MicroVue™ Complement MULTIPLEX

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A symbols glossary can be found at quidel.com/glossary.

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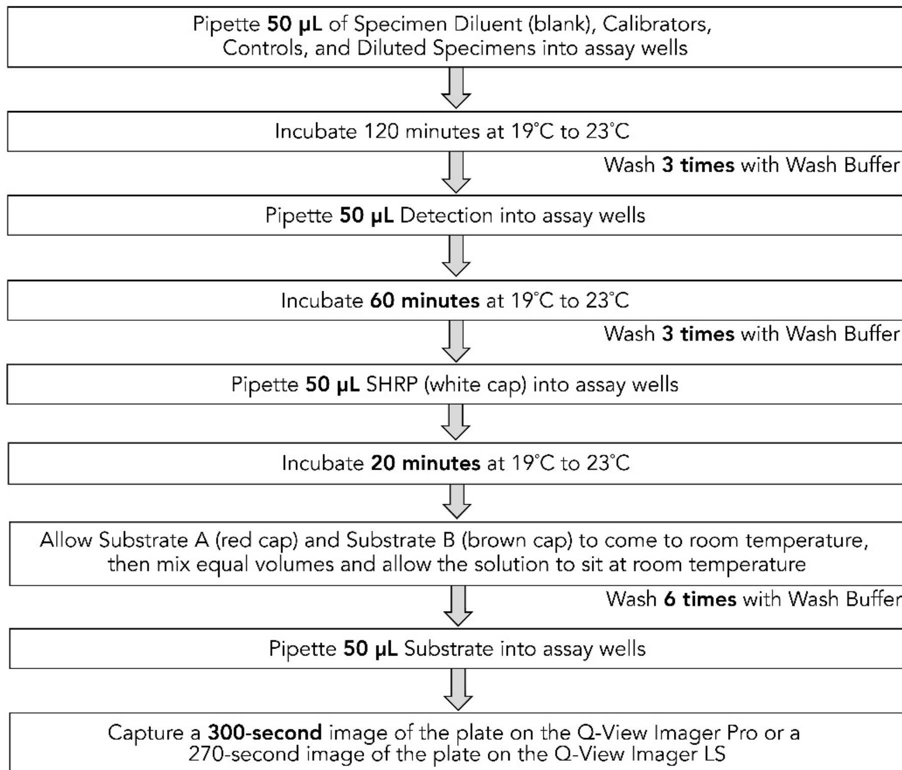
SUMMARY

Reagent and Sample Preparation

- ☐ Dilute Wash Buffer Concentrate (clear cap) 1:20 with DI Water
- ☐ Dilute 2X Specimen Diluent with DI Water: 40 mL 2X Specimen Diluent with 40 mL DI Water.
- ☐ Dilute Samples 1:1000 with Specimen Diluent.
- ☐ Reconstitute Calibrators (silver caps) and Controls (red caps) in Specimen Diluent.

Assay Procedure



SUMMARY AND EXPLANATION

The Complement System consists of more than 40 fluid and membrane-bound proteins and protein fragments organized into three primary pathways: Classical, Alternative, and Lectin.[1] By a series of specific activation steps via either the classical, alternative, or lectin pathway, the complement proteins mediate a set of activities ranging from the initiation of inflammation, clearance of immune complexes, disruption of cell membranes, and regulation of the immune response.[1] Deficiencies in the complement cascade can predispose individuals to infection through ineffective opsonization or defects in the ability to lyse invading pathogens.[1] Besides its essential role in maintaining health, the importance of the complement system in medicine lies in the fact that many acute and chronic diseases are associated with abnormalities in its function.[1] Accordingly, quantitative analysis of different complement proteins and their activation by-products are of great practical importance in experimental and clinical medicine.

There are numerous immunological methods for complement testing, including individual protein ELISA-based kits measuring complement cleavage products as markers of complement activation. However, the ability to measure multiple complement proteins in an individual sample constitutes a major step forward in research-based tools. The MicroVue Complement Multiplex assays can measure the amount of individual complement

proteins C1q, C2 Intact, C3 Intact, C4 Intact, C5 Intact, Factor D, and Factor P, providing a comprehensive overview of complement system homeostasis and activation.

