**Question 1**

The introduction of a new method is a common occurrence in the clinical laboratory. Method selection and evaluation are key steps in the process of implementing new methods. A new method must be selected carefully and its performance evaluated thoroughly in the laboratory before being adopted for routine use.

Evaluation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications.Method validation provides an assurance of reliability during normal use, and is sometime referred to as “the process for providing documented evidence that the method does what it is intended to do.” The main objective of the validation is to demonstrate that the analytical method is suitable for its intended purpose, is accurate, specific and precise over the specified range that an analyte will be analyzed. Analytical Method Validation is to be performed for new analysis methods or for current methods when any changes are made to the procedure, composition of the drug product and synthesis of the drugs substances.

1. **Trueness and Accuracy**

The accuracy of an analytical method is the closeness of the test results obtained by that method to the true value.This is sometimes termed trueness. It is recommended that accuracy should be determined using a minimum of nine determinations over a minimum of the three concentration levels, covering the specified range (3 concentrations/3 replicates each of total analytical procedures).[4]

It is measured as the percent of analyte recovered by assay. The recovery can be determined by the equation:

Recovery = Analytical Result x 100%
  True Value

The recovery should be in the range of Control limit.

1. **Precision**

The precision of an analytical method is the degree of agreement among individual test results when the method is repeated to multiple samplings of a homogeneous sample.[6] The precision of an analytical procedure is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements. It is indicated by Relative Standard Deviation, RSD, which is determined by the equation:



Where xi is an individual measurement in a set of n measurement and  is the arithmetic mean of the set. Generally, the RSD should not be more than 2%.

Precision is specified as follows:

* Repeatability: closeness of agreement between results of successive mesurements carried out under the same conditions (i.e., corresponding to within-run precision).
* Reproducibility: closeness of agreement between results of measurements performed under changed conditions of measurements e.g., time, operators, calibrators and reagent lots.
* Intermediate precision: Intermediate precision is the results from within lab variations due to random events such as different days, different analysts, different equipment, etc. The standard deviation, relative standard deviation (coefficient of variation) and confidence interval should be reported for each type of precision investigated.

1. **Specificity**

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix such as impurities, degradation products and matrix components. It must be demonstrated that the analytical method is unaffected by the presence of potentially interfering substances or factors in the simple matrix (e.g., hyperlipemia, hemolysis, bilirubin, anticoagulants, antibodies, and degradation products).

1. **Sensitivity**

Analytical sensitivity is the ability af an analytical method to assess small variations of the concentration of the analyte. This is often expressed as the slope of a calibration curve. Analytical sensitivity depends on the ratio between standard deviation of the calibration function and the slope.

1. **Linearity**

This refers to the relationship between measured and expected values over the analytical measurement range. Linearity is the ability of the method to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to analyte concentration within a given range.It should be established initially by visual examination of a plot of signals as a function of analyte concentration of content. If there appears to be a linear relationship, test results should be established by appropriate statistical methods. Data from the regression line provide mathematical estimates of the degree of linearity. The correlation coefficient, y-intercept, and the slope of the regression line should be submitted.

It is recommended to have a minimum of five concentration levels, along with certain minimum specified ranges. For assay, the minimum specified range is from 80% -120% of the target concentration.

 Regression line, y = ax + b

Where, a is the slope of regression line and b is the y- intercept.

Here, x may represent analyte concentration and y may represent the signal response.

**Question 2**

To determine whether an analytical run has been properly performed or not in laboratory testing, we make use of “control”. A control material is a device, solution, lyophilized preparation, or pool of collected human or animal specimen, or artificially derived material, intended for use in the quality control process. In the routine operation of clinical laboratories worldwide, the performance of analytical methods is routinely monitored by analyzing specimens whose concentration are known followed by comparing the observed values with the known values.

