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**15/MHS06/057**

**MLS516 ASSIGNMENT**

**ANSWER**

 **NUMBER ONE**

Methods used in the laboratory for any analysis, must be evaluated and tested to ensure that they produce valid results suitable for their intended purpose, i.e. they must be validated.

The validation or verification of a method follows a standardized set of experimental tests which produce data relating to accuracy, precision etc. The validation practice demonstrates that an analytical method measures the correct substance, in the correct amount, and in the appropriate range for the intended samples. It allows the analyst to understand the behavior of the method and to establish the performance limits of the method. The process by which this is done should be written down as a standard operating procedure (SOP). Assay validation provides an assurance of reliability during normal use, and is sometime referred to as "the process of providing documented evidence that the method does what it is intended to do’’, that is, analyse the sample for glucose accurately by getting similar or very close result to the known old methods; for instance, estimation of glucose, using glucose-peroxidase method.

In order to determine the suitability of a new method of glucose estimation which is being promoted by Yeenx Inc, the following must be considered;

**Assay Optimization (pre-validation):** Assay optimization and pre-validation are experiments that determine how a range of matrix and sample elements, as well as assay conditions, effect assay parameters and assay performance. These data, along with scientific judgment, set the acceptance criteria for the assay validation. It is important to establish acceptance criteria before executing the validation protocol.

**Assay Qualification:** Assay qualification is an experimental protocol that demonstrates that an accepted method will provide meaningful data for the specific conditions, matrix and samples that the procedure is intended for. Assay Qualification may not require validation of accuracy and reliability of the method (sensitivity), but merely verify the suitability of the protocol under actual conditions (generally, specificity).

**Assay Validation:** Comprehensive experiments that evaluate and document the quantitative performance of an assay, including sensitivity, specificity, accuracy, precision, detection limit, range and limits of quantitation. Full Assay Validation will include inter-assay and inter-laboratory assessment of assay repeatability and robustness.

The steps for validation of this new method are;

* **Specificity**; is the ability to assess unequivocally the target analyte (glucose) in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc. This definition has the following implications:

IDENTIFICATION: To ensure the identity of the target analyte.

ASSAY (Content or Potency): To provide an exact result which allows an accurate statement on the content or potency of the target analyte in a sample.

* **Accuracy**; The closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value, and the value found.

NOTE: When measuring accuracy, it is important to spike control preparations with varying concencentration value. If the control cannot be obtained, then a sample should be spiked at varying levels. In both cases, acceptable recovery must be demonstrated.

* **Precision**; The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the homogeneous sample under the prescribed conditions.

Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Intermediate Precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

Reproducibility expresses the precision between laboratories (collaborative studies usually applied to standardization of methodology).

Precision should be investigated using homogeneous, authentic (full scale) samples. However, if it is not possible to obtain a full-scale sample it may be investigated using a pilot-scale or bench-top scale sample or sample solution.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

* **Detection Limit;** The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.
* **Quantitation Limit;** The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.
* **Linearity**; The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

NOTE: Measurements using clean standard preparations should be performed to demonstrate detector linearity, while method linearity should be determined concurrently during the accuracy study.

* **Range;** The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.
* **Robustness;** The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters and provides an indication of its reliability during normal usage.

NOTE: Ideally, robustness should be explored during the development of the assay method. One might investigate: sample sonication or mixing time; mobile phase organic solvent constituency; mobile phase pH; column temperature; flow rate; modifier concentration; etc. It is through this sort of a development study that variables with the greatest effects on results may be determined in a minimal number of experiments. The actual method validation will ensure that the final, chosen ranges are robust.

* **System Suitability**; In addition, prior to the start of laboratory studies to demonstrate method validity, some type of system suitability must be done to demonstrate that the analytical system is performing properly. Examples include: standardization of a volumetric solution followed by assays using the same burette for titrimetric methods, etc.

When the method in question utilizes an automated system such as a chromatograph or an atomic absorption spectrophotometer, a suitable standard preparation should be intermittently measured during the sample analysis run. The responses generated by the standard should exhibit a reasonable relative standard deviation.

This is done primarily to demonstrate the stability of the system during sample measurements.

* **Protocols**; Prior to initiating a validation study, a well-planned validation protocol should be written and reviewed for scientific soundness and completeness by qualified individuals. The protocol should describe the procedure in detail, and should include pre-defined acceptance criteria and pre-defined statistical methods. Following approval by the appropriate Quality Control authorities, the protocol should be executed in a timely manner.

A typical assay validation will require the preparation of product placebo(s), standards, and many samples.

