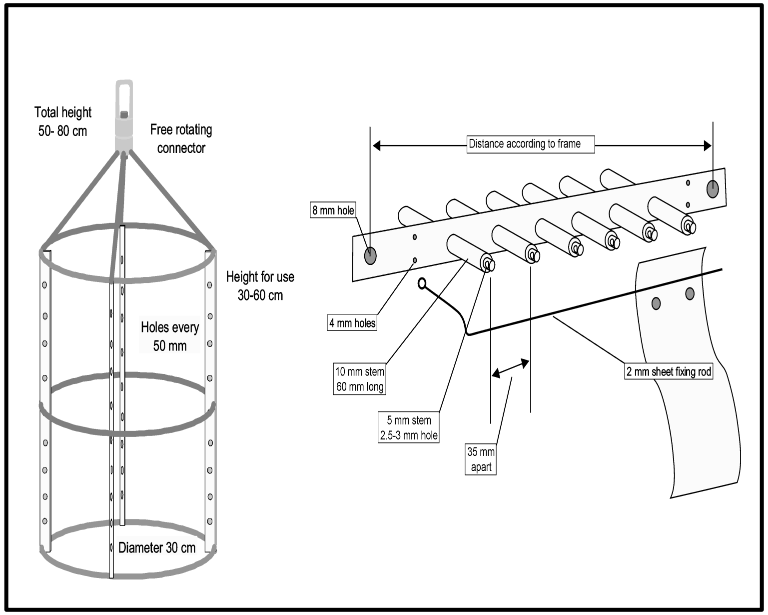
- ICES TIMES no. 52* for PDMS
- EA lab/NLS guidelines for POCIS

Record

- GPS co-ordinates
- Date and time of deployment
- Salinity
- Water temperature

*ICES TIMES no. 52. 2012. Guidelines for passive sampling of hydrophobic contaminants in water using silicone rubber

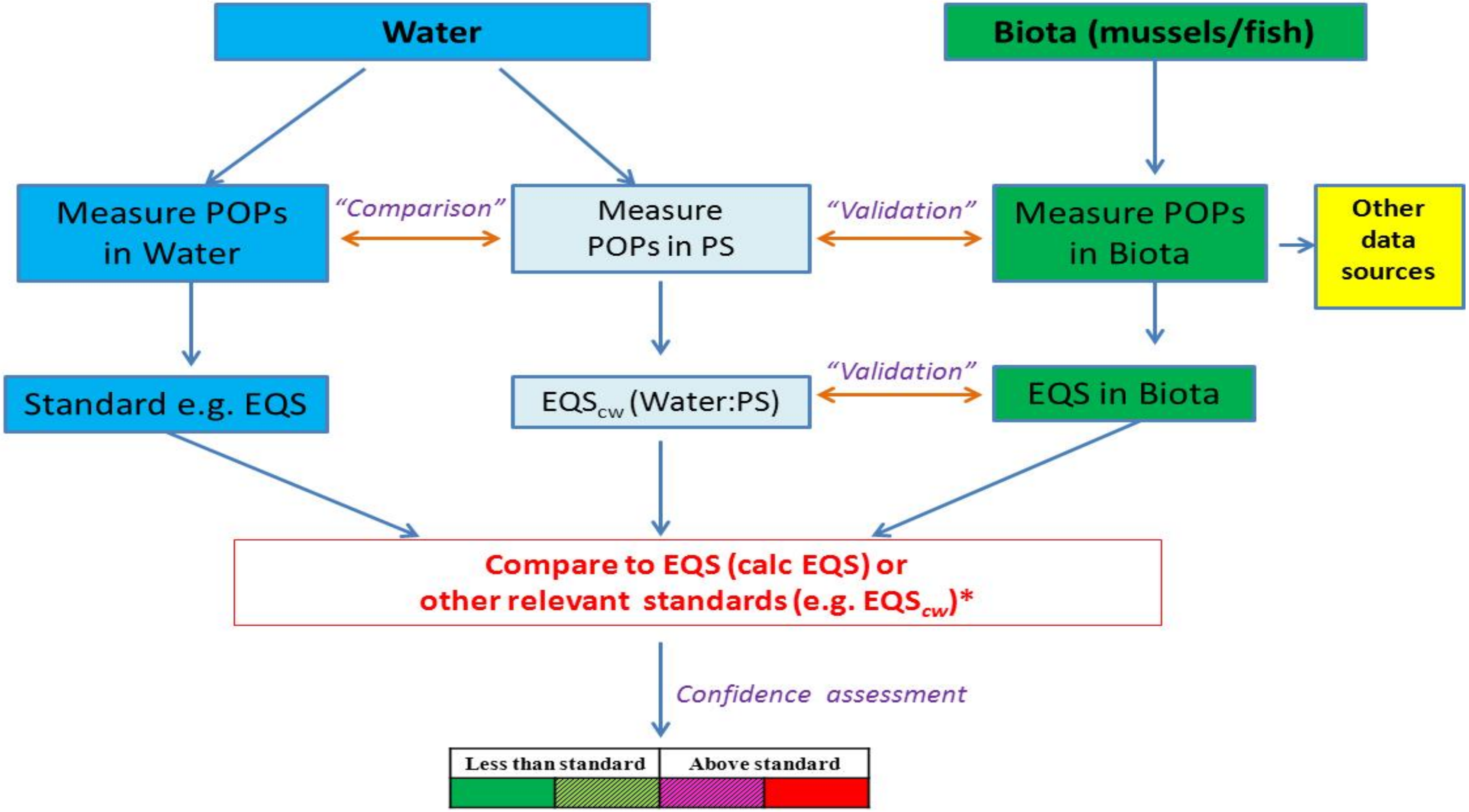
**Environmental Sampling Technologies lab: http://www.est-lab.com/pocis.php



PDMS sheet attachment*

POCIS canister**

Passive sampling

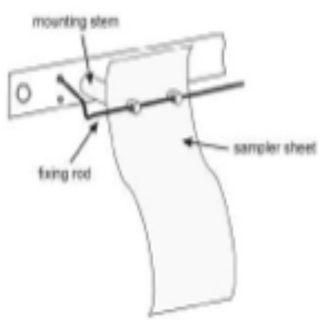


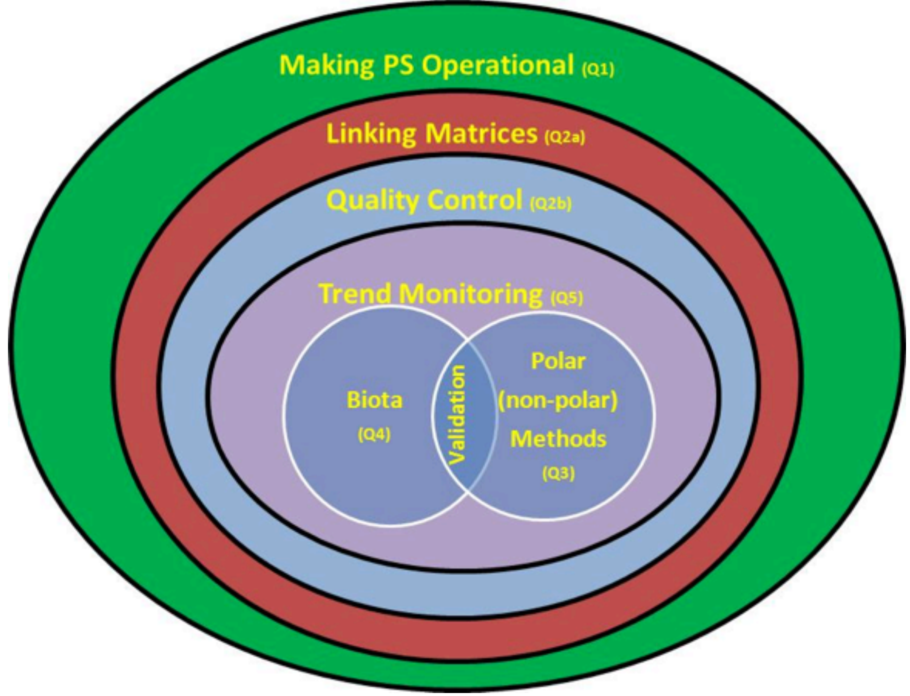
* *Based on the potential derivation of a passive sampling EQS equivalent* EQS(*PS*).

Summary of project approach to further incorporating PS into operational monitoring programmes.

Overview of sites selected

County	Site	Rationale	POCIS	PDMS	Water	Mussels	Fish (IFI)
Cork	Inchigeelagh	Upstream river	✓	✓	✓		✓
	Inniscarra	Downstream river	✓	✓	✓		✓
	Shandon	Riverine/transitional	✓	✓	✓		✓
	Lough Mahon	Riverine/transitional	✓	✓	✓	✓	
	Outer bay	Riverine/transitional	✓	✓	✓	✓	
Dublin	Poolbeg	High pressure coastal	✓	✓	✓	✓	
	Osberstown	Riverine/transitional	✓	✓	✓	✓	
	Lucan Bridge	Downstream river	✓	✓	✓		✓
	Kilcullen Bridge	Upstream river	✓	✓	✓		✓
Galway	Kilkieran Bay	Coastal reference	✓	✓	✓	✓	
Mayo	Burrishoole	Upstream river	✓	✓	✓		✓
Donegal*	Glen Lackagh 1	Cypermethrin study	SPMD	✓	✓	Benthic kick sampling	
	Glen Lackagh 2	Cypermethrin study	SPMD	✓	✓		





	Matrix		Glen Lackagh U/S	Midstream A	Midstream B	Midstream C	Glen Lackagh D/S
Analyte		*Units*	2014				
Cypermethrin 29/4/14	Water	ng L^{-1}	1.17	NA	NA	NA	1.08
Cypermethrin 22/5/14	Water	ng L^{-1}	1.47	1.67	1.38	1.73	1.78
Cypermethrin	PDMS	ng L^{-1}	++	NA	NA	NA	+++
Cypermethrin	SPMD	ng L^{-1}	<70	NA	NA	NA	<70

