2.54 mmol/L to 2.62 mmol/L (2 × SD) is within the limits of expected analytical variation, whereas an increase from 2.54 to 2.70 (4 × SD) is not. However, to decide whether an analytical change is **clinically significant** it is necessary to consider the extent of natural **biological variation**. The effects of analytical and biological variation can be assessed by calculating the overall standard deviation of the test, given by:

$$SD = \sqrt{SD_A^2 + SD_B^2}$$

in the above equation, SD_A and SD_B are the SDs for the analytical and biological variation respectively. If the difference between two test results exceeds 2.8 times the SD of the test, the difference can be regarded as of potential clinical significance—the probability of this difference being a result of analytical and biological variation is <0.05. It should be appreciated, however, that setting the level of significance at a probability of <0.05 is arbitrary (albeit conventional). It does not mean that a difference of less than that equating to this probability cannot be of significance, nor that a greater difference necessarily is significant. If undertaking a major intervention depends on a result, it may be desirable only to make this decision if the probability that the change is not the result of innate variation is considerably greater.

Is this Change Clinically Significant?

If the result is consistent with clinical findings, it is evidence in favor of the clinical diagnosis. If it is not consistent, the explanation must be sought. There may have been a mistake in the collection, labelling or analysis of the sample, or in the reporting of the result. In practice, it may be simplest to request a further sample and to repeat the test. If the result is confirmed, the utility of the test in the clinical context should be considered and the clinical diagnosis itself may have to be reviewed.

The Clinical Utility of Laboratory Investigations

In using the result of a test, it is important to know how reliable the test is and how suitable it is for its intended purpose. Thus, the laboratory personnel must ensure, as far as is practicable, that the data are accurate and precise, and the clinician should appreciate how useful the test is in the context in which it is used. Various properties of a test can be calculated to provide this information.

Specificity and Sensitivity

The specificity is an ability of a measurement to correctly identify those who do not have the condition in question. The word 'specificity' refers to how narrowly a test is targeted; does it only identify people with that particular type of disease (is it specific to that condition?), i.e. 'true negative' (TN). Sensitivity is the ability of a measurement or screening test to identify those who have a condition, i.e. 'true positive' (TP). A specificity of 90% implies that 10% of disease-free people would be classified as having the disease on the basis of the test result; they would have a 'false positive' (FP) result. A sensitivity of 90% implies that only 90% of people known to have the disease would be diagnosed as having it on the basis of that test alone; 10% would be 'false negatives' (FN).

Specificity and sensitivity are calculated as follows:

$$\text{Specificity} = \frac{\text{TN}}{\text{All without disease [FP + TN]}} \times 100$$

$$\text{Sensitivity} = \frac{\text{TP}}{\text{All with disease [TP + FN]}} \times 100$$

An ideal diagnostic test would be 100% sensitive, giving positive results in all subjects with a particular disease, and also 100% specific, giving negative results in all subjects free of the disease. Because the ranges of results in quantitative tests that can occur in health and in disease almost always show some overlap, individual tests do not achieve such high standards. Factors that increase the specificity of a test tend to decrease the sensitivity, and vice versa. To take an extreme example, if it were decided to diagnose hyperthyroidism only if the plasma free thyroxine concentration were at least 32 pmol/L (the upper limit of the reference range is 26 pmol/L), the test would have effectively 100% specificity; positive results (>32 pmol/L) would only be seen in thyrotoxicosis (an exception is a very rare condition in which patients are resistant to thyroid hormones). On the other hand, the test would have a low sensitivity in that many patients with mild hyperthyroidism would be misdiagnosed. If a concentration of 20 pmol/L were used, the test would be very sensitive (all those with hyperthyroidism would be correctly assigned) but have low specificity, because many normal people would also be diagnosed as having the condition. These concepts are illustrated in Fig. 1.4.

