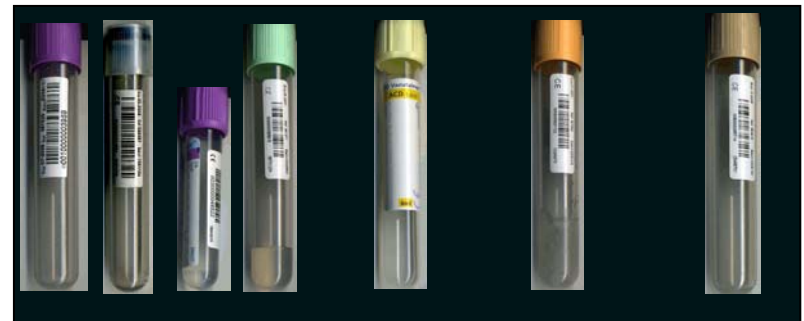
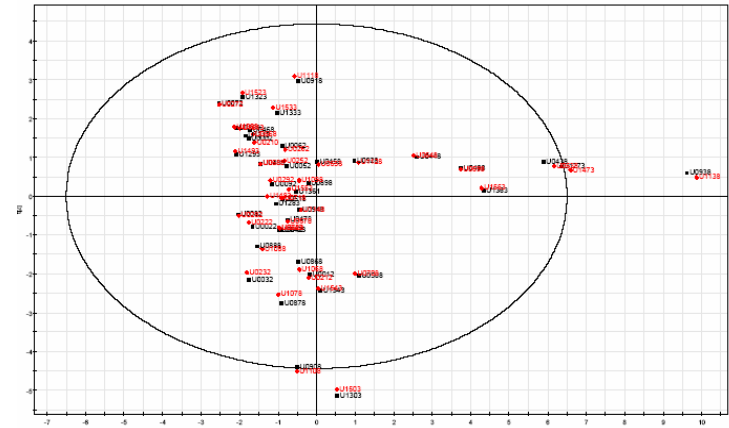
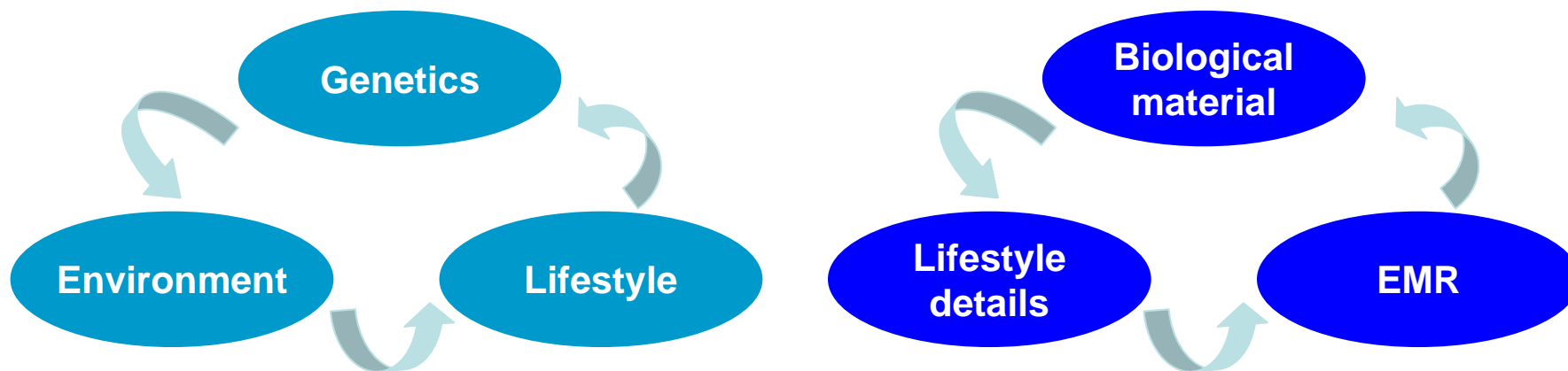


Selecting and optimising a biobank sample handling Protocol – experience from UK Biobank



What is UK Biobank?