PRINCIPLE OF THE PROCEDURE

The MicroVue Multiplex Complement Array for Panel 2 is based on the microplate sandwich enzyme immunoassay technique for the measurement of C1q, C2 Intact, C3 Intact, C4 Intact, C5 Intact, Factor D, and Factor P.

The C1q, C2 Intact, C3 Intact, C4 Intact, C5 Intact, Factor D, and Factor P assays use two different antibodies specific for their respective targets. Human serum, plasma, controls, or assay calibrators are added to microplate wells arrayed with analyte specific antibodies that capture C1q, C2 Intact, C3 Intact, C4 Intact, C5 Intact, Factor D, and Factor P, thereby immobilizing C1q, C2 Intact, C3 Intact, C4 Intact, C5 Intact, Factor D, and Factor P to their respective locations within the array. After washing away any unbound protein, a mixture that contains biotinylated analyte specific antibodies is added. The biotinylated antibodies complete the sandwich for each specific arrayed analyte. After washing away unbound biotinylated antibody, streptavidin- horseradish peroxidase (SHRP) is added. Following an additional wash, the amount of SHRP remaining on each location of the array is proportional to the amount of C1q, C2 Intact, C3 Intact, C4 Intact, C5 Intact, Factor D, and Factor P initially captured.

The amount of conjugated enzyme on each location of the array is measured with the addition of a chemiluminescent substrate. The intensity of the light generated at each of the specific locations within the array is proportional to the concentration of C1q, C2 Intact, C3 Intact, C4 Intact, C5 Intact, Factor D, and Factor P present in the test samples, controls, and calibrator.

The assay procedure described here and in detail below is for the MicroVue Complement Multiplex Panel 2 series of products, including a standard 7-plex (Cat. #A916), the Focused 4-plex (Cat. #A917) as well as the custom sized kits (Cat. #s A918, A919, A920, and A921).

REAGENTS AND MATERIALS PROVIDED

MicroVue Multiplex Complement Array Panel 2 kit contains the following:

Component	Part No.	Storage of opened/ reconstituted material
6 Multiplexed Calibrators (Silver Caps) Lyophilized. Each contains purified human complement proteins with an assigned protein concentration in TBS (Tris-buffered saline) with protein stabilizers.	7356100HU 7356101HU 7356102HU 7356103HU 7356104HU 7356105HU	2°C to 8°C until kit expiration Discard unused reconstituted reagents after use.
High/Low Controls (Red Caps) Lyophilized. Each contains purified human complement proteins with an assigned protein concentration in TBS with protein stabilizers.	7356106HU 7356107HU	
Specimen Diluent 2X (Blue Cap) 40 mL/vial of TBS with protein stabilizers and preservatives (<0.0085% ProClin® 300).	725657HU	2°C to 8°C until kit expiration. 2°C to 8°C for 24 hours or <–20°C for 4 weeks if opened or reconstituted.

Component	Part No.	Storage of opened/ reconstituted material
Detection Mix (Green Cap) Liquid. 6 mL/vial of biotinylated antibodies in PBS with protein (<0.0015% Kathon™ LX).	735656HU	
Arrayed Microplate Arrayed and blocked 96-well polystyrene microtiter plate in a resealable foil pouch.	735652HU	2°C to 8°C until kit expiration
Wash Buffer Concentrate (20X, Clear Cap) 50 mL/vial of a concentrated solution of buffered surfactant.	605658HU	2°C to 8°C until kit expiration
Streptavidin-HRP (White Cap) Liquid, 6 mL/vial of streptavidin-conjugated horseradish peroxidase.	605673GR	Do not expose to UV light. 2°C to 8°C until kit expiration
Substrate A (Red Cap) Liquid, 3 mL/vial of stabilized hydrogen peroxide.	605659GR	Do not expose to UV light. Store mixed substrate solution at room temperature (20°C to 25°C) for up to 1 week. Store unmixed solutions at 2°C to 8°C until kit expiration.
Substrate B2 (Brown Cap) Liquid. 3 mL/vial of stabilized signal enhancer.	605628GR	
Plate Seals (3) Adhesive strips		Non-perishable

