

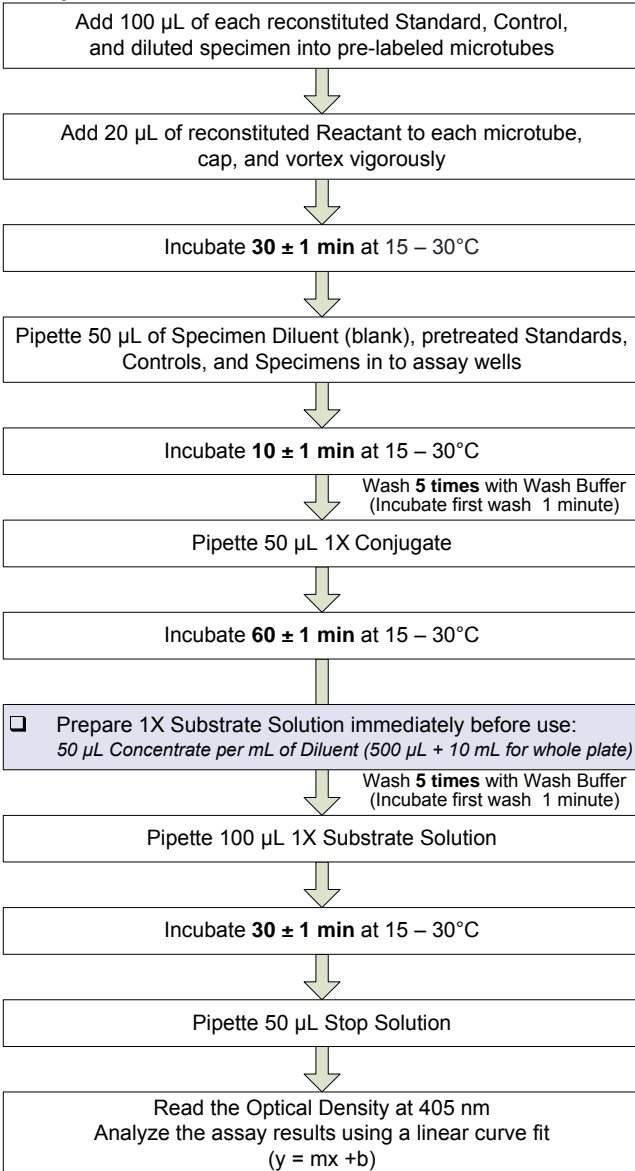
Enzyme immunoassay to measure the amount of functional C1-Inhibitor protein in human plasma or serum

## C1-Inhibitor EIA Summary

### Reagents, Standards, Controls, and Samples Preparation

- Dilute Wash Buffer Concentrate 1:20 with DI Water
- Dilute Specimen Diluent Concentrate 1:5 with DI Water
- Dilute Conjugate Concentrate 1:100 with Conjugate Diluent
- Reconstitute each Standard and Control with 1.0 mL Hydrating Reagent (*let sit for 15 minutes then mix thoroughly*)
- Reconstitute C1-Inhibitor Reactant with 0.5 mL Hydrating Reagent (*swirl gently and let sit for 15 minutes*) (*1 vial will treat about 25 test samples.*)
- Dilute Specimens 1:101 with 1X Specimen Diluent (*e.g. 10 µL + 1 mL*)

### Assay Procedure



### INTENDED USE

The MicroVue C1-Inhibitor Enzyme Immunoassay measures the amount of functional C1-Inhibitor protein in human plasma or serum.

### SUMMARY AND EXPLANATION

C1-Inhibitor (C1-INH) is a multispecific, protease inhibitor that is present in normal human plasma and serum and that regulates enzymes of the complement, coagulation, fibrinolytic, and kinin-forming systems.<sup>1</sup> The enzymes (proteases) regulated by this protein include the C1r and C1s subunits of the activated first component of complement, activated Hageman factor (factor XIIa), Hageman factor fragments, activated plasma thromboplastin antecedent (PTA or factor XIa), kallikrein (Fletcher factor) and plasmin.<sup>2</sup>

