Sample Collection

Ronda Greaves

NATA

- National Association of Testing Authorities
- Accredits laboratories to ISO 15189:2003
- Accreditation required to receive government funding
- Medical Testing field application document dated August 2007
  - Section 5.4 details requirements for “pre examination procedures”

http://www.nata.asn.au/index.cfm?objectid=DBCABA6C-B07C-AD52-0EB6E2E877F61885&productId=0870C458-AAF4-3D9F-9D3B55C80FDD17EF

Section 5.4.3

- Order of draw e.g. EDTA can interfere with calcium result
- Self collection of samples e.g. Information sheets need to be given out for 24 urine collections
- Labeling containers: Pre labeling of containers is dangerous and can result on patient mix-up

5.4 Pre-examination procedures

5.4.3

(i) The laboratory's collection procedures must include 'order of draw'.

Note: Blood collection tubes must be drawn in a specific order to avoid cross contamination of additives between tubes.

(ii) Documented instructions should be available for self-collect samples (e.g. midstream urine, semen) in languages appropriate for the patient population.

5.4.3 c) 5) Sample collection containers must not be labelled before collection.

5.4.5 - Positive patient identification

5.4.5

(i) On presentation for collection, all patients must be positively identified by the collector without prompts (e.g. by asking 'What is your name?').

Note: Where identification of the patient by the above means is not possible, e.g. unconscious patients, non-English speakers etc, other mechanisms would be necessary to ensure correct identification.

(ii) The minimum requirements for labelling samples are two identifiers attributable to the patient. Generally these will be patient's full name and either date of birth or medical record number. Where point-of-care testing is performed, labelling requirements may be relaxed; where a delay in testing occurs there must be labelling of the syringe or tube as above. Samples which are not labelled with two identifiers are considered to be inadequately labelled.

(iii) Where inadequately labelled samples are received and accepted for testing, the laboratory must assure itself of the identity of the sample. If samples that do not meet minimum acceptability criteria are accepted and tested, a record must be kept of any subsequent action taken.

(iv) Where the labelling of a sample has been corrected or amended by collection staff, a comment indicating the original identification of the sample must be recorded.

(v) There may be special circumstances where the identity of the patient will not be revealed to the laboratory. In such cases, adequate precautions must be taken to maintain unique identification of the sample at all stages.

When samples are received by lab

- Lab reception staff must confirm two patient identifiers on sample and paper work i.e.
  - 1. first + surname
  - 2. date of birth or unique hospital record number.
- Unique lab number needs to be applied to each collection on receipt to lab
- Once lab no. applied sample and paper work can then be separated

5.4.7 Samples and associated records (worksheets, slides etc) must be uniquely identified during all stages of testing.

Note: This may be achieved by the use of a unique laboratory number. This is usually the most practical option especially where large numbers of samples are processed. Alternatively, samples and associated records can be uniquely identified by the use of two patient identifiers (e.g. patient's name and either date of birth or medical record number). The uniqueness of a numbering system should take into consideration the sample storage time and ensure two samples with the same number cannot be in the laboratory at the one time.

5.4.8 Documented sample reception procedures must include the action to be taken in the event that an unsuitable sample is received.

Sample Collection - Biochemistry

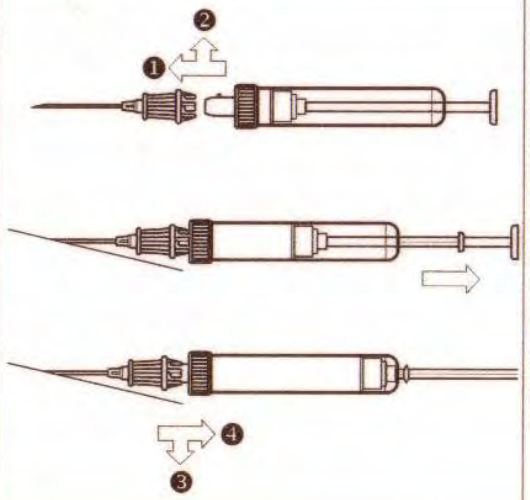
- Blood
- Urine
- Faeces
- Saliva
- Fluids
- Breath
- Sweat
- Hair

- Who should collect samples?
- Collection tubes
- Site of collection
- Difficulty of collection
- Stability of analyte

Blood: Who should collect samples?

