A couple of decades back, heart disease spelt doom, not anymore. Now many predictors of impending coronary disease are freely available, the disease process can not only be arrested but also reversed. Coronary artery blockages can be removed physically or grafts be placed instead of the diseased arterial segment. However, Ischaemic Heart Disease (IHD) still continues to be a significant reason for human morbidity and mortality. Genetic factors, sedentary life styles coupled with poor dietary habits and stressful living conditions are to be blamed. Incidence of IHD varies geographically; it is 1.7% amongst Chinese, 4.6% in Europeans and 10.7% amongst South Asians. Consider the “French Paradox”, where inspite of high caloric nutrition the incidence of IHD is significantly low! The credit is given to the drinking, eating and genetic factors at play amongst the French.

An accurate, fast diagnostic tool (with high sensitivity and specificity) has been felt missing for diagnosing Acute Myocardial Infarction. The markers used earlier (enzymes) could arise from many locations. A marker that only the cardiac muscle could produce was supposed to be ideal. In came the cardiac troponins! Of the two currently in use cTnT and cTnl, the latter is a better diagnostic tool for ruling in Acute Myocardial Infarction. Presentation in device format that can accept whole blood makes the availability of the test at rural and primary health center setups possible. Needless to say that Nurses in Cardiology Hospitals can perform the test by the bedside and report to the cardiologist immediately, thereby considerably reducing the morbidity and mortality. This issue’s DISEASE DIAGNOSIS portion considers the emerging cardiac markers - Troponins to be precise.

Overflow from the previous issues’ INTERPRETATION section is carried on here too. Specific tumour markers are considered along with their reference values and indications for estimating them. There are tumours that require multiple markers to be tested for. The prognosis and therapeutic regimen is to a great extent dictated by the values of the tumour markers in particular malignancies.

What if you are constantly working with biohazard III samples all the time? How do you prevent yourself from getting infected? What are the universal work precautions for laboratory personnel especially in relation to HIV transmission? Consider all samples as potentially infective. An HIV patient may come for LFTs and would usually not disclose that he/she is HIV positive. What are set or established norms for handling such samples? The TROUBLE SHOOTING segment of this issue considers these very important aspects of day-to-day Laboratarians’ practice.

Shall we say that BOUQUET is as important as the rest of the serious articles! Well it is and has not been forgotten.
INTRODUCTION

Coronary artery disease (CAD) is the most important cause of morbidity and mortality in the industrialized world and now even in the third or the so-called developing world. Genetic, dietary and lifestyle differences have revealed that with regards to both morbidity and mortality (vis-à-vis Acute Myocardial Infarction - AMI) significant regional differences exist. The risk of coronary artery disease is significantly higher in Northern Europe than in Central and Southern Europe. The risk of coronary artery disease is especially high in Eastern Europe, whereas Canada, USA and Australia show a midlevel risk. Also the risk of CAD is comparatively higher in males as compared to females. In the more recently reported SHARE study the overall prevalence of coronary artery disease was 10.7% among South Asians as against 4.6% in Europeans and 1.7% in Chinese population. Projections based on global burden of disease estimate that by year 2020, the burden of atherothrombotic cardiovascular disease in India would surpass that in any other region in the world.

EARLY AND ACCURATE DIAGNOSIS OF AMI IS OF PRIME IMPORTANCE AND A LIFE SAVING MEASURE.

Triageing of patients with or without AMI

According to W.H.O. criteria, diagnosis of Acute Myocardial Infarction (AMI) is based on the detection of at least two out of three infarction specific findings:

- Chest pain > 20 minutes, resistant to nitro derivatives.
- Infarction specific ECG changes (ST segment elevation, development of abnormal Q wave) in at least two leads of the standard 12 lead ECG within the same vascular area.
- Serial enzyme changes (cardiac markers) with initial rise and subsequent reduction in level of concentration.

The triaging of patients presenting in emergency department is the major diagnostic challenge the physicians face today.

If an ECG reveals ST segment elevation or abnormal Q wave, the probability of acute myocardial infarction is high and further management is well established. However, the sensitivity of ECG may be as low as 50% and even less in patients with an evolving myocardial infarction i.e. in Non-ST segment elevated myocardial infarction and Non-Q wave myocardial infarction. Though these patients may present with chest pain because of the absence of diagnostically specific ECG changes they could be discharged undiagnosed.

On the other hand, many patients with symptoms of chest pain suffer from severe unstable angina pectoris. Also in these patients ECG changes may be less specific and fail to provide conclusive diagnostic information.

Besides a subset of patients with absence of chest pain but with myocardial infarction i.e. silent infarction is especially common in diabetic patients. With advancing age the course of AMI is often atypical (without chest pain) but presenting with shortness of breath.