A deficiency of functionally active C1-INH may lead to life-threatening angioedema. Two major forms of C1-INH deficiency have been reported: the congenital form, termed hereditary angioedema (HAE)<sup>3,4</sup> and the acquired form, which is associated with a variety of diseases, including lymphoid malignancies.<sup>5</sup> Hereditary angioedema is characterized by transient but recurrent attacks of nonpruritic swelling of various tissues throughout the body. The symptomatology depends upon the organs involved. Intestinal attacks lead to a diversity of symptoms including pain, cramps, vomiting, and diarrhea. The most frequent cause of death in this disease is airway obstruction secondary to laryngeal edema occurring during an attack. There are two types of hereditary angioedema that can be distinguished biochemically. Patients with the more common type (85% of HAE patients) have low levels of functional C1-INH and C1-INH antigen. Patients with the second form (15% of HAE patients) have low levels of functional C1-INH but normal or increased levels of C1-INH antigen, which is associated with a dysfunctional protein.<sup>6</sup>

The variable nature of the symptoms at different time-periods during the course of the disease precludes definitive diagnosis based solely on clinical observation. Hereditary or acquired angioedema can only be definitively diagnosed by laboratory tests demonstrating a marked reduction in functional C1-INH levels in a patient's plasma or serum.

Several methods have been reported to measure C1-INH functional or antigenic levels. These methods include enzyme inhibition assays,<sup>7,8</sup> radial immunodiffusion,<sup>9</sup> immunoelectrophoresis, and inhibition of immune hemolysis.<sup>10</sup> Each of these methods has disadvantages. The enzyme inhibition assays<sup>7</sup> are difficult to set up and conduct on a routine basis, the immunochemical methods which measure total antigen cannot distinguish between functional and nonfunctional C1-INH protein, and the anti-C1r immunodiffusion method,<sup>9</sup> which was developed to measure functional C1-INH activity, is not quantitative. The MicroVue assay is able to measure quantitatively the level of functionally active C1-INH protein present in a patient's plasma or serum by utilizing a convenient, standardized and reproducible EIA procedure.

## PRINCIPLE OF THE PROCEDURE

The MicroVue C1-Inhibitor Enzyme Immunoassay for the quantitation of functional C1-Inhibitor protein (a protease inhibitor) in human serum or plasma is a four-step procedure. In the first step, Standards, Controls and test specimens are incubated with C1-Inhibitor Reactant (biotinylated, activated C1 $\bar{5}$ ). During this incubation, functionally active C1-INH present in the Standards, Controls, and test samples will bind to the C1-Inhibitor Reactant to form complexes.

In the second step, an aliquot of the incubation mixtures containing C1-Inhibitor Reactant is added to microtiter wells precoated with avidin. C1-Inhibitor Reactant: C1-INH complexes present in the Standards, Controls or specimens will bind to the avidin-coated microassay wells. After incubation, a wash cycle removes unbound material.

In the third step, horseradish peroxidase (HRP)-conjugated goat anti-human C1-INH is added to each test well. During this step, the HRP-conjugated anti-C1-INH binds to the C1-Inhibitor Reactant: C1-INH complexes that were captured on the surface of the avidin-coated microassay wells. After incubation, a wash cycle removes excess conjugate.

In the fourth step, a chromogenic enzyme substrate is added to each microassay well. The bound HRP-conjugate reacts with the substrate forming a green color. After incubation, the enzyme reaction is stopped chemically, and the color intensity is measured spectrophotometrically at 405 nm. The color intensity of the reaction mixture is proportional to the concentration of functional C1-INH protein present in the test specimens, standards, and controls.

## REAGENTS AND MATERIALS PROVIDED

**C1-Inhibitor Enzyme Immunoassay contains the following:**

- A**
- B** C1-INH Standards      Parts A4469 – A4473      2 ea., 1 mL
- C** (lyophilized) When reconstituted, each contains a known amount
- D** of C1-Inhibitor in human plasma, PBS, stabilizers
- E**

