CIBMTR collects information regarding disease and relapse assessment on the TED level and comprehensive report forms in several places. Several different techniques are commonly used to assess patients for evidence of disease – either residual or progressive disease for those who never achieved remission, or recurrence of disease for those who have achieved a remission. These assessments are crucial, as they represent one measure of success of the transplant.

In general, disease can be assessed by several different methods. Techniques to assess continue to evolve. Since different techniques have different levels of sensitivity (different abilities to detect whether or not the disease is present), CIBMTR now collects information on when the disease was evaluated (or recurrence was detected), and the technique(s) employed. This also becomes important since, in some cases, physicians are not always certain that minimal evidence of disease detected by a method with high sensitivity represents enough evidence to consider the disease to have relapsed or to act upon. In some cases, the physician will wish to repeat the sensitive test in a few months to determine whether there is increasing evidence of disease by the same method, or whether the disease becomes evident by another less sensitive method. This situation is accommodated on the CIBMTR forms by providing space to record whether disease was detected by a certain method, AND, an indicator for whether disease that was detected was considered to represent a relapse or progression.

In general, the categories of disease/recurrence detection provided on the TED and cRF forms are divided by level of sensitivity: hematologic/clinical, cytogenetic/FISH and molecular. Below, the level of sensitivity and types of testing that belong in each category are described. Not all techniques are applicable to all diseases for which HCT is performed. In other cases, new techniques are emerging but have not yet achieved acceptance for routine use.

**Hematologic/clinical:**

These techniques are the least sensitive at detecting evidence of disease. Testing that generally would be considered clinical or hematologic include:

- Clinical examination to determine lymph node enlargement.
Example: A physician feels a lymph node mass of 3 cm in the left neck that was not present a few months ago. (In general, this would also be biopsied to confirm lymphoma)

**Clinical examination** of a mass lesion.
- Example: A physician sees and feels a new nodule involving the skin and subcutaneous tissue that on biopsy shows leukemia cells (leukemia cutis).

**Radiographic examinations**, including x-rays, CT scans, MRI scans and PET scanning.
- Examples:
  - A physician reviews a CT scan for a patient with lymphoma that demonstrates a lymph node mass in the mid chest region, or enlargement of a mass previously known to represent lymphoma.
  - A PET scan is performed and demonstrates new areas of lymph node enhancement believed to represent recurrent lymphoma.
  - A Chest x-ray shows new lytic bone lesions in several ribs of a patient with multiple myeloma.

**Histologic and immunohistochemical staining** of biopsy tissues (including mass lesions, lymph nodes or bone marrow biopsy).
- Examples:
  - Bone marrow biopsy with histologic staining revealing 31% lymphoid blasts in a patient with ALL.
  - Lymph node biopsy with histologic staining showing follicular lymphoma, and immuno staining positive for CD 20.
  - Bone marrow biopsy with 60% plasma cells, and special staining demonstrating kappa light chain clonality.

**Some blood/serum and urine tests**, including detection of circulating malignant cells (blasts, plasma cells) by *routine histology, serum or urine protein electrophoresis* (plasma cell disorders).
- Examples:
  - Routine CBC followed by microscopy of peripheral blood smear with circulating myeloid blasts.
  - Serum quantitative immunoglobulins demonstrating increase in levels of IgG in patient being followed for IgG myeloma.

**Cytogenetic/FISH:**

These tests, generally performed on blood or bone marrow specimens, are more sensitive than clinical/hematologic testing. However, there is a range of sensitivity within this category of tests. In general, the range of detection is between 1 in 20 to as much as 1 in
1,000 cells. Routine cytogenetics are performed by culturing cells until they are in dividing phase, then doing techniques to visualize the chromosomes during division so that various bands/reconfigurations can be seen. This is called karyotyping. Typically, karyotyping is done for leukemia and other hematologic malignancies, most often using a bone marrow specimen. A more sensitive technique that assesses a larger number of cells is fluorescent in situ hybridization (FISH). In this technique special “probes” are mixed with cells from blood which recognize and bind to fragments of DNA that are commonly found in certain diseases. The binding of the probe to the disease cells is then visualized using a fluorescent ‘tag.’ This technique, capable of detecting 1 in 100 or as much as 1 in 1,000 cells is commonly used for some hematologic malignancies, like MDS, myeloma and leukemia. However, the abnormality to be detected by the probe must be known to be present in the patient from the time of diagnosis. Note, FISH testing for sex chromosome after sex mismatched allogeneic HCT should not be considered disease assessment, by itself.

- **Cytogenetics** for detection of AML:
  - Examples:
    - Bone marrow aspirate specimen with 10 of 20 cells positive for trisomy 8 by routine karyotyping.
    - Bone marrow aspirate specimen with 456 of 1,000 cells positive for translocation 8:22 by FISH testing.

- **Flow cytometry** is a technique that can be performed on blood, bone marrow or tissue preparations where cell surface markers can be quantified on cellular material. Flow cytometry is routinely performed to better characterize lymphomas, for instance. Its most common application is in establishing a diagnosis, however, these techniques are increasingly being used to evaluate for disease recurrence. Although newer techniques are emerging to increase the sensitivity of flow cytometric testing, in general the level of sensitivity is 1 in 1,000 to 1 in 10,000 cells. As such, CIBMTR is collecting this method of assessment/detection in the same category as cytogenetics.

A future version of CIBMTR data collection forms will capture flow cytometry as a specific method of disease detection. In the meantime, since the level of sensitivity is similar to that of FISH, flow cytometry assessments may be reported in the same category as FISH cytogenetics on CIBMTR forms.

- Example: Flow cytometry of peripheral blood to detect AML. The flow cytometry results could then be reported in Q76-79 “Was the disease status assessed via FISH?” on F2110 (Post-HSCT AML).
Molecular testing:

This technique is the most sensitive available to evaluate presence of disease. It most often applies to hematologic malignancies. For these tests, material from blood, bone marrow or other specimens is treated in a way to amplify the expression of the marker (usually a protein) being tested. This is usually done using polymerase chain reactions (PCR). Then, a probe is applied, and subsequently quantified. Using this technique, the level of sensitivity is generally 1 in 100,000 or 1 in 1,000,000 cells detectable. However, in many cases these techniques are so sensitive that there may be questions as to whether, and at what level, a positive result represents clinically meaningful recurrence of disease. In many cases, a single measure is not considered meaningful by itself, but rather in the context of repeated measures that represent a trend.

The best example of molecular testing is use of RT PCR for detection of bcr/abl protein in serum of patients with CML.