Cork POCIS and water estrogens

Upstream → **Downstream**

	Matrix		Lough Allua Inchigeelagh	Iniscarra	Shandon	Lough Mahon	Cork Outer Harbour
Analyte		***Units***			**2013**		
EE2	POCIS	ng L^{-1}	<0.2	**1.39**	<0.2	<0.2	<0.2
E2		ng L^{-1}	<0.5	<0.5	<0.5	**2.36**	**1.98**
EE2	Water	ng L^{-1}*	nd	nd	nd	nd	nd
E2		ng L^{-1}*	nd	nd	nd	nd	nd
Analyte		***Units***			**2014**		
E1	POCIS	ng L^{-1}	< 0.51	0.24	0.37	**0.48**	**0.37**
EE2		ng L^{-1}	< 0.12	< 0.04	< 0.04	< 0.04	**0.07**
E2		ng L^{-1}	< 0.13	< 0.04	< 0.04	**0.06**	**0.09**
E1	Water	ng L^{-1}*	nd	**0.41**	nd	**0.41**	**0.54**
EE2		ng L^{-1}*	nd	nd	nd	nd	nd
E2		ng L^{-1}*	nd	nd	nd	nd	nd

*LOD water samples by LC-MS/MS: E1: 0.07 ng L^{-1} E2: 0.07 ng L^{-1}, EE2, 0.11 ng L^{-1}. 5 L sample n = 2
Effective sampling rates POCIS (ng/sampler/day)*: E1: 0.39, E2: 0.46, EE2: 0.235

Dublin POCIS/water estrogens

Upstream → **Downstream**

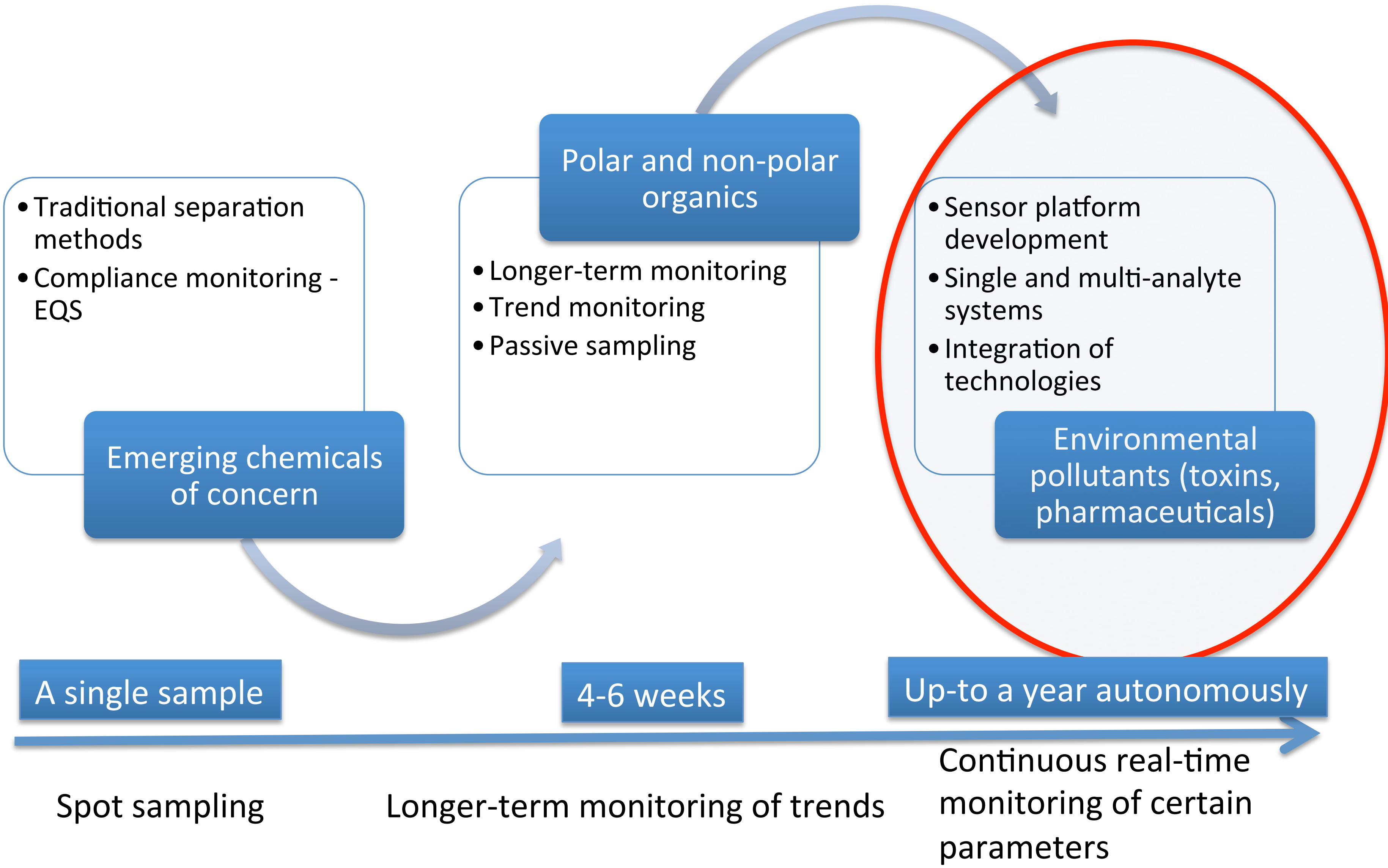
	Matrix		**Kilcullen**	**Osberstown**	**Lucan**	**Poolbeg**
Analyte		***Units***	**2014**			
E1	POCIS	ng L^{-1}	<0.23	0.29	0.37	
E2	POCIS	ng L^{-1}	<0.06	<0.06	<0.06	
EE2	POCIS	ng L^{-1}	<0.06	<0.06	<0.06	
E1	Water	ng L^{-1}*	<0.07		0.33	1.92
E2	Water	ng L^{-1}*	nd	0.33	0.43	0.23
EE2	Water	ng L^{-1}*	nd	nd	nd	nd
Analyte		***Units***	**2015**			
E1	POCIS	ng L^{-1}	<0.23	0.31	0.42	0.41
E2	POCIS	ng L^{-1}	<0.06	<0.06	0.06	0.07
EE2	POCIS	ng L^{-1}	<0.06	<0.06	<0.06	<0.06

*LOD water samples by LC-MS/MS: E1: 0.07 ng L^{-1} E2: 0.07 ng L^{-1}, EE2, 0.11 ng L^{-1}. 5 L sample n = 2
Effective sampling rates POCIS (ng/sampler/day)*: E1: 0.39, E2: 0.46, EE2: 0.235

Dublin Bay PAHs

Analyte	Estimated Water Concentrations ($ng\ L^{-1}$) SPMD
Acenaphthene	<1.19
Acenaphthylene	<1.47
Anthracene	<0.84
Benzo(a)anthracene	<0.63
Benzo(a)pyrene	<0.74
Benzo(b)fluoranthene	<0.61
Benzo(ghi)perylene	<1.00
Benzo(k)fluoranthene	<0.69
Chrysene	0.84
Dibenzo(a,h)anthracene	<0.80
Fluoranthene	2.25
Fluorene	<0.98
Indeno(1,2,3-cd)pyrene	<0.91
Naphthalene	<5.73
Phenanthrene	1.04
Pyrene	3.73