- L** C1-INH Abnormal Control (Human)  
**Part A9524**      **2 x 1 mL**  
 (lyophilized) When reconstituted, each contains human plasma with a low level of C1-Inhibitor in PBS, stabilizers
- N** C1-INH Normal Control (Human)  
**Part A9523**      **2 x 1 mL**  
 (lyophilized) When reconstituted, each contains human plasma with a normal level of C1-Inhibitor in PBS, stabilizers
- 1** Microassay Plate      **Part 4634**      **12 each**  
 Eight-well strips coated with avidin in a resealable foil pouch
- 2** Stop Solution      **Part A3673**      **6 mL**  
 Contains 250 mM oxalic acid
- 3** 20X Wash Solution Concentrate  
**Part A9957**      **2 x 50 mL**  
 When diluted, each contains phosphate buffered saline (PBS), 0.05% Tween-20<sup>®</sup>, and 0.035% ProClin<sup>®</sup> 300
- 4** 5X Specimen Diluent Concentrate  
**Part A9519**      **25 mL**  
 When diluted, contains PBS, stabilizers, 0.035% ProClin 300
- 5** Substrate Diluent      **Part A3672**      **25 mL**  
 Contains 0.1M citrate buffer and 0.05% H<sub>2</sub>O<sub>2</sub>
- 6** Substrate Concentrate      **Part A3671**      **1.5 mL**  
 Contains 0.7% 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), diammonium salt
- 7** C1-Inhibitor Conjugate (100X)  
**Part A9525**      **0.25 mL**  
 Contains a 100X concentrate of peroxidase-conjugated (goat) anti-human C1-Inhibitor in PBS, stabilizers, 0.01% thimerosal
- 8** Hydrating Reagent      **Part A3675**      **25 mL**  
 Contains 0.035% ProClin 300
- 9** Conjugate Diluent      **Part A9526**      **10 mL**  
 Contains PBS, stabilizers, 0.01% thimerosal
- 10** C1-Inhibitor Reactant      **Part A9527**      **4 x 0.5 mL**  
 When reconstituted, each contains biotinylated (biotin conjugated), activated C1 $\bar{5}$  in PBS with stabilizers
- Tween<sup>®</sup> 20 is a registered trademark of ICI Americas Inc.  
 ProClin<sup>®</sup> is a registered trademark of Rohm and Haas Company.

## MATERIALS REQUIRED BUT NOT PROVIDED

- Timer (60 minute range)
- Calculator or other computational method to validate the assay
- Clean, unused microassay plates and/or test tubes and racks
- Container for wash buffer dilution
- Wash bottle or other immunoassay washing system
- Adjustable multichannel pipette (8 or 12 channels) or repeating micropipettes (optional)
- Clean pipettes, 1 mL, 5 mL, and 10 mL
- Micropipettes and pipette tips
- Plate reader capable of optical density readings between 0.0 and 2.0
- Deionized or distilled water

## WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
2. Follow Universal Precautions when handling contents of this kit and any patient samples.
3. Dispose of containers and unused contents in accordance with Federal, State and Local regulations.

4. Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
5. Wear suitable protective clothing, gloves, and eye/face protection when handling contents of this kit.
6. Store assay reagents as indicated.
7. When adding or aspirating liquids from the microassay wells, do not scrape or touch the bottom of the wells.
8. Using incubation times and temperatures other than those indicated in the Procedure section may give erroneous results.
9. Do not allow microassay wells to dry once the assay has begun.
10. Do not use a microassay well for more than one test.
11. Use of multichannel pipettes or repeat pipettors is recommended to ensure timely delivery of reagents.
12. For accurate measurement of samples, add samples and standards precisely. Pipette carefully using only calibrated equipment.
13. Proper collection and storage of test specimens are essential for accurate results.
14. Avoid microbial or cross-contamination of specimens, reagents, or materials. Incorrect results may be obtained if contaminated.
15. Thimerosal is used as a preservative. Incidental contact with or ingestion of buffers or reagents containing thimerosal can lead to increased hypersensitivity reactions including irritation to the skin, eyes, or mouth. Seek medical attention if symptoms are experienced. Exposure to thimerosal may have potential mutagenic effects. Avoid contact with strong acids and bases.
16. ProClin 300 is used as a preservative. Incidental contact with or ingestion of buffers or reagents containing ProClin can cause irritation to the skin, eyes or mouth. Use good laboratory practices to reduce exposure. Seek medical attention if symptoms are experienced.
17. The Substrate Concentrate is light sensitive. Avoid prolonged exposure to bright or direct light. Store reagents in the dark when not in use.
18. To avoid aerosol formation during washing, use an apparatus to aspirate the wash fluid into a bottle containing household bleach.
19. Heat-inactivated, hyperlipemic or contaminated specimens may give erroneous results.

