

Patient Name: _____ DOB: _____ MRN: _____

Please see page 3 for sample handling requirements - No Weekend Deliveries

Functional Testing Panels Requested

- C3G Functional Panel** (serologies for DDD, C3GN)
 (CH50eq, APFA, Hemolytic Assay, FHAA, FBAA, Nephritic Factors (Nef Activity Assay – IFE, C3Nef-C3CSA, C5Nef-C3CSAP, C4Nef), C3, C3c, C4, FB, Ba, Bb, C5, Properdin, soluble C5b-9, FI and FH levels)
 - 2 mL frozen serum
 - 2 mL frozen EDTA plasma
- TMA Functional Panel** (serologies for TTP, aHUS, HUS)
 (CH50eq, APFA, Hemolytic Assay, FHAA, C3, C4, FB, Bb, soluble C5b-9, FI and FH levels)
 - 2 mL frozen serum
 - 2 mL frozen EDTA plasma
- Autoantibody Micro-Panel**
 (FHAA, FBAA, Nephritic Factors (Nef Activity Assay – IFE, C3Nef-C3CSA, C5Nef-C3CSAP, C4Nef)
 - 2 mL frozen serum
- Complement Biomarker Micro-Panel**
 (Ba, Bb, C3, C3c, C4, C5, FB, FH, FI, Properdin levels and soluble C5b-9)
 - 2 mL frozen EDTA plasma
- Complement Activity Micro-Panel**
 (CH50eq, APFA, Hemolytic Assay)
 - 2 mL frozen serum

Genetic Testing Requested

- Genetic Renal Panel** (DNA test for TTP, aHUS, HUS, DDD, C3G and other complement diseases)
 (CFH, CFI, MCP, CFB, CFHR5, C3, THBD, ADAMTS13, PLG, DGKE, G6PD, MMACHC and MLPA)
 - 8-10 cc EDTA whole blood (room temp or refrigerated)
OR
 - 10 µg DNA, minimum concentration 50 ng/µl
- MLPA Testing ONLY** (screening for copy number variations in the CFH-CFHR5 genomic region)
 - 8-10 cc EDTA whole blood (room temp or refrigerated)
OR
 - 10 µg DNA, minimum concentration 50 ng/µl

- Familial Testing** (site specific analysis to screen for variants previously identified in a family member)

Familial Testing Details:

Gene/s: _____

MORL ID# or Variant/s: _____

Relationship to previously tested person: _____

a La Carte Testing Requested

Autoantibody Tests – 2 mL Frozen Serum

- FH autoantibody (FHAA)** (appropriate for aHUS & C3G)
- FB autoantibody (FBAA)** (appropriate for aHUS & C3G)
- Nef Activity Assay (IFE)** (appropriate for aHUS & C3G)
- C3Nef (C3CSA)** (appropriate for C3G)
- C5Nef (C3CSAP)** (appropriate for C3G)
- C4Nef** (appropriate for C3G)

Biomarker Tests – 1 mL Frozen EDTA Plasma

- C3 ELISA Level** **C3c Level**
- C4 Level** **FB Level**
- Ba Level** **Bb Level**
- C5 ELISA Level** **Properdin Level**
- Soluble C5b-9** **FI ELISA Level**
- FH Level**

Complement Pathway Function Tests – 1 mL Frozen Serum

- CH50eq** **APFA** (Alternative Pathway Functional Assay)
- Hemolytic Assay** **C3b Deposition Assay**

ADAMTS-13 Tests – 1 mL Frozen Citrate Plasma

- ADAMTS-13 Activity**
- ADAMTS-13 Activity with reflex to Inhibitor** (when activity <25%)

Important Information for ALL Requests

If you are interested in ordering Custom Testing please contact Amy Weaver at 319-335-6623 or amy-weaver@uiowa.edu

All serum and plasma samples **MUST** be processed and frozen down to -80° C immediately after collection (please see instructions on page 3). Sample **type must be clearly labeled (either serum or plasma)** and shipped out **overnight** on at least 5 lb dry ice (Monday – Thursday).

If samples arrive thawed they will be **REJECTED**.

