‘Atypical Body Fluids – How Should We Assess Quality of Biochemical Testing?

Presentation by:

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"We're all experts in one thing or another. For instance I consider myself something of an expert in fluids."
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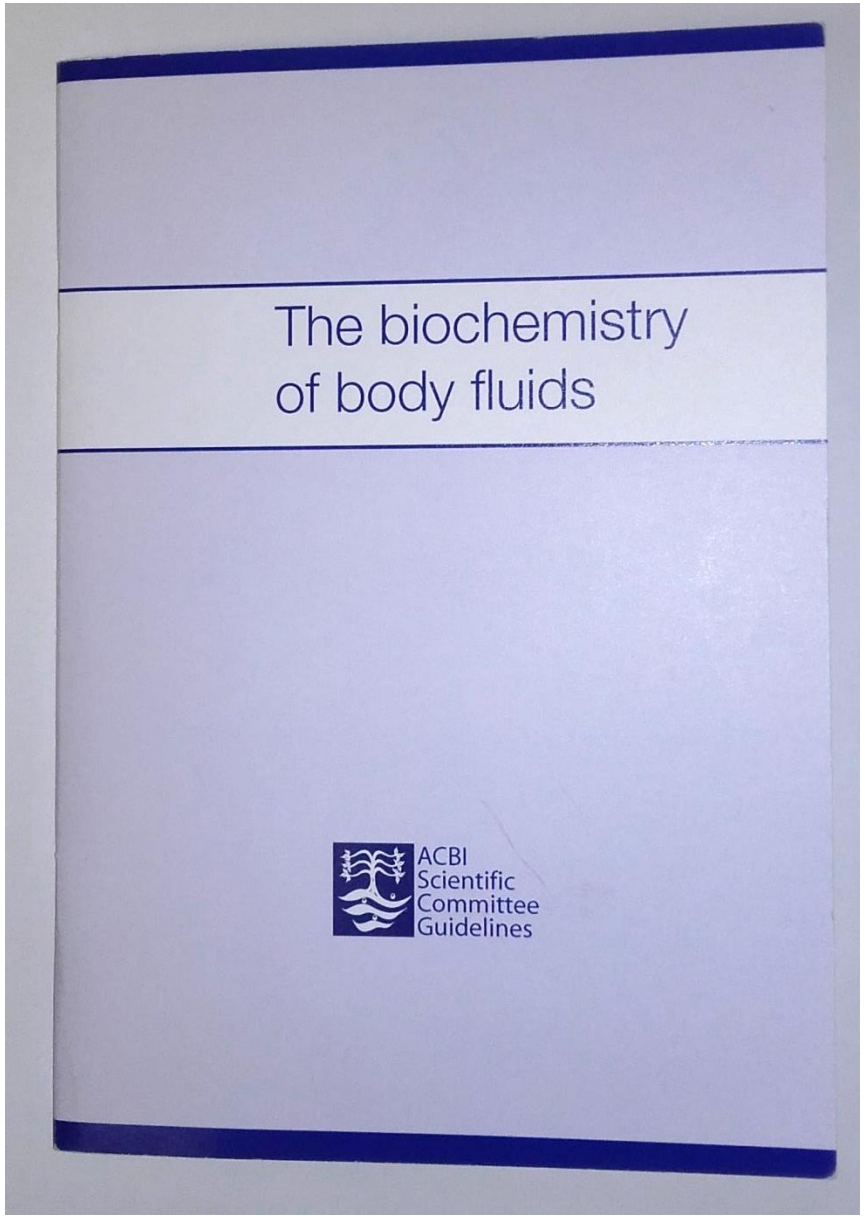
- 