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer
- Clean, unused polypropylene microassay plates or tubes for preparation of calibrator and samples
- Container for wash buffer dilution
- Microplate washer or wash bottle
- Adjustable multichannel pipette (8 or 12 channels) or repeating micropipettes (optional)
- Micropipettes and pipette tips
- Deionized or distilled water
- Q-View Imager and Software

WARNINGS AND PRECAUTIONS

- For Research Use Only. Not for use in diagnostic procedures.
- Treat specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any patient samples.
- Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
- Store assay reagents as indicated.
- Set up and practice using the Q-View Imager before starting the assay.
- When adding or aspirating liquids from the microassay wells, do not scrape or touch the bottom of the wells.
- Using incubation times and temperatures other than those indicated in the Procedure section may give erroneous results.
- Do not allow microassay wells to dry once the assay has begun.
- Do not use a microassay well for more than one test.
- Use of multichannel pipettes or repeat pipettors is recommended to ensure timely delivery of reagents.
- Use reconstituted calibrators within 30 minutes of reconstitution. See the Calibrators and Controls section if additional reagents are required.

- All calibrators, controls, and samples should be added to the microplate within 10 minutes of initial pipetting.
- For accurate measurement of samples, add samples and calibrators precisely. Pipette carefully using only calibrated equipment.
- Ensure all equipment has been calibrated before use.
- Proper collection and storage of test specimens are essential for accurate results (see SPECIMEN COLLECTION AND PREPARATION, pages 6 and 7).
- Avoid microbial or cross-contamination of specimens, reagents, or materials. Incorrect results may be obtained if contaminated.
- Each donor unit used in the preparation of the Calibrator and Control sera was tested by an FDA-approved method for the presence of antibody to human immunodeficiency virus, (HIV 1 and 2) and hepatitis C virus, as well as for hepatitis B surface antigen and found to be negative (were not repeatedly reactive). However, because no test method can offer complete assurance that infectious agents are absent, these reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Micro-biological and Biomedical Laboratories" 1999.
- ProClin 300 and Klathon LX are used as preservatives. Incidental contact with or ingestion of buffers or reagents containing ProClin and Kathon LX can cause irritation to the skin, eyes, or mouth. Use good laboratory practices to reduce exposure. Seek medical attention if symptoms are experienced.
- The Substrates and Streptavidin-Horseradish Peroxidase are light sensitive. Avoid prolonged exposure to bright or direct light. Store reagents in the **dark** when not in use.
- To avoid aerosol formation during washing, use an apparatus to aspirate the wash fluid into a bottle containing household bleach.
- Heat-inactivated, hyperlipemic, or contaminated specimens may give erroneous results.
- For proper data analysis ensure that the product software code contained on the provided Product Card is used with this kit only.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State, and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

SPECIMEN HANDLING AND PREPARATION

Handle and dispose of all specimens using Universal Precautions.

All specimen-handling operations should be carried out at 2°C to 8°C.

Specimen Collection

The proper collection, processing, and storage of specimens is essential since complement proteins may be generated in improperly handled specimens through artifactual complement activation. For optimal plasma results, K_2 EDTA collection tubes are recommended (Fisher Catalog Number: 22 040-161).[2]

Values for serum samples will typically be higher than those obtained with EDTA plasma samples. The complement levels in EDTA plasma may therefore more accurately represent the *in vivo* concentrations.

Serum and EDTA plasma specimens should be collected aseptically using standard techniques.

The specimens should be tested immediately or stored on ice for no longer than two hours before being assayed. If the specimen cannot be tested within two hours under the guidelines detailed above, the specimen should be frozen at –70°C, or below.

Thawing Frozen Specimens

To minimize specimen handling time, set up a dilution plate (or tubes) and add the appropriate volume of diluent (as described in the Specimen Dilution section below) prior to thawing specimens for evaluation.

Thaw frozen specimens rapidly at 37°C until just thawed. Transfer thawed specimens immediately to ice to prevent complement activation prior to dilution. Keep specimens on ice for no longer than two hours. Do not leave specimens at 37°C, as complement activation may occur. Do not thaw specimens at room temperature or on ice as this can lead to complement activation and affect results. Specimens should be tested as soon as possible after thawing. Only one freeze/thaw cycle may be performed without affecting the samples. If samples need additional freezing for further analysis, Quidel suggests freezing multiple aliquots of the specimen to prevent multiple freeze/thaw cycles.