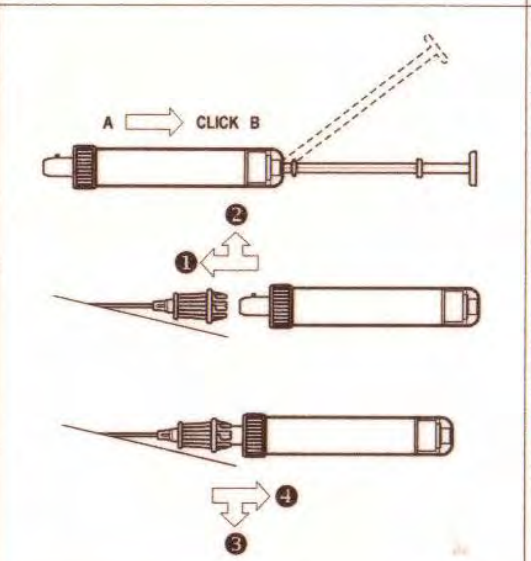
- Dedicated pathology collection teams
- Trained in phlebotomy
- Protocols for labeling tubes and positive sample identification
- Consistency in sample tubes and collection techniques
- Standardised procedures for use of tourniquet and posteur
- Protocols in place to deal with difficult collections
- Report to head of laboratory
- Accountable for collections
- Can on train ward staff for after hour collections

CONTENTS	VOLUME	APPLICATION	CAPS
GEL	0.5ml 7.5ml	Antibiotic Levels, Anticonvulsant Levels, Autoantibodies, Bile Acids, Creatinine, CRP, Digoxin, Ferritin, Hormones, Immunoglobulins, Iron Studies, LFT, RAST, SBR, Hepatitis/HIV/Microbial/Viral/Serology, Urea and Electrolytes, Vitamin A, D, E	BROWN
EDTA	0.5ml 2.7ml	Cyclosporin, Factor V Leiden, ESR, FBE, Film, G6PD Screen/Assay, Hb, Hba1c, Platelets, PTH *(must fill to line)*, Prothrombin Gene Mutation, Red Cell Folate, Renin, Tacrolimus, WCC	RED
EDTA BLOOD BANK SAMPLES	2.7ml 4ml 9ml	SEPARATE TUBE REQUIRED Antibody Screen, ASBT Protocol, Blood Group, Cord Blood, Cross Match, DAT, Kleihauer *Birth - 6 months: 2.7ml tube with min of 1.5ml of blood* *Paediatrics: 4ml tube* *Adults and Cord Bloods: 9ml tube*	RED
SODIUM CITRATE	1.4ml 3ml	MUST FILL TO LINE APCR, APTT, ATIII, Clotting Factors, Coagulation Profile, D Dimer, Fibrinogen, INR, Multiple Factor Assays *(Collect 2-3 tubes)*, Protein C&S, PT, Vitamin K	GREEN
LITHIUM HEPARIN	0.5ml 7.5ml	Amino Acids, Ammonia, Chromosomes, Flecainide, Glutathione Peroxidase, Homocysteine, Lead, Lymphocyte Function, Neutrophil Function, Trace Metals, Vitamin C	ORANGE
GLUCOSE	0.3ml 1.2ml	Glucose, Lactate	LIGHT GREY
CPDA	9ml	HLA B27 Tissue Typing	YELLOW



