SAMPLE STABILITY

Improving the Future

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IN OUR PREVIOUS CONFERENCE WHAT WAS IDENTIFIED AS THE MOST CRUCIAL STEP TO HARMONISE

A. Patient preparation
B. Transport and storage
C. Quality indicators
D. Sampling
E. Management of Unsuitable specimens
F. Test ordering
G. Patient identification
H. Paediatric/neonatal sampling
IN OUR PREVIOUS CONFERENCE WHAT WAS IDENTIFIED AS THE MOST CRUCIAL STEP TO HARMONISE
WHERE DOES STABILITY SIT

Impact

Cause

Effect
GARBAGE IN GARBAGE OUT

- Laboratory results can only be as good as the sample allows.
- Awareness of the impact of the preanalytical phase on sample quality has grown hugely over recent decades.
- Monitoring of this the preanalytical phase is growing.
- ISO 15189 is encouraging us to improve this area.
- Laboratory staff need to know which samples are of sufficient quality for analysis.
- To do this we need to know what a sample has been subjected to on its journey to the laboratory.
  - AND how long for.
DEFINITIONS

• VIM defines stability for an instrument as
  • ‘property of a measuring instrument whereby its metrological properties remain constant in time’

This can be measured in two ways
1. Stability limit = time interval over which a metrological property changes by a stated amount exceeding the MPD
2. Instability equation = change of a property over a stated time interval under given conditions
   \[ y = mx + c \]

MPD = Maximum permissible difference
WHERE DO YOU MOST COMMONLY GET YOUR STABILITY DATA WHEN SETTING UP A NEW ASSAY?

A. Use manufacturer kit insert
B. Search the literature
C. Use a database
D. Perform a study
E. All of the above
Currently we tend to state that an analyte is stable for a set period of time in a defined matrix

- We actually mean that instability cannot be demonstrated:
  1. In a defined matrix
  2. Under defined conditions
  3. For a defined time

- Specific guidance on how to perform stability studies is currently lacking
- Tends to be part of wider documents and lacking in specifics
CURRENT SITUATION(2)

- Stability databases currently exist in Spain, Norway and Germany.
  - Differ in their approach
- Many studies in existence
  - Why so many?
- Inability to transfer data between healthcare settings
- Contradicting data
- Old data
  - Now new tubes, equipment, reagents etc.
- Insufficient detail to determine suitability
BARRIERS (1)

- Stability is influenced by a multitude of factors making it very complex
- Many studies look only at stability once in serum/plasma
  - Most labs need stability in whole blood
- No universally accepted deviation from the true baseline
  - Can be arbitrary percentage
  - Can be based on biological variation
    - Acceptable quality goal $B_A = 0.25(CV_i^2 + CV_G^2)^{0.5}$
      - i.e. a quarter of the group biological variation

Fraser CG, Hyltoft Petersen P, Libeer JC, Ricos C. Proposals for setting generally applicable quality goals solely based on biology. Ann Clin Biochem 1997;34:8-12
BARRIERS (2) – THE VARIABLES

- Sample type
  - Whole blood, serum, plasma, fluid saliva, csf etc
- Temperature
  - Should be defined and specific
- Centrifuge conditions
  - RCF, time, temperature
- Tube type
  - Additive
  - Light exposure
- Biological factors
  - Interindividual variation
  - Mixing technique
  - Additive distribution and cell damage
- Sample volume
- Evaporation
- Laboratory instrumentation and reagents
WHAT IS YOUR STABILITY LIMIT FOR POTASSIUM IN WHOLE BLOOD

- 1-2hrs
- 2-4hrs
- 4-6hrs
- 6-8hrs
- 8-12hrs
- >12hrs
- Always allow
AIMS

- Aim is to develop a checklist against which future studies should be reported to ensure:
  - Standardisation
  - Transferability