REAGENT AND MICROPLATE PREPARATION

Bring all reagents and materials to 19°C to 23°C before use.

After removing the needed reagents and materials, return the unused items to their appropriate storage temperatures (see REAGENTS AND MATERIALS PROVIDED for storage recommendations).

Wash Buffer

Mix the 20X Wash Buffer Concentrate (clear cap) by inverting the bottle several times. If the 20X Wash Buffer Concentrate has been stored at 2°C to 8°C, crystals may have formed. To dissolve the crystals, warm the bottle in a 37°C to 50°C water bath until all crystals have dissolved, and follow by mixing thoroughly. Prepare the Wash Buffer by diluting the entire contents of one of the bottles of 20X Wash Buffer Concentrate and adjusting the total volume to one liter (1 L) with deionized water. Mix thoroughly.

The Wash Buffer is stable for 30 days when stored in a clean container at room temperature (19°C to 23°C). If discoloration or cloudiness occurs, discard the reagent.

Specimen Diluent

Dilute the 2X Specimen Diluent concentrate by adding 40 mL of deionized or distilled water to 40 mL of diluent.

Specimen Dilution

CAUTION: Treat all specimens as potentially infectious. Use Universal Precautions. Do not use heat-inactivated, contaminated, or improperly stored specimens.

NOTE: See Thawing Frozen Specimens for important notes on proper methods to thaw frozen specimens. Proper sample handling is essential for accurate results.

CRITICAL NOTE: Perform specimen collection and dilution correctly to avoid complement activation and resultant complement protein generation in specimens.

Specimens must be diluted so that values observed are above the LLOQ and do not exceed the assay ULOQ. Specimens with concentrations outside this range should be re-assayed at a new dilution.

Determine the number (N) of specimens to be tested. Label 2 sets of test tubes #1 through #N, and record which specimen corresponds to each tube. Alternatively, a 96-well dilution plate may be used for making the dilutions. Prepare an appropriate dilution (see the following section) of each specimen using the Specimen Diluent. For each dilution, mix gently to avoid formation of foam and bubbles. Do not store or reuse diluted specimens.

Dilution Method

Plasma

Dilute plasma specimens 1:1000 using the prepared Specimen Diluent.

For example, for each test specimen, pipette 90 µL of the Specimen Diluent into one tube or preparation plate well and 990 µL of the Specimen Diluent into a second tube or well.
Add 10 µL of the Test Specimen into 90 µL of the Specimen Diluent, mix thoroughly, then transfer 10 µL of the first dilution into 990 µL of the Specimen Diluent for the final 1:1000 dilution. Mix gently.

Serum

Dilute serum samples 1:1000 using the prepared Specimen Diluent.

All samples must be diluted such that observed concentrations are above each of the assay's LLOQ and below the ULOQ for all the array's targeted complement factors. Samples with levels of complement factors outside of these ranges should be re-assayed with an appropriate sample dilution that allows for measurements between the LLOQ and ULOQ of each assay.

When sample dilutions are complete, add diluted specimens to the Microtiter Wells.

Complete the addition of diluted specimens to the Microtiter Wells within 10 minutes of the application of the first specimen.

Calibrators and Controls

Reconstitute Calibrators (1-6) (silver caps) and Controls (High and Low) (red caps) using the Specimen Diluent. Refer to the Product Card which accompanies each kit for diluent volumes. Allow Calibrators and Controls to sit for 5 minutes. Mix thoroughly.

Add calibrators to microplate within 30 minutes of reconstitution.

Additional Calibrators and Controls

In the event that only a portion of the plate will be utilized, or that further calibrators and controls are required, additional calibrators and controls are available, MicroVue Complement Multiplex Calibrators, Cat. #A951. Refer to the ASSISTANCE section for ordering details.

All procedures for preparation for use of Calibrators and Controls should be followed.

Substrate

Allow Substrate A (red cap) and B2 (brown cap) to come to room temperature (19°C to 23°C). In a separate container, combine 3 mL of Substrate A with 3 mL of Substrate B2, mix gently. Do not expose to UV light. Store at room temperature (19°C to 23°C) after mixing.

Microplate Preparation

If using the entire plate, follow the ASSAY PROCEDURE steps.

