Procedure & Instrument Evaluation

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Introduction

- A key role of a laboratory is to decide what test should be offered and to select the best method.
- The test chosen should improve the quality of care to the patient.
- Prior to the procurement of a chosen method, the method or test must be evaluated.
- Following an evaluation prior to the implementation of a new test they should be validated.
- Validations should aim to show that the new method is as good as or an improvement on existing methods.
- Validation is an evidence-based assessment of how a test performs in the laboratory, and shows suitability for intended purpose.

Definitions

- **Evaluation is a generic term to describe the measurement of the performance capabilities of a system.**
- **Validation, examines the whole process that is being used to produce the correct result.**
- **Accuracy** – The closeness of agreement between the average values obtained from a large series of test results and an accepted reference value.
- **Reproducibility** – Conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment
- **Sensitivity** – is the number of true positives recognized by the assay.
- **Specificity** – is the number of true negatives recognized by the assay.
- **Reliability** – The ability of a system or component to perform its required functions under stated conditions for a specified period of time.

Procedure Evaluation

- Why do we need procedure evaluation?
  - Laboratory Requirement
  - Manufacturer Requirement
  - Medical Requirement

Laboratory Requirements (1)

- Problems with current method
  - ✓ In accuracy
  - ✓ In precision
  - ✓ In specificity
  - ✓ In sensitivity
  - ✓ Laborious
  - ✓ Costly
  - ✓ Time consuming

Laboratory Requirements (2)

- Automation

  - ✓ Change from manual to autoanalyzer Methods
  - ✓ Change of autoanalyzer

Laboratory Requirements (3)

- Measurement of a new analyte
- New kit introduced
- New method developed (Reported in Scientific Journals)

Manufacturer Requirements

- Manufacturer of kits, reagents, instruments are required to publish the analytical performance of their products due to;

  - ✓ Law Requirements
  - ✓ Competition
  - ✓ Defense ….etc.

Medical Requirements

- Physician require tests that are able to detect analytes at *decision level concentrations* (Xc) within certain *allowable error* (E_A).

- Decision level concentration (Xc): the concentration at which medical decision is made.

- Allowable error (E_A): the error that can be tolerated without affecting clinical judgment.

Allowable Error (E_A) According to Barnett (1968, 1977)

Analyte	Xc	E_A
Bicarbonate	20 mmol/l	2
	30 mmol/l	2
Chloride	90 mmol/l	4
	110 mmol/l	4
Glucose	3 mmol/l	0.5
	6.7 mmol/l	0.5
Na	130 mmol/l	4
	150 mmol/l	4
K	3 mmol/l	0.5
	6 mmol/l	0.5

Method Evaluation (1)

- Every lab must perform procedure evaluation whenever a new method is to replace an old one.

- Detection of errors in a procedure
  - ✓ Random Error (RE)
  - ✓ Systematic Error (SE)

Method Evaluation (2)

- ✓ RE →occurs by chance and fluctuates about the mean. Every procedure & instrument has some degree of RE due to various factors such as;
  - Pipetting
  - Temperature
  - Humidity
  - handling by different technologists
  - Method of operation
- ✓ RE → measure of the precision of the instrument. CV = sd/mean X100 (Replication experiments)

Method Evaluation (2)

- ✓ SE → should not occur in the procedure, it indicates the presence of gradual deterioration or permanent fault or interference. (Comparison of methods & linearity check experiments).

  - Constant (CSE): the magnitude of the error is independent of the conc. Of the analyte being measured (shift), (measured by Comparison of methods, interfence & linearity check experiments).

  - Proportional (PSE): the magnitude of the error is proportional to the conc. Of the analyte being measured, (measured by Comparison of methods, linearity check & recovery experiments).

Method Evaluation (3)

- Types of evaluations

1) Comprehensive

Applied by the lab when it is measuring a new analyte or developing a new method.

2) Limited

Applied by the lab when a new method is to replace an old one for the same analyte.

Procedure Evaluation

- Experiments used to determine type and size of errors (accuracy and precision)

1) Replication experiments
2) Comparison of method experiments
3) Linearity check experiments
4) Recovery experiments
5) Interference experiments

Replication Experiments (1)

- To detect RE.

  In this type of experiments, high, normal and low control samples are analyzed at least 20 times, to calculate the following for each parameter;

  - ✓ Mean (X)
  - ✓Standard Deviation (SD)
  - ✓Coefficient of Variation (CV)

  (RE = 1.96 SD)

Replication Experiments (2)

- Replication experiments can be carried out as;

a) Within Run
b) Run to Run (between runs)
c) Day-to-Day

a) Within Run

- Measurements are made within single run.

  - Detects least amount of RE
  - Underestimates real RE
  - For initial RE evaluation experiment

b) Run to Run (Between Runs)

- Measurements are made in different runs during the same day.