Outline

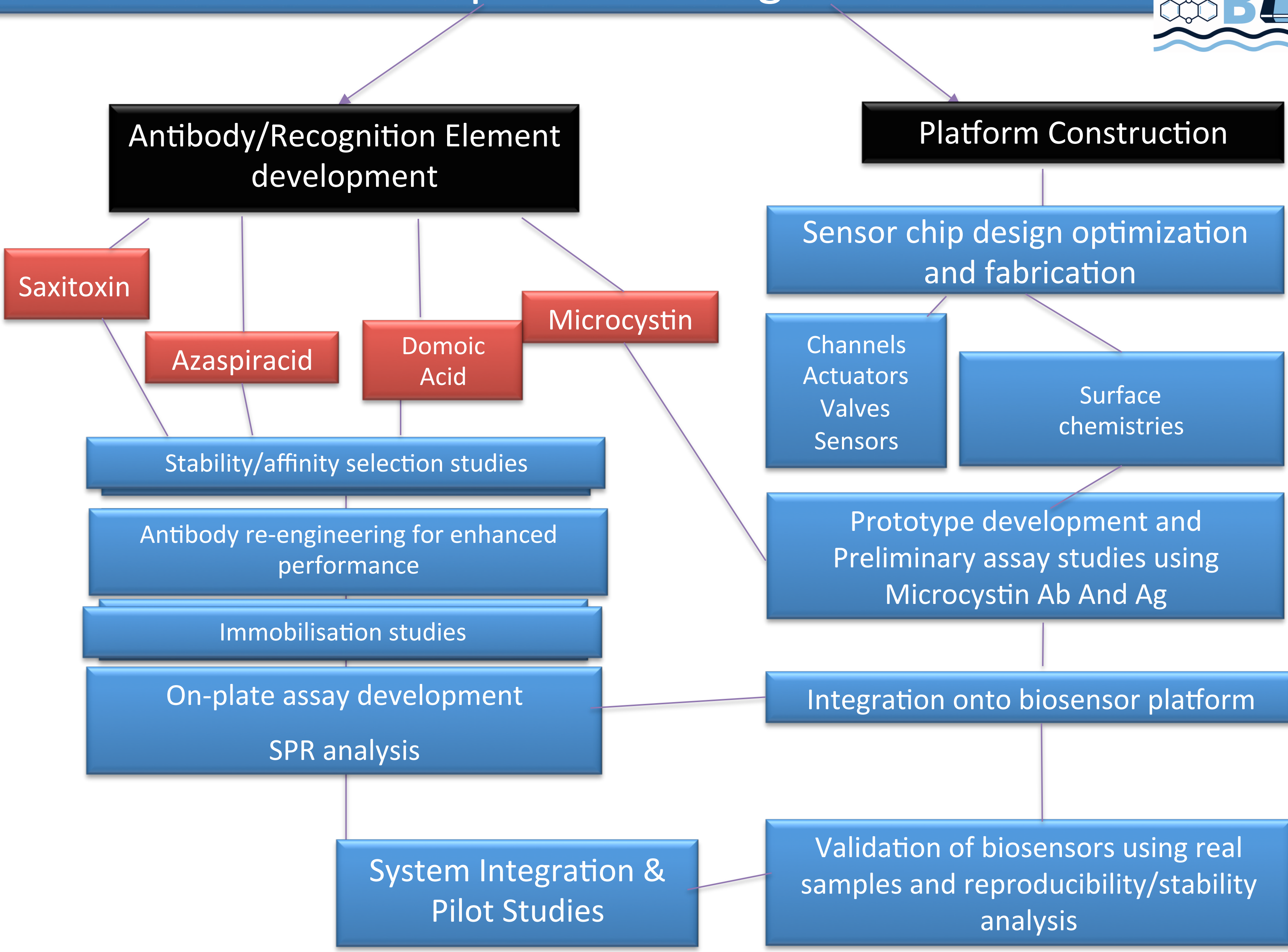


Traditional separation methods
Compliance monitoring - EQS
Emerging chemicals of concern
Polar and non-polar organics
Longer-term monitoring
Trend monitoring
Passive sampling
Sensor platform development
Single and multi-analyte systems
Integration of technologies
Environmental pollutants (toxins, pharmaceuticals)
A single sample
4-6 weeks
Up-to a year autonomously
Spot sampling
Longer-term monitoring of trends
Continuous real-time monitoring of certain parameters

Antibody based sensors:

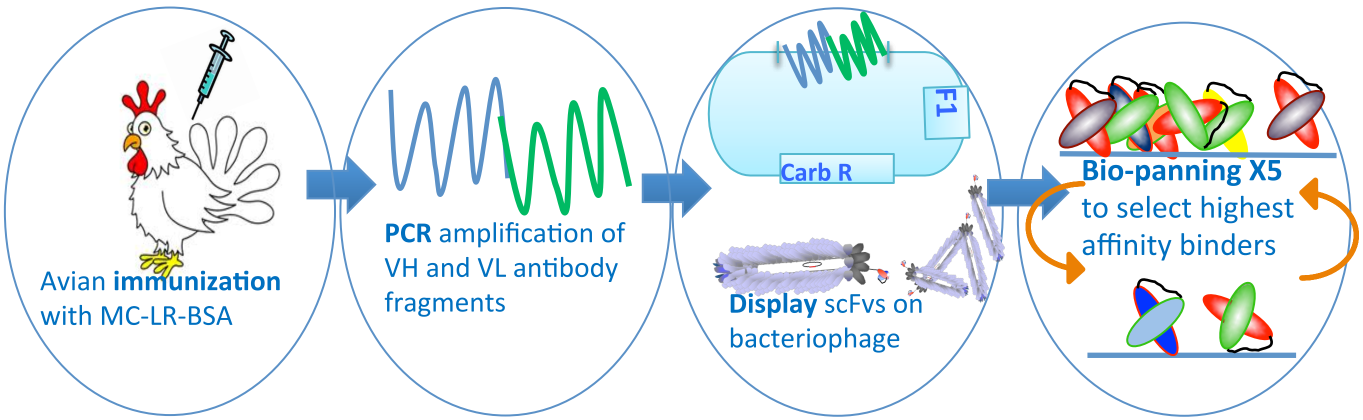
- Immunoassays have increased in popularity and are routinely used for analyte identification today.
- They are highly sensitive and capable of detecting toxins at levels similar levels to HPLC.
- Attempts to miniaturize the detection systems and to develop *in-situ* monitoring systems have been made, a lateral flow 'dipstick' style assay for toxins developed
- EU FP7 project Mariabox – Oceans of Tomorrow call → algal toxin monitoring and micro-pollutant detection.