**Discharge or Test**

- Rule-out AMI
- Normal History
- Normal cardiac biomarkers

**Admission**

- Positive History
- Inconclusive ECG
- Cardiac markers questionable

**Diagnostic Challenge**

- Positive History
- Inconclusive ECG
- Cardiac markers questionable

**Rule-in AMI**

- Positive History
- ECG consistent with AMI

**CCU**

- Continue treatment

**CHEST PAIN**

**Importance of accurate triaging**

Coronary ischaemia is the root cause of acute myocardial infarction, hence early and reliable detection of myocardial ischaemia is a prerequisite for appropriate triage decision in emergency room so as to initiate the right therapy. It has been observed that only 10-15% of patients presenting in emergency room with the cardinal symptom of chest pain develop acute myocardial infarction. Hence early diagnosis of AMI has to be so sensitive that all suitable patients with myocardial infarction can be treated with thrombolytic therapy and yet so specific that patients with chest pain but without myocardial infarction are not unnecessarily exposed to the risks of such therapy. This is the most important decision the clinicians have to take when the patients present in the emergency rooms.

**Role of cardiac markers**

Following are the important applications of cardiac markers for management of patients with acute coronary syndrome:

- Confirm the diagnosis of AMI in the presence of diagnostically specific ECG changes.
- Diagnosis of AMI in the absence of unequivocal ECG changes (NSTEMI and Non-Q wave MI).
- Identification of high-risk patients with unstable angina pectoris in the absence of unequivocal ECG changes.
- For monitoring patients with AMI undergoing thrombolytic therapy i.e. success of therapy or reperfusion.

From a clinical point of view, an ideal cardiac marker that detects myocardial injury should satisfy the following properties:

- It should be present in myocardium in high concentration and absent in other tissues thereby ensuring high cardiac specificity.
- It should be released rapidly in blood stream after myocardial injury, so as to achieve optimal sensitivity in early phase after the onset of myocardial injury.
- It should remain abnormal for several days thereby offering wide diagnostic window time.
- It should be assayed with a rapid turnaround time.

**Cardiac Markers**

**Creatine Kinase (CK)/Creatine Kinase MB (CK-MB) activity**

Three different isoenzymes of CK exist namely CK-MM, CK-MB and CK-BB. Skeletal muscle approximately consists of CK-MM (97-99%) and CK-MB (1-3%). The cardiac muscle approximately contains CK-MM (95%) and CK-MB (5%). CK-BB is found primarily in brain and contributes very little to the total CK level. After myocardial infarction CK and CK-MB levels rise after 2-6 hours; peak levels are observed at 12-24 hours. CK returns to normal levels after 3-4 days whereas CK-MB because of shorter half-life returns to normal level after 2-3 days. The calculation of CK-MB/CK ratio improves the specificity of CK-MB for acute myocardial infarction in patients accompanying with skeletal muscle damage. CK-MB returns to normal levels within 2-3 days after myocardial infarction, hence it is useful in detecting reinfarction.

**Limitation:** Though CK-MB/CK ratio improves specificity of CK-MB, however small myocardial necrosis may be missed (unstable angina pectoris may show the presence of micro infarcts / minor myocardial injury).

**CK-MB isoforms**

In an attempt to improve sensitivity of CK-MB, high voltage electrophoresis technique was developed to separate CK-MB into its two isoforms: CK-MB2 and CK-MB1. Serum of healthy individuals CK-MB2/CK-MB1 ratio of approximately 1 is present. The reference range of this ratio is 1.5. A higher ratio indicates acute myocardial infarction.

**Limitation:**
- This technique is labour intensive.
- Requires high level of technical skill.
- Long delay in reporting of results.

**CK-MB mass**

Here CK-MB is detected immunologically by using a combination of CK-B and CK-M specific monoclonal antibodies or with CK-MB specific monoclonal antibodies.

**Limitation:** Interference in these assays is observed because of CK-MM, CK-BB, and CK-B autoantibodies.

**CK-MB immunoinhibition method**

The theoretical basis for the clinical application of immunoinhibition method is the assumption that only CK-MM and CK-MB are released into the blood stream after muscle damage. The reagent contains anti CK-M antibodies, which completely inhibit all CK-M activity i.e. both M subunits in CK-MM and the single M subunit in CK-MB. The remaining non CK-M activity corresponding to the CK-B activity of CK-MB is measured. Since only CK-B of the dimeric CK-MB molecule is measured, multiplication by a factor of 2 gives the CK-MB activity in the specimen.
Limitation: In case of macro CK, which contains no CK-M subunits immuno-inhibition cannot take place.