Whether it is desirable to maximize specificity or sensitivity depends on the nature of the condition that the test is used to diagnose and the consequences of making an incorrect diagnosis. For example, sensitivity is paramount in a screening test for a harmful condition, but the inevitable false positive results mean that all positive results will have to be investigated further. However, in selecting patients for a trial of a new treatment, a highly specific test is more appropriate to ensure that the treatment is being given only to patients who have a particular condition. In some cases, this decision may not be straightforward. For example, in the

The critical values notifications should be made by one of the team members involved in performing the procedure using a call center for critical value notifications. Several hospitals have implemented the use of automated notification systems for critical value reporting by transmitting the critical values from the LIS to a hospital clinical information system trigger the generation of text messages directed to the responsible clinician's mobile phone and computer. If the clinician does not confirm receipt in the clinical information system within 60 minutes, results are communicated by telephone. This approach improved the speed of communication and another automated paging system was developed for critical value notification. In that program, critical values transmitted from the LIS, generate a page containing the patient name, medical record number, collection time, critical result, and reference range. The clinician must confirm receipt of the critical value by dialing a phone number listed in the message. If the clinician does not respond within 10 minutes (or rejects the notification), the call is escalated to a trained group of operators which proceed with telephone notification. Implementation of that system increased documentation of critical value receipt by physicians and decreased the median time for notification.

It should be emphasized that automated solutions should allow for an *escalation policy* to ensure communication of critical results, when clinicians do not acknowledge receipt.

Laboratory contact information should also be available so that clinicians with additional questions can ask a laboratory professional or medical director as appropriate. Patient privacy requirements should also be considered with automation.

To whom should critical values be reported?

Notification should be done to a physician or the individual or entity requesting the test and the individual responsible for using the test results.

Types of Specimens

Types of biological specimens that are analyzed in clinical laboratories include (1) whole blood, (2) serum, (3) plasma, (4) urine, (5) feces, (6) saliva, (7) spinal, synovial, amniotic, pleural, pericardial, and ascitic fluids, and (8) various types of solid tissues.

Plasma and Serum

Plasma, the liquid component of blood, comprises 55% of the total blood volume. It can separate by artificially spinning or centrifuging the blood at high rotations of 3000 rpm or higher (Fig. 1.6). The blood cells and platelets that make up about 45% of the blood are separated by centrifugal forces to the bottom of a specimen tube, leaving the plasma as the upper layer. Plasma consists of 90% water along with various substances required for maintaining the body's pH, osmotic load, and for protecting the body. The plasma also contains the coagulation factors and antibodies.

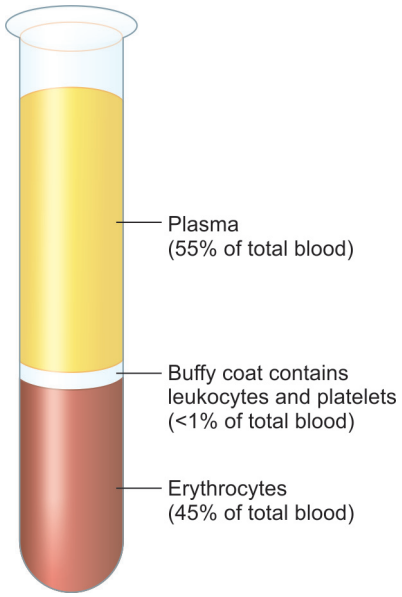

Fig. 1.6: Blood sample after centrifugation—the liquid components of blood called plasma (yellow section), can be separated from the erythrocytes (red section) and platelets (white section) by using a centrifuging or spinning the blood

Serum, the plasma component of blood which lacks coagulation factors, is similar to interstitial fluid in which the correct composition of key ions acting as electrolytes is essential for normal functioning of muscles and nerves. Other components in the serum include proteins, which assist with maintaining pH and osmotic balance while giving viscosity to the blood; antibodies, or specialized proteins that are important for defense against viruses and bacteria; lipids, including cholesterol, which are transported in the serum; and various other substances including nutrients, hormones, metabolic waste, and external substances, such as drugs, viruses, and bacteria.

Human serum albumin, the most abundant protein in human blood plasma, is synthesized in the liver. Albumin, which constitutes about one-half of the blood serum protein, transports hormones and fatty acids, buffers pH, and maintains osmotic pressures. Immunoglobulin, a protein antibody produced in the mucosal lining, plays an important role in antibody-mediated immunity.

Handling of Specimens for Analysis

Steps that are important for obtaining a valid specimen for analysis include (1) identification, (2) preservation, (3) separation and storage, and (4) transport.