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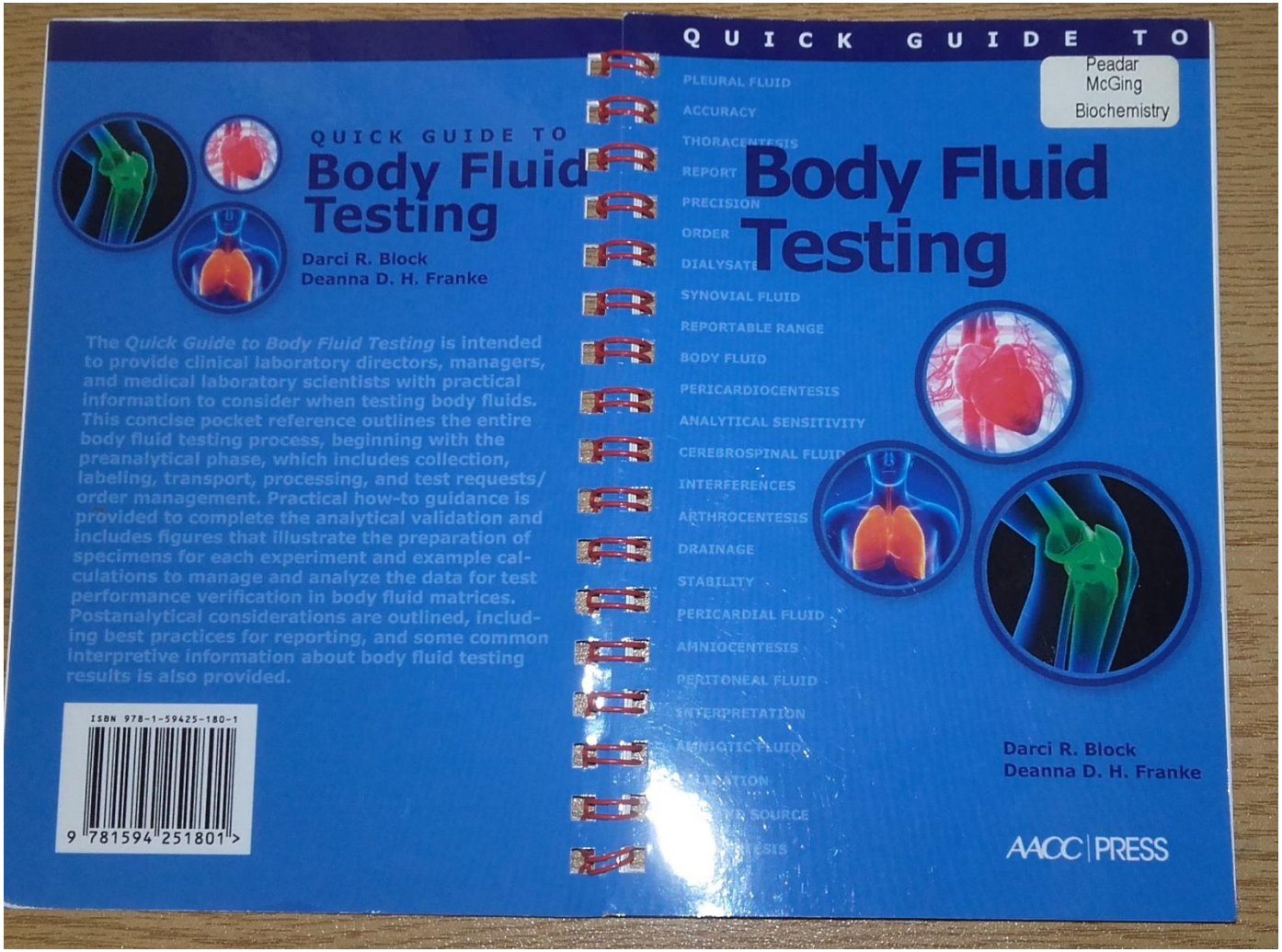

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From ACB Mailbase

Cc:
Subject: FW: Protocols for validating assays for use in fluids
Message | Validation of Tests on Non-Standard Fluid Types - v1 SB.docx (911 KB)

Referring to your query below, I have received permission from our lab network and the lead author of this doc, Fred San Gil et al, to share the attached local protocol with the mail base. Hope you find this useful. Feedback and comments from the mail base to Fred (cc-d in) are welcome as they help us refine our own procedures.