Common limitations of CK-MB assays:
- As CK-MB is also present in skeletal muscle it is not absolutely specific to cardiac muscle damage.
- Evaluation of CK-MB levels may present problems in conditions such as extensive skeletal muscle injury with small infarction, chronic skeletal muscle injury and myocardial infarction after coronary artery bypass graft.
- Determination of CK and CK-MB activity alone is not suitable for assessment of risk in patients with unstable angina pectoris (minor myocardial damage).

Lactate Dehydrogenase
Lactate Dehydrogenase is also an enzyme released by ischemic heart muscle. Out of the 5 isoenzymes only two of them LD1 and LD2 are useful in the diagnosis of AMI. Usually in normal healthy individuals the amount of LD2 in blood is higher than LD1 but patients with AMI show more of LD1 than LD2.

Limitation:
- LD1 and LD2 are not cardiospecific markers
- Elevated levels of LD1 and LD2 are observed in leukemia, renal and hemolytic diseases.

Myoglobin
Myoglobin, the oxygen binding haem protein constitutes about 2% in both skeletal and cardiac muscle. The low molecular weight of Myoglobin (17.8 kDa) facilitates its rapid release in circulation and is the first marker to exhibit rising levels after AMI. The advantages of Myoglobin in early diagnosis of myocardial infarction are its high early sensitivity and the possibility of rapidly assessing the success of thrombolytic therapy.

Limitation:
- Since Myoglobin is also present in skeletal muscle it is not a cardiospecific marker.
- The extremely short biological half life (10-20 minutes) restricts the usage of myoglobin to detect unstable angina pectoris (minor myocardial injury or micro infarcts).

Typical characteristics of cardiac markers

<table>
<thead>
<tr>
<th>Cardiac Marker</th>
<th>M.Wt (KDa)</th>
<th>Half life (hours)</th>
<th>Increase (hours)</th>
<th>Peak* (hours)</th>
<th>Normalization (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD - 1</td>
<td>135</td>
<td>110</td>
<td>6 - 12</td>
<td>48 - 144</td>
<td>7 - 14</td>
</tr>
<tr>
<td>CK</td>
<td>86</td>
<td>17</td>
<td>3 - 12</td>
<td>12 - 24</td>
<td>3 - 4</td>
</tr>
<tr>
<td>CK - MB</td>
<td>86</td>
<td>13</td>
<td>3 - 12</td>
<td>12 - 24</td>
<td>3 - 4</td>
</tr>
<tr>
<td>CK - MB mass</td>
<td>86</td>
<td>13</td>
<td>2 - 6</td>
<td>12 - 24</td>
<td>3</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>17.8</td>
<td>0.25</td>
<td>2 - 6</td>
<td>6 - 12</td>
<td>1</td>
</tr>
</tbody>
</table>

*Strongly dependent on the timing of reperfusion of the infarct-related blood vessel

Average diagnostic sensitivities (%) of cardiac markers during early phase of AMI

<table>
<thead>
<tr>
<th>Cardiac Marker</th>
<th>2-3</th>
<th>3-4</th>
<th>5-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK activity</td>
<td>15</td>
<td>35</td>
<td>70</td>
</tr>
<tr>
<td>CK-MB activity</td>
<td>10</td>
<td>25</td>
<td>55</td>
</tr>
<tr>
<td>CK-MB mass</td>
<td>30</td>
<td>70</td>
<td>90</td>
</tr>
<tr>
<td>CK-MB isoform ratio</td>
<td>25</td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>35</td>
<td>80</td>
<td>95</td>
</tr>
</tbody>
</table>

Cardiac Troponins (cTn)-emerging cardiac marker of choice

Limitations of existing cardiac markers led to the search for markers uniquely expressed by the myocardium. The cardiac troponins T and I (cTnT and cTnI) have excellent sensitivity and specificity and are superior to CK-MB in indicating minor myocardial injury. The advent of cardiac Troponins T and I, unarguably the most sensitive and specific markers encompass all the requirements that physicians and laboratarians require for accurate triaging and better risk stratification of patients with acute coronary syndrome.

Role of Troponins in muscle contraction

The contractile apparatus of striated muscle fiber is composed of thick and thin filaments. The thick filament is composed mainly of myosin. Actin, troponin I and Troponin comprise of thin filaments. Muscle contraction occurs when thick and thin filaments slide past each other. The interaction between thick and thin filaments is regulated by Troponin complex found on thin filaments. The Troponin complex is composed of three protein subunits: Troponin I (TnI), Troponin T (TnT) and Troponin C (TnC). The calcium mediated contraction of striated muscle (fast-skeletal, slow-skeletal and cardiac muscle) is regulated by the Troponin complex. Contraction of smooth muscle is regulated by calmodulin (intracellular protein that combines with calcium and is involved in smooth muscle contraction). Troponins are proteins that are integral to the functioning of striated muscle. They exist as a complex with actin and tropomyosin on thin filament of the contractile apparatus. The Troponin complex consists of three protein subunits:
- Troponin C, binds with calcium and regulates activation of thin filaments during contraction.
- Troponin T, binds the Troponin complex to tropomyosin.
- Troponin I, prevents the contraction of muscle in the absence of calcium and Troponin C.

