The CA19-9 assay kit is intended to be used as a monitoring and screening test. An abnormal result (i.e. an elevated serum CA19-9) suggests the need for further clinical management. This test has been found useful for patients in clinical remission, as post-operative serum CA19-9 values which fail to return to normal strongly suggest the presence of residual tumor and tumor recurrence is often accompanied by a rise of serum levels before progressive disease is clinically evident.

**Intended Use**

The CA19-9 assay kit is intended to be used as a monitoring and screening test. An abnormal result (i.e. an elevated serum CA19-9) suggests the need for further clinical management. This test has been found useful for patients in clinical remission, as post-operative serum CA19-9 values which fail to return to normal strongly suggest the presence of residual tumor and tumor recurrence is often accompanied by a rise of serum levels before progressive disease is clinically evident.

**Materials and Components**

Materials provided with the test kits:
- Murine monoclonal anti-CA19-9 coated plate with 96 wells.
- Assay Buffer, 12 mL.
- Enzyme conjugate reagent, 12 mL.
- CA19-9 reference standards containing 0, 15, 30, 60, 120, and 240 U/mL CA19-9 (liquid, ready for use) or lyophilized form.
- TMB Substrate, 12 mL.
- Stop solution, 12 mL.
- Wash Buffer Concentrate (50X), 15 mL.

Materials required but not provided:
- Precision pipettes and tips, 0.04~0.2mL, 1.0mL
- Distilled water.
- Vortex mixer
- Absorbent paper or paper towel
- Graph paper
- A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at a wavelength of 450nm

**Specimen Collection and Preparation**

1. Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as practical. Avoid grossly hemolytic, lipemic or turbid samples.

2. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.

3. Specimens should be capped and may be stored up to 48 hours at 2-8°C prior to assaying. Specimens held for a longer time can be frozen at -20°C. Thawed specimens must be mixed prior to testing.

**Storage of test kits and instrumentation**

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. One year from the date of manufacture. Refer to the package label for the expiration date.

2. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above.

3. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

**Reagent Preparation**

1. All reagents should be brought to room temperature (18-22°C) before use. All reagents should be mixed by gently inverting or swirling prior to use. Do not induce foaming.

2. If reference standards are lyophilized, reconstitute each standard with 0.5 mL distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be sealed and stored at 2-8°C.

3. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 mL of Wash Buffer (50x) into 735 mL of distilled water to prepare 750 mL of washing buffer (1x). Mix well before use.

**Calculation of Results**

Calculate the mean absorbance value for each set of CA19-9 reference standards, specimens, and controls. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in units per mL on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of CA19-9 in units per mL from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.
Results of typical standard run with optical density reading at 450nm shown in the Y axis against CA19-9 concentrations shown in the X axis.

### Performance characteristics

1. **Accuracy:** Comparison between Our Assay and commercial available Kits provide the following data
   - N = 48
   - Correlation Coefficient = 0.966
   - Slope = 0.908
   - Intercept = 2.32
   - Mean (Our Kits) = 36.10
   - Mean (Abbott) = 33.18

2. **Precision.**

1. **Intra-Assay**

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>N</th>
<th>Mean</th>
<th>S.D.</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level</td>
<td>14</td>
<td>11.76</td>
<td>1.098</td>
<td>9.81</td>
</tr>
<tr>
<td>Level I</td>
<td>14</td>
<td>33.15</td>
<td>2.160</td>
<td>6.52</td>
</tr>
</tbody>
</table>

2. **Inter-Assay**

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>N</th>
<th>Mean</th>
<th>S.D.</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level</td>
<td>14</td>
<td>11.76</td>
<td>1.098</td>
<td>9.81</td>
</tr>
<tr>
<td>Level I</td>
<td>14</td>
<td>33.15</td>
<td>2.160</td>
<td>6.52</td>
</tr>
</tbody>
</table>

3. **Linearity**

Two patient sera were serially diluted with 0 U/mL standard in a linearity study. The average recovery was 102.7 %.

<table>
<thead>
<tr>
<th>Sample A</th>
<th>Dilution</th>
<th>Expected</th>
<th>Observed</th>
<th>% Recov.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted</td>
<td>192.43</td>
<td>192.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2x</td>
<td>96.22</td>
<td>98.11</td>
<td>102.0</td>
<td></td>
</tr>
<tr>
<td>4x</td>
<td>48.10</td>
<td>50.01</td>
<td>104.0</td>
<td></td>
</tr>
<tr>
<td>8x</td>
<td>24.05</td>
<td>25.98</td>
<td>108.0</td>
<td></td>
</tr>
<tr>
<td>16x</td>
<td>12.02</td>
<td>13.11</td>
<td>109.1</td>
<td></td>
</tr>
</tbody>
</table>

**Average Recovery:** 105.8 %

<table>
<thead>
<tr>
<th>Sample B</th>
<th>Dilution</th>
<th>Expected</th>
<th>Observed</th>
<th>% Recov.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted</td>
<td>220.77</td>
<td>220.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2x</td>
<td>110.39</td>
<td>106.31</td>
<td>96.3</td>
<td></td>
</tr>
<tr>
<td>4x</td>
<td>55.19</td>
<td>56.03</td>
<td>101.5</td>
<td></td>
</tr>
<tr>
<td>8x</td>
<td>27.60</td>
<td>26.92</td>
<td>97.5</td>
<td></td>
</tr>
<tr>
<td>16x</td>
<td>13.80</td>
<td>14.25</td>
<td>103.3</td>
<td></td>
</tr>
</tbody>
</table>

**Average Recovery:** 99.7 %

### References


### Limitations of the Procedure

There are some limitation of the assay. We should let our customers know about that.

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee to eliminate all the effects of that.
3. The wash procedure (steps 6-8) is critical. Insufficient washing will result in poor precision and falsely elevated absorbance. The use of tap water for washing could result in a higher background absorbance.

### Expected Values and Sensitivity

Healthy individuals are expected to have CA19-9 assay values below 35 U/mL. The minimum detectable concentration of CA19-9 in this assay is estimated to be 5 U/mL.