Kind Regards,
Rita

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From: Clinical biochemistry discussion list [ACB-CLIN-CHEM-GEN@JISCMAIL.A 3f2af2ac7f-dmarc-request@JISCMAIL.AC.UK]
Sent: Wednesday, April 25, 2018 6:55 PM
To: ACB-CLIN-CHEM-GEN@JISCMAIL.AC.UK
Subject: Protocols for validating assays for use in fluids

Dear All,
Does anyone have a protocol they would be willing to share with me on how they managed to validate an assay for use with fluids other than those that have already been CE marked by the manufacturer?
Thanks,
Robyn

Procedure

Validation of tests on non-standard body fluids

Insert the unique identification number

1. Purpose

To provide a consistent approach for validating commercially available test methods for use with body fluids that have not been validated by the assay manufacturer.

2. Background

In Australia, method validation is required if a diagnostic pathology test is modified or used differently to its original intended purpose or the original manufacturer's validation claims.

Such methods are classed as Class 3 in-house IVDs.

Most commercially available assays are validated for use in serum and urine (and occasionally CSF). Testing of a wide range of alternate body fluids with commercially available assays is also widespread.

Testing in most alternate body fluids has not been validated for these commercially available assays, either by the manufacturer or by other third parties.

Types of Fluids for Analysis

- **Cerebral Spinal Fluid**
  - Query CSF
- **Pleural Fluid**
- Pericardial Fluid
- **Ascitic / Peritoneal Fluid**
  - PET Dialysis Fluid
- Saliva
- Sweat
- Amniotic Fluid
- Seminal Fluid
- Synovial Fluid
- Cyst Fluids
  - Pancreatic cyst fluid
- Faecal Water
- Vitreous humour
- *Drain fluids*

Fluid collection for testing

- Blood readily available, but minimally invasive,
- Urine can be readily available (timing-dependent)

- Transcellular fluid may be difficult –
  - Increasingly Ultrasound guided,
  - Sometimes just diagnostic tap
  - Often fluid drainage is therapeutic

An informal audit

- By show of hands –
- How many of your labs perform chemistry and/or IA tests on fluids other than plasma/serum, urine, or CSF?
- How many of your labs have formal verification / validation for any of such assays performed?
- How many of your labs have formal verification / validation for all such assays your lab performs?

Pleural Effusion

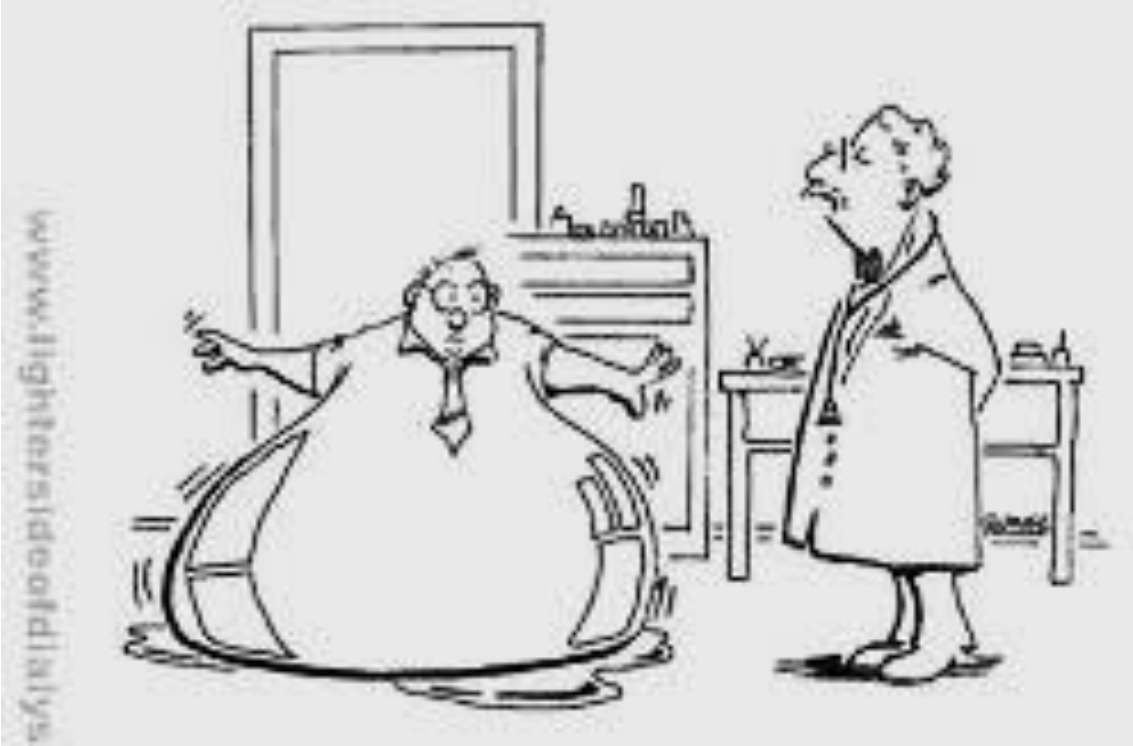
- “Pleural fluid is not just there to annoy respiratory physicians but has a purpose. It allows us to breathe.”
  - Opening remark of Consultant Respiratory Physician at Medical Grand Rounds presentation ‘A fluid situation. Myths, pearls, and reality of pleural effusions’.