During the functioning of the contractile apparatus depolarization of muscle leads to intracellular release of calcium, which binds with Troponin C. A conformational change occurs in Troponin-Tropomyosin complex in such a way that actin molecules can then interact with myosin, resulting in muscle contraction.

Troponin holds tropomyosin in position to block myosin-binding sites on actin.

Types of Cardiac Troponins

- Troponin C exists as two isoforms, fast and slow. The fast isoform is found only in skeletal muscle, but the slow isoform is found both in skeletal and cardiac muscles. The molecular weight of cardiac isoform (cTnC) is 18 kDa.
- Troponin T is also found in fast and slow skeletal muscle, cardiac muscle Troponin T present in skeletal muscle exists as a slightly different subform. The cardiac isoform (cTnT) has a molecular weight of 37 kDa.
- Three isoforms of Troponin I have been identified, one each in fast and slow skeletal muscles and one isoform in cardiac muscle. The cardiac isoform of Troponin I (cTnl) has a molecular weight of 22.5 kDa. cTnI has an extra 30 amino acid, sequence at the N terminal portion of molecule making it absolutely specific to cardiac muscle. cTnI is mostly bound to contractile apparatus in myocardium, But about 8% is found free in cytoplasm.

Characteristics of cardiac troponin (I and T) in AMI

<table>
<thead>
<tr>
<th>Cardiac Troponin</th>
<th>M.Wt (KDa)</th>
<th>Half life (hours)</th>
<th>Increase (hours)</th>
<th>Peak* (hours)</th>
<th>Normalization (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cTnl</td>
<td>22.5</td>
<td>2 - 4</td>
<td>3 - 8</td>
<td>12 - 24</td>
<td>7 - 10</td>
</tr>
<tr>
<td>cTnT</td>
<td>37</td>
<td>2 - 4</td>
<td>3 - 8</td>
<td>12 - 24</td>
<td>7 - 14</td>
</tr>
</tbody>
</table>

*Strongly dependent on the timing of reperfusion of the infarct-related blood vessel

Graphical representation-Levels of cardiac markers in AMI

Cardiac Troponins (cTnI and cTnT)-sensitivity & specificity

Any damage or injury to myocardial cells results in the release of cardiac Troponins into the circulation. Concentration of cTnl and cTnT in the circulation initially increases with the number of hours after the onset of chest pain and decreases as the enzymes are cleared from the circulation. The most important take home message is that sensitivity of cardiac Troponin tests, like any other cardiac marker is dependent on the number of hours after the onset of chest pain. Internationally a
lot of scientific research work has been done to evaluate important parameters of sensitivity, specificity and predictive values of cTnl and cTnT in clinical settings.

Various Studies Indicate that:
- Sensitivity of cardiac Troponin tests (cTnl and cTnT) is dependent on the number of hours since the onset of chest pain.
- cTnl appears to be better at ruling in MI than cTnT. A positive cTnT value is only moderately useful at ruling in AMI at 6 hours from the onset of chest pain whereas cTnl values appear to be very useful in AMI at 6 hours from the onset of chest pain.
- cTnl and cTnT are very useful at ruling out AMI when the value is negative at 10 or more hours from the onset of chest pain.
- A normal cTnT or cTnl value at 8 or more hours after the onset of chest pain is strong evidence against the presence of AMI.
- Abnormal values of cTnT and cTnl at 8 or more hours after the onset of chest pain are moderately strong evidence in favour of presence of AMI.

cTnl versus cTnT

The debate continues as to which of the two, cTnl or cTnT is better for management of patients with acute coronary syndromes. Since assays of cTnT were commercially available few years before cTnl, more peer-reviewed publications on theclinical utility of cTnT might have appeared in the past. However, recent studies have questioned the diagnostic specificity of cTnT assays in patients with myocardial injury and chronic renal failure, muscular dystrophies and skeletal muscle damage. cTnl indeed scores over cTnT in the assays in patients with myocardial injury and chronic renal failure, muscular dystrophies and skeletal muscle damage. However, recent studies have questioned the diagnostic specificity of cTnT in these patients.

Thus cTnl determination promises higher diagnostic efficacy because of the following unique characteristics:
- Wide diagnostic window time with early appearance and prolonged presence in circulation.
- Allows detection of minor myocardial injury because cTnl levels is almost absent in normal healthy individuals.
- No cross-reactivity with skeletal muscle isomers.
- Virtually absent in skeletal muscle tissue.