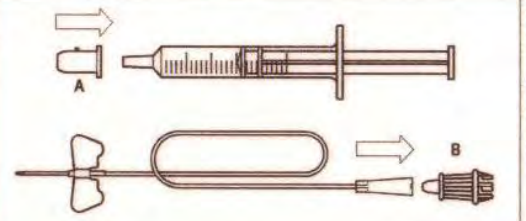
Syringe Principle

a. Push Monovette® onto needle and secure by twisting clockwise (see ❶ + ❷).
b. Puncture vein and withdraw plunger slowly. Wait until blood flow stops.
c. Remove Monovette® from needle by twisting anticlockwise (see ❸ + ❹). Needle remains in vein.
d. When multiple sampling, secure subsequent Monovettes® onto needle and collect further samples as described above.
Completion of blood collection:
e. Remove final Monovette® from needle (see ❸ + ❹), then withdraw needle from vein.
Remember: ***REMOVE MONOVETTE® THEN WITHDRAW NEEDLE.***
f. Mix sample(s) thoroughly.
g. For transportation and centrifugation, lock piston into Monovette® base and break off plunger.



Vacuum Principle

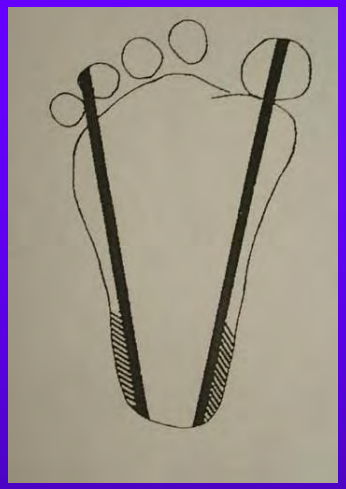
Prior to blood collection, the Monovette® needle must already be in the vein. Either puncture the vein directly with the needle or collect the first sample using the syringe principle - then apply the vacuum principle.
a. Prior to collection, lock piston into base (see **A** + **B**). Break off plunger.
b. Push Monovette® onto needle and secure by twisting clockwise (see ❶ + ❷).
c. Wait until blood flow stops.
d. Remove Monovette® from needle by twisting anticlockwise (see ❸ + ❹)Needle remains in vein.
e. When multiple sampling, secure subsequent Monovettes® onto needle and collect further samples as described above
Completion of blood collection:
f. Remove final Monovette® from needle, then withdraw needle from vein.
Remember: ***REMOVE MONOVETTE® THEN WITHDRAW NEEDLE.***
g. Mix sample(s) thoroughly.

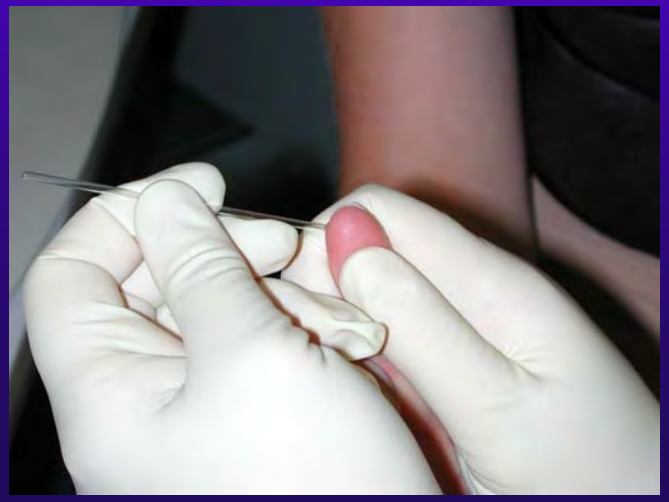


Special Applications

a. After blood collection, if injection though the Monovette® needle is required, the membrane adapter (**A**) may be used.
b. The membrane seal of the Monovette® can be adapted to a luer cone by use of the multi-adapter (**B**). This is particularly relevant to blood collection from 'butterflies', etc.