Table 2.1 Body buffer systems

Site	Buffer system	Comment
ISF	Bicarbonate	For metabolic acids
	Phosphate	Not important because concentration too low
	Protein	Not important because concentration too low
Blood	Bicarbonate	Important for metabolic acids
	Hemoglobin	Important for carbon dioxide
	Plasma protein	Minor buffer
	Phosphate	Concentration too low
ICF	Proteins	Important buffer
	Phosphates	Important buffer
Urine	Phosphate	Responsible for most of 'titratable acidity'
	Ammonia	Important—formation of NH_4^+
Bone	Calcium carbonate	In prolonged metabolic acidosis

ICF, intracellular fluid; ISF, interstitial fluid

important buffer is hemoglobin, which contributes to buffering of hydrogen ion generated from the carbonic anhydrase reaction. Hydrogen ion is neutralized by intracellular buffers, mainly proteins and phosphates.

The bicarbonate buffering system is especially a key, as carbon dioxide (CO_2) can be shifted through carbonic acid (H_2CO_3) to hydrogen ions (H^+) and bicarbonate ions (HCO_3^-):

$$H_2O + CO_2 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

Acid-base imbalances that overcome the buffer system can be compensated in the short term by changing the rate of ventilation. This alters the concentration of carbon dioxide in the blood, shifting the above reaction according to Le Chatelier's principle, which in turn alters the pH.

The kidneys are slower to compensate, but renal physiology has several powerful mechanisms to control pH by the excretion of excess acid or base. In response to acidosis, tubular cells reabsorb more bicarbonate from the tubular fluid, collecting duct cells secrete more hydrogen and generate more bicarbonate, and ammoniagenesis leads to increased formation of the NH_3 buffer. In response to alkalosis, the kidneys may excrete more bicarbonate by decreasing hydrogen ion secretion from the tubular epithelial cells, and lowering rates of glutamine metabolism and ammonium excretion.

ACID-BASE HOMEOSTASIS

Acid-base homeostasis is one of the homeostatic mechanisms required to maintain health. It refers to the equilibrium between acids and bases; it is also referred to as body pH. An acid is a substance capable of giving up a hydrogen ion during a chemical exchange, and a base is a substance that can accept it. The positively charged hydrogen ion (H^+) is the active constituent of all acids.

Most of the body's metabolic processes produce acids as their end products, but a somewhat alkaline body fluid is required as a medium for vital cellular activities. Therefore, chemical exchanges of hydrogen ions must take place continuously in order to maintain a state of equilibrium. An optimal pH (hydrogen ion concentration) between 7.35 and 7.45 must be maintained; otherwise, the enzyme systems and other biochemical and metabolic activities will not function normally.

The body's response to a change in acid-base homeostasis has three components (Fig. 2.2):

1. **The chemical buffer systems (immediate response):** Buffering is a rapid physicochemical phenomenon. The body has a large buffer capacity. The buffering of fixed acids by bicarbonate changes the $[HCO_3^-]$ numerator in the ratio (in the Henderson-Hasselbalch equation).

   $pH = pK'a + \log_{10} ([HCO_3^-]/0.03 \times pCO_2)$

2. **The respiratory regulation (alteration in ventilation):** Adjustment of the denominator pCO_2 (in the Henderson-Hasselbalch equation) by alterations in ventilation is relatively rapid (minutes to hours).

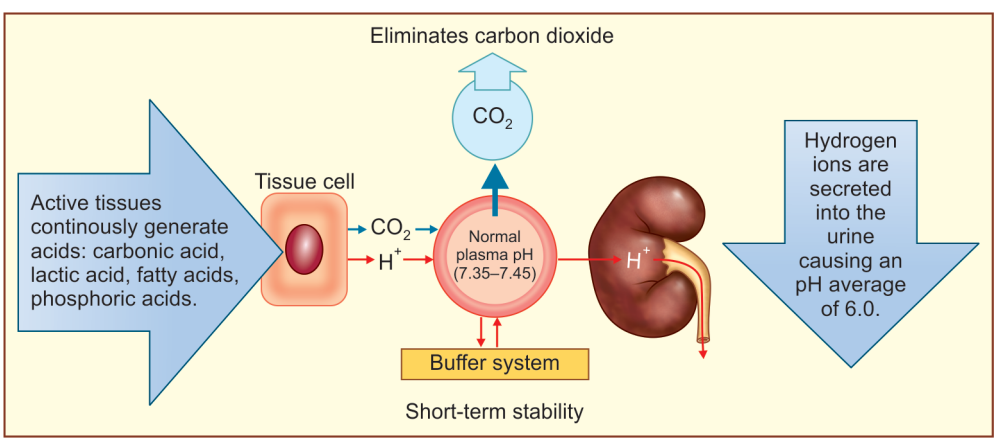

Fig. 2.2: The body response to a change in acid-base homeostasis has three components

Symptoms and Signs

Symptoms and signs depend on the rate and degree of pCO_2 increase. CO_2 rapidly diffuses across the blood-brain barrier. Symptoms and signs are a result of high CNS CO_2 concentrations (low CNS pH) and any accompanying hypoxemia.