Numerous international reports highlight the superior diagnostic efficacy of cTnl.

cTnl-Cut off levels

The National Academy of Clinical Biochemistry (NACB), USA and International Federation of Clinical Chemistry (IFCC), Germany have recommended the use of two decision cut off limits for cardiac Troponins. A low limit that establishes the presence of myocardial injury and a high limit that establishes injury to the extent that qualifies as AMI. Based on literature data and clinical assessments cTnl levels greater than 0.1 ng/ml places a patient with unstable angina in the high-risk category for short-term risk of death or non-fatal MI. The cut off for the definition of AMI is taken to be greater than 1.2 ng/ml. Thus cTnl levels with a cut-off of 0.1 ng/ml identify patients at higher risk for very early adverse outcomes.

Graphical representation - two decision cut off limits of cTnl

Applications of cTnl Test

Following are the important applications of cTnl test in management of patients:

Protocol For Use Of Cardiac Troponin I Test

Presentation with chest pain possibly of cardiac origin in the emergency room
- Is pain of cardiac origin?
  - Not cardiac cause?
    - Consider history and electrocardiogram
  - Cardiac origin
    - Clearly cardiac origin
      - Etiology unclear
        - Assess cTnl at 6 h post-onset
          - Positive
            - Diagnose acute myocardial infarction (AMI)
            - Perform baseline assay of creatine kinase
            - Treat
            - Monitor creatine kinase
          - Negative
            - Assess cTnl at 12 h post-onset
              - Positive
                - AMI unlikely
                - Reconsider diagnosis - acute coronary syndrome but not AMI
              - Negative
                - Not cardiac cause?

with acute coronary syndrome:
- Patient with ECG specific findings and elevated cTnl test confirms the diagnosis of AMI.
- An elevated cTnl level is of immense diagnostic value in patients with symptoms of chest pain, absence of diagnostically significant ECG change and normal CK-MB level. cTnl test value ≥ 1 ng/ml classifies these patients under NSTEMI. A negative cTnl test classifies the patient as UAP.
- Also elevated cTnl value provides prognostic value in identifying patients with unstable angina pectoris. Symptoms of chest pain, absence of diagnostically significant ECG change, normal CK-MB level and cTnl value > 0.1 ng/ml is classified under UAP. If the test is negative retesting probably at 12 hours after post onset of chest pain is important to rule out diagnosis of acute coronary syndrome.
- Patients presenting with chest pain after trauma or surgery and elevated CK-MB assay value (to rule out true elevation of CK-MB).
- Patients presenting with chest pain 2 to 6 days prior to admission may have sustained acute myocardial infarction but CK-MB would have returned to normal levels.

Superior diagnostic efficacy of cTnl over CK-MB in detecting microinfarcts

Patients with normal CK-MB levels and elevated cTnl levels could be attributed probably to the low sensitivity and specificity of CK-MB in detecting micro infarction. Since CK-MB is present in skeletal muscle and normal healthy individuals, diagnostic cut off values are typically set above the upper limit of reference range for CK-MB assay. Cardiac troponin I is not present in the blood of normal healthy individuals and is approximately 13 times more abundant in myocardium than CK-MB on a weight basis. Hence the signal to noise ratio (increased sensitivity) associated with cTnl is more favorable for detection of micro infarction (NSTEMI and UAP).

Limitations
- cTnl levels remain elevated for about 7 days hence for serial monitoring of patients undergoing thrombolytic therapy cardiac markers such as CK-MB and myoglobin may be used for successful reperfusion. Also in cases of reinfection markers with shorter half-life such as CK-MB should be used for accurate diagnosis.
- Since cardiac Troponins (both cTnl and cTnT) are sensitive markers for myocardial damage, they are detected in many other cardiac conditions such as acute pericarditis, acute myocarditis, congestive heart failure (CHF), perioperative myocardial infarction and cardiac contusion.
Furthermore, it may be used as a second-line marker, after CA 19-9, for pancreatic cancer. It cannot be recommended for other malignant diseases due to its low clinical sensitivity and specificity.