Capillary collections





Standardise: Collection tube

	Cap tube – analyser 1	Syringe – analyser 1	Syringe – analyser 2
pH (7.35-7.45)	7.49	7.41	7.41
pCO2 (35-45)	29	24	22.8
pO2 (80-100)	97	97	104.5
HCO3-	22.1	15.2	14.0
hct	36	21	
Ica++	0.79	<0.10	
Na+	135	137	
K+	2.6	1.3	
lactate	1.6	1.0	

Standardise: Tourniquet & posture

TEST	RESULT	RESULT	RR	UNITS
	Plasma 0830	Plasma 0845		
Na+	141	140	135-145	mmol/L
K+	3.7	3.7	3.5-5.0	mmol/L
HCO3-	26	24	22-32	mmol/L
Urea	3.6	3.5	3.5-8.5	mmol/L
Creat	0.10	0.09	0.5-0.12	mmol/L
Protein	82	**75**	60-80	g/L
Alb	51	**47**	35-50	g/L
TBil	6	5	<20	umol/L
ALT	17	17	5-50	U/L
ALP	82	**75**	40-120	U/L
Chol	4.6	4.4	<5.5	mmol/L
Trig	1.7	**1.2**	<2.0	mmol/L

Standardise: Time of collection

- Fasting
  - Glucose
  - Insulin
  - Cholesterol
  - Triglercerides
- Diural variation
  - Cortisol
  - LH, FSH, prog & E2
- Medication
  - Thyroxine
  - Drugs
- Dynamic Function Tests
  - Synacthen stimulation test
  - Glucose tolerance test

Difficult collections

- Often associated with dehydrated patients
- Trauma to patient
- Small volume collected
- Breakdown of cells
- False results for some analytes where there is a significant difference between intracellular and extracellular concentration
  - Potassium
  - Phosphate
- Haemolysis
  - Identify if haemolysis is present on report
  - Can affect some measurement systems

- Important for pathology collector to indicate if the collection was difficult on request card.

Do we miss hypokalaemia because of difficult collections?

- Consequences
  - Skeletal muscle - Weakness, paralysis
  - Gastrointestinal - Paralytic Ileus
  - Kidney - Impaired concentrating ability, Tubular defects
  - Cardiac - Conduction defects, arrhythmias, Digoxin toxicity

Most usual causes

- renal loss (diuretics), vomiting or diarrhoea

Sample stability: e.g. ammonia

- Ammonia is a by product of amino acid metabolism and is toxic to the brain (encephalopathy)
- It is detoxified via the urea cycle to urea which is excreted in the urine
- Hyperammonaemia in the newborn usually occurs following the introduction of protein feeds.

- Specimen handling is important for accurate results = heparin on ice to lab and analysed within 30 minutes of collection

- Why is plasma used rather than serum?

Urine

- Valuable
  - an overall estimation of production / metabolism c.f. a single time point (blood sample)
  - Metabolites often have a longer half life than parent compounds
- Considerations include:
  - Diurnal variation
  - Timed vs. random
  - Completeness of timed collections
  - Use of creatinine ratio
  - Addition of preservatives/ stabilizers
  - Dietary considerations
  - Solubility of analyte e.g. uric acid and calcium

Faeces

- Main problem – people hate collecting faeces
- Difficult to collect!
- Random or timed
- Relate to symptoms
- Consider which part of faeces is important to collect!
- Stability of sample

Faeces: Sugar malabsorption

- Can lead to:
  - diarrhoea (due to the osmotic activity of sugars)
  - failure to thrive (due to lack of sugar precursors)
  - abdominal distention and pain
    - large volumes of gas within the gut
    - bacteria in the large intestine will metabolise the malabsorbed sugar producing H_2 and methane gas
- Conditions assoc with malabsorption include:
  - congenital deficiencies of disaccharidases
  - coeliac disease
  - food allergy
  - bacterial overgrowth
- Lactase deficiency the most common