When using only a portion of the plate, the unused portion should be protected from exposure to moisture. Place a plate seal over the unused wells and ensure that the plate seal remains sealed while the assay is being run. After the assay is complete, the substrate should be completely removed from the used portion of the plate by aspirating the substrate then tapping the plate upside down on paper towels. Leave the plate upside down on absorbent materials for 10-20 minutes to facilitate drying, then the plate seal can be removed from the unused portion of the plate and the entire plate should be returned to the foil plate bag with the desiccant. The plate should be stored at 2°C to 8°C for up to one month.

ASSAY PROCEDURE

Read entire Product Insert before beginning the assay. See REAGENT PREPARATION and WARNINGS AND PRECAUTIONS before proceeding.

1. Record the Microassay well positions corresponding to the blank well(s), all Test Specimens, Calibrators, and Controls as well as the indicated lot numbers from the vial labels. Label one corner of the Microassay Plate for orientation.
    a. If using a portion of the Microassay Plate, refer to the Microplate Preparation section for instructions on sealing the plate.
2. Label dilution plate/tubes to correspond to all Test Specimens.
3. Add Specimen Diluent to the dilution plate/tubes.
4. Thaw Test Specimens and dilute immediately.
5. Add 50 µL of each Multiplexed Calibrator (1-6) to duplicate wells.
6. Add 50 µL of both the Multiplexed Low Control and Multiplexed High Control to duplicate wells.
7. Add 50 µL of each diluted specimen to its assigned microassay well (in duplicate recommended). (See Specimen Dilution).
8. Incubate at 19°C to 23°C for 120 ± 10 minutes.
9. Wash the Microassay Wells as follows:
    a. After the incubation in step 8, remove the liquid from each well.
    b. Add 300 µL to 400 µL Wash Solution to each well using a wash bottle or automated filling device.
    c. Remove the liquid from each well and tap firmly on absorbent paper (if manual washing is used).
    d. Repeat steps b-c two additional times for a total of three washes.
    e. After the third wash cycle, invert the plate, and tap firmly on absorbent paper twice to remove any remaining liquid. See Appendix A for additional instructions on plate washing using an automatic plate washer or multichannel pipette.
10. Using a multichannel or repeating pipette, dispense 50 µL of prepared Detection Mix into each washed test well.
11. Incubate the Microassay Plate at 19°C to 23°C for 60 ± 10 minutes.
12. Wash the Microassay Wells after the 60-minute incubation, as described under ASSAY PROCEDURE, step 9.
13. Immediately following the wash procedure, use a multichannel or repeating pipette to dispense 50 µL of the Streptavidin-HRP (white cap) solution into each well.
14. Incubate the Microassay Plate at 19°C to 23°C for 20 ± 1 minutes.
15. Wash the Microassay Wells after the 20-minute incubation, as described under ASSAY PROCEDURE, step 9a through 9c, repeating for a total of six washes.
16. Add 50 µL of the previously prepared Substrate (red and brown cap) to each well. Wait no longer than 5 minutes to commence imaging.
    Note: If imaging cannot commence immediately, protect the plate from drying for up to 15 minutes by dispensing 100 µL of wash buffer into each well of the plate prior to adding the mixed Substrate. When ready to image, remove the wash buffer from the plate and add the Substrate.
17. Place the plate in the Q-View Imager.
18. Open Q-View Software, create or open a project, and click Acquire Image.
    a. Refer to the imager manual for the required image exposure time.

19. Click the Capture Image button. Users may continue on to Well Assignment while images are being captured.
    Note: Details about these imaging steps are available in the Q-View Software Manual viewable at quidel.com or within Q-View Software under **Support > Manual**.
20. Dispose of the remaining Diluted Specimens and Controls and the used Microassay Plate (see WARNINGS AND PRECAUTIONS).

ANALYZING A Q-PLEX™ IMAGE

The following summarizes a general workflow for analyzing a Q-Plex image in Q-View Software. Each of these steps is described in greater detail in the Q-View Software and Imager Manual, viewable at quidel.com, or within Q-View Software under **Support > Manual**.

