BR-MA, GI-MA and OM-MA: Immunoassays for the Tumor Markers CA15-3, CA19-9 and CA125

Paul E. C. Sibley, Ph.D.
EURO/DPC Limited
BR-MA, GI-MA and OM-MA:
Immunooassays for the Tumor Markers CA15-3, CA19-9 and CA125

During the past decade, a number of new tumor marker tests have been evaluated. Three of the most clinically important markers to emerge for treatment follow-up are CA15-3 in breast cancer, CA19-9 in pancreatic and gastrointestinal cancer, and CA125 in ovarian cancer. These are valuable for patient monitoring and are in routine use worldwide.1 All three markers are high-molecular-weight molecules: CA15-3 and CA19-9 are mucins, and CA125 is a glycoprotein.

Diagnostic Products Corporation has recently introduced automated, chemiluminescent assays — IMMULITE® BR-MA, IMMULITE GI-MA and IMMULITE OM-MA — for the detection of CA15-3, CA19-9 and CA125, respectively. DPC has also released two assays in an immunoradiometric format: IRMA-Count® BR-MA and IRMA-Count OM-MA; a third immunoradiometric assay, for CA19-9, is expected soon.

This report contains comparison data between the DPC assays and other methods for measuring CA15-3, CA19-9 and CA125, as well as information on the character and clinical use of each of these markers.

BR-MA for the Detection of CA15-3

The MUC-1 breast cancer antigen known as CA15-3 is one of the most commonly used circulating tumor markers for monitoring metastatic breast cancer. It is a high-molecular-weight, polymorphic epithelial mucin (300–450 kDa) with a repeated protein core sequence encapsulated by carbohydrate.

Both the IMMULITE and the IRMA-Count BR-MA assays use a pair of monoclonal antibodies — Ma552 and Ma695 — for the detection of CA15-3. Epitope mapping studies of these antibodies have shown that one is specific for the hexapeptide [-TRPAPG-], while the other recognizes a sialylated carbohydrate epitope on the MUC-1 mucin.2 The antibodies have been extensively characterized by immunohistochemistry, immunoblotting and inhibition studies.3

Several studies have shown CA15-3 to be a more sensitive and specific marker than carcinoembryonic antigen (CEA) in breast cancer follow-up. Elevated levels of CA15-3 can be detected in a high proportion of sera from breast cancer patients; in some disease stages, 98 percent of patients show raised levels. Higher values have also been noted in lung cancer (63 percent) and ovarian cancer (80 percent).

The primary use of this marker is in following the clinical course of breast cancer, detecting metastatic disease progression, and monitoring response to therapy.

Two recently published studies have evaluated DPC’s BR-MA assays for CA15-3 in breast cancer patients during therapy.4,5

In the first study, longitudinal samples (n = 77) were taken from four breast cancer patients followed for a period of 28 to 35 months. The two DPC assays were compared to the Sorin CA15-3 K IRMA, which uses the monoclonal antibodies designated 115D8 and DF3 to measure CA15-3. Method comparisons are shown in Figures 1 and 2.

![Figure 1](image-url)
It is evident from the graphs that both of the DPC BR-MA assays correlate closely with the Sorin CA15-3 assay, but also that the IRMA-Count BR-MA assay can be expected to yield somewhat lower absolute values than the other two assays. This is clearly the case for one patient from this series shown in Figure 3, where the pattern of the markers follows the same course for each assay, in spite of marked assay-to-assay differences in absolute values.

Another patient from the series (Figure 4) shows a cyclical pattern of the CA15-3 marker by all three methods; and there was close agreement between the isotopic and nonisotopic assays, and even between assays using different antibodies.

The results shown above illustrate some of the difficulties involved in comparing tumor marker assays. Differences in absolute values are due to a variety of factors, including assay format, calibration, choice of antibodies and antigenic determinants, and the presentation of antigenic sites in patient samples. These problems have been well documented in the case of a related antigen, CA125, and are partly responsible for there being no International Reference Preparation for any of the CA series of tumor markers. Attempts, however, are being made to rectify this situation.

In a larger patient series, comparisons were made between the two DPC assays for BR-MA, the CIS ELSA CA15-3 IRMA, the Abbott IMx CA15-3 and the Boehringer Mannheim ES300 CA15-3. The study consisted of 32 breast cancer patients followed longitudinally.

Linear regression analysis of the two DPC kits against the CIS CA15-3 IRMA for all samples (n = 206) in this series yielded the following relationships.

**Linear regression equation:**

\[(\text{IMMULITE}) = 1.47 \times (\text{CIS}) - 4.5 \text{ kU/L}\]

\[r = 0.94 \quad n = 206\]

Range: 10–10,000 kU/L
(IRMA-Count) = 0.97 (CIS) – 2.3 kU/L
r = 0.97
n = 206
Range: 10–10,000 kU/L

Three examples from this series are shown in Figures 5, 6 and 7.