Saliva

- Popular sample type for hormone and drug testing
- Stimulated vs. non stimulated collection
- Swabs often used for collection
- Sample preparation often difficult due to extra “bits” in sample e.g. food

Fluids

- Cerebrospinal Fluid
- Pleural Fluid
- Ascites Fluid
- Cyst Fluid
- Vitreous Humour

Collection of fluids

- Fluids are collected by medical staff
- Often under local anesthetic
- Occasionally doctors are unsure about the type of fluid collected
- There are some simple ways of differentiating between fluids
- Specific questions often asked of laboratory

Differentiation between fluids

- **Is the CSF contaminated with blood?**
  - Blood Stained
    - Traumatic tap
    - If all the specimens are uniformly blood stained = Sub-arachnoid haemorrhage (< 12 hours ago).
  - Xanthochromic (yellow)
    - Subarachnoid haemorrhage more than 12 hours ago
    - Patient is jaundiced.
- **Is the fluid CSF or nasal secretions?**
  - β -2-transferrin (asialotransferrin) by protein electrophoresis
    - Only reliable test
    - Not present in nasal secretions
    - Present in CSF and serum
- **Is the fluid transudate or exudate?**
  - Plural fluid exudates have protein > 30g/L
- **Is the fluid ascites or urine?**
  - If the fluid is urine, or contaminated by urine, the urea and creatinine will be higher than in the plasma.

Vitreous Humour

- Collected at post mortum
- 1 – 2 mL usually collected
- Viscous fluid behind the retina
- Stagnant fluid – very slow exchange and equilibration between the systemic circulation and vitreous humour
- Sample collected when other body fluids not available or when there is a delay in collection post death i.e. where substances would be degraded or metabolised.
- Originally used to analyse alcohol in the 1960's
- Often analysed for metabolic diseases & drugs
- Qualitative analysis by GCMS

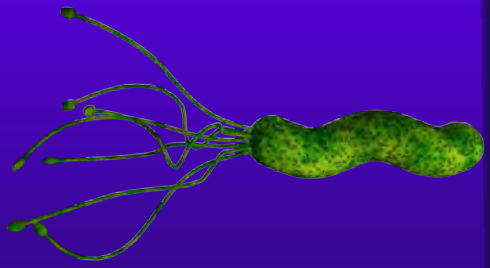
Breath

Breath tests

- Non invasive
- Breath samples can be collected on babies, children and adults
- Point of care test or laboratory
- Quantitative or qualitative
- Screening or diagnostic
- First line test in some cases may be followed up by a confirmatory test

Common breath tests

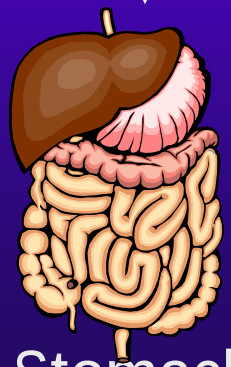
Analyte	Assessment
CH_3CH_2OH	Alcohol consumption
CO	Smoking status
Isotope CO_2	H. Pylori infection
H_2 +/- Methane	Sugar intolerance
	Bacterial overgrowth



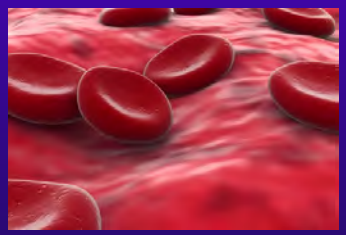
http://www.hpylori.com.au/hpylori-16.png

General mechanism

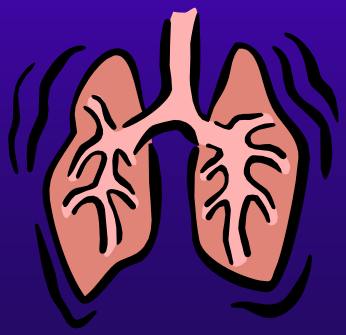
Oral intake



Stomach/ intestine



Absorbed into blood stream



Lung aveoli

Exhaled in breath

Alcohol breath test

http://www.ferret.com.au/

- Commenced in Victoria in 1976
  - Positive change to driving habits
  - Significant decrease in road deaths
  - 1977 – 49% of drivers killed had BAC >0.05%
  - 1992 – 21% of drivers killed had BAC > 0.05%
  - Legal limit = 0.05% BAC