## STORAGE

Store unopened kit at 2–8°C. After the kit is opened, the 20X Wash Solution Concentrate and Hydrating Reagent may be stored at 2–30°C.

After selecting the reagents or materials to be used in the assay, return the unused reagents immediately to their appropriate storage temperatures. Bring reagents and materials to room temperature (15–30°C) before use.

## INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

The Substrate Concentrate may range in color from colorless to pale or dark green. This condition will not influence performance. However, the freshly prepared Substrate Solution should be colorless to pale green. A dark green color indicates that the prepared Substrate Solution has deteriorated, must be discarded, and new Substrate Solution prepared in clean glassware.

Cloudiness or discoloration of the diluted Wash Solution indicates a deterioration of this reagent. If this occurs, the solution should be discarded.

## SPECIMEN COLLECTION AND STORAGE

### Handle and dispose of all specimens using Universal Precautions.

The assay requires at least 10 µL of serum or EDTA plasma. All specimens should be collected aseptically and prepared using standard techniques for clinical laboratory testing. A freshly drawn, non-hemolyzed specimen is preferred. An EDTA plasma sample may be held at room temperature (15–30°C) for up to 24 hours. A serum sample should not be stored at room temperature for longer than six hours. If extended storage is anticipated, the plasma or serum sample must be stored frozen (-20°C or below). Avoid repeated freezing and thawing of the sample. Any particulate matter should be cleared from the specimen by low speed centrifugation before testing.

## REAGENT PREPARATION

Refer to tables 1 and 2 for the amounts of reagents and materials required. After removing the needed reagents and materials, return the unused items to their appropriate storage temperatures (see *STORAGE*). Bring all reagents to room temperature (15–30°C) before use. After use, return the kit to the refrigerator (2–8°C).

1. **Wash Solution.** Mix the 20X Wash Solution Concentrate by inverting the bottle several times. If the 20X Wash Solution Concentrate has been stored at 2–8°C, crystals may have formed. To dissolve the crystals, warm the bottle in a 37–50°C water bath until all crystals have dissolved. Mix thoroughly. Prepare the Wash Solution for washing the microassay wells by diluting the entire contents of one of the bottles of 20X Wash Solution Concentrate up to one liter with distilled or deionized water. Mix thoroughly. The Wash Solution is stable for 30 days when stored in a clean container at 2–8°C. If discoloration or cloudiness occurs, discard the reagent.
2. **Selecting the Microassay Strips.** Determine the number of specimens to be tested and add fifteen (15) wells for the five Standards, the Normal and Abnormal Controls to be tested (in duplicate), and one blank well. It is recommended that duplicate Standards and Controls be tested in separate microassay strips when possible. Based on the number of required wells, remove the desired number of strips. Secure the selected strips that are to be used in the plate frame. Place the unneeded strips back into the storage bag, seal it, and store at 2–8°C.

- Reconstitution of C1-Inhibitor Standards, Controls, and C1-Inhibitor Reactant.** Add 1 mL of Hydrating Reagent to each Standard vial (A–E) and to each Control. Add 0.5 mL Hydrating Reagent to each required vial of C1-Inhibitor Reactant. (One vial will treat approximately 25 test samples.) Allow the reconstituted vials to rehydrate for at least 15 minutes at 15–30°C, followed by thorough mixing. Avoid formation of foam or bubbles during mixing. Reconstituted Standards and Controls are stable for 30 days when stored at 2–8°C. **NOTE: Further dilution of reconstituted standards and controls is not required prior to running the assay.** Because four (4) vials of C1-Inhibitor Reactant are provided, the user is able to perform up to four (4) different assay runs with the materials supplied in the kit. **Reconstituted C1-Inhibitor Reactant is stable for up to twenty-four (24) hours at 2–8°C and up to two (2) hours at 15–30°C.**
- Preparation of 1X Specimen Diluent.** To determine the required amount of 1X Specimen Diluent, refer to Table 1. Prepare the required amount of 1X Specimen Diluent by mixing the indicated volumes of distilled or deionized water and 5X Specimen Diluent Concentrate.