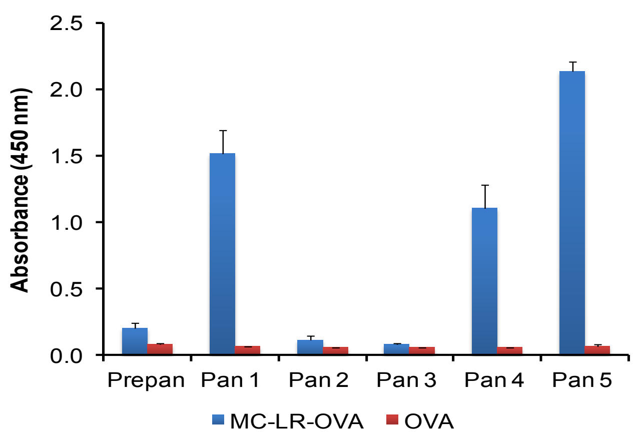
Sensor platform Integration Plan



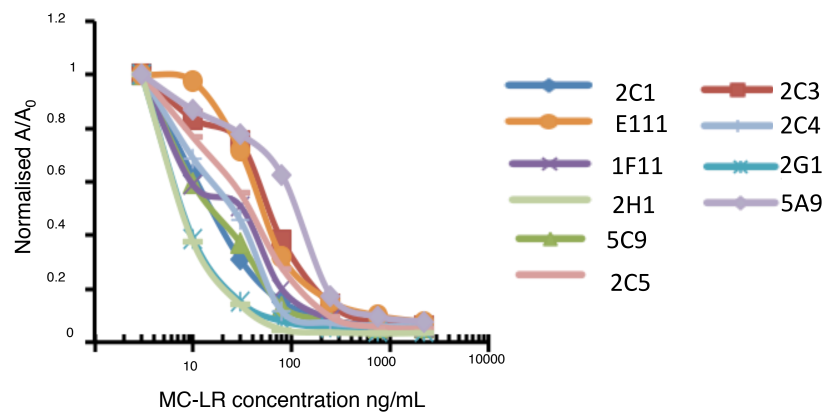
Methodologies

Development of anti-microcystin recombinant antibodies



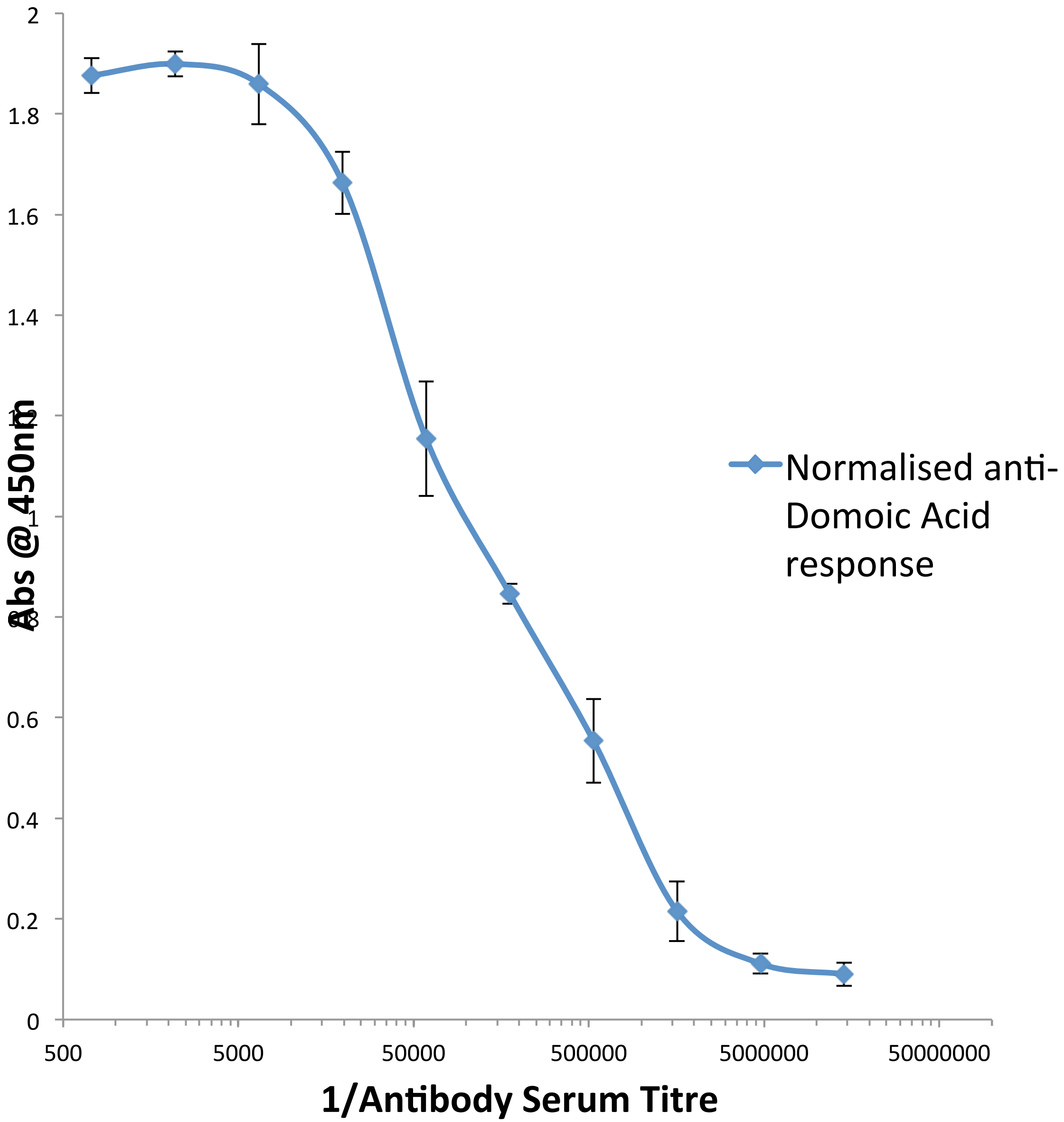


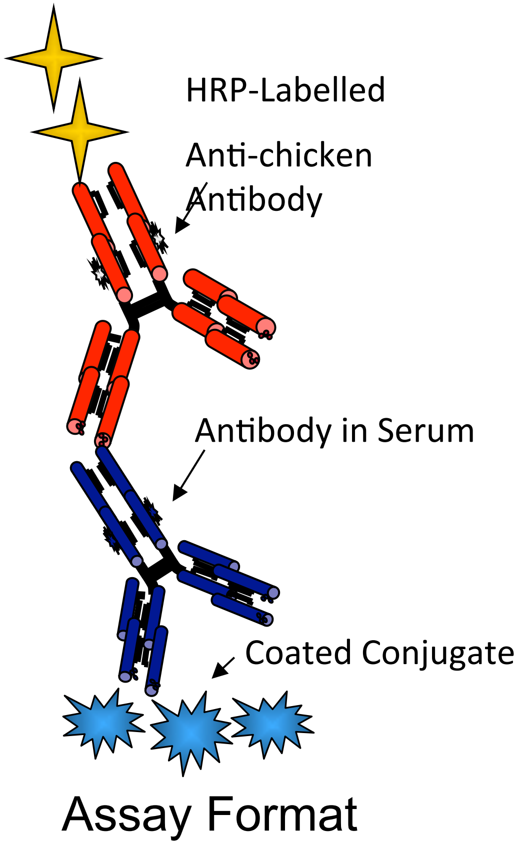
Antibodies produced from each round of biopanning



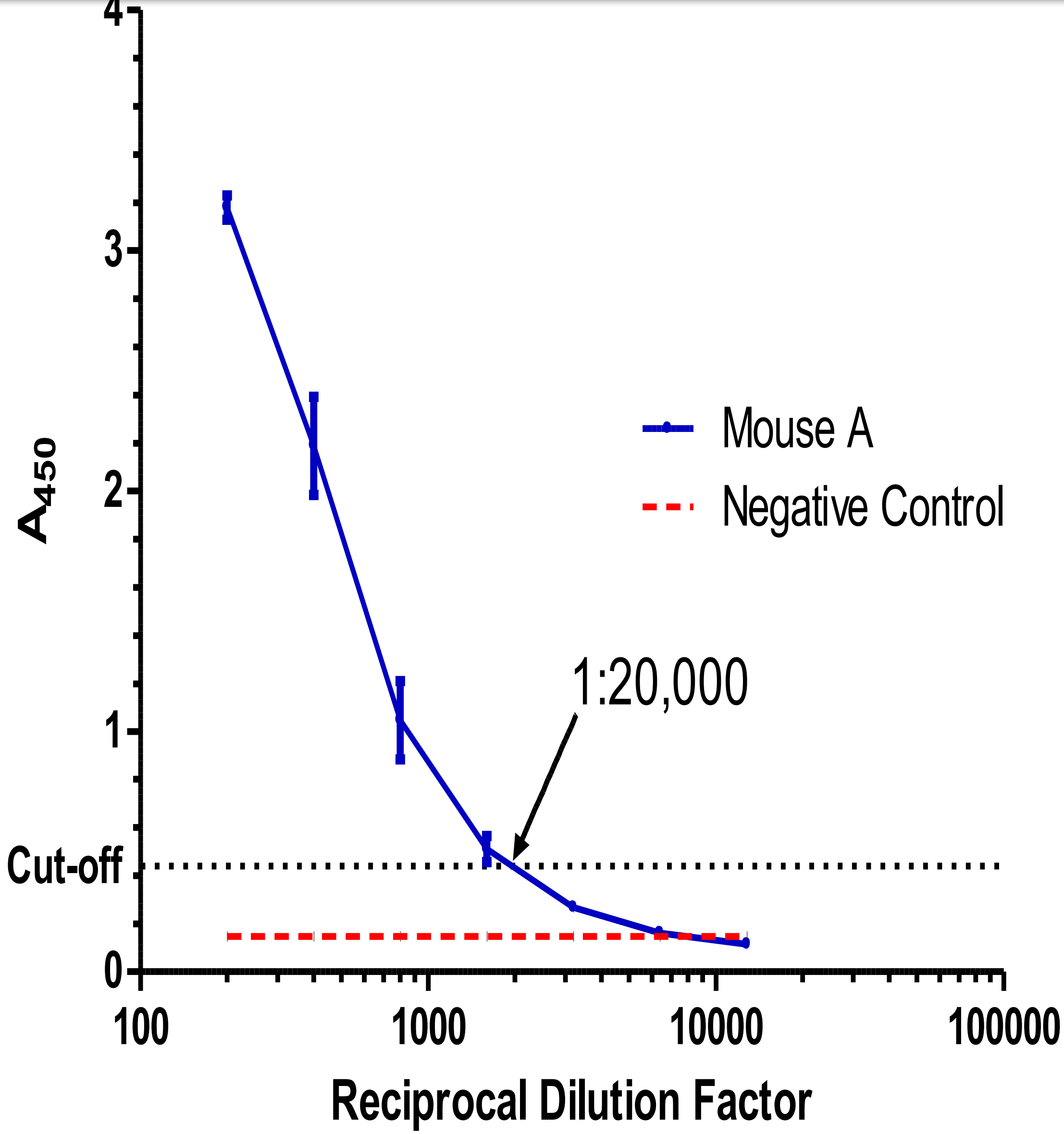
The most sensitive binder was determined by inhibition ELISA