**Reference interval**
- Serum, plasma: ≤ 35 U/ml

**Indication**
- Absolute indications
  - Suspected ovarian cancer
  - Monitoring the treatment and the course of ovarian cancer

**Relative indication**
- Suspected pancreatic cancer; second-line marker after CA 19-9

### CA 72-4 (TAG-72)

**Reference interval**
- Serum, plasma: ≤ 6 U/ml

**Absolute indication**
- First-line tumor marker for monitoring treatment and disease course in patients with gastric cancer; CA 19-9 or CEA as second-line markers

**Relative indication**
- Second-line tumor marker for mucinous ovarian cancer

### CA 15-3

**Reference interval**
- Serum, plasma: ≤ 40 U/mL

**Indication**
- Monitoring the outcome of treatment and disease course in patient with breast cancer

### CA 549

**Reference interval**
- Serum, plasma: ≤ 12 U/mL

**Indication**
- Monitoring treatment outcome and disease course in patients with breast cancer

### hCT (Human calcitonin)

- A peptide hormone secreted by the parafollicular C cells of the thyroid gland. It serves as a specific and sensitive tumor marker for the diagnosis and monitoring of medullary thyroid carcinoma (C-cell carcinoma).

**Reference interval**
- Basal hCT Level: Males < 2-48, Females < 2-10
- Maximal levels post pentagastrin: Up to 79 for Males, Up to 50 for Females

**Indication**
- Diagnosis of clinically overt medullary thyroid carcinoma in patients with:
  - Thyroid nodules which appear cold upon thyroid scanning, are hypoechoic or extraglandular tumors
  - Follow-up of patients after treatment for germ-cell tumors or primary liver cancers of or of those still undergoing treatment, e.g. postoperatively or during/after irradiation and chemotherapy

### Diagnosis of clinically overt medullary thyroid carcinoma in patients with:

- Thyroid nodules which appear cold upon thyroid scanning, are hypoechoic during sonography and cytologically suspicious: medullary thyroid carcinoma makes up 10% of all thyroid cancers.
- Diarrhea refractory to therapy: 10-20% of patients with advanced medullary thyroid carcinoma suffer from this type of diarrhea
- Unexplained CEA elevation: clinically overt medullary thyroid carcinoma is associated with an elevation of both hCT and CEA
- Thyroid cancer characterized by lack of radioactive iodine uptake and inconclusive histological findings, e.g. anaplastic thyroid cancer with an extraordinarily long survival time.

### Family screening in cases of hereditary medullary thyroid carcinoma:

- Relatives of patients with the hereditary multiple endocrine neoplasia syndrome type 2 or so-called MEN 2, e.g. by measurements of hCT after stimulation (pentagastrin test). 25% of medullary thyroid carcinomas occur as part of MEN 2 with an autosomal dominant pattern. Nowadays, in these cases, the molecular-genetic analysis of a mutation in the RETproto oncogene is the diagnostic method of first choice.
- Patients with bilateral/unilateral and/or familial pheochromocytoma. 50% of patients with MEN 2 will develop pheochromocytoma which may precede the clinical manifestation of medullary thyroid carcinoma. In such cases (MEN 2, von Hippel-Lindau syndrome, neurofibromatosis), if indicated, molecular genetic
testing for possible underlying typical mutations should be initially performed.

**Monitoring of medullary thyroid carcinoma:**
- Postoperatively in patients with histologically confined medullary thyroid carcinoma. If the hCT value is within the reference interval and there is no Stimulation by pentagastrin the patient can be assumed to be disease free.
- Elevated postoperative hCT values suggest tumor persistence or recurrence.
- The mediastinum and the liver.
- Carcinoma may be associated with paraneoplastic hCT secretion. In these cases serial hCT determinations can help in the follow-up.

**Diagnostic test for localization of medullary thyroid carcinoma:**
- Localization of medullary thyroid carcinoma can be accomplished by selective venous catheterization during which calcitonin is determined after obtaining systematic and selective blood samples from veins in the neck region, the mediastinum and the liver.
- Relative indication
  - Neuroendocrine tumors, e.g. carcinoid, insulinoma, VIPoma, small cell lung carcinoma may be associated with paraneoplastic hCT secretion. In these cases serial hCT determinations can help in the follow-up.
  - Cold nodules during thyroid scanning have potential risk for underlying malignancy (< 1-5%), 10% of all thyroid cancers are of the medullary type.
  - The clinical sensitivity for medullary thyroid carcinoma is 0.1-0.5% when hCT is determined after detection of a cold nodule. hCT is used because of low analytical specificity and sensitivity.

**CEA (carcinoembryonic antigen)**

**Reference interval**
- Serum or plasma - Upper limit 1.5 to 5.0 µg/L depending on method

**Indication**
- Detection of tumor recurrence in the postoperative monitoring of colorectal carcinomas
- Differential diagnosis of liver tumors

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**In Lighter Vein**

**The ways to grade the final exams**

- Dept of Statistics: All grades are plotted along the normal bell curve.
- Dept of Psychology: Students are asked to blot ink in their exam books, close them and turn them in. The professor opens the books and assigns the first grade that comes to mind.
- Dept of History: All students get the same grade they got last year.
- Dept of Religion: Grade is determined by God.
- Dept of Philosophy: What is a grade?
- Law School: Students are asked to defend their position of why they should receive an A.
- Dept of Logic: If and only if the student is present for the final and the student has accumulated a passing grade then the student will receive an A else the student will not receive an A.
- Dept of Computer Science: Random number generator determines grade.
- Music Department: Each student must figure out his grade by listening to the instructor play the corresponding note (+ and - would be sharp and flat respectively).
- Dept of Physical Education: Everybody gets an A.