**TABLE 1  
1X SPECIMEN DILUENT  
(Requirements and Preparation)**

# Strips	1X Specimen Diluent Needed (mL)	Volume of Reagents Required	
		Water (mL)	5X Specimen Diluent (mL)
2	6	4.8	1.2
3	15	12.0	3.0
4	22	17.6	4.4
5	30	24.0	6.0
6	38	30.4	7.6
7	46	36.8	9.2
8	54	43.2	10.8
9	62	49.6	12.4
10	70	56.0	14.0
11	78	62.4	15.6
12	86	68.8	17.2

- Specimen Dilution.** Determine the number (N) of specimens to be tested. Label test tubes #1 through #N and record which specimen will correspond to each tube. Prepare 1 mL of a 1:101 dilution (10 µL sample into 1 mL of 1X Specimen Diluent) of each specimen using 1X Specimen Diluent. Mix thoroughly, but avoid formation of foam and bubbles. Do not store or re-use diluted specimens.
- Preparation of 1X Conjugate.** For each three strips to be assayed, add 25 µL of 100X Conjugate to 2.5 mL of Conjugate Diluent. 1X Conjugate is stable for up to 12 hours at 2–8°C.

- Preparation of Substrate Solution. Prepare just prior to use.** Do not prepare the Substrate Solution until step 8 of the Assay Procedure. Determine the required volume of Substrate Solution from Table 2. **Prepare the Substrate Solution by adding 50 µL of Substrate Concentrate to each mL of Substrate Diluent.** Mix thoroughly. If the substrate solution turns dark green prior to use, discard it, and make a fresh solution in a clean container.

**TABLE 2  
1X SUBSTRATE SOLUTION  
(Requirements and Preparation)**

# Strips	Substrate Solution Needed (mL)	Volume of Reagents Required	
		Substrate Diluent (mL)	Substrate Concentrate (µL)
2	2	2	100
3	3	3	150
4	4	4	200
5	5	5	250
6	5	5	250
7	6	6	300
8	7	7	350
9	8	8	400
10	9	9	450
11	9	9	450
12	10	10	500

## ASSAY PROCEDURE

**Read the entire product insert before beginning the assay.**

See *REAGENT PREPARATION* before proceeding.

- Record the microassay well positions corresponding to all test samples, Standards, and Controls, as well as the indicated lot numbers from the vial labels on a data sheet. Label one corner of the Microassay Plate for orientation.
- Treatment of Standards, Controls, and test specimens with C1-Inhibitor Reactant:
  - Add 100 µL of each reconstituted C1-Inhibitor Standard (A, B, C, D, E) to pre-labeled micro-tubes.
  - Add 100 µL of C1-Inhibitor Abnormal Control and 100 µL of C1-Inhibitor Normal Control to pre-labeled micro-tubes.
  - Add 100 µL of the 1:101 dilution of each patient specimen (see *Specimen Dilution*, item 5 under *REAGENT PREPARATION*) to a pre-labeled micro-tube.
  - Add 20 µL of freshly reconstituted C1-Inhibitor Reactant to the micro-tubes containing Standards, Controls, and diluted test specimens. Vigorously vortex each micro-tube.
  - Incubate micro-tubes at 15–30°C for 30 ± 1 minutes.
- Depending upon the EIA plate reader requirements, select one or more wells to serve as a blank, and add 50 µL of 1X Specimen Diluent to these microassay wells.