The assay should be repeated to ensure "validity"; 3 sequential replicates are often considered the "magic number," however a far more definitive number is one produced by a sound scientific rationale, usually with the assistance of statistical analyses. Subsequent to the execution of the protocol, the data must be analyzed with results, conclusions and deviations presented in an official validation summary report. Provided the pre-defined acceptance criteria are met, and the deviations (if any) do not affect the scientific interpretation of the data, the method can be considered valid. A statement of the method's validity should be placed at the beginning of the final summary report, along with the signatures and titles of all significant participants and reviewers.

 **NUMBER TWO**

It is important to note that, sources of error, can occur in any of these analytical phase due to;

* Preanalytical phase; this includes the biological variation, the test ordered (relationship/correlation of test ordered with diagnosis), patient preparation (if the patient was properly prepared for the test, for instance for Fasting blood sugar test, it is important to know if the patient is aware that he/she is not supposed to eat before the sample is being collected), sample collection (it is important to know if the sample was taken into the right sample container), transportation of samples also is considered (it is important to know, if the sample was properly preserved, or for samples that should be analysed immediately, are in the right condition; for example; Bilirubin sample), etc
* Analytical phase; sample identification (there is a possibility of running several samples at the same time, so, proper labelling and identification of samples are important), calibration is essential too, measurement of samples and reagent (Pipetting method), the type of reagent used (to know if it is the right one), the state of the tubes used, wavelength selection, use of control samples and control materials (SOP), etc
* Post analytical phase; Quality control use, interpretation of results in full clinical context, clinical response to result.

Steps to consider whether an analytical run has been properly performed, includes;

1. Use of control

Quality control materials should have the following characteristics.They should have the same matrix as patient specimens, including viscosity, turbidity, composition, and color.For example, a method that assays serum samples should be controlled with human serum based controls. Quality control material should be simple to use because complicated reconstitution procedures increase the chance of error.

If an equivalent control is included in the method, it should be checked that it was performed as specified.

* Check if the batch or sequence was done using a Quality Control sample.
* Meet system suitability requirements; System suitability is intended to determine whether the ‘system’ including instruments, analysts, etc. is capable of performing a particular process, test, or assay.
* Analyze a blank, i.e. reagent, matrix to assess contamination from the laboratory environment and demonstrate low system background. The blank must be less than the determined MDL.
* Analyze matrix spike and/or reagent spike (match matrix and analyte) to assess and demonstrate accuracy.
* The accuracy expressed as percent recovery must be 80-120% unless otherwise specified, i.e. by in-house statistical analysis.
* Analyze duplicate matrix/reagent spike or sample duplicate to assess and demonstrate precision.
* Analyse calibration, check sample/standard
1. Match the result with provisional diagnosis

It is important to take note of the correlation of the result, with the suspected diagnosis, by the clinician; this basically means, the result obtained, will be investigated, to see if it relates with the diagnosis provided on the request form.

1. Match the result with other related analytes.

It is also important to compare the result obtained, with other the analytes. For instance, a patient whose urea result is abnormal, is most likely to have an abnormal creatinine level also, and this is due to the relationship between the effect of urea and creatinine, particularly known to be products of metabolism, also the glomerular filtration rate can also be checked to confirm renal function. This will help to determine that the analytical run has been properly done. This is mostly effective in liver function test; results are matched with other related analyte.

 NUMBER THREE

Coronavirus RNA synthesis occurs in the cytoplasm via a negative-strand RNA intermediate. The virion RNA is infectious and functions as an mRNA, having a 5’ terminal cap followed by a leader sequence and an untranslated region. At the 3’ end of the genome, there is an untranslated region followed by a poly (A) tail. Coronaviruses have a polycistronic genome organization and synthesize multiple sub genomic mRNAs, all overlapping at the 3’ end (nested set of sub genomic RNAs) and all containing the same 5’ leader sequence derived from the 5’ end of the genome. Each mRNA is translated to generate the protein product of its most 5’ gene, but some-times is translated into a second, downstream protein as well. Coronaviruses replicate by a unique discontinous transcription mechanism that is not completely understood. Discontinuous transcription of sub genomic mRNAs is believed to be regulated by transcription regulating sequences (TRSs, also referred to as intergenic sequence) at the 5’end of each transcriptional unit. The current model is that discontinuous transcription occurs during the synthesis of sub genomic negative-sense RNAs; this model is supported by data that demonstrate the existence of transcriptionally active, sub genomic-size negative RNA strands containing the anti-leader sequence.