- Screening test
  - Follow-up confirmatory blood test if required
  - Battery operated device
  - Oxidize sensors: When sensor exposed to alcohol molecules electrical current generated
  - Predicts BAC – based on ratio of 2100:1 blood:breath

Breath hydrogen tests

- Fasting patients are given a sugar solution and breath samples are collected at 1/2 hourly intervals for 2 ½ hours.
- If the patient is malabsorbing the sugar, the bacteria in the large bowel digest it and form H_2 as a by-product.
- The H_2 is absorbed in the blood stream and exhaled in the breath.

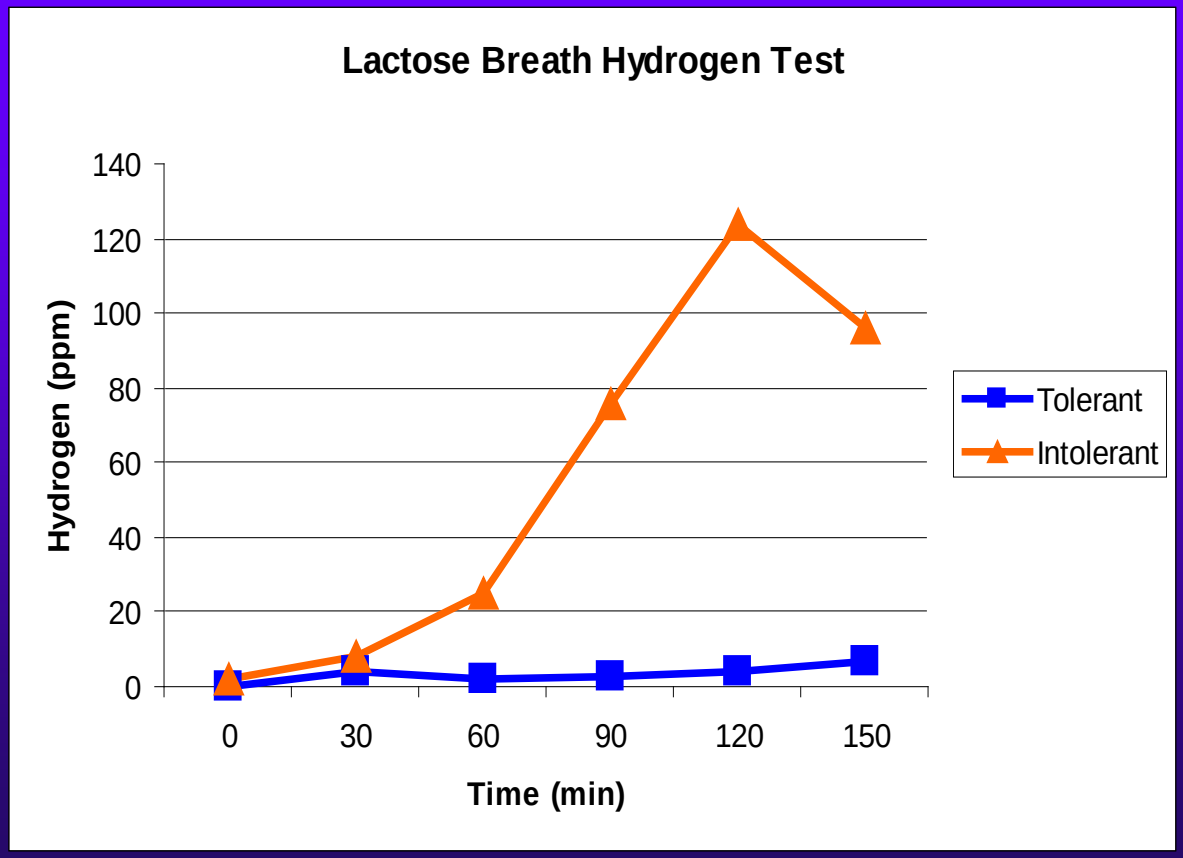
Electrochemical fuel cell

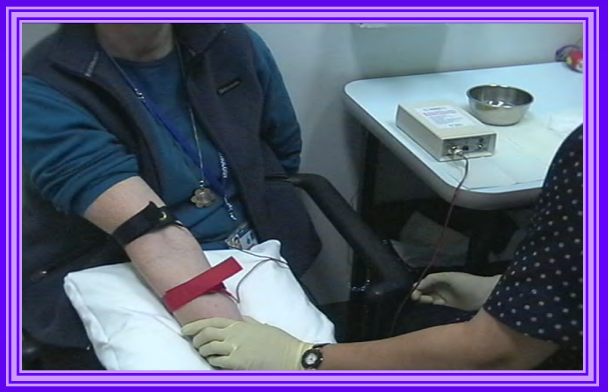
Hydrogen plus methane

- Gas Chromatograph
- Detects
  - Hydrogen
  - Methane
- Increased specificity
- >50% of labs in Melbourne use this approach*