1. Acquire or import an image into Q-View Software as previously described.
2. Enter the Product Code (found on the Product Card) into the Product Code field of the software.
3. Image Processing: Align the plate overlay as follows:
    a. Set the overlay: If using the Auto-Set Plate Overlay feature, this will occur automatically. Otherwise, go to **Overlay Options > Set Plate Overlay**.
    b. To visualize bright or dim spots, optimize the display using **Image Options > Adjust Gamma.**
    c. Optimize overlay alignment: Go to **Overlay Options > Adjust Plate Overlay** to pivot the overlay, **Adjust Well** and **Adjust Spot** to move individual wells and spots, then **Auto-Adjust Spots** to automatically snap each circle of the overlay to the nearest spot of the image beneath.
4. Well Assignment: Label wells as samples, controls, calibrators, or negatives, and specify their dilution factors. Use Templates to quickly assign layouts that are repeated often, or export the layout as a .csv file.
5. Data Analysis: Once you have completed Image Processing and Well Assignment, select Data Analysis. Click Perform Analysis to generate charts, concentrations, and statistics based on optimal regression settings provided by the product definition.

Note: Tips for data analysis are available from Quidel. We take great care to ensure that customers have success using our products and services. If you have further questions about running the assay, data analysis, troubleshooting, or our products or services, please contact us at 800.874.1517 or technicalsupport@quidel.com.

QUALITY CONTROL

Quality control ranges are provided in the software and are associated with the Product Code supplied with each kit. The control values are intended to verify the validity of the curve and sample results. Each laboratory should establish its own parameters for acceptable assay limits. If the control values are NOT within your laboratory's acceptance limits, the assay results should be considered questionable, and testing should be repeated.

TYPICAL PERFORMANCE CHARACTERISTICS

Limits

LOD: The limit of detection (LOD) for each analyte is determined by the upper 3SD limit in a zero-standard study.
LLOQ: The lower limit of quantitation (LLOQ) for each analyte is the lowest concentration that had a coefficient of variation of <15%.
ULOQ: The upper limit of quantitation (ULOQ) for each analyte is the highest concentration that had a coefficient of variation of >15%.

Analyte	units	LOD	LLOQ	ULOQ
C1q	µg/mL	0.00038	0.003	0.44
C2 Intact	ng/mL	0.30	0.710	362.43
C3 Intact	µg/mL	0.0027	0.102	13.10
C4 Intact	µg/mL	0.024	0.044	6.80
C5 Intact	µg/mL	0.00017	0.002	0.47
Factor D	ng/mL	0.014	0.015	4.60
Factor P	ng/mL	0.22	0.7283	154.5

Cross-Reactivity

C1q cross-reacts with the Factor P assay at ~1%. Samples with C1q levels greater than 0.188 µg/mL may impact Factor P results.

Interfering Substances

The following substances were tested in the MicroVue Multiplex Complement Array and found not to interfere with the assay using serum, EDTA plasma, or Heparin Plasma.

- Bilirubin, conjugated (0.1 mg/mL)
- Cholesterol (5 mg/mL)
- Glucose (12 mg/mL)
- Hemoglobin (lysed RBCs) (5 mg/mL)
- Protein, total (120 mg/mL)
- Protein, albumin (60 mg/mL)
- Protein, gamma-globulin (60 mg/mL)
- Triglycerides (lipemia) (30 mg/mL)

Precision

Within-run precision was determined by assaying 20 replicates of 2 EDTA plasma samples and 2 serum samples. Between-run precision was determined by assaying 4 replicates of 2 plasma and 2 serum samples on 20 different runs.

Analyte	Units	Sample	Conc.	Within Run %CV	Between Run %CV
C1q	µg/mL	Serum 1 Serum 2 Plasma 1 Plasma 2	123 123 126 38	14% 12% 13% 13%	15% 15% 14% 15%
C2 Intact	ng/mL	Serum 1 Serum 2 Plasma 1 Plasma 2	27,544 21,939 22,362 9,515	7% 6% 6% 8%	12% 14% 12% 12%
C3 Intact	µg/mL	Serum 1 Serum 2 Plasma 1 Plasma 2	3,339 2,217 1,423 1,469	7% 6% 8% 6%	13% 13% 13% 13%
C4 Intact	µg/mL	Serum 1	1,069	8%	14%