Acute (or acutely worsening chronic) respiratory acidosis causes headache, confusion, anxiety, drowsiness, and stupor (CO_2 narcosis). Slowly developing, stable respiratory acidosis (as in chronic obstructive pulmonary disease) may be well tolerated, but patients may have memory loss, sleep disturbances, excessive daytime sleepiness, and personality changes. Signs include gait disturbance, tremor, blunted deep tendon reflexes, myoclonic jerks, asterix is, and papilledema.

Maintenance

A rise in arterial pCO_2 is a potent stimulus to ventilation, so a respiratory acidosis will rapidly correct unless some abnormal factor is maintaining the hypoventilation.

This feedback mechanism is responsible for the normal tight control of arterial pCO_2. The factor causing the disorder is also the factor maintaining it. The prevailing arterial pCO_2 represents the balance between the effects of the primary cause and the respiratory stimulation due to the increased pCO_2.

Other than by ventilatory assistance, the pCO_2 will return to normal only by correction of the cause of the decreased alveolar ventilation.

An extremely high arterial pCO_2 has direct Anesthetic effects and this will lead to a worsening of the situation either by central depression of ventilation or as a result of loss of airway patency or protection.

Metabolic Effects

1. **Depression of intracellular metabolism:** As CO_2 rapidly and easily crosses lipid barriers, a respiratory acidosis has rapid and generally depressing effects on intracellular metabolism.

   Hypercapnia will rapidly cause an intracellular acidosis in all cells in the body. The clinical picture will be affected by the arterial hypoxemia that is usually present. The effects described below are the metabolic effects of hypercapnia rather than respiratory acidosis. Patients with respiratory acidosis can be hypocapnic if a severe metabolic acidosis is also present.
2. **Importance of cerebral effects:** The cerebral effects of hypercapnia are usually the most important. These effects are:
   - Increased cerebral blood flow
   - Increased intracranial pressure
   - Potent stimulation of ventilation

   This can result in dyspnea, disorientation, acute confusion, headache, mental obtundation or even focal neurologic signs. Patients with marked elevations of arterial pCO_2 may be comatose, but several factors contribute to this:
   - Anesthetic effects of very high arterial pCO_2 (i.e. >100 mmHg)
   - Arterial hypoxemia
   - Increased intracranial pressure

   As a practical clinical example, the rise in intracranial pressure due to hypercapnia may be particularly marked in patients with intracranial pathology (e.g. tumor, head injury) as the usual compensatory mechanism of CSF translocation may be readily exhausted. Any associated hypoxemia will contribute to an adverse outcome.
3. **Effects on cardiovascular system:** The effects on the cardiovascular system are a balance between the direct and indirect effects. Typically, the patient is warm, flushed, sweaty, tachycardic and has a bouncing pulse.

   The clinical picture may be modified by effects of hypoxemia, other illnesses, and the patient's medication. Arrhythmias may be present particularly if significant hypoxemia is present or sympathomimetic have been used.

   Acutely, the acidosis will cause a right shift of the oxygen dissociation curve. If the acidosis persists, a decrease in red cell 2, 3 diphosphoglycerate (DPG) occurs which shifts the curve back to the left.

Compensation

1. **The compensatory response is a rise in the bicarbonate level:** This rise has an immediate component (due to a resetting of the physicochemical equilibrium point) which raises the bicarbonate slightly.

   Next is a slower component where a further rise in plasma bicarbonate due to enhanced renal retention of bicarbonate. The additional effect on plasma bicarbonate of the renal retention is what converts an 'acute' respiratory acidosis into a 'chronic' respiratory acidosis.

   As can be seen by inspection of the Henderson-Hasselbalch equation (below), an increased $[HCO_3^-]$, will counteract the effect (on the pH) of an increased pCO_2, because it returns the value of the $[HCO_3^-]/0.03\ pCO_2$ ratios towards normal.

   $$\mathbf{pH = pK'a + \log_{10}([HCO_3^-]/0.03 \times pCO_2)}$$
2. **Buffering in acute respiratory acidosis:** The compensatory response to an acute respiratory acidosis is limited to buffering. By the law of mass