Upon mixing monoclonal biotinylated antibody, and a serum containing the monoclonal antibody reaction between the native antigen and the antibody, forming an antibody-antigen complex. The interaction is illustrated by the following equation:

\[ \text{Ag}_{\text{CA15-3}} + \text{Ab}_{\text{Bi1}} + \text{Ab}_{\text{Bi2}} \xrightarrow{k_a} \text{Ag}_{\text{CA15-3}} - \text{Ab}_{\text{Bi1}} - \text{Ab}_{\text{Bi2}} \]

**4.1 Required But Not Provided:**
1. Pipette capable of dispensing 0.025ml (25 µl) and 0.050ml (50 µl) with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 ml (100 µl) and 0.350 ml (350 µl) with a precision of better than 1.5%.
3. Pipette (100 µl) used for serum diluent in patient dilutions.
4. Microplate washer or a squeeze bottle (optional).
5. Microplate Reader with 450nm and 600nm wavelength absorbance capability.
6. Absorbance Paper for blotting the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
9. Pipette (optional) for the appropriate diluted calibrator, control or specimen into the assigned well.
10. Quality control materials.

**5.0 PRECAUTIONS**

For In Vitro Diagnostic Use

**6.0 SPECIMEN COLLECTION AND PREPARATION**

The specimens shall be blood serum or heparinized plasma in type and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal ranges, a fasting sample should be obtained. The blood should be collected in a redtop (with or without additive) vial for plasma use evacuated tube(s) containing heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells. Store the sample at 2-8°C before use. Do not use reagents beyond the kit expiration date.

**7.0 QUALITY CONTROL**

Each laboratory should assay controls at levels in the low, normal, and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in each assay run. Immediate interpretation of control chart should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trend. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

**8.0 REAGENT PREPARATION**

1. Wash Buffer: Dilute stock of wash solution to 1000 ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at room temperature (2-30°C) for up to 60 days.

2. Patient Sample Dilution (1:20): Dilute 0.025ml (25 µl) of each control and/or patient specimen into 0.500ml (500 µl) of CA-15-3 dilution matrix appropriately labeled, clean container(s) and mix thoroughly before use. Store refrigerated at 2-8°C for up to 48 hours.

**9.0 TEST PROCEDURE**

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

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicating fashion. Place an unused microwell strip across the aluminum bag, seal and store at 2-8°C.
2. Place the appropriate diluted calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100 µl) of the biotinylated labeled antibody to each well. This step is very important to dispense all reagents close to the bottom of the coated well.
4. Swirl the microplate gently for 20-30 seconds to mix and cover. Incubate for 60 minutes at room temperature (20-27°C).
5. Decant the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
6. Add 0.350ml (350 µl) of wash buffer (see Reagent Preparation Section) to each well, mix and cover. Incubate 30 minutes at room temperature (20-27°C).
7. Add 0.100 ml (100 µl) of the CA-15-3 Enzyme Reagent to each well. Incubate at room temperature for thirty (30) minutes.
8. Add 0.100 ml (100 µl) of the CA-15-3 Enzyme Reagent to each well. Incubate at room temperature for thirty (30) minutes.

**DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION**

9. Cover and incubate 60 minutes at room temperature. 10. Add 0.100 ml of each dilution matrix to the microwells.
11. Add 0.100 ml of each calibration matrix to the microwells.
12. Add 0.350ml of wash buffer (see Reagent Preparation Section) to each well, mix and cover. Incubate 30 minutes at room temperature (20-27°C).
13. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times, and add 0.350ml of substrate wash and the contents of the microplate can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1998, HHS Publication No. (CDC) 88-8395.

**10.0 CALCULATION OF RESULTS**

A dose response curve is used to ascertain the concentration of CA15-3 in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader for each sample.
2. Plot the absorbance for each duplicate serum reference versus the corresponding CA-15-3 concentration in U/ml (see Figure 1). Each microplate contains three duplicates of the serum reference (see Figure 1).
3. Connect the points with a best-fit curve.
4. To determine the concentration of CA-15-3 for an unknown, locate the average absorbance of the duplicates for each unknown, and plot it on the graph. The intersection point on the curve, the concentration (in U/ml) which is indicated by the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the absorbance (0.721) intersects the dose response curve at (58.4U/ml) CA-15-3 concentration (See Figure 1).

**Note:** The EC50 value is defined as the concentration of the analyte that produces a 50% response of the maximum observed absorbance.

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The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.0 RISK ANALYSIS

1. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

13. 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. Measurement 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 maximal 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 antibodies; a problem for all immunoassays'. Clin. Chem. 1988;34:273). For diagnostic purposes, the results from this assay should be in concert with clinical examination, patient history and all other clinical findings.

4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.

5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.

6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the expected values.

7. It is important to keep in mind that establishment of a range of "normal"-persons is dependent upon a multiplicity of conditions.


13. 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.

14.5 Specificity

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 reactivity was found. Percent cross-reactions for some of these additions are listed below in Table 5.

Table I

<table>
<thead>
<tr>
<th>Sample</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
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<tbody>
<tr>
<td></td>
<td>N</td>
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<td>C.V.</td>
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It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a specific analyte is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons tightly controlled populations are essential. The number of expected values established by the Manufacturer only until an in-house range can be determined by the analyst using the system described in this Manual. The range tested is dependent upon the population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

12.1.2 Sensitivity

The CA 15-3 procedure has an analytical sensitivity of 0.2 U/ml at three (3) SD from the zero calibrator. The functional sensitivity (20% CV) was found to be 1.25U/ml.

14.1 Precision

The within and between assay precision of the CA 15-3 AccuBind® ELISA test system was determined by analyses on three different levels of control sera. The number, mean value, standard deviation (σ) and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

Table II

<table>
<thead>
<tr>
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15.0 REFERENCES


12. NCCLS, 'Assessment of Laboratory Tests When Proficiency Testing is Not Available; Approved Guidelines. 2008.'