Domoic Acid Chicken Serum Titre



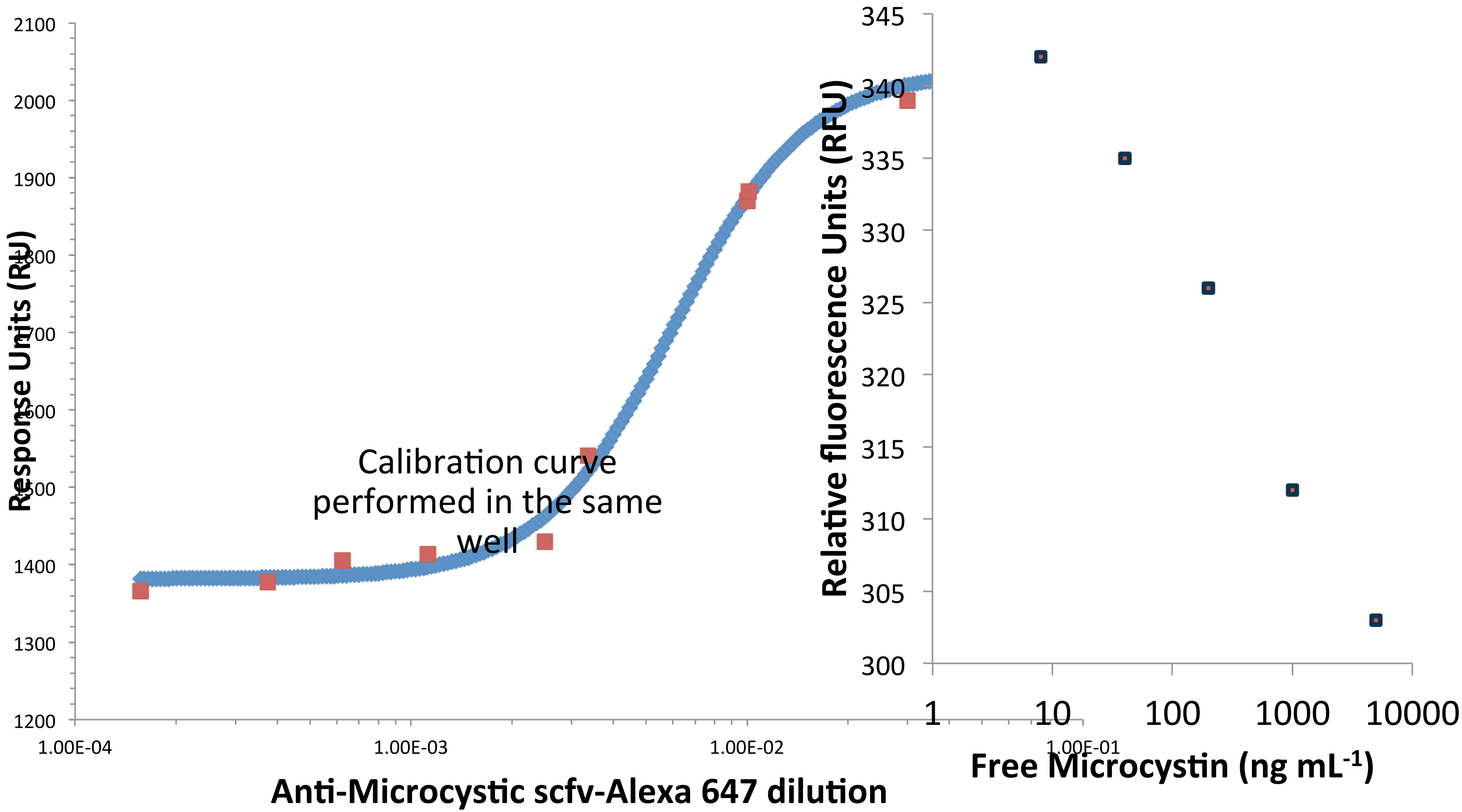


Azaspiracid

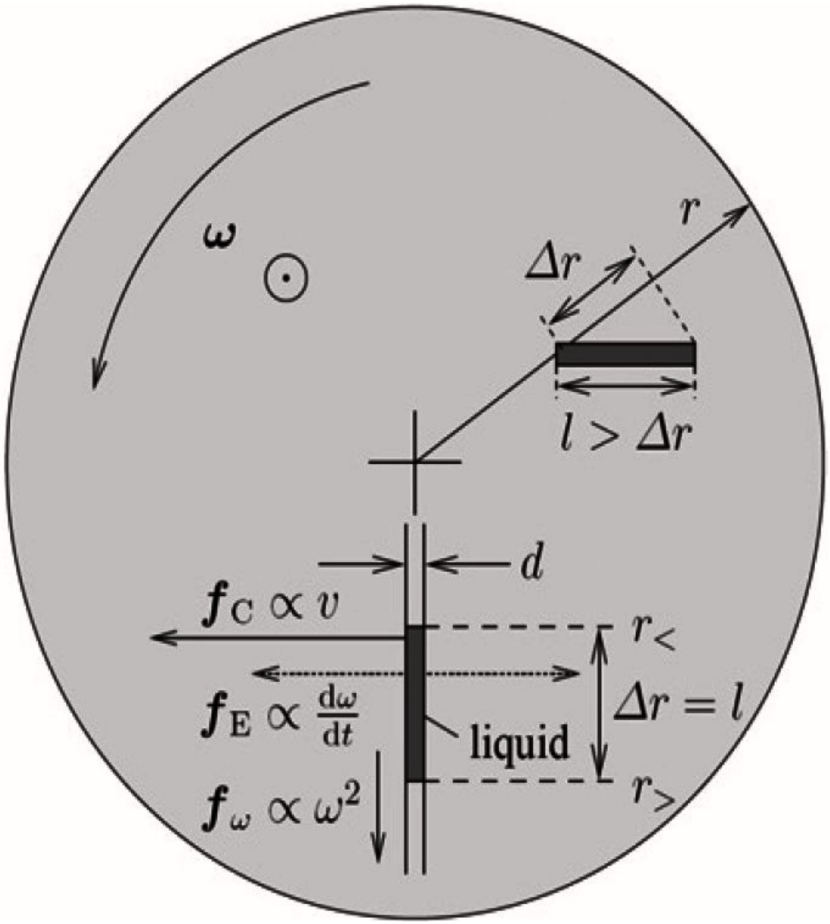


- ScFv library successfully constructed
- Preparation of conjugates underway for biopanning
- Polyclonal antibodies successfully isolated

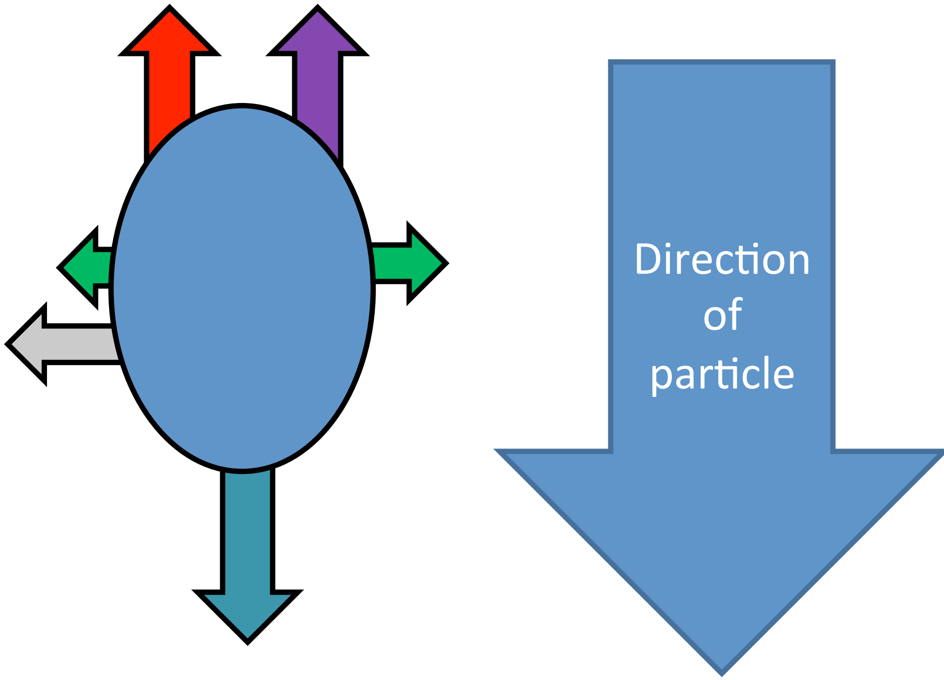
Analysis of capture raw optical data generated