**Figure 5.** Progressive disease diagnosed initially, with lymph node involvement. Treatment: anti-estrogen therapy.

**Figure 6.** Metastatic disease diagnosed initially. Treatment with chemotherapy was followed by rapid disease progression.

For each of the three patients shown here, the pattern of marker changes is essentially the same by all methods. Indeed, very few discrepancies were observed throughout the entire 32-patient series. In Figure 7, the higher CA15-3 values generated by the IMMULITE BR-MA assay result in steeper rises and falls in the marker levels, which could be a distinct advantage in patient monitoring.

It was concluded that essentially the same clinical information is obtained with the IMMULITE and IRMA-Count BR-MA assays as compared to other methods for CA15-3, even those using different antibodies.

**GI-MA for the Detection of CA19-9**

The monoclonal antibody, C192, used in the IMMULITE GI-MA assay detects a sialylated Lewis\(^a\) blood group antigen designated CA19-9. This antigen is expressed in the circulation of some colorectal and pancreatic cancer patients as a secreted, high-molecular-weight, mucin glycoprotein. The C192 monoclonal antibody has been extensively characterized in specificity and inhibition studies.

Dr. Arie van Dalen has recently compared the IMMULITE GI-MA assay to the CIS ELSA CA19-9 IRMA on a total of 47 samples from 17 patients with gastrointestinal (GI) malignan-
cies, followed longitudinally.\textsuperscript{9} Linear regression analysis of the results yielded the following relationship.

\[
\text{(IMMULITE)} = 0.89 \times (\text{CIS}) + 4.0 \text{ kU/L} \\
r = 0.96 \\
n = 47 \\
\text{Range: 1–500 kU/L (CA19-9)}
\]

In this series, a close correlation was observed between the IMMULITE GI-MA assay and the CIS assay. (The latter uses the Centocor monoclonal antibodies to detect CA19-9.) Still more important to the evaluation of marker assays, the two methods exhibited comparable patterns. Three colon carcinoma cases, representative examples from this patient series, are shown below.

\textbf{Figure 8.}

In Figure 8, sample 1 is preoperative and sample 2 postoperative, with the CA19-9 marker showing a characteristic decrease in serum concentration. Following 13 months with no evidence of disease, sample 3 showed an increase in CA19-9 as detected by both assays. This increase continued during the next 16 months, at which point there was clinical evidence of liver metastases.

\textbf{Figure 9.}

In Figure 9, sample 1 is preoperative. Samples 2 and 3 were taken 5 months and two months, respectively, before liver metastases became clinically apparent. High CA19-9 levels were detected by both the IMMULITE GI-MA assay and CIS assay.

\textbf{Figure 10.}

In Figure 10, sample 1 is preoperative. Sample 2 was taken 2 months before liver metastases were diagnosed (sample 3). Sample 4 was taken 2.5 months after the diagnosis of metastases.

These figures show that the IMMULITE GI-MA and CIS CA19-9 results exhibited similar patterns during follow-up for all three patients, although
the absolute values were not always identical: for example, in Figure 10, a more significant rise was observed with the IMMULITE GI-MA assay for CA19-9 than was seen with the CIS assay.

From the full series of patients, Dr. van Dalen concluded that the two assays behave identically with respect to the interpretation of marker changes during patient follow-up. Even though one assay might yield CA19-9 values higher or lower than the other, comparable clinical information is obtained from both.

**OM-MA for the Detection of CA125**

The CA125 determinant is a high-molecular-weight (>1,000 kDa), nonmucinous glycoprotein. The antigen detected by assays for CA125 is repeated in the protein core of the molecule, and was characterized as having three epitopes by the 1994 ISOBM CA125 Workshop. The monoclonal antibody used in the DPC IMMULITE and IRMA-Count OM-MA assays is OV185, which detects the M11 epitope of CA125.

Elevated levels of CA125 have been reported in patients with cancers of the ovary, pancreas, stomach, colon and rectum; in benign gynecological conditions, such as endometriosis; and in normal pregnancy. The primary use of CA125 assays is for the follow-up of ovarian cancer patients, where the serum concentration correlates with tumor mass.

Two recent European studies have evaluated both the IMMULITE and the IRMA-Count OM-MA assays against a variety of other assays for CA125.5,11

In the first study, longitudinal samples (n = 55) were taken from four ovarian cancer patients who were followed for a period of 5 to 13 months. A comparison of the two DPC assays was made against the Sorin CA125 II IRMA, which uses antibodies against both the M11 and OC125 epitopes of CA125.

Figure 11a depicts the relationship between IMMULITE and Sorin results across the entire concentration range exhibited by the samples; while Figure 11b displays the results for the 27 samples in this study with both IMMULITE and Sorin values less than 900 kU/L.
Figure 12 shows how IRMA-Count and Sorin results in this study compared throughout the entire range of the samples.

![Figure 12](image)

Figure 13 shows one patient from the series whose CA125 (OM-MA) results exhibited rapid increases, as measured by all three assays. It was reported that both isotopic and nonisotopic assay methodologies can provide similar information. The automated IMMULITE system is particularly convenient for analyses of this type.