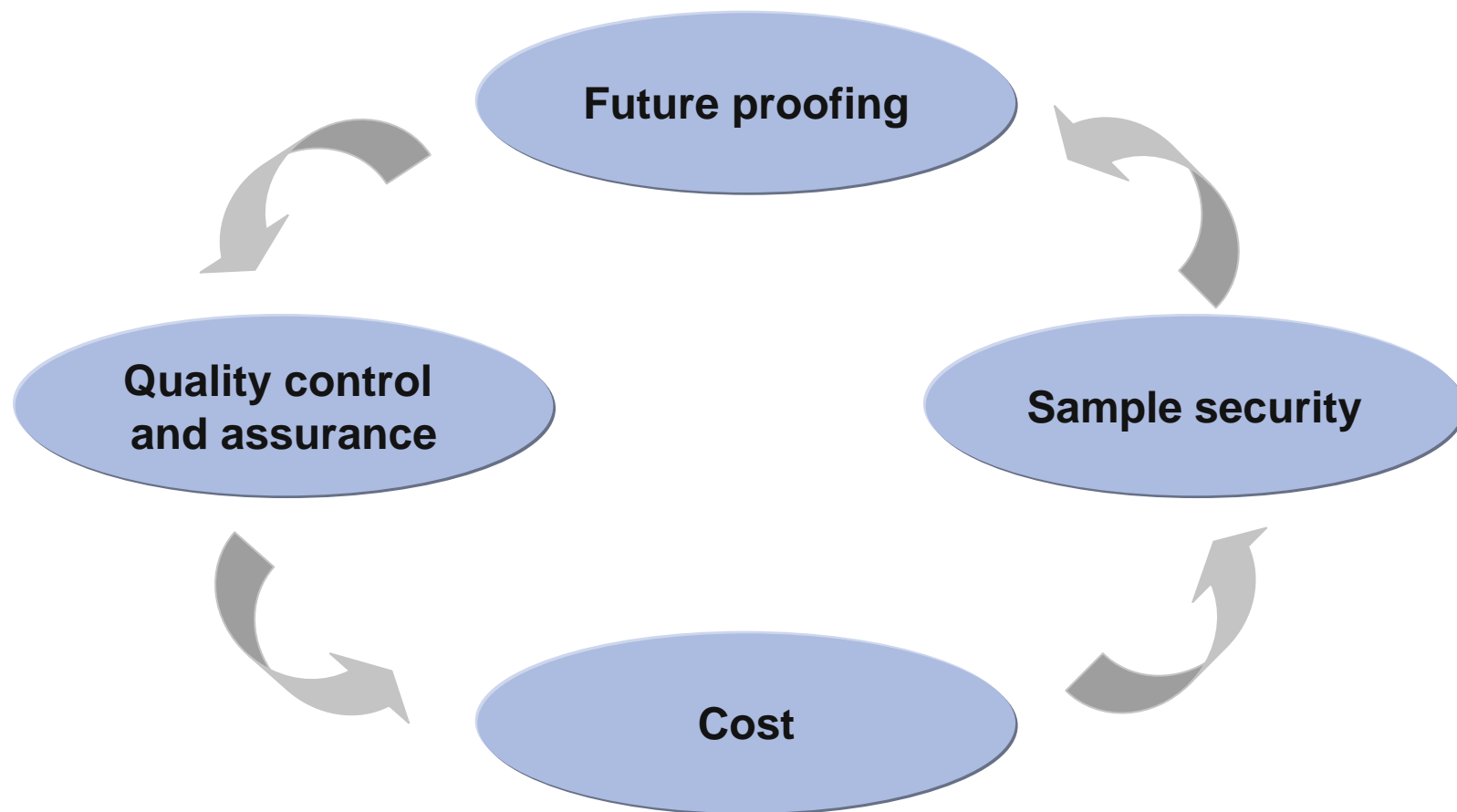
- 500K volunteer participants
- 40-69 years



SCOTTISH EXECUTIVE



Key principles



Protocol development

- Sample Handling and Storage development sub-group
- Made recommendations on
 - Samples collected
 - Collection protocols and volumes
 - Cross sectional assays
 - Processing protocols
 - Archiving of sample aliquots
 - Optimal structure of UK Biobank laboratory organization (central vs local processing)
 - Testing and validation of recommended protocol

Which sample types should be collected?