*Data from Shepherd Works Pty Ltd 2008

BHT: Lactose


Lactose Breath Hydrogen Test
Hydrogen (ppm)
140
120
100
80
60
40
20
0
0
30
60
90
120
150
Time (min)
Tolerant
Intolerant



Sweat

Sweat glands

- Apocrine
  - Associated with the production of body odour
  - Less glands in Asians c.f. Europeans
- Eccrine
  - Number of eccrine glands constant from birth
  - Found all over body
  - Density of glands varies over the body
  - Chloride channel in eccrine glands

Sweat: 1938 to today

Progress in Pediatrics

CYSTIC FIBROSIS OF THE PANCREAS AND ITS RELATION TO CELIAC DISEASE

A CLINICAL AND PATHOLOGIC STUDY

DOROTHY H. ANDERSEN, M.D.
NEW YORK

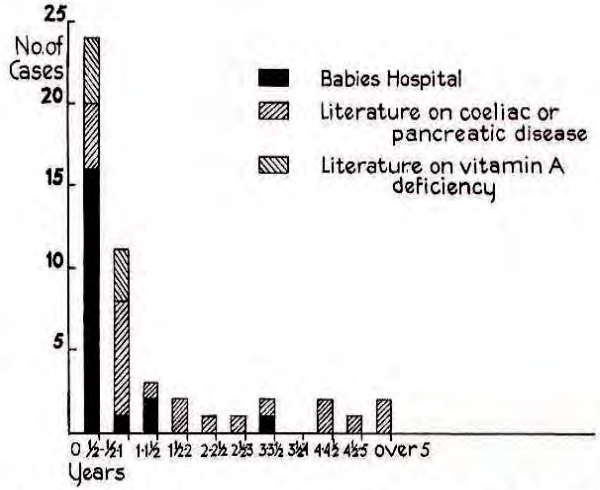


Fig. 1.—Chart showing the age at death in 49 cases of cystic fibrosis of the pancreas. The shading of the columns indicates the sources from which the cases were collected.