Fluidic movement on rotating platform

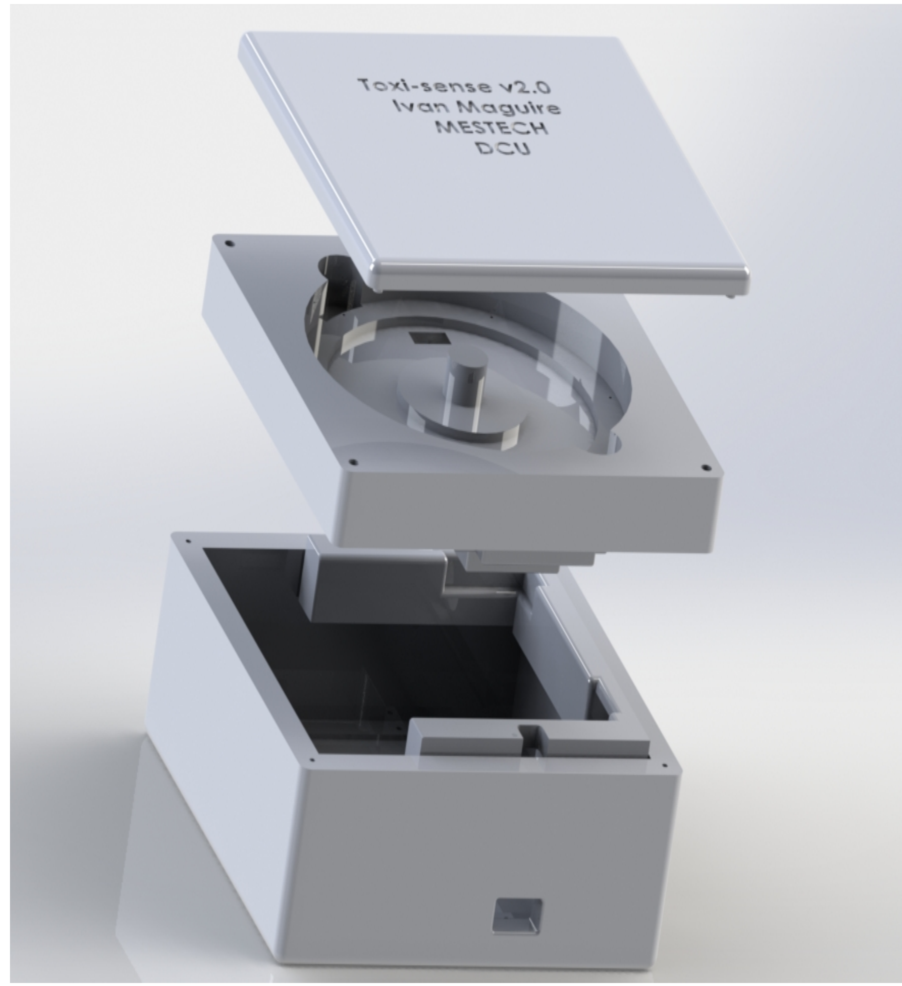


Forces on acting on a rotating disc

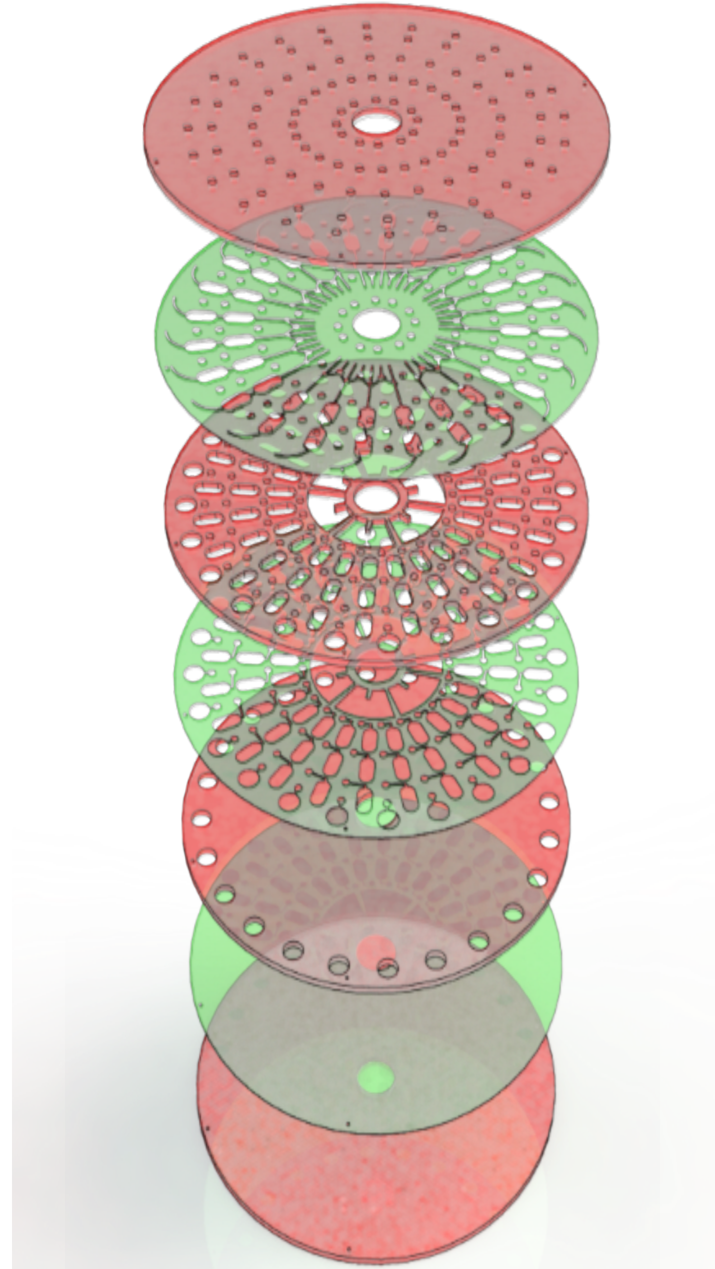


Particle sedimentation through a fluid on anti-clockwise rotating disc

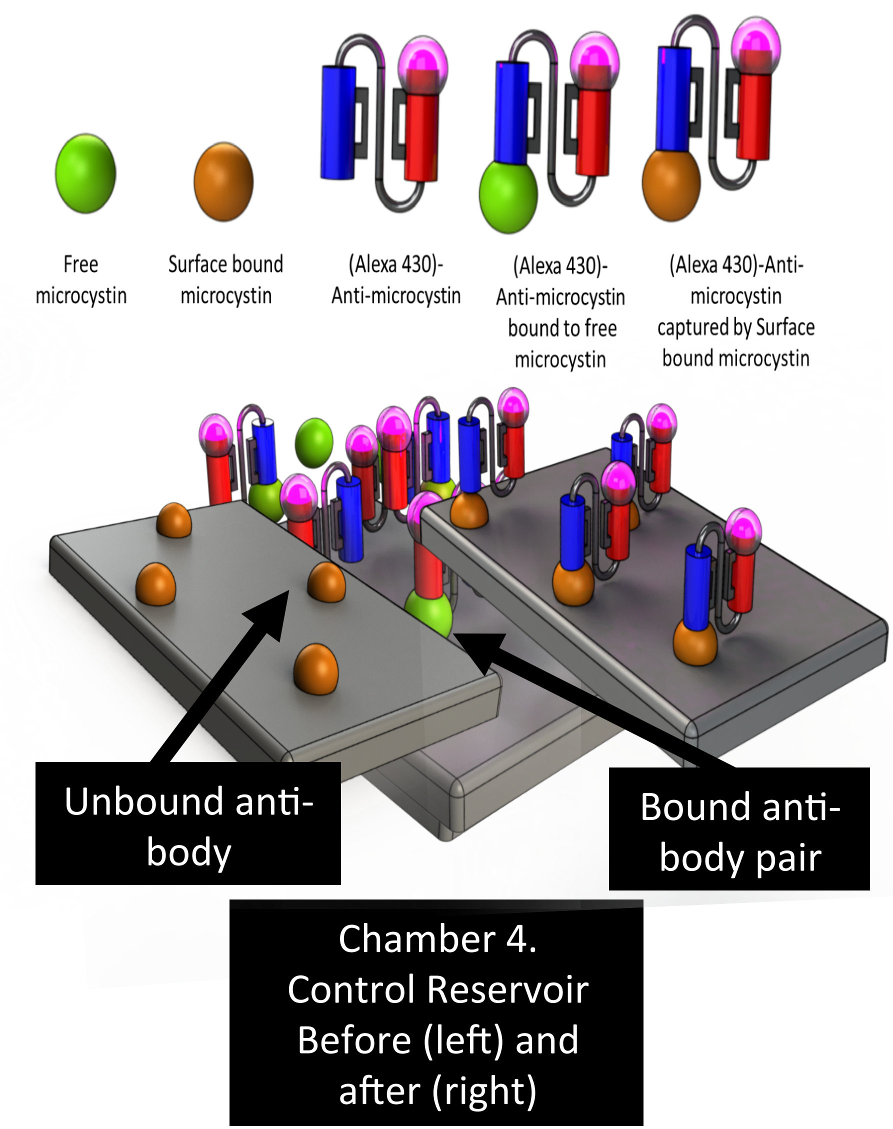
ToxiSense microfluidic System

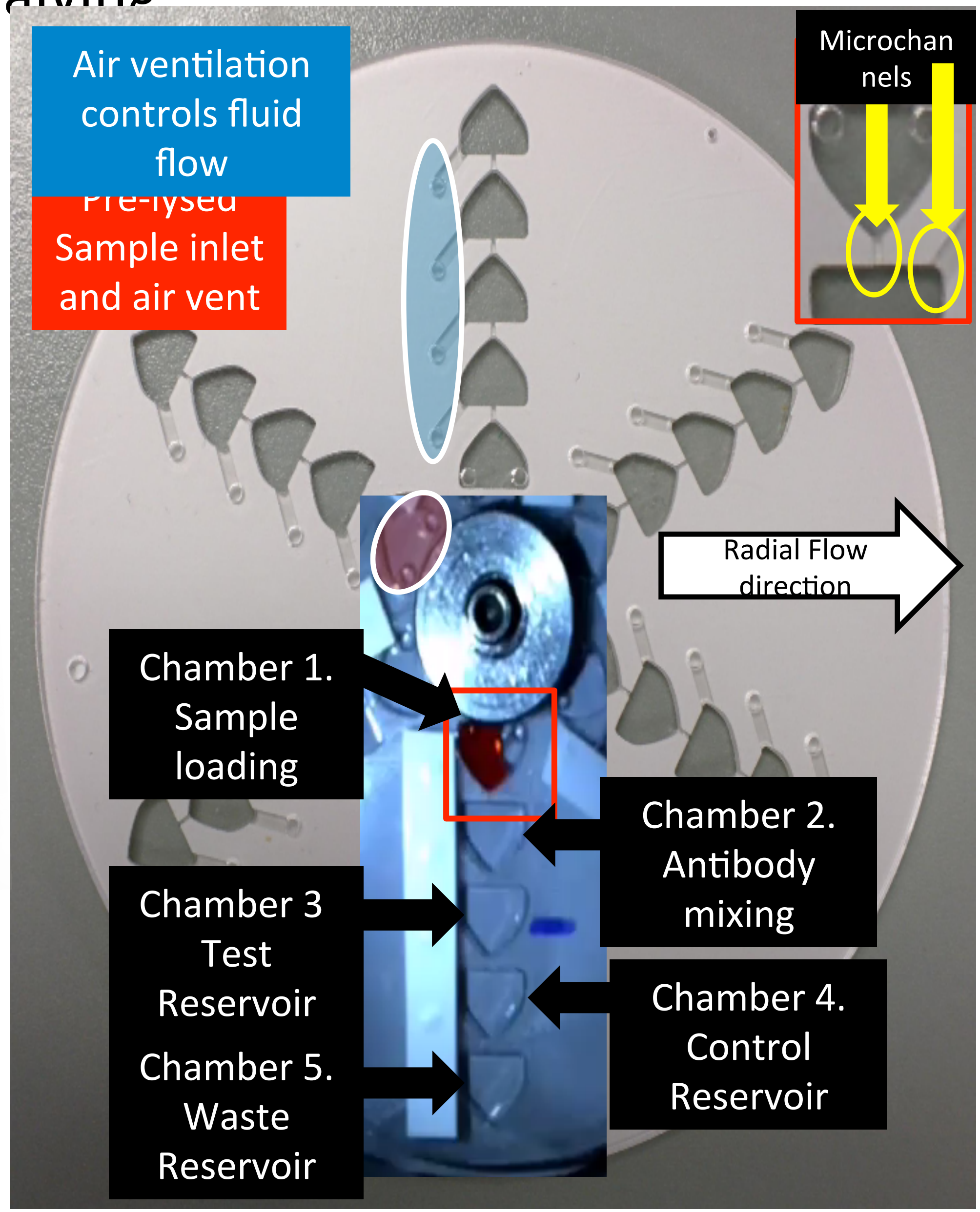


- On-board microfluidics (Lab-On-A-Disc platform)
- *Manufactured from poly(methyl methacrylate) (PMMA) (Red) (Radionics™) and pressure sensitive adhesive (PSA) (Green)(Adhesives Research Inc. ™)*
- Easily modifiable
- Microcystin-LR detection: Proof of concept
- High sensitivity
- Low sample size
- Cheap to manufacture