Sample type	Inclusion criteria
Blood	<ul style="list-style-type: none">• Variety of fractions• Wide range of biomolecules• Suitable for wide range of assays• Ease and cost of collection
Spot Urine	<ul style="list-style-type: none">• Range of biomolecules• Physiological coverage (inc gut microbiome)• Suitable for many assays• Ease and cost of collection

Which sample types are excluded?

Sample type	Exclusion criteria
Faeces, Hair, Nails, (Saliva)	<ul style="list-style-type: none">• Additional information from sample• Difficulty in collecting and processing• Potential impact on recruitment• Complexity and cost of storage• Complicating effects of cosmetics and toiletries• Consistency of sample collection

Consideration of pre-analytical variables

- Anticoagulants
- Haemolysis
- Clot accelerators
- Preservatives
 - RNase
 - Protease inhibitors
 - Fluoride oxalate
 - Borate
 - Azide
- Cryoprotectants
- (Central vs local processing)
- Transport and archiving temperature

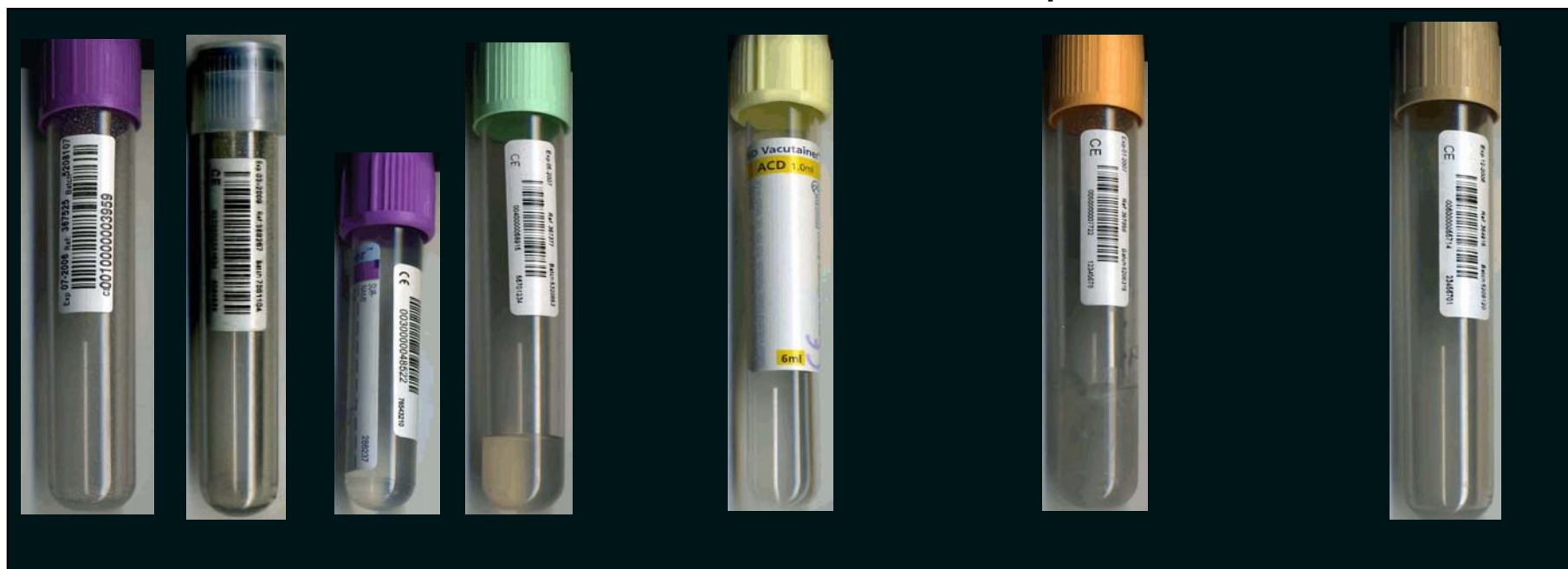
The following tubes will be used to collect blood from participants