Pleural Effusions

- The most common causes of *exudative* pleural effusions are
  - parapneumonic effusions (particularly bacterial pneumonia), and
  - malignancy.
- The most common causes of *transudative* pleural effusions are
  - left ventricular failure (very common) and
  - cirrhosis.

Transudate v Exudate

- Fluid Protein strongly suggests
- ➢ Transudate if Fluid Protein <25 g/L
- ➢ Exudate if Fluid Protein >35 g/L



- "Your tests reveal you are retaining fluids."

Transudate v Exudate

- Fluid Protein strongly suggests
  - Transudate if Fluid Protein <25 g/L
  - Exudate if Fluid Protein >35 g/L

- Fluid Protein equivocal if 25-35 g/L
  - Further testing required, including
    - Fluid LDH, or
    - Light's Criteria

The Light Criteria

The Beginning and Why they are Useful 40 Years Later

Richard W. Light, MD

KEY POINTS

- The Light criteria serve as a good starting point in the separation of transudates from exudates.
- The Light criteria misclassify about 25% of transudates as exudates, and most of these patients are on diuretics.
- If a patient is thought likely to have a disease that produces a transudative pleural effusion but the Light criteria suggest an exudate by only a small margin, the serum–pleural fluid protein gradient should be examined.
- If this is greater than 3.1 gm/dL, the patient in all probability has a transudative effusion.
- If the gradient is less than 3.1 gm/dL, either the NT-pro-BNP level in the pleural fluid or the serum–pleural fluid albumin gradient can be measured.
- Either an NT-pro-BNP greater than 1300 pg/mL or an albumin gradient greater than 1.2 gm/dL indicate that the effusion is a transudate.

It has been 40 years since I published the article[1] describing what came to be known as the Light criteria. I thought that it might be appropriate to begin this article by detailing how that article came about.

THE DEVELOPMENT OF THE LIGHT CRITERIA

When I was an intern in medicine at Johns Hopkins Hospital in Baltimore, Maryland, in 1968 to 1969, there was a period when a large percentage of my patients had a pleural effusion. The chief resident, Dr Richard Winterbauer, made rounds around midnight and always asked me what the thoracentesis revealed. At that time, we routinely measured the cell count and differential, glucose, and protein, and performed smears and cultures on the pleural fluid. I asked Dr Winterbauer the significance of the various pleural fluid findings and, for the most part, neither he nor anybody else had a scientific answer.

It was at this time that additional measurements were first being made on blood, such as the lactic dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). At about the same time, blood gas machines became available that would allow the accurate measurement of pH, P_{CO_2}, and P_{O_2} of body fluids. I theorized that some of these new measurements might be useful in the differential diagnosis of pleural effusions. After doing a literature review, I developed 2 hypotheses. The first was that the pH of pleural fluid would be lower in tuberculous pleural effusions than in other exudative pleural effusions. The basis for this hypothesis was an article in the *Scandinavian Journal of Respiratory Disease* that purported to show this.[2] My second hypothesis was that LDH isoenzymes would be

Disclosures: The author has nothing to disclose related to this publication.
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0272-5231/13/$ – see front matter ©

Clin Chest Med 34 (2013) 21-26.

Light's criteria (Pleural Fluid)

- Pleural fluid is an exudate if one or more of the following criteria are met:
  - Pleural fluid protein divided by serum protein is >0.5
  - Pleural fluid lactate dehydrogenase (LDH) divided by serum LDH is >0.6
  - Pleural fluid LDH >2/3 the upper limits of laboratory normal value for serum LDH.