4. Add 50  $\mu\text{L}$  of each C1-Inhibitor Reactant-treated (step 2) Standard and Control to duplicate assigned microassay wells. Add 50  $\mu\text{L}$  of each C1-Inhibitor Reactant-treated (step 2) specimen to its assigned microassay well.
5. Incubate at 15–30°C for 10  $\pm$  1 minutes.
6. Wash the microassay wells as follows:
 

**Note: Washing of the microassay wells is a critical step. Follow the wash procedure instructions carefully.**

  - a. After the incubation in step 5 (or in step 8 below) remove the contents from each well.
  - b. Fill all wells with Wash Solution (approximately 300  $\mu\text{L}$ ) using a wash bottle or other filling device.
  - c. Incubate the wells for 1 minute at 15–30°C.
  - d. Remove the contents from each well.
  - e. Fill all wells with Wash Solution (approximately 300  $\mu\text{L}$ ).
  - f. Remove the contents from each well.
  - g. Repeat steps e-f three additional times.**
  - h. After the fifth wash cycle, invert the plate, and tap firmly on absorbent paper twice to remove any remaining liquid.

**Do not allow the wells to dry.**
7. Using a multichannel or repeating pipette, dispense 50  $\mu\text{L}$  of diluted C1-Inhibitor Conjugate (see step 6 under *REAGENT PREPARATION*) into each washed test well, including the blank well(s).
8. Incubate the microassay strips at 15–30°C for 60  $\pm$  1 minutes. **Prepare the Substrate Solution during this incubation (see REAGENT PREPARATION step 7).**
9. Wash the microassay wells after the 60-minute incubation (step 8), as described under *ASSAY PROCEDURE*.
10. Immediately following the wash procedure, dispense 100  $\mu\text{L}$  of the freshly prepared Substrate Solution into each well, including the blank(s).
11. Incubate the microassay strips at 15–30°C for 30 minutes.
12. Add 50  $\mu\text{L}$  of Stop Solution to each well to stop the enzymatic reaction. The Stop Solution should be added to the wells in the same order and at the same rate as was the Substrate Solution. Gently tap the plate on the bench top to disperse the substrate color development evenly.
13. Determine the absorbance reading at 405 nm ( $A_{405}$  value) of each test well within one hour after the addition of the Stop Solution (step 12), making a blank correction in accordance with the spectrophotometric system in use.
14. Dispose of the remaining diluted specimens, Controls, substrate, conjugate, C1-Inhibitor Reactant, and the used microassay strips (see *WARNINGS AND PRECAUTIONS*). Keep the strip holder and strip retainer for future use.

## QUALITY CONTROL

Good laboratory practice recommends the use of controls to ensure that the assay is performing properly. Each C1-Inhibitor kit contains Normal and Abnormal Controls that can be used for this purpose. These Controls should be tested at least once for each batch of specimens, i.e., for each separate run. The Controls, when used as instructed, should give a percentage of mean normal values within the ranges specified on their vial labels. Since these Controls are to be Reactant-treated and tested exactly like a typical specimen, they serve as Controls for each C1-Inhibitor run. External controls, prepared by your laboratory, may also be used to help ensure that the assay is performing properly.

In addition, the product insert requires that the standard curve generated with the kit A-E Standards meet stringent validation requirements (see *INTERPRETATION OF RESULTS*). Standards should be tested in duplicate for each assay run. If the assay does not meet these requirements, repeat the assay or contact Quidel Technical Assistance.

## INTERPRETATION OF RESULTS

### Calculation of Results

The standard curve is generated using the blank-subtracted  $A_{405}$  values for each Standard (on the y axis) and the assigned concentration for each Standard (along the x axis). The standard curve must meet the validation requirements. Most computers and calculators are capable of performing this calculation. An example of a typical standard curve is shown in Figure 1.

The percent concentration for each sample is calculated from the standard curve using linear regression analysis.

### Validation

Determine the slope, intercept, and correlation coefficient of the derived best-fit line. The values must be within the following ranges to qualify the assay:

correlation coefficient (r):	Greater than 0.95
Slope (m):	0.798 to 1.944
y-intercept (b):	(-)0.115 to (+)0.047

### Interpretation

The concentration of functional C1-INH in a given sample is reported as the percentage of the mean level in normal specimens. Based on a sample of 100 normal subjects assayed by each of three technicians, a mean normal level of functional C1-INH was determined with this assay (see *PERFORMANCE CHARACTERISTICS, Accuracy*). The percentage of mean normal for any given test specimen diluted 1:101 is determined as specified in the *Calculation of Results* section.