Am J Dis Child.1938;56:344-399

A TEST FOR CONCENTRATION OF ELECTROLYTES IN SWEAT IN CYSTIC FIBROSIS OF THE PANCREAS UTILIZING PILOCARPINE BY IONTOPHORESIS

Lewis E. Gibson and Robert E. Cooke

Pediatrics 1959;23;545-549

Review Article

The Relevance of Sweat Testing for the Diagnosis of Cystic Fibrosis in the Genomic Era

Avantika Mishra[1,2], Ronda Greaves[3], *John Massie[4]

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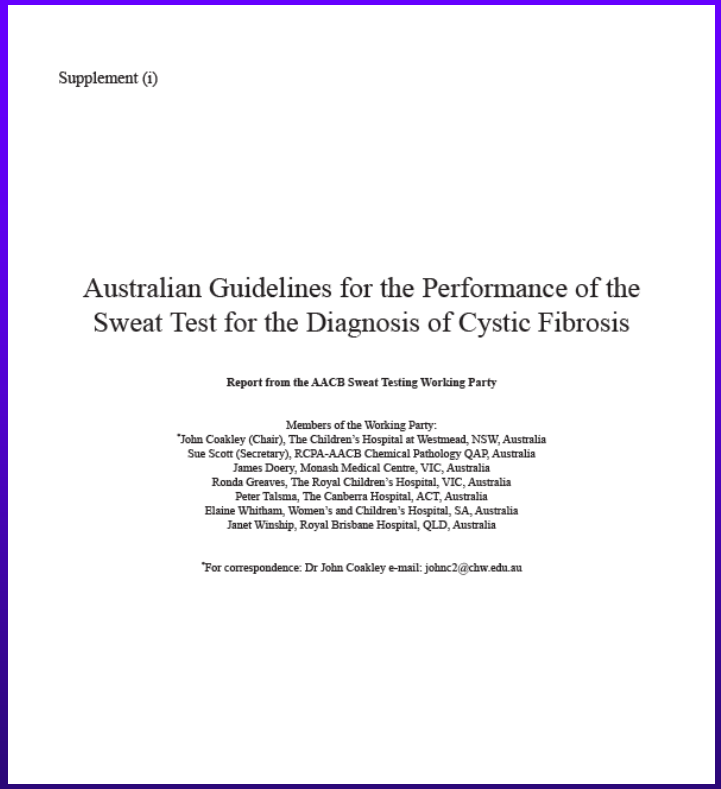
Considerations for Interpretation of a Sweat Test

- Pre-analytical contamination
- Insufficient Sample – often associated with patients hydration status
- Biological Variation
- Sweat electrolytes increase with age

Sweat Collection

- In house collection system or Commercial Wescor method
- Pilocarpine iontophoresis
- Following published guidelines
  - USA: 1990
  - UK: 2002
  - Australia: 2006



Supplement (i)

Australian Guidelines for the Performance of the Sweat Test for the Diagnosis of Cystic Fibrosis

Report from the AACB Sweat Testing Working Party

Members of the Working Party:
*John Coakley (Chair), The Children's Hospital at Westmead, NSW, Australia
Sue Scott (Secretary), RCPA-AACB Chemical Pathology QAP, Australia
James Doery, Monash Medical Centre, VIC, Australia
Ronda Greaves, The Royal Children's Hospital, VIC, Australia
Peter Talsma, The Canberra Hospital, ACT, Australia
Elaine Whitham, Women's and Children's Hospital, SA, Australia
Janet Winship, Royal Brisbane Hospital, QLD, Australia

*For correspondence: Dr John Coakley e-mail: johnc2@chw.edu.au

Hair

- Alternative medium
- Used for assessment of long term exposure or deficiency
- Limitations:
  - –Site of hair collection – root or ends
  - –Inter individual rate of growth of hair
  - –Semi-quantitative
- Analytes:
  - –Drugs use
  - –Smoking
  - –Metal analysis
- Analysis:
  - –Weigh hair
  - –Liquid extraction of analyte of interest with e.g. dichloromethane
  - –GCMS or AA

In Summary:

- Samples are collected to aid the clinician in treating the patient
- Appropriate sample must be collected on the correct patient at the correct time!
- Sample must be received and process by laboratory within time and temperature constraints for analyte of interest.
- Biochemical measurements must be made with appropriate “in control” methods.
- Any biochemical value must be interpreted in the light of the **appropriate** reference interval.