Pleural Effusions

- Where clinical suggests transudate but Light Criteria suggest exudate:
- Serum:fluid protein gradient >31 g/L suggests Transudate,
- If protein gradient <31 g/L then
  - Fluid NT-Pro BNP >1300ng/L, or
  - Serum:fluid Albumin gradient >12 g/L

  suggests Transudate.

Biochemical Tests performed in specific clinical circumstances.

- **Query Chylothorax**
  - triglycerides and cholesterol +/- chylomicrons
- **Query TB**
  - (Adenosine deaminase)
- **Query Malignancy**
  - value of tumour markers is questionable.
- **Query Pancreatitis**
  - amylase.

Clinical Audit (Bio / Respiratory)

AUDIT

HOSPITAL DOCTOR OF IRELAND

Adequacy of pleural fluid tests in a hospital setting

The aim of this audit was to examine current practice in relation to tests requested when analysing pleural fluid and to highlight any deficiencies, if they exist, write

Dr Sarmad Waqas and **Dr Peadar McGing**

Audit findings

- 34 consecutive pleural fluids

Fluid test	n	%
Culture	32	94
LDH	29	85
Gram stain	29	85
Protein + albumin	27	79
pH	26	76
Glucose	21	62
Cytology	14	41

Serum / Plasma	n	%
Blood with fluid	17	50
Protein + albumin	14	41
LDH	8	24
Glucose	7	21

Pre-analytical

- The correct sample
- Correctly labelled
  - Including fluid source
- All tests requested
  - Including paired blood tests
- All test samples taken
  - and in correct tubes
- All samples presented in timely manner
- All samples processed in timely manner

CLINICAL Chemist

EWSLETTER OF THE AMERICAN ASSOCIATION OF CLINICAL CHEMISTS

ol. 1 No. 3 November 1949

nical fields. Clinical chemists were reminded of their re-
nsibilities to the physician and the patient, and of their o
ations to recruit and train chemists with superior qualifica
ns for this important field.

Dr. Archibald reviewed the lamentable performance of
pital laboratories in the examination of known solutions by
mical methods disclosed by the Belk and Sunderman survey. He
cussed factors important in the selection of chemical method
clinical laboratories and described ways by which errors ma
avoided. Among these are the following:

adequate methods
clear directions
regular inclusion of an aliquot of a large stock sample
checking the calculations by an individual other than the analyst.

Of great importance are:

adequate number of properly trained analysts
a director who has the "feel" of a quantitative chemist

Very useful reference:

Clinical Biochemistry 58 (2018) 44–52

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journal homepage: www.elsevier.com/locate/clinbiochem

An approach to analytical validation and testing of body fluid assays for the automated clinical laboratory

Darci R. Block[a,*], Lucas J. Ouverson[a,1], Craig A. Wittwer[a], Amy K. Saenger[b], Nikola A. Baumann[a]

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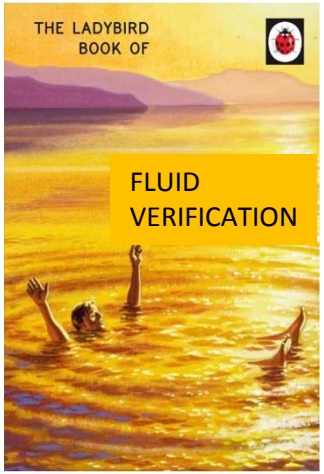
[b] University of Minnesota, Department of Laboratory Medicine and Pathology, 1200 Washington Ave. S, Minneapolis, MN 55415, USA

- Clinical Biochemistry 58 (2018) 44-52.
  - Block D, et al.

From *The Ladybird Book of Fluid Verification (2019)*



1. Gather surplus fluid & freeze aliquots (mark spec type) – 'A',
2. Gather surplus plasma/serum with high levels of analyte of interest; freeze – 'B'
3. Add known amount of B to 500µL A,
4. Add same amount of B to 500µL anonymised plasma/serum (analyte concentration known),
5. Compare measured levels of B in fluid A versus plasma/serum.
6. Also perform dilution study on the above.
7. Repeat on some more fluid samples.