K₂EDTA

Li/Hep PST

Acid citrate dextrose

Clot activator and gel for serum separation



4°C Transport

Plasma, Buffy coat, Red cells

Haematology

Plasma

18°C Transport

PBLs

4°C Transport

Serum

4°C Transport

Urine analytes

A variety of samples will be collected and fractionated

Vacutainer tube	Fractions	Number of aliquots	
		-80°C	Liquid N ₂
EDTA (9ml) x 2	Plasma	6	2
	Buffy coat	2	2
	Red cells	1	1
Lithium heparin (PST)	Plasma	3	1
Silica clot activator (SST)	Serum	3	1
Acid Citrate Dextrose	DMSO blood	-	2
EDTA (4 ml)	Haematology (immediate)	-	-
Urine	Urine	4	2
TOTAL ALIQUOTS		19	11

The operation will be high throughput,
reproducible and standardized.....



**660
Participants
per day**



**4620
Vacutainers
per day**

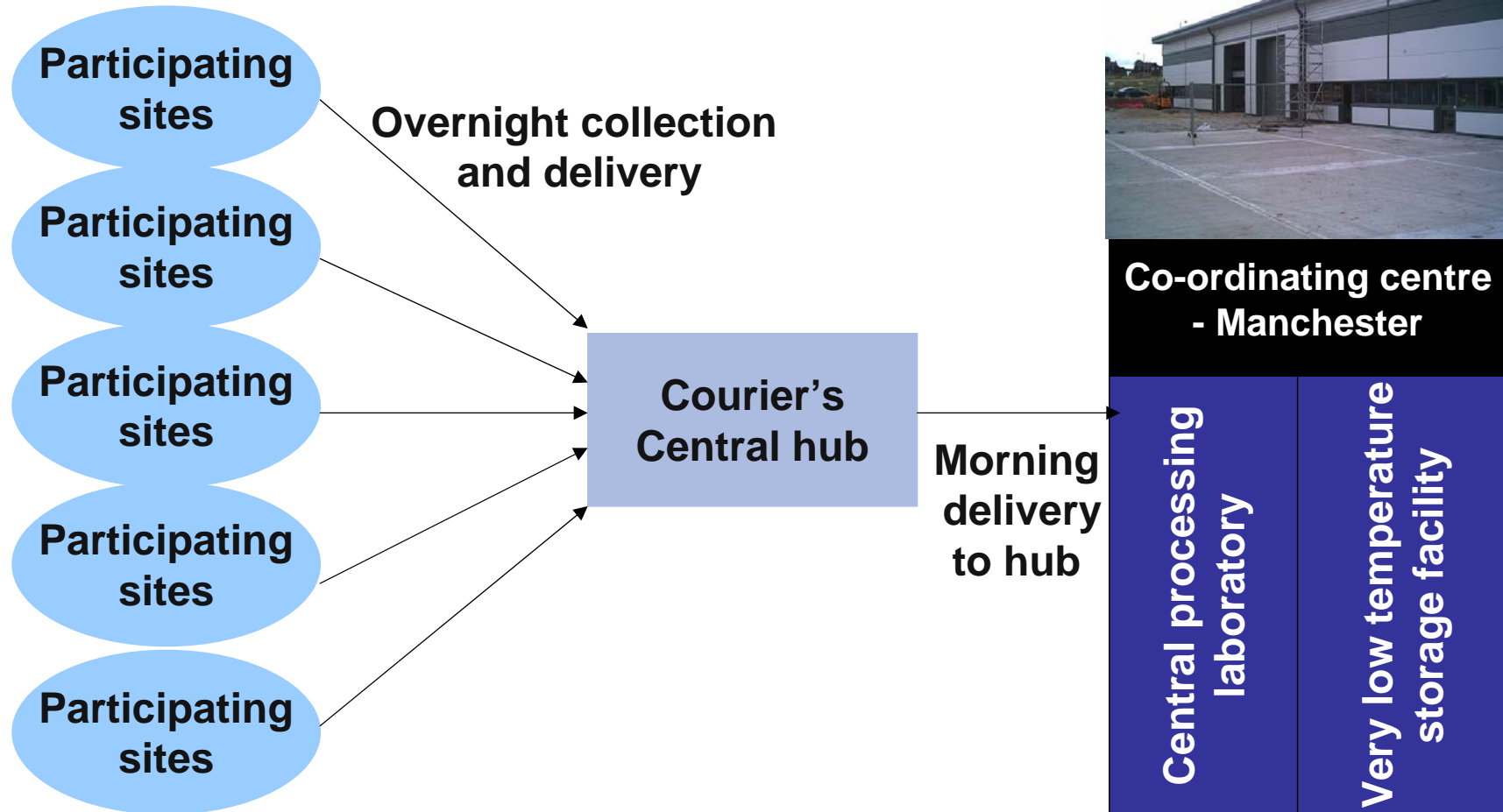


**19,800
1ml Samples
per day**



**15 million
1ml aliquots**

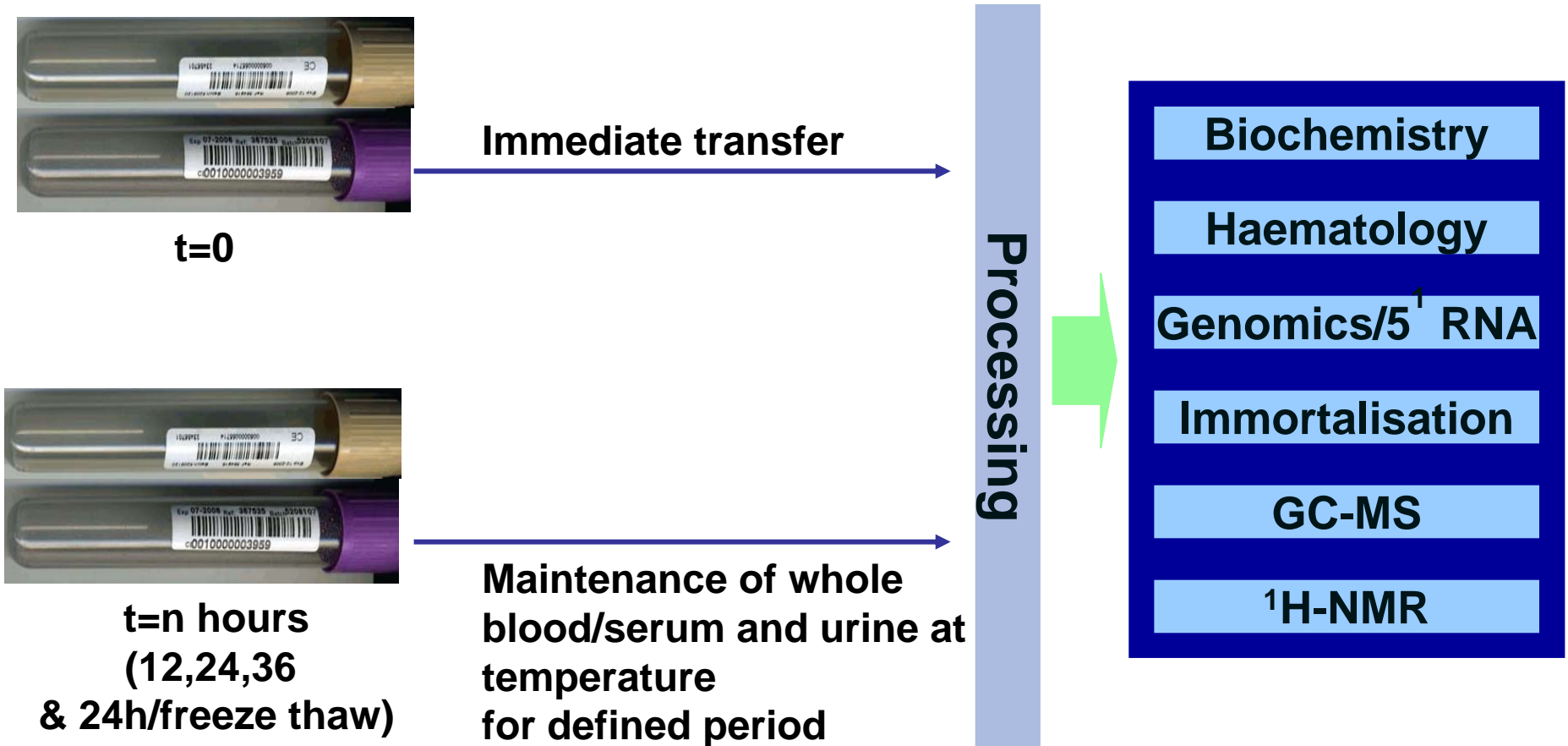
Samples will be processed centrally:



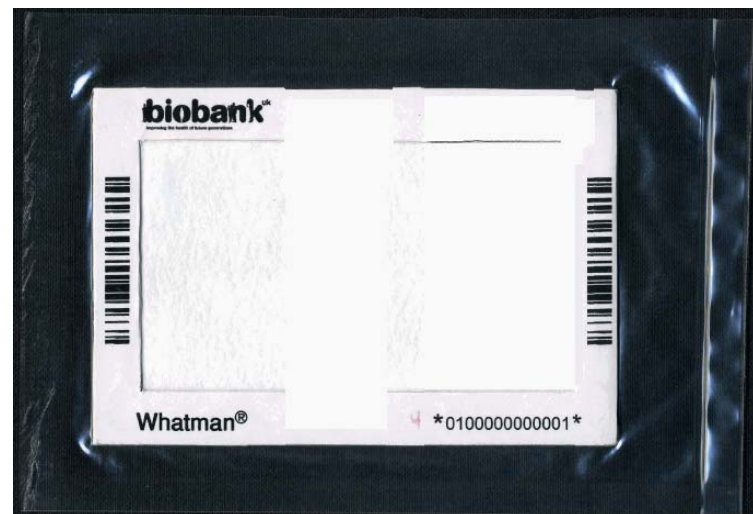
The sample handling pilot

- Demonstrate that the protocol produces samples that are fit for purpose
 - No general degradation
 - Samples are fit for a wide range of current assays
- Impact of short term freezing and controlled thawing
- Impact of delay between sample collection and cryopreservation
- Is a separate Fluoride Oxalate tube required for measures of plasma glucose concentration?

The sample handling pilot - principal



Analysis of DNA extracted from white cells and paper media



- DNA yield
- DNA purity
- Fragment length
- Performance in Whole Genome Amplification, Sequencing and SNP mapping



At 0 and 24 hours

Analyses by Dr S. Little *et al.* DxS,
Manchester

Biochemistry/haematology

Ions	Enzymes	Lipid/ cholesterol cycle	Other analytes	Other protein
<u>Sodium</u> <u>Potassium</u> Chloride <u>Calcium</u> Magnesium Phosphate Bicarbonate	ALT AST Alkaline phosphatase Gamma GT Amylase Creatinine kinase	Cholesterol HDL Triglycerides	Creatinine Uric acid Bilirubin Fibrinogen Glucose HbA1c <u>Urea</u>	Total protein Albumin Insulin CRP

Cell count	Physical characteristics
Red cells, platelets, white cells, lymphocytes, monocytes, neutrophils, eosinophils, basophils	Haemoglobin (total), Packed cell volume, mean cell volume, MCHC, MCH