  - Within day precision
  - Does not give the realistic RE of the procedure

c) Day-to-day

- Measurements are made every day (different times) over a period of 20 –30 days

  - Provide the true assessment of RE

Replication Experiments (3)

- **To accept the performance of the procedure or instrument in any Replication Experiments (i.e. Within Run, Run to Run and/or Day-to-Day replication experiments), RE should be less than E_A (i.e. 1.96 SD < E_A).**

- **Within Run and Run to Run replication experiments are optimistic evaluations of the method's precision but are useful in determining if the method is performing properly. The Day-to-Day replication experiment is a better indication of the method's overall precision.**

Comparison of Methods Experiments (1)

- The analytical performance of the instrument or procedure is compared to a *Reference* procedure or instrument or a comparative instrument e.g. a new instrument is compared to the current instrument.

- At least 20 times low, normal and high patient samples are tested by both procedures or instruments, then the results are plotted against each other (comparative or current on X axis and new one on the Y axis)

Comparison of Methods Experiments (2)

- Linear regression analysis used to find out the correlation coefficient (r), the intercept (a), and the slope (b).

$$CSE = bias = \bar{X} - \bar{Y}$$

$$SE = (a + b\,Xc) - Xc$$

Where,

X= mean of determinations by the comparative procedure or instrument (Reference = Gold Std. or the current procedure)

Y= mean of determinations by the New procedure or instrument

a= intercept b= slope Xc= Critical decision level

Comparison of Methods Experiments (3)

- SE due to either or both methods *Except* if the comparative method is the reference method then SE is due to the new method.

- For the procedure or instrument performance to be accepted CSE and SE must be less than E_A.

Linearity Check Experiments (1)

- To measure PSE and CSE.
- To confirm the procedure or instrument is linear over its published working range.
- Method:-

  - A known control or standard of high conc. is diluted to obtain a series of sample of lesser but known conc.

  - Analyze the sample and compare the results to the expected values by constructing a scatter plot

N.B: **- For dilution use volumetric pipetting from the stock.**
- Do not carry out serial dilution.
- Analyze each sample in triplicate and average the results.

Linearity Check Experiments (2)

- Plot observed conc. Vs dilution and check visually for the analytical limit of the assay.

- Compare the calculated value (after correction for dilution) with the expected value.

- Find out PSE and CSE →for each dilution.

✓ CSE = $\bar{n}$ - $\bar{y}$

$\bar{n}$ = mean for undiluted sample

$\bar{y}$ = mean for diluted sample

Linearity Check Experiments (3)

- ✓ For PSE find % Recovery then use the PSE formula;

  PSE = [(% Recovery –100)/100] Xc

- ❑ Interpretation;

- If PSE < E_A and CSE< E_A → Accept procedure

- If PSE > E_A and CSE> E_A → procedure not accepted

Recovery Experiments (1)

- To evaluate a test method's proportional systematic error in the absence of a reliable comparative or reference method.

- Method:-

  - A patient sample is analyzed for the analyte in question and a baseline value is determined.

  - A small, carefully measured (not more than 10% of the sample volume) amount of pure standard is added to the sample and it is reananalyzed.

Recovery Experiments (2)

- % Recovery = Conc. Recovered / Conc. Added x 100

- PSE = [(% Recovery –100)/100] Xc

- **Conc. Added = Std. Conc. X vol. Std / vol. Std + vol. Sample**

- **Conc. Recovered = Conc. diluted test – Conc. baseline**

Interference experiments (1)

- To measure CSE
- An interfering substance can be either;
  - ✓ React with the same reagents (competition)
  - ✓ Alter the reaction between the analyte and the reagents (inhibition).

- Two types of interfering substances;
  - ✓Endogenes (within specimen) such as; haemolysis, lipaemia, creatinine…etc.
  - ✓Exogenes (from outside the specimen) such as; drugs, diet, drinks, temp., humidity…etc.

Interference experiments (2)

- Method:-

  - A vol. (not exceeding 10% of the sample vol.) of known conc. Of the interfering material (maximum conc.) in a diluent is added to the patient sample e.g. 0.1 ml to 1 ml of patient sample

  - A base-line sample (same patient specimen) is used by adding same vol. Of diluent only (H_2O) to same vol. of sample i.e. o.1 ml H_2O to 1 ml of patient sample.

Interference experiments (3)

- Method cont. :-

  -Both samples (test & base-line) are analyzed by the test procedure in triplicates

  - Interference = test Conc. – base-line Conc.

  smallest Conc. That causes interference is studied.

Interference experiments (4)

- Effect of haemolysis, lipaemia….etc. must also be studied.

  - A whole blood sample is divided into two aliquots.

    - **One (base-line) centrifuged and the resulting plasma (serum) is analyzed.**
    - **The second, the RBCs are ruptured physically (using a swab stick) to obtain elevated amount of serum haemoglobin then the haemolyzed sample is centrifuge and analyzed.**

      - Difference is attributed to haemolysis

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