MMUH Fluids SOP

- **6.2 External QA**

- Other than for CSF (and the schemes for blood and urine) there is no EQA scheme covering analysis of biochemical parameters in Body Fluids.
- Details of the EQA scheme for CSF are to be found in the SOP for EQA [QP-CCE-0001]
- CSF Protein is also specifically covered by RIQAS Urine Scheme (as well as CSF-specific scheme).
- We use the EQA schemes for blood and urine to monitor quality of performance for all other tests carried out on miscellaneous body fluids.

EQA of Fluids - Options

- EQA of specific fluid
  - Plasma / serum
  - CSF
  - Other
- Assurance from plasma/serum
  - Testing the analyte
  - Testing the system
- EQA by sample exchange

Glucose EQA

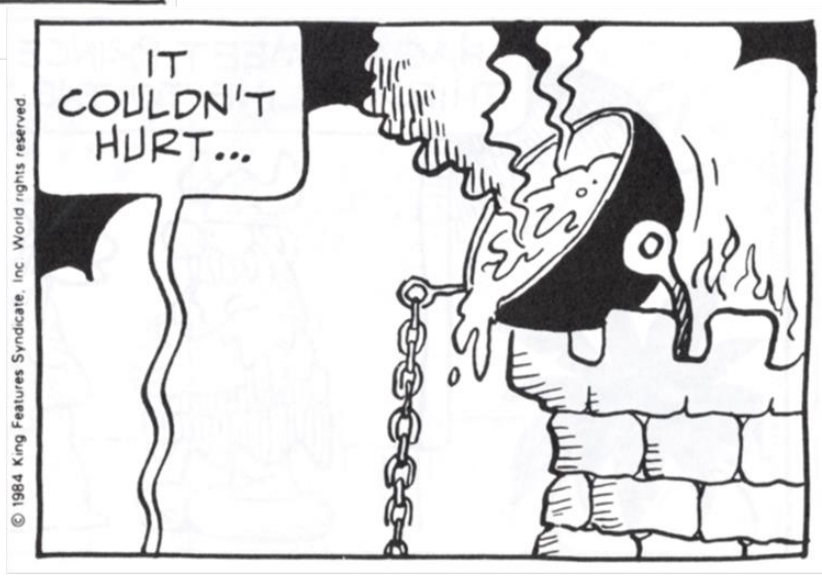
- MMUH Routine Glucose assay = Hexokinase/G-6-PDH (on Abbott Architect)
  - EQA in Plasma / Serum – Good
  - EQA in Urine – Good
  - EQA in CSF – Good
- MMUH POCT Glucose assay = Glucose sensor (electrode) (on ABG machine)
  - EQA in Whole Blood – Good

Lactate EQA

- MMUH lactate assay = Lactate sensor (electrode) (on ABL90 'blood gas' machine)
  - EQA in Whole Blood – Good
  - EQA in Plasma / Serum – Good

- Used CSF EQA as initial test of validity of our Lactate assay if applied to CSF.
  - EQA in CSF – Poor → Decision = refer CSF Lact

Remember testing may not be always appropriate.



Summary (1 of 3)

- Many body fluids can accumulate in disease
- In some cases laboratory testing, including biochemical, may be of value.
- Testing of other fluids (not pathological accumulations) can also be helpful.
- The number of samples and analytes so tested is a very small part of our workload
- Despite this, fluid analyses can be difficult and labour intensive.

Summary (2 of 3)

- Matrix differences versus serum/plasma/urine means some verification needed
- Full verification / validation is probably not clinically necessary (usually),
- Matrix effect MUST be tested.
- Some interpretation should be provided either on report, via electronic link, or via reference to hospital intranet /internet.
- Message that test is used off-label must be attached to report (unless not appropriate)

Summary (3 of 3)

- The difficulties associated with verification of fluid analyses should not deter laboratories from using them.
  - All tests used should be verified to some extent, either
    - In-house, or
    - In parallel with user of same assay system,
  - Some form of External Quality Assessment will be needed,
  - IEQAS will consider suggestions / proposals for Irish surveys – both questionnaire and analytical.

Open to Floor

- Do you think IEQAS can help with quality assurance of atypical fluids?

- Why / why not?

- If yes – how?
- If no – who can?
  Or, is help needed?