![Figure 13](image)

In a larger patient series, comparisons were made between the DPC IMMULITE OM-MA and IRMA-Count OM-MA, the CIS CA125 IRMA, Abbott IMx CA125 and Boehringer Mannheim ES 300 CA125. This was a collaborative study by Drs. A. van Dalen, A. C. W. Swart and E. Sanders at three hospitals in Holland. Thirty-two patients were followed longitudinally, and samples from 55 normal female subjects were assayed to establish a reference range, tabulated below.

<table>
<thead>
<tr>
<th></th>
<th>IMMULITE</th>
<th>IRMA-Count</th>
<th>CIS ELSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>1.3</td>
<td>1.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Median</td>
<td>5.0</td>
<td>12.0</td>
<td>15.0</td>
</tr>
<tr>
<td>95th Percentile</td>
<td>16.7</td>
<td>26.0</td>
<td>51.0</td>
</tr>
<tr>
<td>Maximum</td>
<td>24.9</td>
<td>41.0</td>
<td>55.0</td>
</tr>
</tbody>
</table>

Based on the tabulated results, both the IMMULITE OM-MA and the IRMA-Count OM-MA would be expected to yield lower values than the CIS assay in longitudinal studies of patient samples. Three examples from the series are shown below.

![Figure 14](image)

Diagnosis at time of first sample was multi-focal extra-ovarian carcinoma (FIGO Stage III). Following chemotherapy, stable disease was clinically confirmed.
First sample was from a pretreated, endometroid-type, ovarian carcinoma (FIGO Stage II). Following a debulking operation, no treatment was given until chemotherapy courses were started in Sep-93, based on rising CA125 levels.

First sample was from a preoperative, serous-type, ovarian carcinoma (FIGO Stage III). The patient was in remission until Feb-92, but subsequently relapsed (clinically confirmed in Jun-92).

It is quite apparent that all of the assays yield the same information, closely related to the clinical data, even though the absolute values do vary.

Conclusions

A variety of studies have clearly demonstrated that DPC’s IMMULITE and IRMA-Count BR-MA, GI-MA and OM-MA assays (for CA15-3, CA19-9 and CA125, respectively) yield essentially the same information as other assays for these markers in longitudinal patient follow-up. Moreover, good agreement among methodologies has been demonstrated for all three markers by linear regression analysis across a wide range of antigen concentrations. However, as expected for these high-molecular-weight tumor markers, results are not always quantitatively the same from method to method. This is due mainly to differences in assay formats and antibodies. There are no International Reference Preparations (IRPs) for these markers; consequently, external quality control schemes often show large intermethod variation. Cutoff levels are not always the same, and have been known to change. Accordingly, a period of duplicate measurement is essential when switching from one assay format to another.

The IMMULITE and IRMA-Count BR-MA, GI-MA and OM-MA assays are both effective and convenient in the long-term follow up of breast, ovarian, and gastrointestinal cancer patients. A number of other clinical studies are progressing with these tumor marker assays. All of the assays are becoming widely accepted throughout the world.

References


DPC’s Tumor Marker Assays for CA15-3, CA19-9 and CA125

For treatment monitoring of breast, pancreatic and gastrointestinal, and ovarian cancer patients.

DPC has recently introduced three new kits for the IMMULITE, a continuous random access, chemiluminescent immunoassay system.

- BR-MA for the detection of CA15-3
- GI-MA for the detection of CA19-9
- OM-MA for the detection of CA125

**IMMULITE® BR-MA, GI-MA and OM-MA Kits: Essential Assay Features**

<table>
<thead>
<tr>
<th>Feature</th>
<th>BR-MA (CA15-3)</th>
<th>GI-MA (CA19-9)</th>
<th>OM-MA (CA125)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalog Number</td>
<td>LKBR</td>
<td>LKGI</td>
<td>LKOM</td>
</tr>
<tr>
<td>Configuration</td>
<td>Two-Step</td>
<td>Two-Step</td>
<td>Two-Step</td>
</tr>
<tr>
<td>Sample volume</td>
<td>5 µL</td>
<td>50 µL</td>
<td>50 µL</td>
</tr>
<tr>
<td>Incubation time</td>
<td>60 min</td>
<td>60 min</td>
<td>60 min</td>
</tr>
<tr>
<td>Working range</td>
<td>1.0 – 300 U/mL</td>
<td>2.5 – 1000 U/mL</td>
<td>1.0 – 500 U/mL</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.5 U/mL</td>
<td>2.0 U/mL</td>
<td>0.3 U/mL</td>
</tr>
<tr>
<td>Hook</td>
<td>&gt; 23,500 U/mL</td>
<td>&gt; 34,000 U/mL</td>
<td>&gt; 95,400 U/mL</td>
</tr>
</tbody>
</table>

In addition, the following immunoradiometric assays have been released:

- IRMA-Count BR-MA for the detection of CA15-3
- IRMA-Count OM-MA for the detection of CA125

An immunoradiometric GI-MA for detection of CA19-9 is expected soon.