No Weekend Deliveries

Molecular Otolaryngology & Renal Research Laboratories

For test inquiries please call: 319-335-6623 • Fax: 319-353-5869
 For billing inquiries please call: 319-335-6653 • Fax: 319-353-5869
 Email: morl@uiowa.edu • <https://morl.lab.uiowa.edu>

Ship to:

Dr. Richard Smith
 Molecular Otolaryngology & Renal Research Laboratories
 The University of Iowa
 285 Newton Rd., 5270 CBRB
 Iowa City, IA 52242-1078

Monday – Friday ONLY – No Weekend Deliveries

Molecular Otolaryngology & Renal Research Laboratories Sample Requirements

Questions? Contact Amy Weaver at 319-335-6623 or amy-weaver@uiowa.edu

Genetic Renal Panel/MLPA/DNA Testing Sample Requirements:

- 8-10 cc. EDTA whole blood (minimum amount of 3-4 cc. is accepted for children under age 4)
- **OR** 10 µg DNA, minimum concentration 50ng/µl (A260/A280 1.8-2) resuspended in 0.1mM EDTA (10mM Tris HCl, 0.1mM EDTA, pH 8, Teknova Cat# T0220)
Note: MORL is not responsible for broken tubes.
- ***Please note: blood samples drawn from a bone marrow transplant patient will result in genetic results for the donor rather than the patient.**
- Overnight delivery, Room temperature (**DO NOT FREEZE WHOLE BLOOD**)
- Samples are accepted Monday-Friday.
- Samples may be refrigerated if delivery is delayed (stability – 1 week)

Serum (FB & FH autoantibody, Hemolytic Assay, APFA, CH50eq, Nef Activity Assay, C3Nef, C4Nef, C5Nef) Collection Protocol (minimum volume 2ml) ***PLEX treatments will affect serum tests, please wait ~14 days after PLEX to draw samples:**

1. Follow standard phlebotomy techniques to collect at least **6 cc** of whole blood drawn in a **red-top** vacutainer tube. *Note: Serum separators with “clot activators” should **not** be used for the serum samples.*
2. Allow the blood in the **red-top** tube to clot at room temperature for 30 minutes.
3. Centrifuge the clotted blood at room temperature (1000 x g for 10 minutes).
4. **Label “Serum” or “Red-top”** on clean screw top-tube(s).
5. Pipette **cell-free supernatant** (at least 2 mL) to each labeled tube(s).
6. **Place the tube immediately at -80°C (or on dry ice). Sample must remain deep frozen.**
Note: Do not transfer cells with serum. If necessary centrifuge a second time.

Plasma (soluble C5b-9, C3, C3c, Ba, Bb, Properdin, C4, C5, FB, FH, FI levels) Collection Protocol (minimum volume: 2ml)

***PLEX treatments will affect plasma tests, please wait ~14 days after PLEX to draw samples:**

1. Follow standard phlebotomy techniques to collect at least **6 cc** of whole blood drawn in a **lavender-top** (EDTA) vacutainer tube.
2. Centrifuge at room temperature immediate after blood draw (1000 x g for 10 minutes).
3. **Label “Plasma” or “Lavender-top”** on clean cryovial screw-top tube(s).
4. Pipette **cell-free supernatant** (at least 2 mL) to each labeled tube(s).
5. **Place the tube immediately at -80°C (or on dry ice). Sample must remain deep frozen.**
Note: Do not transfer cells with plasma. If necessary centrifuge a second time.

Plasma (ADAMTS-13 Activity/Inhibitor) Collection Protocol (minimum volume: 0.5ml):

