The discovery of a monoclonal antibody clone (116NSN 19-9), which exhibited selective reactivity with human gastrointestinal antigens, is summarized in the following equation:

\[ Ag(CA 19-9) + E n z y m e - a n t i b o d y \rightarrow \text{Immunocomplex} + E n z y m e - a n t i b o d y \]

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. The interaction is illustrated below:

\[ Ag(CA 19-9) + E n z y m e - a n t i b o d y \rightarrow \text{Immunocomplex} + E n z y m e - a n t i b o d y \]

After a suitable incubation period, the antigen-antibody bound fraction is separated from unbound antigen by precipitation or adsorption. Another enzyme (biotinylated antibody) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled antigen-biotinylated antibody complex on the surface of the wells. The excess, if any, is washed away via a wash step. A substrate suitable for the enzyme is added. Color measurement is made using a microplate spectrophotometer. The enzymatic activity, determined by reaction with a substrate (luminol) that generates light, is measured. The ratio of enzyme activity, determined by reaction with a substrate (luminol) that generates light, is measured. The ratio of enzyme activity, determined by reaction with a substrate (luminol) that generates light, is measured.

The specimens shall be blood serum in type, and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting serum sample should be obtained. The blood should be collected in a plain red-top tube. The samples shall be separated from the serum within one hour. The specimens shall be stored at 2-8°C. Centrifuge the specimen to separate the serum from the cells. One (1) vial contains 1.0 ml/vial - Icons A-F.

Samples may be refrigerated at 2-8°C for a maximum period of 4 days (if made). DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION. The results should be read within 30 minutes after mixing. If complete utilization of the reagents is anticipated within 2 hours, the contents of Signal Reagent B into Signal Reagent A and label accordingly.

The results should be read within 30 minutes after mixing. If complete utilization of the reagents is anticipated within 2 hours, the contents of Signal Reagent B into Signal Reagent A and label accordingly.

Note: Do not use reagents that are contaminated or have bacterial growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C). Performing the test should be performed by a skilled individual or trained professional**

1. Format the microtiter wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. Place the test plate with the wells facing up into the microplate washer. Attach the cover. Pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

2. Pipette 0.025 ml (25 µl) of the biotinylated labeled antibody to each well. It is very important to dispense all reagents down the bottom of the well.

3. Place the cover on the plate and proceed to step 4.

4. Swirl the microtiter plate gently for 20 seconds to mix and cover. Inoculate 30 minutes at room temperature.

5. Place the cover on the plate and proceed to step 6.

6. Wash the plate by decantation or aspirate. Follow the manufacturer's instructions for proper usage. If actual buffer is used, filter each well before the buffer to the container (avoiding air bubbles) to ensure the wash.

7. Discard the plate and blot the plate dry with paper towels.

8. Add 0.350 ml (350 µL) of wash buffer. Store at 2-8°C (see Reagent Preparation Section).

9. After the last pipetting of strips has occurred, pour the contents of Reagent Preparation into the microplate washer. DO NOT SHAKE THE PLATE AFTER TRACER ADDITION.

10. Discard the sample from the microplate by decantation or aspirate. Repeat four (4) additional times.

11. Add 0.350 ml (350 µl) of wash buffer. Store at 2-8°C (see Reagent Preparation Section). DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION.

12. Incubate for five (5) minutes in the dark. After the incubation period is completed, a white precipitate is formed. The precipitate may be observed in any form. After a 10 minute incubation, pour the contents of Reagent Preparation into the microplate washer. DO NOT SHAKE THE PLATE AFTER TRACER ADDITION.

13. Filter off the precipitate and continue to incubate for 30 minutes at room temperature.

The results should be read within 30 minutes of adding the signal solution.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of CA19-9 in unknown specimens. A standard curve is plotted from the printout of the microplate reader as outlined in Example 1.

2. Plot the absorbance for each duplicate serum reference versus the concentration of CA19-9 (μg/ml) in the unknown sample. The concentration of CA19-9 in the unknown sample is determined by interpolation of the unknown curve on the graph. The results should be read within 30 minutes of adding the signal solution.
1. The Dose Response Curve should be within established ranges. This conversion minimizes differences in "greatest light output." The MSDS and Risk Analysis Form for this product are available on request from Monobind.

12. Risk Analysis - as required by CE Mark IVD Directive 98/79/EC for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation
1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate minimal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988;34:273-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.

12.3 Accuracy
1. The CA 19-9 AccuLite® CLIA test system has a sensitivity of 0.04 U/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.4 Specificity
1. In order to test the specificity of the antibody pair used massive concentrations of possible cross-reactants were added to known serum pools and assayed in parallel with the base sera. No cross reactions was found. Percent cross-reactions for some of these additions are listed below in Table 5.

15.0 REFERENCES