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**BOUQUET**

**Wisdom Whispers**

- We do not remember days; we remember moments.
- Let him who would enjoy a good future waste none of his present.
- Look at life through the windshield, not the rear-view mirror.
- To look backward for a while is to refresh the eye, to restore it, and to render it more fit for its prime function of looking forward.
- Forgiveness does not change the past, but it does enlarge the future.
- Never think of the future - it comes soon enough.
- Learn from yesterday, live for today, hope for tomorrow. The important thing is not to stop questioning.
- Time is at once the most valuable and most perishable of all our possessions.
- The only limit to our realization of tomorrow will be our doubts of today.

**Brain Teasers**

1. Granulomas are typically soft in
   - A) Tuberculosis  B) Gumma  C) Sarcoidosis  D) Leprosy
2. Which of the following stains confirms Glycogen?  
   - A) PAS  B) Von Kossa  C) Methyl Violet  D) Sudan Black B
3. Intranuclear vacuolation in hepatocytes is seen in
4. Flame cells are observed in
   - A) Multiple myeloma  B) Bronchogenic carcinoma  C) Ewing's sarcoma  D) Retinoblastoma.
5. Which of the following is not a trace element as related to human body?
   - A) Iron  B) Zinc  C) Magnesium  D) Copper
Waste Disposal
Sterilization (for non-disposable items)

Biosafety Regulations for Laboratory Procedures
Guidelines of Basic Practices and Procedures

Introduction
Health care personnel (HCP) can acquire certain illnesses beyond those acquired by all others who live and work in our society, by virtue of their profession. HCPs are at risk of acquiring any of the whole gamut of infections from patients/ specimens, which may be viral, bacterial, parasitic or fungal. However this risk due to occupational exposure can be minimized if not obliterated altogether, if we follow universal work precautions.

Today with WHO estimates of above 4 million HIV positive persons in India, there is an urgent need to review UWP. Besides HIV, there is the very real danger of acquiring Hepatitis B and Hepatitis C in exactly the same way as HIV and could also be fatal. Hepatitis B is 100 times more infectious than HIV. Besides Hepatitis B is also far more prevalent in India in comparison to HIV with estimated carriers being between 30 and 40 million, a considerable number being infectious. However fortunately effective vaccination is available for Hepatitis B therefore it is strongly recommended for all levels of health care workers.

Much of the contamination in the laboratory occurs as a result of penetrating injuries caused by sharp objects and the spilling and splashing of specimen materials.

Components of UWP
1. Hand-washing
2. Barrier precautions (Mask, cap, plastic apron and protection of feet)
3. Careful handling of all kinds of sharps and needles
4. Effective disinfection
5. Sterilization
6. Correct disposal of different kinds of wastes generated in a health care facility

Guidelines of Basic Practices and Procedures

- Prevention of puncture wounds, cuts and abrasions and protection of existing wounds skin lesions, conjunctiva and mucosal surfaces.
- Application of simple protective measures designed to prevent contamination of the person and his/her clothing.
- Good basic hygiene practices, including regular hand washing.
- Control of surface contamination by containment and disinfection procedures.
- Safe disposal of contaminated waste.

Biosafety Regulations for Laboratory Procedures

- Wear gloves when handling infectious materials or where there is a possibility of exposure to blood and other body fluids. All laboratories that work with material that is potentially infected with HIV require a generous possibility of exposure to blood and other body fluids. All laboratories that work with material that is potentially infected with HIV require a generous possibility of exposure to blood and other body fluids.

Precautions for Laboratory Personnel

- Use autoclaving for other reusable items (e.g. needle holders, gowns etc.). Wherever autoclaving is not possible boiling must be for 30 minutes at the least.
- Safe disposal of contaminated waste.
- Sterilization (for non-disposable items)

- Sharp, reusable blades, cystoscopy instruments, endoscopy instruments use CiDEX (2% Glutaraldehyde) or 5% Korsolex. Disinfection in 30 minutes.
- Use autoclaving for other reusable items (e.g. needle holders, gowns etc.).
- Wherever autoclaving is not possible boiling must be for 30 minutes at the least.