Analyte	Units	Sample	Conc.	Within Run %CV	Between Run %CV
		Serum 2	538	6%	15%
		Plasma 1	2,249	7%	11%
		Plasma 2	341	9%	14%
C5 Intact	µg/mL	Serum 1	208	6%	13%
		Serum 2	154	6%	14%
		Plasma 1	154	7%	12%
		Plasma 2	71	10%	13%
Factor D	ng/mL	Serum 1	1,417	7%	15%
		Serum 2	1,146	9%	15%
		Plasma 1	853	9%	14%
		Plasma 2	548	7%	14%
Factor P	ng/mL	Serum 1	31,663	7%	11%
		Serum 2	30,326	8%	12%
		Plasma 1	37,303	8%	9%
		Plasma 2	10,286	7%	12%

Linearity

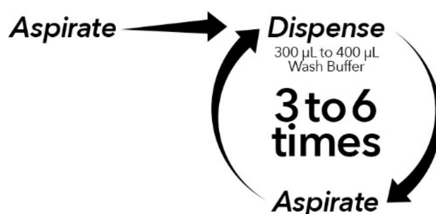
Three of each type of sample (serum, EDTA plasma, and Li Heparin Plasma) were assayed at dilution factors 500, 1000, 2000, 4000, and 8000. The observed value was compared to the expected value for each sample. The % Recovery is reported below.

	Serum			EDTA Plasma			Li Hep Plasma		
	Max	Min	Average	Max	Min	Average	Max	Min	Average
C1q	109%	89%	102%	119%	90%	107%	110%	92%	103%
C2	119%	86%	105%	110%	98%	103%	116%	99%	108%
C3	116%	85%	106%	106%	89%	95%	109%	101%	81%
C4	107%	89%	101%	108%	99%	101%	115%	81%	93%
C5	102%	98%	100%	105%	89%	98%	102%	84%	95%
FD	104%	84%	94%	108%	92%	100%	112%	87%	97%
FP	105%	99%	102%	101%	96%	99%	108%	89%	99%

APPENDIX A: PLATE WASHING METHODS

Automated Wash Method

1. Use a program that will aspirate and dispense 300 µL to 400 µL wash buffer.



2. Ensure that the plate washer has a program that will not scratch the bottom of the microplate and will prevent plate drying. This is critical to prevent damage to the capture antibody arrays. The simplest method to avoid plate drying is to leave a small, uniform amount (1-3 µL) of wash in the well after the final aspiration and add the next reagent to the plate as quickly as possible.
3. Leaving a uniform amount of wash in the wells can be accomplished with an automated washer by slightly increasing the aspiration height of the washer head.

For example:

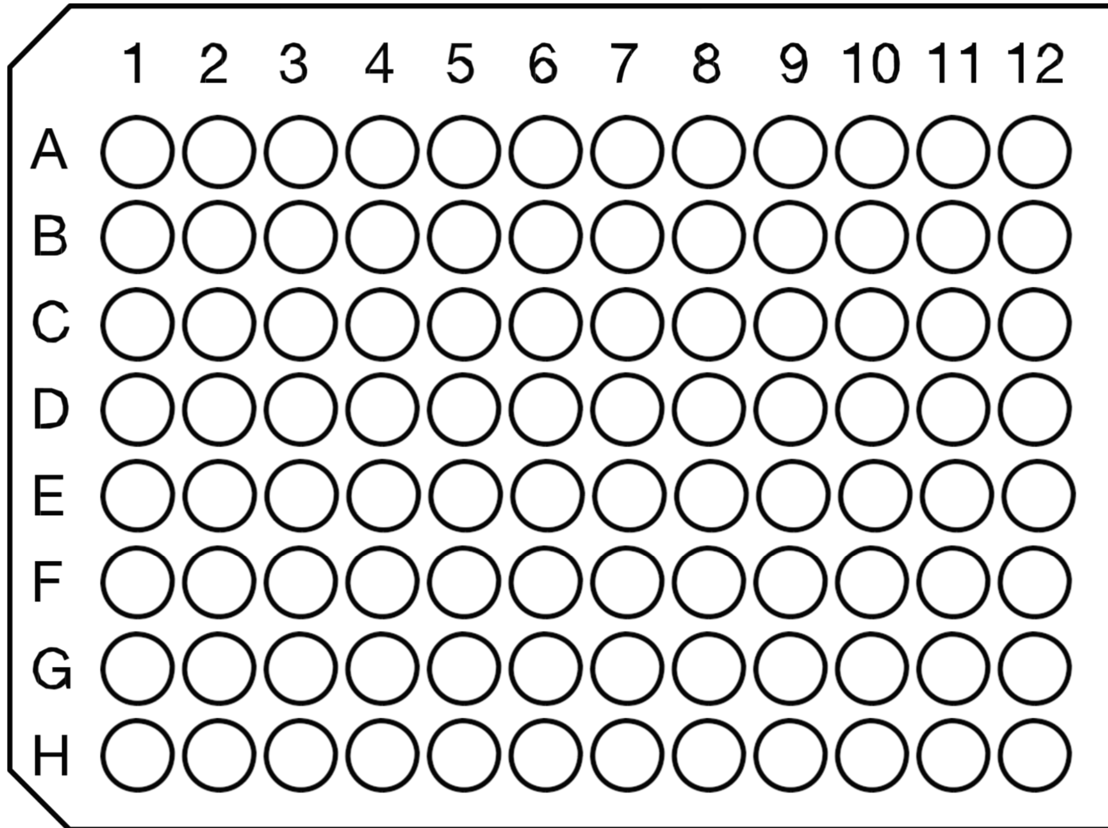
Process	**Distance**	**Steps on a Biotek ELX-405**
Aspiration Height	3.810 mm	30
Aspiration Position	1.28 mm from center	–28
Dispense Height	15.24 mm	120
Each wash step should have a 20-40 second soak with wash buffer		