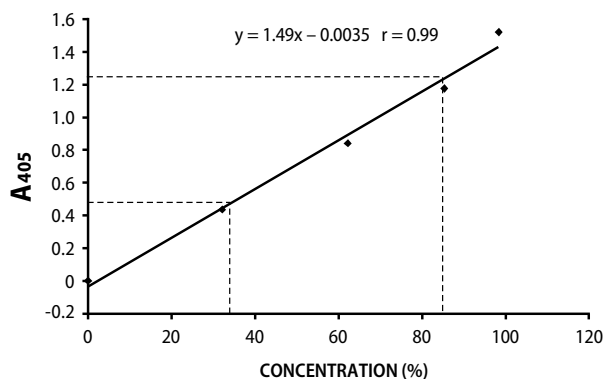
**Abnormal Results:** C1-INH concentrations less than or equal to 40% Mean Normal are considered significantly lower than normal and should be considered abnormal. Specimens that repeat-test as equivocal (see below) may also be considered abnormal.

**Equivocal Results:** C1-INH concentrations within the range of 41%–67% Mean Normal, although lower than expected for normal, are not significantly lower than normal and are considered equivocal results. These specimens may be repeat-tested or a new sample drawn and tested. If an equivocal specimen repeats as equivocal, the specimen is then considered significantly lower than normal and may be reported as abnormal.

**Normal Results:** Concentrations greater than or equal to 68% Mean Normal are considered normal.

#### Example of Standard Curve

**FIGURE 1**



#### LIMITATIONS OF THE PROCEDURE

The MicroVue C1-Inhibitor Enzyme Immunoassay has been used to test specimens collected as serum or as plasma in EDTA. Anticoagulants other than EDTA have not been tested.

#### EXPECTED VALUES

One hundred (100) normal serum specimens consisting of forty-nine (49) pediatric and fifty-one (51) adult subjects were tested in the MicroVue C1-Inhibitor Enzyme Immunoassay. There was no significant difference between pediatric and adult samples. The average concentration of C1-INH protein in these samples was defined to be 100% Mean Normal (standard deviation = 15.8%).

Paired EDTA plasma and serum samples were collected from fifteen (15) normal adult subjects and tested in the assay. There was no significant difference between these specimen types.

Specimens from twenty-eight (28) different documented C1-Inhibitor deficient patients were tested in the assay. These specimens were collected from several facilities located in different geographical areas of the United States. All twenty-eight patients tested had significantly lower levels of functional C1-INH than the mean normal level. These data are presented in Table 3.

**TABLE 3**  
**ANGIOEDEMA PATIENTS**

Patient No.	Site	Percentage of Mean Normal (%)	Interpretation
1P*	A	19	abnormal
1S*	A	37	abnormal
2**	A	0	abnormal
2**	A	6	abnormal
3	A	0	abnormal
4	A	17	abnormal
5	A	6	abnormal
6	A	0	abnormal
7	A	0	abnormal
8†	A	56	abnormal
9†	A	61	abnormal
10	B	8	abnormal
11	C	0	abnormal
12	D	28	abnormal
13	D	14	abnormal
14	D	32	abnormal
15	D	21	abnormal
16†	D	45	abnormal
17	D	3	abnormal
18	D	24	abnormal
19	E	0	abnormal
20	E	0	abnormal
21	E	2	abnormal
22	E	13	abnormal
23	E	17	abnormal
24	E	19	abnormal
25	E	4	abnormal
26	E	3	abnormal
27†	E	44	abnormal
28	E	0	abnormal

\* 1S is serum and 1P is EDTA plasma collected from a patient at the same time

\*\* The two samples from patient 2 were obtained three years apart.

† Upon repeat, these patients tested equivocal, and were therefore judged abnormal

#### PERFORMANCE CHARACTERISTICS

##### Accuracy

Using a linear regression model, the C1-Inhibitor concentrations measured in the EIA assay for fifteen normal sera were assigned based on their concentrations as determined by a radial immunodiffusion technique. The concentration of C1-INH in the primary standard was determined using value-assigned kit standards based on the linear regression model. To test the accuracy of the model, six C1-INH concentration determinations using the radial immunodiffusion technique were also made for the primary standard. These two methods that measure C1-INH concentrations were not significantly different. The mean value obtained for 100 normal serum specimens in the MicroVue C1-Inhibitor Enzyme Immunoassay was 182 µg/mL. This value agrees favorably with the published normal concentration of 180 µg/mL.