Several studies have also reported evidence of COVID-19-associated clotting disorders, and post-mortem studies have indicated pathological changes to the lung microvasculature, including microthrombi and hemorrhagic necrosis. “Although the pathophysiology underlying severe COVID-19 remains poorly understood, accumulating data suggest that a lung-centric coagulopathy may plan an important role,” the team noted. “Moreover, emerging data suggest that severe COVID-19 is also associated with a significant risk for developing deep vein thrombosis and pulmonary embolism.”

Most published data on COVID-19-related coagulopathy have been derived from studies on Chinese patients, but race and ethnicity have major effects on thrombotic risk, the investigators continued. Epidemiological studies have found that the incidence of venous thromboembolism (VTE) is 3–4 fold lower in Chinese patients than it is in Caucasians, and is significantly higher in African-Americans than it is in Caucasians.

Blood clots are a serious condition: Untreated, they can cause damage to your brain, heart and lungs. Death or long-term complications are a real concern.Covid-19 patients are riddled with blood clots in the smallest vessels of the body. Lungs seem to be especially hard-hit. Their clots appear to have cut off blood flow to the small air sacs where blood cells would be exchanging oxygen and carbon dioxide.

“There’s no ability for the blood to flow through and exchange oxygen like it should.

The findings suggest early testing for D-dimer, a protein fragment in the blood associated with increased blood clotting (thrombosis) in COVID-19 patients, could enable clinicians to prescribe specific treatments, including anticoagulants ("blood thinners"), at a much earlier stage, which might reduce the number of people subsequently having further strokes or blood clots elsewhere in the body.

Use of personal protective equipment is important

All specimens collected for laboratory investigations should be regarded as potentially infectious.

FACE AND EYES PROTECTION

This may be fluid resistant shields, face mask, face shields. Selecting the most suitable eye and face protection should take into consideration the following elements:

• Ability to protect against specific workplace hazards

• Should fit properly and be reasonably comfortable to wear

• Should provide unrestricted vision and movement

• Should be durable and cleanable

• Should allow unrestricted functioning of any other required PPE

LABCOATS

Wear protective clothing that resists physical and chemical hazards when exposure may occur. Lab coats are appropriate for minor chemical splashes and solids contamination, while plastic or rubber aprons are best for protection from corrosive or irritating liquids. Disposable outer garments may be useful when cleaning and decontamination of reusable clothing is difficult.

GLOVES

Wear gloves protect against skin absorption of chemicals, chemical burns, thermal burns, lacerations, and cryogenic liquid exposure. Choosing the appropriate hand protection can be a challenge in a laboratory setting.

Wear gloves when handling hazardous materials, chemicals of unknown toxicity, corrosive materials, rough or sharp-edged objects, and very hot or very cold materials. Disposable nitrile or neoprene gloves are usually appropriate as protection from incidental splashes or contact with lab chemicals. However, the SDS should be consulted to verify chemical compatibility with the gloves being used.

FOOT PROTECTION

Wear closed-toe shoes at all times in buildings where chemicals are stored or used. Do not wear perforated shoes, sandals or cloth sneakers in laboratories or where mechanical work is conducted. These shoes offer no barrier between you and chemical and physical hazards.

Chemical resistant overshoes or boots may be used to avoid possible exposure to corrosive chemical or large quantities of solvents or water that might penetrate normal footwear (e.g., during spill cleanup). Leather shoes tend to absorb chemicals and may have to be discarded if contaminated with a hazardous material.

RESPIRATORY PROTECTION

Inhalation is one of the principal routes by which harmful materials can enter the body. If an individual is exposed to a hazardous airborne concentration of such a material, then undesirable health effects can result. So, NIOSH-approved N95 filtering face piece respirator can be used.

Investigation can be done by performing the following analysis for diagnosis for different organs;

* Liver: the test includes albumin test, aspartate amino transferase (AST), alanine amino transferase (ALT), gamma – glutamyl transferase (GGT), N-nucleotidase, lactate dehydrogenase (LD),bilirubin, etc.
* Kidney: Electrolytes test, urea test, creatinine test, glomerular filtration rate (eGFR).
* Heart: high density lipid, low density lipid, cholesterol, triglycerides, Troponin, interlukin-6, etc.
* Lungs; **Arterial blood gas tests, Pulse oximetry, Spirometry**
* Other tests includes; urinalysis (for protein), proclacitonin,, ferritin, D-dimer using ELISA method, fibrin degradation fragment test, Nucleic acid amplication test Rt-PCR,etc