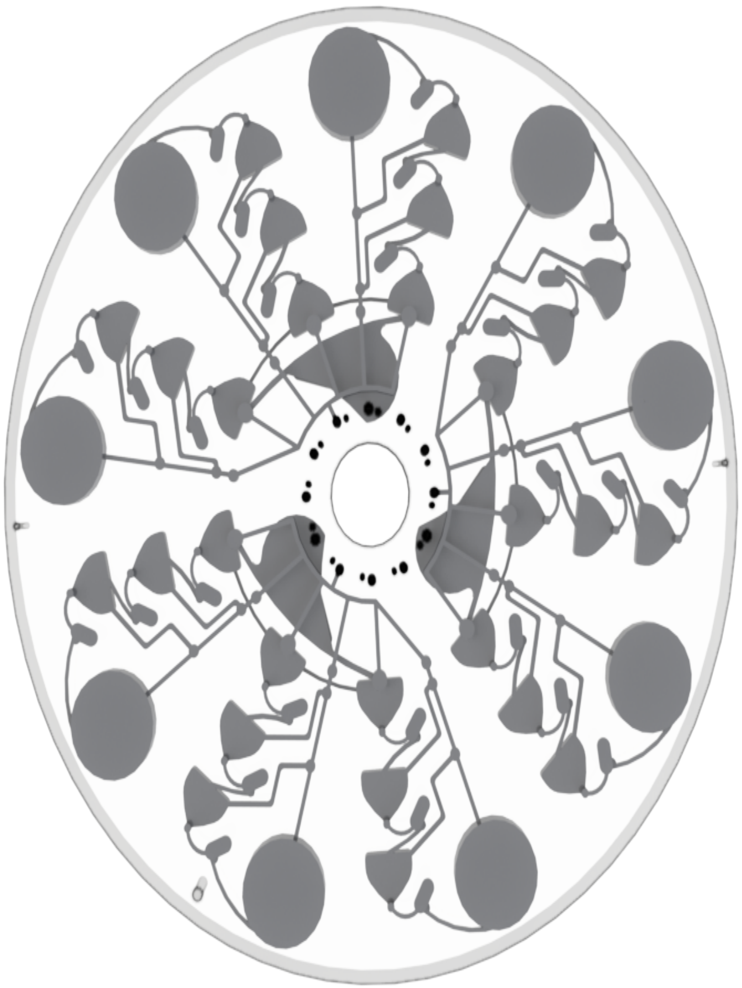


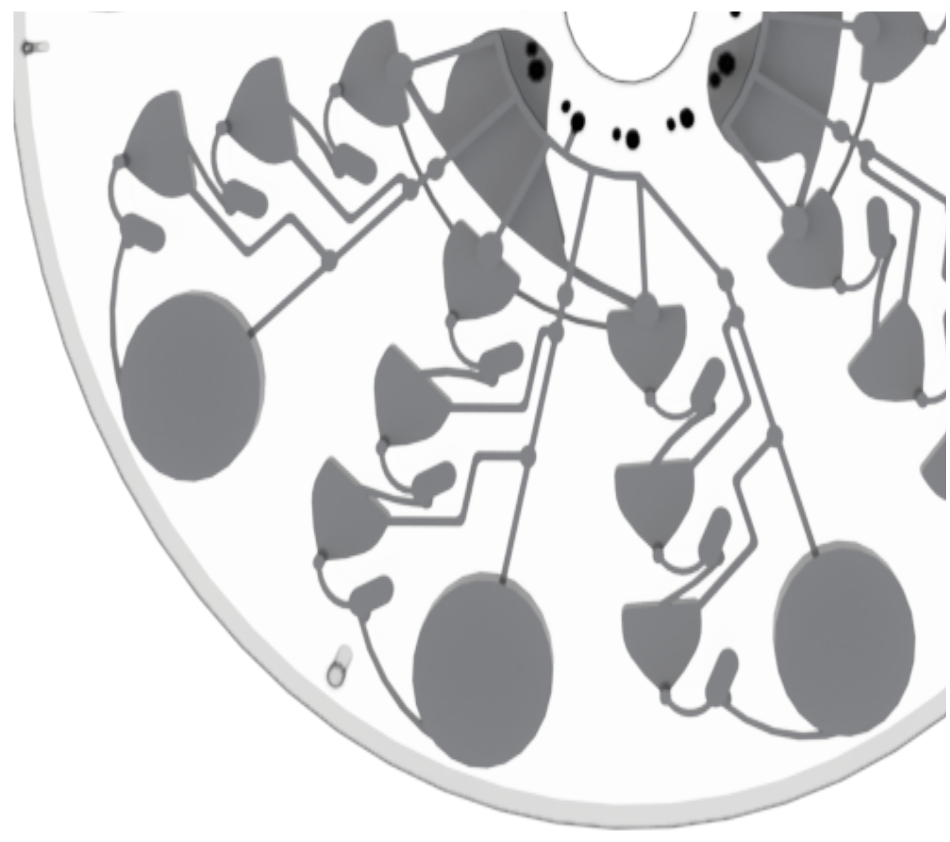
Single assay proof of concept using pneumatic pressure valving

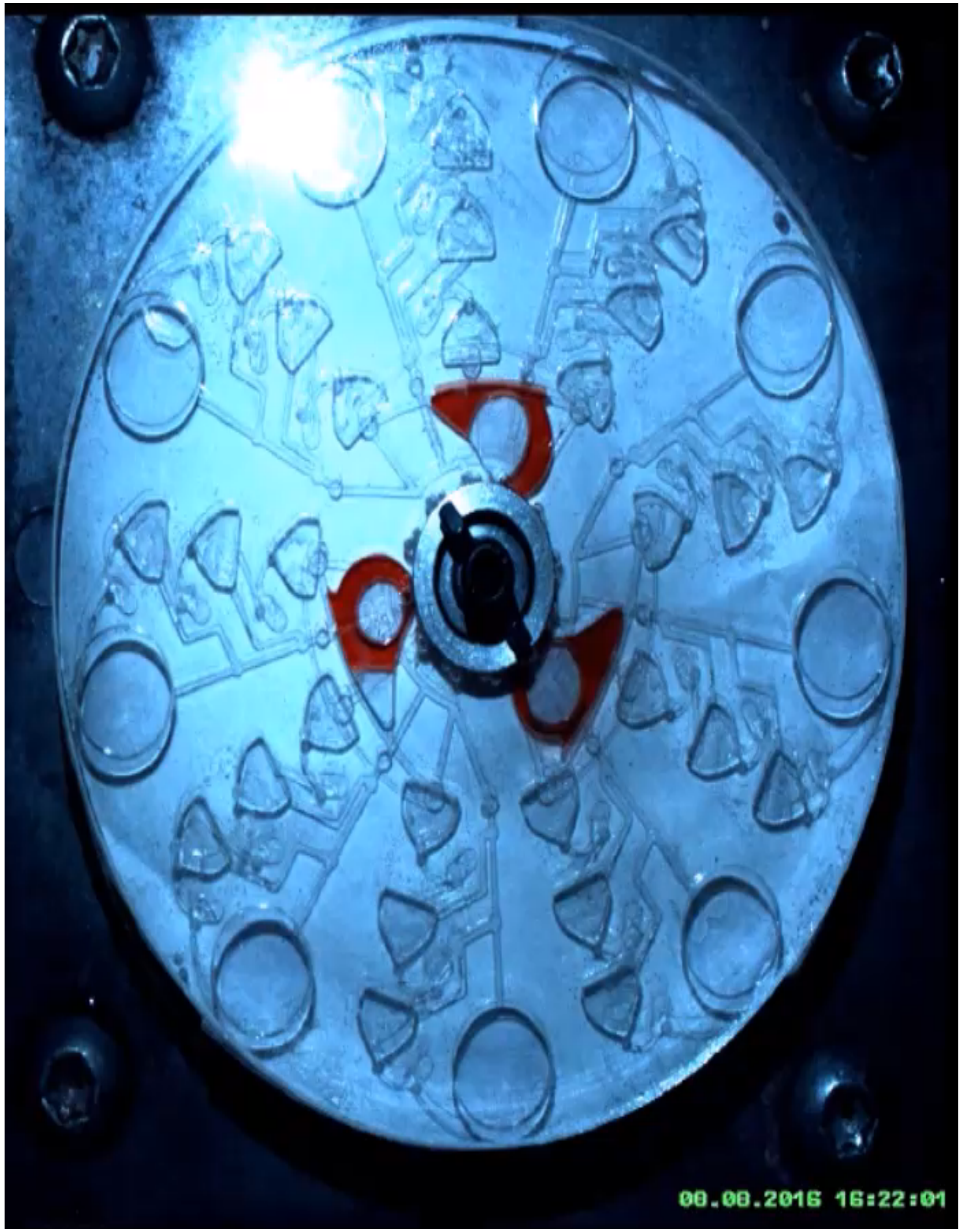




3 day – three analyte detection disc



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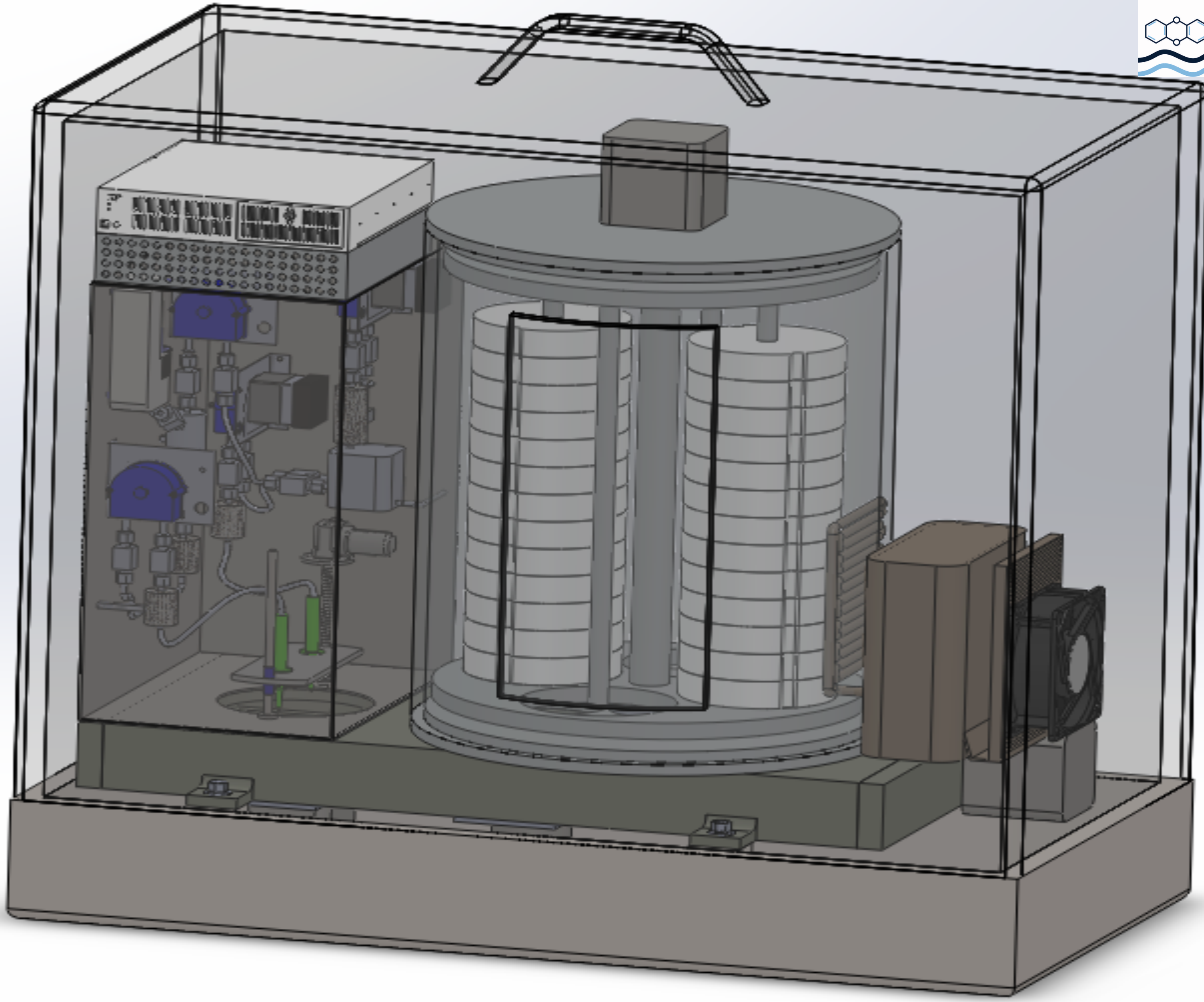
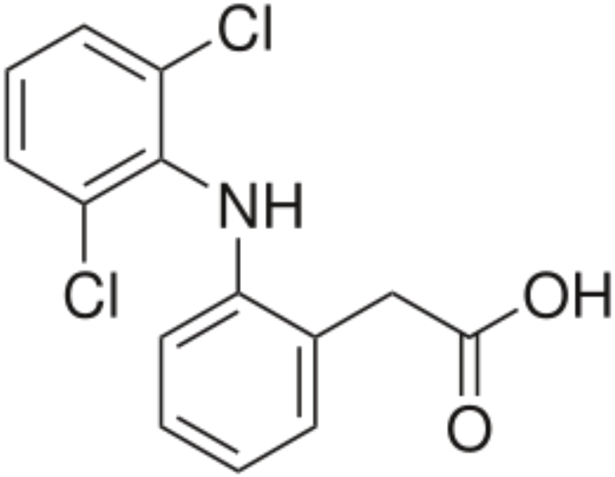
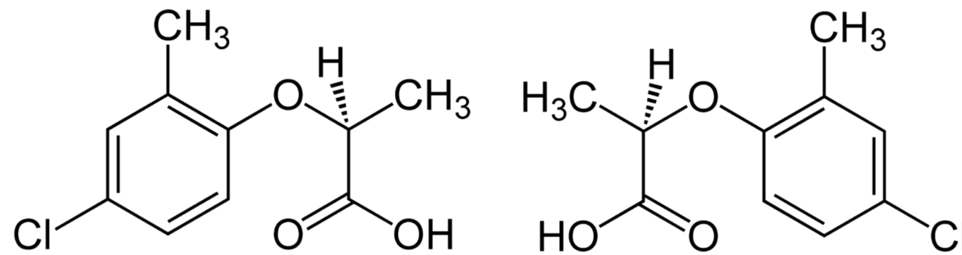