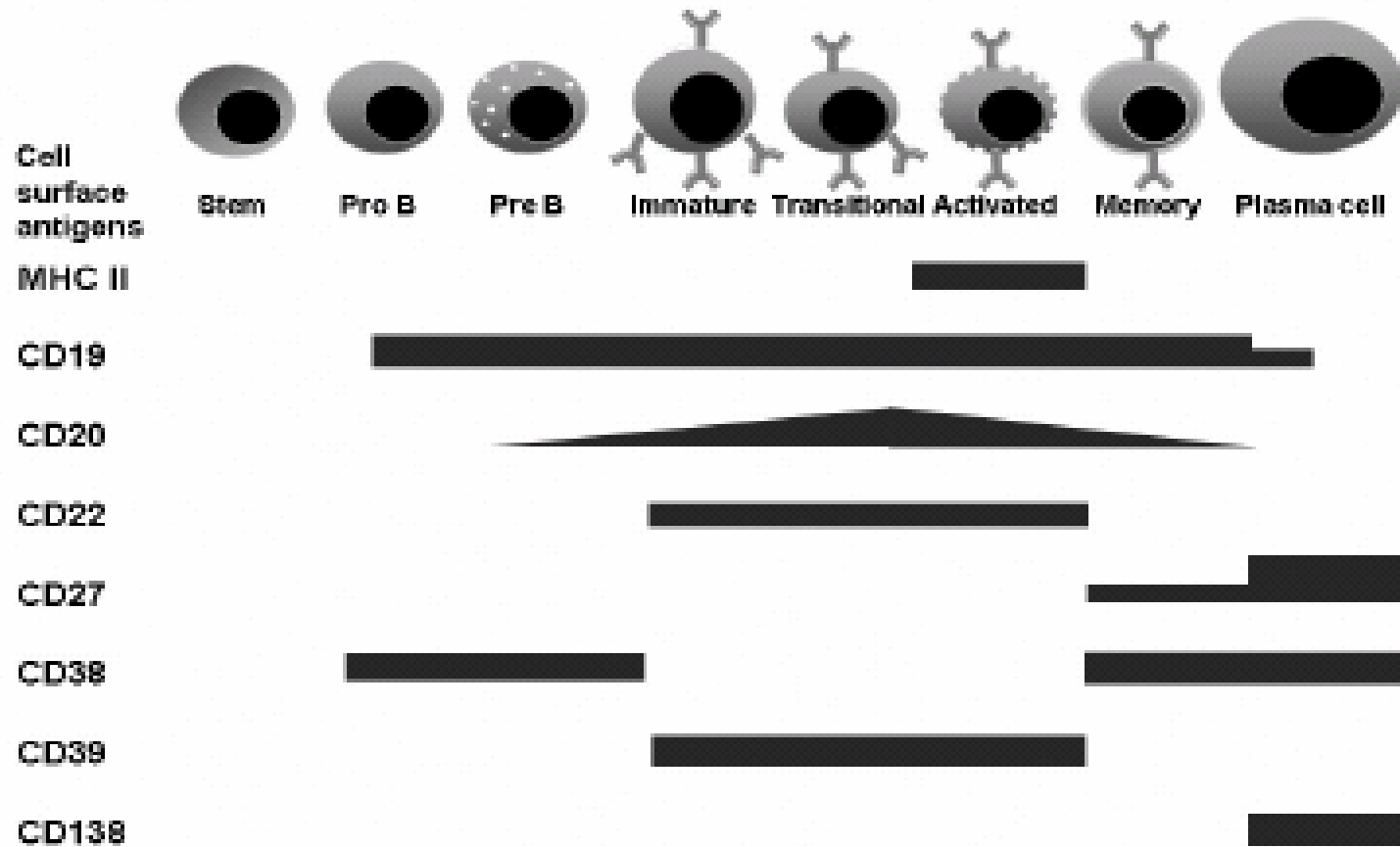
Biochemistry/haematology

- 42 haematological and chemistry parameters in blood and 4 in urine on all time points, plus a freeze thaw.
- Looked at variation over time (RELR), assay variation and inter-person variability
- Small negative biases over time (0.1-3%/12hrs)
- No positive biases
- Compared to inter-person heterogeneity on baseline measurements and inherent measurement variability from assay error, these are negligible in statistical and absolute terms

EBV immortalization of B-lymphocytes

- Integration of EBV into B-cell genome via CD21
- Existing approaches may require relatively large volumes of blood
- Cytotoxic T-cell mediated B-cell death may still be a problem (despite use of mitogens and super antigens)
- Use B-cell selective sorting approach on small volumes of peripheral blood (0.5ml)