4. The dispense rate of any automatic washer should be low to avoid washing bound material off the bottom of the plate. A dispense rate of 3, on a scale of 1-10 where 1 is the lowest, is recommended.
5. Ensure that each wash step has a 20-40 second soak with the wash buffer.
6. Connect the prepared wash buffer to your automatic plate washer.
7. Run 1-2 priming cycles to make sure that the wash buffer is running through the plate washer. When the buffer has run through the machine, the waste will be foamy.
8. To ensure that all pins are functioning, in a spare microtiter plate, dispense 100 µL wash buffer and ensure that all pins dispense uniformly, then aspirate and ensure that all pins aspirate uniformly.
9. Prime the plate washer one time before the first wash step. When running the assay, perform the wash 3 or 6 times according to the protocol.

Multichannel Pipette Wash Method

1. Just prior to washing, pour the prepared wash buffer into a trough or tray.
2. After each incubation, flick the solution out of the plate over a waste container before starting the wash protocol.
3. Using a multichannel pipette, dispense 300 µL to 400 µL of wash buffer into each of the wells used in the test.
4. Aggressively flick the wash buffer out over a waste container.
5. This washes the plate one time. When the assay procedure calls for three or six washes, repeat steps 3-4 accordingly.
6. Tap the plate upside down on a paper towel to remove any residual wash.
7. Proceed immediately to dispense the next solution so drying does not occur.

PLATE DIAGRAM



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1. Merle NS, Noe R, Halbwachs-Mecarelli L, Fremeaux-Bacchi V, Roumenina LT. Complement System Part II: Role in Immunity. Front Immunol. 2015 May 26;6:257. doi: 10.3389/fimmu.2015.00257. PMID: 26074922; PMCID: PMC4443744.
2. Greiner Bio-One™ VACUETTE™ K2EDTA Blood Collection Tubes, Fisher Scientific, REF # 22-040-161

ASSISTANCE

To place an order or for technical support, please contact a Quidel representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern time. Orders may also be placed by fax at 740.592.9820. For e-mail support contact customerservice@quidel.com or technicalsupport@quidel.com.

For services outside the U.S.A., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website quidel.com.

REF A916 – MicroVue Complement Multiplex – Panel 2 - Standard 7-plex
A917 – MicroVue Complement Multiplex – Panel 2 – Focused 4-plex
A918, A919, A920, A921– MicroVue Complement Multiplex – Panel 2 – Custom kits

RUO

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GLOSSARY

REF Catalogue number	LOT Batch code
 Use by	 Manufacturer
 Temperature limitation	 Consult e-labeling instructions for use
RUO For Research use only	 Keep away from sunlight
CONT Contents/Contains	