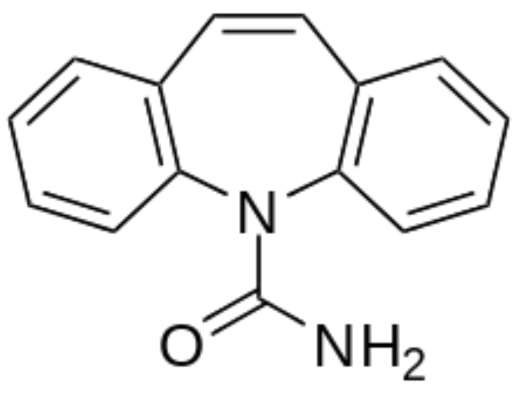
Diclofenac



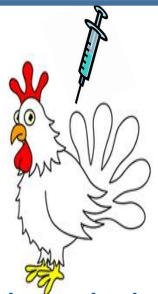
Mecoprop



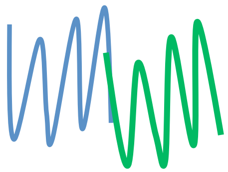
Carbamezapine



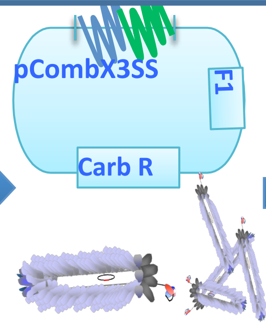
Recombinant antibody-based microfluidic sensor



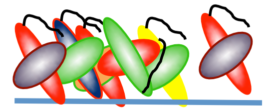
Avian **immunization** with MC-LR-BSA



PCR amplification of VH and VL antibody fragments



Ligation into vector and **display** of scFvs on bacteriophage



Bio-panning X5 to select highest affinity binders

Immunisation

- Diclofenac, mecoprop and carbamapazine were conjugated to proteins and were immunised into an avian host.
- Immune response was identified using a polyclonal ELISA.

Recombinant antibody generation

- Genetic material was then isolated for use in the production of recombinant antibodies (rAbs).
- Generation of three immune antibody libraries for each chemical

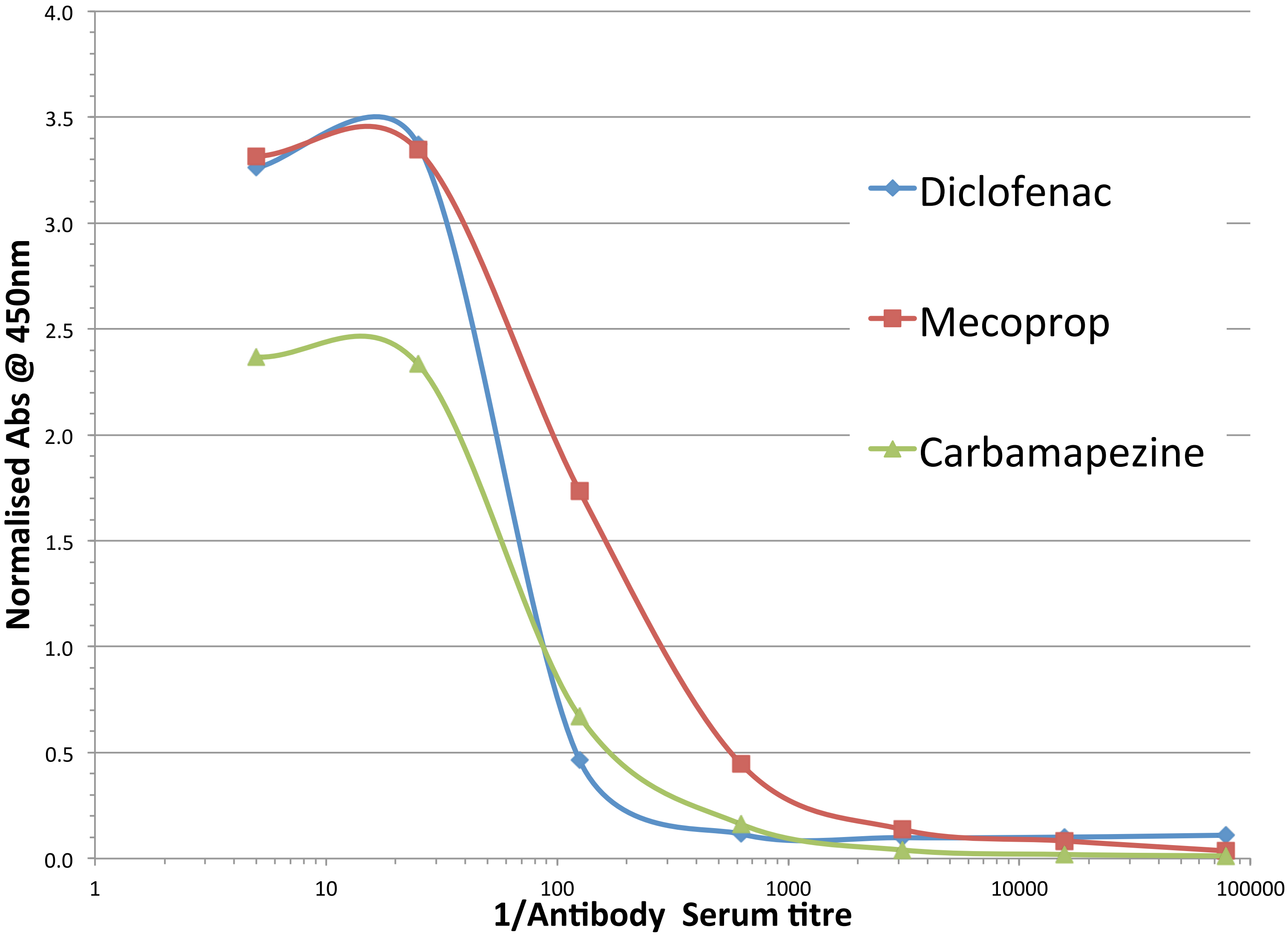
Antibody identification

- **Phage display** and **bio-panning** will be used to isolate most potent binders.
- They will be tested in matrix and cross-reactivity studies.
- Affinity tested using SPR.

Microfluidic disc

- The antibodies and chemical conjugates will be incorporated onto a microfluidic disc- based immunosensor.

Recombinant antibody assessment



Conclusion

Watch List chemicals

Industries with no trade effluent licenses

Continuing need to develop real-time monitoring platforms

Emerging chemicals of concern

- Traditional separation methods
- Compliance monitoring - EQS

Polar and non-polar organics

- Longer-term monitoring
- Trend monitoring
- Passive sampling

Environmental pollutants (toxins, pharmaceuticals)

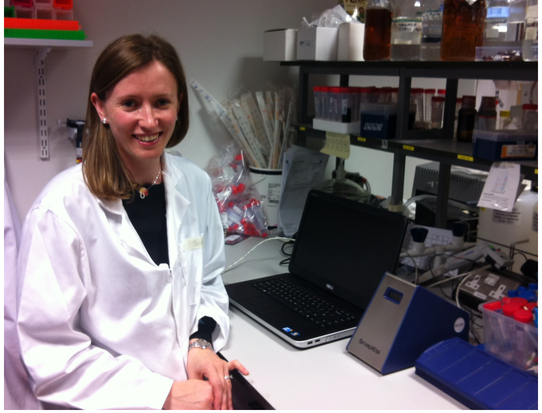
- Sensor platform development
- Single and multi-analyte systems
- Integration of technologies

A single sample — 4-6 weeks — Up-to a year autonomously

Spot sampling

Longer-term monitoring of trends

Continuous real-time monitoring of certain parameters



CIT

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Prof. Jens Ducree, Physics.

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Marine Institute
Foras na Mara

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