- This follows a similar checklist project by the EFLMWG BV and STARD

- Checklist based on expert opinion

Future studies would then allow:
- Better transferability
- Development of a database
- Quick assessment of their suitability for your own laboratory setting
THE CHECKLIST
• Title must clearly indicate that it is a stability study
• It must define
  • Analytes
  • Matrix
  • Time period
• Keywords must be appropriate and include as a minimum
  • (In)Stability
  • Preanalytical
ABSTRACT

• Must indicate that it is a stability study
• Must clearly define the aims of the study
• Should provide an overview of the study design
• As a minimum should define
  • Analytes
  • Tube and matrix
  • Duration
• Headline results must be stated
INTRODUCTION/AIMS

- Should provide a thorough overview of the current situation
- Should define why stability is important
- Should state the knowledge gap being filled
  - Including details about why this important for the analytes involved
- The aims of the study must be defined
- It should also state how the current study will address these aims
METHODS(1) – ANALYTE/CONDITIONS

- The analyte(s) and subject population must be defined
- All parameters associated with the sample up to time point zero must be defined
  - Matrix, Tube type, Phlebotomy technique, Tube mixing, Time to zero, Storage conditions, Transport conditions, Temperature, Centrifuge Conditions, Time of Year etc
- Ideally the sample type and preanalytical conditions should be defined using SPREC (Standard Preanalytical Code)
- Any known additional biases should be stated

https://www.isber.org/page/SPREC
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METHODS(2) - SAMPLES

- As above analytes and conditions should be defined by SPREC coding
- It should state how the number of samples required to ensure an appropriate power of the study was calculated
  - This must allow for intra/inter-individual variability
- The number of replicates should be stated and why
- It should state whether mean or medians were used
- The length of the study should be defined
- Each analyte should be investigated for at least 2 levels covering the clinical range
METHODS(3) - ANALYTICAL PROCEDURE

• Method of analysis should be defined, including:
  • Analytical platform
  • Reagent used
  • Reaction details
  • Deviations from manufacturer recommendations

• Traceability of the method to standards should be defined
• Analytical variation should be minimised
• Within and between batch variation should be stated
METHODS(4) - PATIENT POPULATION

The patient population should be defined and as a minimum should include:

- Gender
- Age
- Geographical location
- Comorbidities
- Relevant medication
- Health status
- Ethnicity
STATISTICS

• The method of data analysis should be defined
• Each analyte should have its own estimate of instability
  • Statistical significance and confidence intervals should be fit for purpose
  • Rational for statistical tools should be stated
• Outlier testing should be performed and outliers removed
  • How the testing was done should be stated
• Spiking studies are not recommended but if necessary details and justification should be included.
ACCEPTABILITY CRITERIA

• The equation used to define the stability limit should be stated
• The level defined as the MPD should be stated alongside justification and evidence for its selection
  • This should follow the Milan criteria for evidence
    • Outcome data
    • Biological variation data
    • State of the art
RESULTS(1)

• Results should be presented clearly in a range of formats
  • Textually
  • Tabulated
  • Visually
• Results should be presented for at least 2 different values covering the clinical range
• An instability equation should be derived from the data in the form $y=mx+c$
  • This allows the inputting of a time to give a given instability or vice versa for a given population and conditions
RESULTS(2)

- Data should be presented in a consistent format using standardised units and terminology
- The format should allow easy and quick interpretation
- Raw data should be provided as a supplemental file
  - This allows others to apply the data to their needs
  - It potentially allows consolidation of data in a central location to improve the power of the data
DISCUSSION

- This must clearly summarise what the data shows
- It should discuss how it can be applied to other healthcare settings
  - If there are any blocks to transferability these should be stated
- Any limitations should be discussed
SUMMARY

- We have produced a guide to how stability studies should be reported
  - Focussing on the information that needs to be included
- This should allow greater transferability of studies between healthcare settings
  - Laboratories should be able to quickly identify suitable studies
- Sharing of well defined data opens the potential to combine studies and produce a larger more powerful dataset
  - Laboratories can then quickly identify/apply data to their own requirements
- Knowledge gaps can also quickly be identified