Distinct stages of B cell maturation



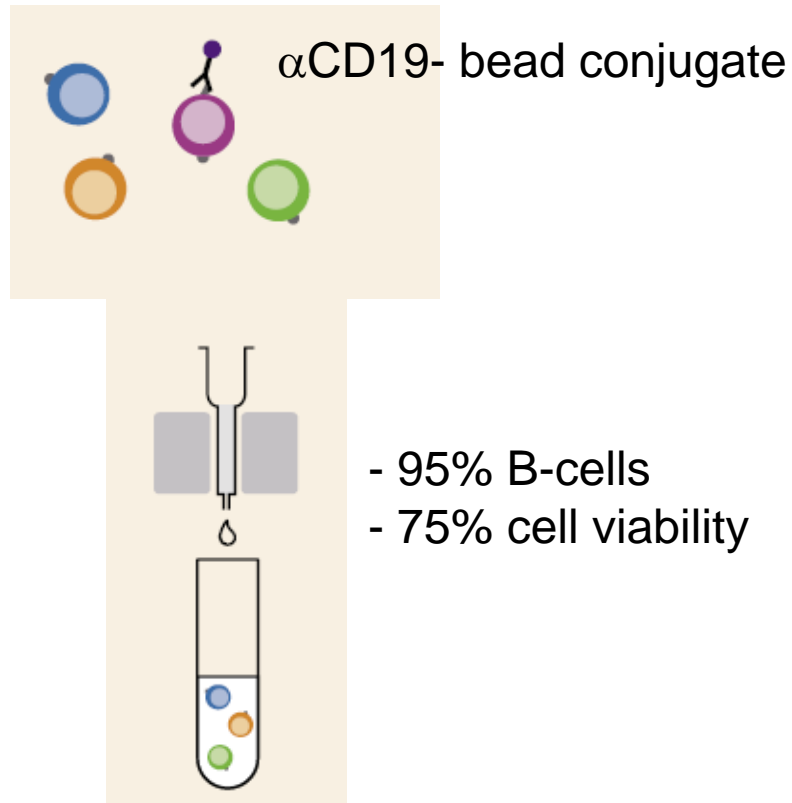
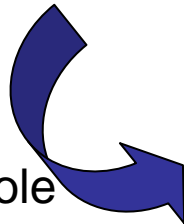
EBV immortalization of B-lymphocytes (24 hours)

40 samples –
24 hours post collection



- 200,000 lymphocytes/ml
- >10% B-cells in all samples

0.5ml whole
blood



Analyses by Dr M Amoli *et al.* – Univ. of,
Manchester

Major findings of sample handling pilot

- Samples maintained at 4°C for up to 36 hours prior to processing and cryopreservation are stable although NMR analysis indicates some systematic instability in 36 hour serum
- Samples maintained at 4°C for up to 36 hours are suitable for a wide range of analyses
- Short term freezing and controlled thawing does not impact sample integrity
- A separate fluoride oxalate tube is not required or measurement of plasma glucose
- Comparative studies of RNA levels (5' ends or whole message) will not be possible from these samples

Acknowledgements

The sample handling and storage group

- Prof. John Newton
- Prof. Paul Elliot (chair)
- Prof. Mark Caulfield
- Prof. Anna Dominiczak
- Prof. Mark McCarthy
- Prof. Bill Ollier
- Prof. Nilesh Samani
- Dr Julian Sampson
- Dr Nick Davies
- Dr Tim Peakman

UK Biobank technology development

- RTS – automated blood fractionation
- TAP - -80°C automated archive
- Jencons PLS – Liquid nitrogen archive
- Whatman – paper storage technology

UK Biobank pilots

- Dr Phil Day *et al.* - Manchester
- Dr Anthony Halsall *et al.* DxS, Manchester
- Drs Chris Jackson and Nicky Best – Imperial
- Prof. Douglas Kell *et al.* – Manchester
- Prof. Jeremy Nicholson *et al.* – Imperial
- Prof. Bill Ollier *et al.* – Manchester
- Ms Claire Wilkinson *et al.* – Medeval, Manchester