- | | |
|--|--|
| <ol style="list-style-type: none"> 1. Follow standard phlebotomy procedure to collect blood in buffered sodium citrate (light blue-top, 3.2%) plastic tubes (available in 4.5 mL, 2.7 mL or 1.8 mL full draw tubes). 2. After collection, invert the tube gently 5 to 6 times. 3. Label “Citrate Plasma” or “Blue-top” on clean cryovial screw-top tubes. 4. Store the blue-top tube upright at room temperature until centrifugation. Samples should be centrifuged between 15 to 60 minutes after blood collection for best results. 5. Re-mix the blood sample immediately prior to centrifugation by gently inverting the tube 5 to 6 times. 6. Centrifuge blood sample at room temperature in a horizontal rotor (swinging bucket rotor) for 15-20 minutes at 1500 to 1800 x g with the <i>brake off</i>. | <ol style="list-style-type: none"> 7. Following centrifugation, transfer the top two-thirds of the plasma layer into a new plastic tube. 8. Re-centrifuge the collected plasma at 1500 to 1800 x g with the <i>brake off</i> for an additional 15-20 minutes to remove any red cells or platelets. 9. Transfer the top two-thirds of the plasma into the previously labeled cryovials, taking care not to disturb any cells at the bottom of the tube. 10. Place the tube immediately at -80°C (or on dry ice). Sample must remain deep frozen.

 <i>Note: if the sample arrives at room temperature a new sample will be required.</i> |
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Serum & Plasma Shipping Requirements:

- Serum and plasma must be **frozen** and U.S. samples must be shipped **OVERNIGHT** with a **minimum** of 3 kg (or 6 lb) dry ice.
- Overseas samples must be shipped with a **minimum** of 6 kg (or 15 lb).
- Cryovials should be put in zip lock bags and completely covered in dry ice to keep the sample frozen until it arrives in the lab.
- Delivery: Monday-Friday. **NO WEEKEND DELIVERIES**
- **Thawed OR unlabeled samples will be REJECTED for testing.**

Ship all samples to:

Dr. Richard Smith
 Molecular Otolaryngology & Renal Research
 Laboratories
 The University of Iowa
 285 Newton Rd., 5270 CBRB
 Iowa City, IA 52242-1078
 Phone: 319-335-6623

DISCLAIMER:

This request to order molecular diagnostic tests from the MORL certifies to the MORL that the ordering physician has obtained informed consent from the patient as required by applicable state or federal laws for each test ordered, that the ordering physician has authorization from the patient permitting the MORL to report results for each test ordered to the ordering physician, and that the ordering physician assumes responsibility for providing the patient with all associated guidance and counseling regarding the test results.

ALL requested information must be provided or testing will not be performed

Patient information: Patient date of birth and gender
Patient ethnicity and race
Patient's clinical information and family history of kidney disease

We request extensive patient demographic and clinical information. This information is required as it is very valuable in the interpretation of your patient's results.

Specimen information: Patient identifiers (**full name, date of birth, sex and medical record number**)
Date of collection
Sample type – frozen samples must be CLEARLY LABELED as either serum or plasma (and type, EDTA or Citrate)
Ordering physician

Billing information: **We will NOT bill insurance, Medicare or patient directly.**
Institutional billing accepted. Visa and Master Card accepted.
Personal checks NOT accepted.
Please include contact information including phone & fax number for billing questions.

Reporting Information: Because of confidentiality issues, reports will only be released to the individual indicated on the page 1 of the testing requisition form.

Research Participation: If your patient's genetic and functional testing results are inconclusive they may qualify for research studies on complement-mediated renal diseases that are ongoing at the MORL. If you would like your patient to be considered for this opportunity please contact Amy Weaver at amy-weaver@uiowa.edu.

IMPORTANT INFORMATION FOR PHYSICIAN OR GENETIC COUNSELOR:

DNA tests may detect an abnormality. Detection methods are greater than 99% accurate. Many of these tests are relatively new. The analysis and interpretation represents our best knowledge and understanding of the genetics of these diseases.

There is a small possibility that a test may not work properly or an error may occur. You may be asked for an additional sample if it is felt that confirmatory testing is needed.

An error in diagnosis may occur if incorrect information is provided with the sample.

Kidney diseases are complex disorders and penetrance of a phenotype (the degree of kidney disease, for example) may be variable. Research to determine whether a genotype-phenotype correlation exists is ongoing.

Because of the complexity of DNA testing, results should be discussed with a genetic counselor or physician.

Note: Kidney diseases are very complex disorders. This complexity means that variants in many different genes can lead to kidney disease. It is possible that no variants will be detected in the variant screens (the genes) you have requested.