## Precision

Three kit lots were evaluated. Each lot was tested three times by a different technician. The samples were tested in replicates of three in each of the nine assay runs. Table 4 presents the resulting intraassay and interassay variation.

**TABLE 4**  
**ASSAY REPRODUCIBILITY**

Type	Mean (A <sub>405</sub> )	Intraassay (%CV)	Interassay (%CV)
Specimen 1	1.26	3	11
Specimen 2	0.68	5	15
Specimen 3	(-) 0.02	26	47
Standard A	(-) 0.02	22	33
Standard B	0.39	5	19
Standard C	1.35	6	13

## Specificity

The goat anti-human C1-INH, used to make the conjugate was compared against another commercially available and FDA-cleared C1-INH antibody. It demonstrated a single line of identity in an immunodiffusion test. In addition, the Quidel antiserum was judged to be mono-specific for C1-INH when tested at various concentrations against freshly drawn normal human serum containing 10 mM EDTA by double immunodiffusion, one-dimensional and two-dimensional immunoelectrophoresis, and rocket immunoelectrophoresis.

## ASSISTANCE

To place an order or for technical assistance, please contact a Quidel Representative at 800-524-6318 or 408-616-4301, Monday through Friday, between 8:00 a.m. and 5:00 p.m., Pacific Time. Orders may also be placed by fax at 408-616-4310.

For services outside the U.S., please contact your local distributor. Additional information about Quidel, our products and our distributors can be found on our website at [www.quidel.com](http://www.quidel.com).

## REFERENCES

1. Ratnoff, O.D., J. Pensky, D. Ogston, and G.B. Naff. The inhibition of plasmin, plasma kallikrein, plasma permeability factor, and C'1r subcomponent of the first component of complement by serum C'1 esterase inhibitor. *J. Exp. Med.* 129:315, 1969.
2. Travis, J. and G.S. Salvesen. Human plasma proteinase inhibitors. *Ann. Rev. Biochem.* 52:655,1983.
3. Donaldson, V.H. and R.R. Evans. A biochemical abnormality in hereditary angioneurotic edema. Absence of serum inhibitor of C'1-esterase. *Am. J. Med.* 35:37, 1963.
4. Rosen, F.S., C.A. Alper, J. Pensky, M.R. Klemperer, and V.H. Donaldson. Genetically determined heterogeneity of the C1 esterase inhibitor in patients with hereditary angioneurotic edema. *J. Clin. Invest.* 50:2143, 1971.
5. Gelfand, J.A., G.R. Boss, C.L. Conley, R. Reinhart, and M.M. Frank. Acquired C1 esterase inhibitor deficiency and angioedema: a review. *Medicine.* 58:321,1979.
6. Kerr, M.A. and A.A.C. Yeung-Laiwah. C1-Inhibitor deficiency and angioedema. In: *Complement in Health and Disease*, ed. K. Whaley, MTP Press Limited, p. 53,1987.
7. Donaldson, V.H. Serum inhibitor of C'1-esterase in health and disease. *J. Lab. Clin. Med.* 68:369,1966.
8. Levy, L.R. and I.H. Lepow. Assay and properties of serum inhibitor of C1-esterase. *Proc. Soc. Exp. Biol. Med.* 101:608, 1959.
9. Ziccardi, R.J. and N.R. Cooper. Development of an immunochemical test to assess C1 inactivator function in human serum and its use for the diagnosis of hereditary angioedema. *Clin. Immunol. Immunopath.* 15:465,1980.
10. Gigli, I., S. Ruddy, and K.F. Austen. The stoichiometric measurement of the serum inhibition of the first component of complement by the inhibition of immune hemolysis. *J. Immunol.* 100(6): 1154, 1968.
11. Centers for Disease Control. Recommendations for prevention of HIV transmission in health-care settings. *MMWR* 1987;36 (suppl no. 2S):001.

## GLOSSARY



Consult Instructions for Use on CDROM



Intended Use

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