**Control charts**

**Levey-Jennings control chart**

1. Analyze samples of the control material by the analytical method to be controlled on at least twenty different days. The mean and standard deviation is calculated.
2. A control chat is constructed on graph paper or manually.
3. The control specimen are introduced into each analytical run, the values are recorded and plotted on the control chart.
4. When the control values fall within the control limits, the run is interpreted as being “in control” and the patient result is reported. When a single control value exceeds the control limit, the method is stopped. The method is then inspected to determine the cause of the error. The problem is resolved and the entire run is repeated for both the specimen and the control samp

**Question 3**

SARS-CoV-2 is an enveloped, positive-sense RNA virus, and belongs to the β-coronavirus genus (sarbecovirus subgenus, orthonavirinae subfamily).It represents the 7th member of the Coronaviridae family known to infect humans. Its counterparts include 4 strains of low pathogenicity (229E, OC43, NL63 and HKU1), as well as 2 other β-coronaviruses, which caused the previous outbreaks of severe and potentially fatal respiratory tract infections – SARS-CoV and MERS-CoV

**Respiratory involvement**

The most frequent, serious manifestation of COVID-19 infection seems to be pneumonia, which is characterized by cough, fever, dyspnoea and bilateral infiltrates displayed on radiographic chest imaging. Unfortunately, there are no specific clinical features that discern COVID-19 from other viral respiratory illnesses.

* **Arterial blood gas tests** to measure levels of oxygen, carbon dioxide, pH, and bicarbonate

**Cardiovascular involvement**

Patients with existing cardiovascular disease (CVD) are at a greater risk of suffering from severe COVID-19 and having poorer prognosis. A meta-analysis comprising of 46,248 patients with confirmed COVID-19 found that the most common co-morbidities were hypertension (17%), diabetes (8%) and CVD (5%). It is widely agreed that COVID-19 can also have adverse effects on cardiovascular health itself, causing or aggravating damage to the heart. There are reports of cardiogenic involvement in patients without known CVD as well as cases with solely cardiac presentations. Surge in cytokine levels due to hyperinflammatory response or secondary hemophagocytic lymphohistiocytosis and increased myocardial demand in the setting of acute infection can lead to atherosclerotic plaque instability and myocardial injury, increasing the risk of acute myocardial infarction. Blood pressure abnormalities can also be seen in response to the illness.Additionally, palpitations due to arrhythmia have been observed.

The following markers are assessed to estimated for myocardial infarction;

* Creatinine kinase isoenzymes and isoforms- CK-MB
* Cardiac troponin
* Myoglobin
* Lactate dehydrogenase
* Brain natruiretic peptide

**Renal involvement**

Acute Kidney Injury (AKI) is the abrupt loss of kidney function that develops within 7 days, its incidence has been observed with SARS and MERS-CoV. AKI in COVID-19 accompanies sepsis, multi-organ failure and shock, suggesting the cause of AKI to be Acute Tubular Necrosis (ATN). To assess the status of the kidney in covid-19 case, the tests include:

* Serum creatinine
* Urinalysis- proteinuria, glucose, ketones, bilirubin
* Blood urea nitrogen
* Glomerular filtration rate- the best overall indicator of the glomerular function is the glomerular filtration rate (GFR). The normal GFR for an adult male is 90 to 120 mL per minute. GFR is the rate in milliliters per minutes at which substances in plasma are filtered through the glomerulus, in other words, the clearance of a substance from the blood. Example is creatinine clearance.

**Gastrointestinal involvement**

A significant number of patients reported gastrointestinal (GI) symptoms such as diarrhea, nausea, vomiting and abdominal pain, with some reporting these symptoms as their sole presenting complaint.The incidence of GI symptoms, alongside the detection of SARS-CoV-2 RNA in stool samples of infected patients,suggest that ACE2 receptors highly expressed in the GI tract are another target for SARS-CoV-2 infection.

**Liver injury**

Mild and transient liver injury, as well as severe liver damage can occur in COVID-19.it is indicated that 14.8-53.1% of COVID-19 patients has abnormal levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin during the course of the disease, with bilirubin showing the smallest elevation. Furthermore, it was reported that severity of liver damage is proportional to that of COVID-19.Gamma-glutamyl transferase (GGT) was elevated in 54% of patients in a study. Assessment of the liver involves the estimation of the following:

* Alanine transaminase (ALT)
* Aspartate aminotransferase (AST)
* Alkaline phosphatase (ALP)
* Albumin
* Bilirubin

 **Immune system involvement**

The immune response is undeniably one of the key determiners of the susceptibility and severity of the disease. While weakened immune system can increase the risk of severe COVID-19, hyperinflammatory response to the infection can be responsible for the commonly seen complications by causing organ damage.

* Serum electrophoresis is done to give a quantitative overview of the electrophoretic groups of serum proteins