Waste Disposal
Divide waste into 3 parts at source.

i) Household type non-infectious waste
   - Not to be decontaminated.
   - To be disposed off as such.

ii) Infected Sharp Waste Disposables (needles/surgical instruments)
   - Place in puncture proof container containing disinfectant (1% bleach prepared every morning).
   - Final disposal

iii) Infected non-sharp waste
   - Is to be decontaminated.
   - Placed in disinfectant 5-10% bleach as the case maybe, (left over blood, tissues etc)

Final Disposal After Infection
- Purchase of needle destroyer if resources permit.
- Incineration of all infected waste.
- Deep burial in controlled landfill sites (protected from all sides)
- Shredding of disposable plastic-ware waste.

Post Exposure Care
- Minor bleed with percutaneous inoculation, open skin wound breached skin, exposed mucous membrane
- First Aid
  - Allow to bleed by squeezing.
  - Wash with water
  - Antiseptic
- Report
  - Employee identification date, time with place of accident.
  - Circumstances around accident.
- Action taken.
  - Post Exposure Care

Initial Consultation
- Easy access to medical advise with counseling. Consult physician for AZT prophylaxis regime if medication available.
- Laboratory Testing
  - After consent with counseling within 2 weeks, 5 weeks, 12 weeks, 24 weeks.
  - Clinical Follow up
    - For fever, pharyngitis, rash, malaise, lymphadenopathy, myalgia, arthralgia within 6 months.

Safety Precautions
- Do not leave the workplace or walk around the laboratory while wearing gloves.
- Wash hands with soap and water immediately after any contamination and after work is finished. If gloves are worn, wash your hands with soap and water after removing the gloves. This is a vital and simple precaution that is often overlooked.
- Wear a laboratory gown or uniform when in the laboratory. Wrap around gowns are preferable. Remove this protective clothing before leaving the laboratory.
- Wear gloves when handling infectious materials or where there is a possibility of exposure to blood and other body fluids.
- Do not touch your eye, nose, or other exposed membranes or skin with your gloved hands.

Sterilization (for non-disposable items)

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Tulip Group Enters The Cardiac Event Marker Segment

Exploring new markets, manufacturing innovative products backed by strong R&D and delivering bench marking in-vitro diagnostic products is synonymous with the Tulip Group of Companies. After extensive Research and Development, Tulip Group of Companies has developed an innovative product in the field of Cardiac event marker,

AMICHECK TROP I - WB manufactured by Zephyr, a Tulip Group Company, is a rapid, two-site sandwich immunoassay for the detection and semi quantification of human cardiac Troponin I (cTnI) levels in serum, plasma and whole blood. This product is presently available in convenient pack sizes of 3 Tests and 10 Tests. Based on internal and external evaluation AMICHECK TROP I - WB is found to be 100% Sensitive and 100% Specific.

FOR SAFE DECISION MAKING AND COST EFFECTIVE USE OF INTENSIVE CARE FACILITIES!

<table>
<thead>
<tr>
<th>FEATURES</th>
<th>BENEFITS</th>
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<tbody>
<tr>
<td>Test system incorporates blend of unique IgG class monoclonal antibodies against cTnI</td>
<td>Highly specific to cTnI released during myocardial injury</td>
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<tr>
<td>Sensitivity adjusted to detect cTnI at 0.1 ng/ml</td>
<td>Indicative of even minor myocardial injury</td>
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<tr>
<td>Reference band calibrated to give signal equivalent to 1 ng/ml of cTnI</td>
<td>cTnI concentration 0.1-1.0 ng/ml indicates increased risk of adverse cardiac events; cTnI 1 ng/ml is suggestive of AMI; Facilitates training of user</td>
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<tr>
<td>CHEMILUMINISCENCE correlated assay</td>
<td>Extremely Reliable</td>
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<tr>
<td>Whole blood can also be used as a sample</td>
<td>No sample preparation required; Ideally suited for point of care testing, at CCU’s, by clinicians</td>
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<tr>
<td>Assay time of 15 minutes</td>
<td>Rapid turnaround time in reporting of results in emergency rooms</td>
</tr>
<tr>
<td>Unique 3Test pack size</td>
<td>Enables sequential testing of patients; Prevents risk of missing an evolving infarct</td>
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INTERPRETATION OF RESULTS

- **Positive**: If the intensity of the Test band is equal to or greater than the Reference band - cTnI concentration is ≥1.0 ng/ml
- **Negative**: Presence of two coloured bands at Reference (R) and Control (C) regions indicates absence of cTnI or the concentration of cTnI in the specimen is below 0.1 ng/ml
- **Invalid**: The test is invalid if the Control band and/or Reference band is not visible at fifteen minutes. Verify the test procedure and repeat the test with a new device.

Truly ... ... The New Gold Standard!