**Data Handling** - All specimens must be logged into the LDMS specimen management and all aliquots must be entered into the storage module of the LDMS and exported weekly. Volumes must be accurate and the # of aliquots entered must be adjusted to match the # of aliquots actually prepared.

<table>
<thead>
<tr>
<th>Visit</th>
<th>Study or Substudy #/ Patient Subset</th>
<th>Primary Spec. Collection and Handling</th>
<th>Tests</th>
<th>Processing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screen</td>
<td>P1068s</td>
<td>50 ul for preparation of thick and thin blood smears directly from a heel or finger stick or from an EDTA anticoagulated venous drawn specimen. <strong>NOTE:</strong> Actual amount is approximately 2 μL for thin smear and 4-6 μL for thick smear. Submit CRF SPW0273 P1068S Note: If smears are made from EDTA anticoagulated venous drawn specimen, gently invert the source of blood in the tube 3 times and then aliquot 5 μL onto each of two slides for thick and thin smear preparation. Also, this MUST be done as soon as possible (no greater than 15 minutes of the blood draw) to preserve the integrity of the slide for parasite identification.</td>
<td>Diagnosis of Malaria parasitemia Test Code: PARBLDSM Note—Thin smears and Thick smears will be made separately. Thin smears will be fixed with methanol prior to Giemsa staining and Thick smears will be stained without methanol. 2 Thin and 2 Thick film smears should be made. PLEASE REFER TO THICK AND THIN SMEARS – CHECKLIST at the end of this LPC for very detailed instructions to follow. Always make smears with frosted side of slide up: Please note that heel or finger stick is the preferred method for preparation of thick and thin smears, wherever possible. Thick and thin smears must be made on separate slides. <strong>Thin Smears:</strong> Place one small drop of blood on slide and using a second clean slide as a “spreader” touch small drop with spreader and allow blood to run along edge. Firmly push the spreader along slide, keeping at 45° angle. After drying, (fixing before drying will cause the slide to be unreadable), fix Thin Film with methanol by taking methanol from stock aliquot (kept sterile), covering slide with methanol, and leaving slide to dry. Do NOT re-use the edge of slides to prepare another thin smear as this may result in cross-contamination of smears. <strong>Thick film:</strong> On a separate slide, using the corner of a clean and new slide spreader, quickly join 2-3 larger drops of blood and spread to make an even thick film. NOTE: Thick smears must be dried overnight prior to staining. Giemsa staining technique – regular method (See step by step instructions at the end of the LPC) - will be used for staining the thick and thin smears. Thick smears are not fixed with methanol prior to Giemsa staining. Each slide should be labeled with patient’s PID and date using soft lead pencil. – Be extremely careful not to contaminate pencil by coming into contact with blood. If thick smears are used for diagnosis and viewed under oil immersion, clean the thick smear before storing by blotting slide gently with a clean tissue (this must be done with great care so as not to wipe the stain off).</td>
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</tr>
<tr>
<td>Section</td>
<td>Code</td>
<td>Steps</td>
<td>Notes</td>
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<td>Screen</td>
<td>P1068s</td>
<td>250μL for Preparation of Dried Blood Spots. Directly from a heel or finger stick or from an EDTA-anticoagulated venous drawn specimen. Submit CRF SPW0273 P1068S</td>
<td>RTQ-PCR malaria parasitemia evaluation; Genotyping - host and parasite DNA isolation Test Code: DNAMALRT Collect 250 μL of blood to fill five spots on 1 Whatman Protein Saver Card (Cat. No. 10534612) (50 μL per spot, 5 spots per card) NOTE: Be careful to fill spots completely by placing blood drops in the center of the circle on the card and do not let blood from one spot touch next spot. See Dried blood spot SOP at the end of the LPC for details. DBS cards should be dried for a minimum of 4 hours on Whatman DBS card rack (Cat. No.: 10537173). and then put in gas impermeable bag with dessicant packet. Bag should be sealed and placed in clean, dry and insect and rodent free area of the lab. Cards prepared using venous blood methods should be logged into the LDMS. LDMS Spec. code: BLD/EDT/DBS. Cards prepared using the heelstick method LDMS spec. code: BLD/NON/DBS. The number of aliquots should equal the number of whole spots per card. One LDMS label should be affixed to the card itself. <strong>Bags containing DBS cards and dessicant stored for &gt;4 weeks but less than 4 months should be stored at -20°C, and storage &gt;4 months should be at -70°C.</strong> DBS cards stored at -20 or -70°C and shipped at ambient temperature: remove bag and allow to equilibrate to room temperature prior to shipping. Check dessicant to be sure it is still functional (note color) and replace dessicant if necessary. DBS shipped on dry ice: Place bag in the shipping box containing dry ice directly from the freezer. All DBS cards should be shipped to NYU. NOTE: This DBS card preparation is in addition to the pre-requisite Whatman Protein Saver cards for P1060.</td>
<td></td>
</tr>
</tbody>
</table>
| Entry | P1068s | 50 μL for preparation of thick and thin blood smears directly from a heel or finger stick or from an EDTA anticoagulated venous drawn specimen. **NOTE:** Actual amount is approximately 2 μL for thin smear and 4-6 μL for thick smear. Submit CRF SPW0273 P1068S | Diagnosis of Malaria parasitemia Test Code: PARBLDSM Note—Thin smears and Thick smears will be made separately. Thin smears will be fixed with methanol prior to Giemsa staining and Thick smears will be stained without methanol. 2 Thin and 2 Thick film smears should be made. PLEASE REFER TO THICK AND THIN SMEARS – CHECKLIST at the end of this LPC for very detailed instructions to follow. Always make smears with frosted side of slide up: Please note that heel or finger stick is the preferred method for preparation of thick and thin smears, wherever possible. Thick and thin smears must be made on separate slides. ThinSmears: Place one small drop of
Note: If smears are made from EDTA anticoagulated venous drawn specimen, gently invert the source of blood in the tube 3 times and then aliquot 5 µL onto each of two slides for thick and thin smear preparation. Also, this MUST be done as soon as possible (no greater than 15 minutes of the blood draw) to preserve the integrity of the slide for parasite identification.

blood on slide and using a second clean slide as a “spreader” touch small drop with spreader and allow blood to run along edge. Firmly push the spreader along slide, keeping at 45° angle. After drying, (fixing before drying will cause the slide to be unreadable), fix Thin Film with methanol by taking methanol from stock aliquot (kept sterile), covering slide with methanol, and leaving slide to dry. Do NOT re-use the edge of slides to prepare another thin smear as this may result in cross-contamination of smears.

**Thick film:** On a separate slide, using the corner of a clean and new slide spreader, quickly join 2-3 larger drops of blood and spread to make an even thick film  NOTE: Thick smears must be dried overnight prior to staining. Giemsa staining technique – regular method (See step by step instructions at the end of the LPC) - will be used for staining the thick and thin smears. Thick smears are not fixed with methanol prior to Giemsa staining. Each slide should be labeled with patient’s PID and date using soft lead pencil. – Be extremely careful not to contaminate pencil by coming into contact with blood.

If thick smears are used for diagnosis and viewed under oil immersion, clean the thick smear before storing by blotting slide gently with a clean tissue (this must be done with great care so as not to wipe the stain off). Slides, once dried (from either staining and/or reading) should be labeled with waterproof marker and will be sent once monthly to NYU., All slides must be shipped in light sensitive slide boxes (Fisher Scientific Catalogue Number S17528) with dessicant beads (in pack, not loose, so they do not scratch slides). See detailed shipping and storage instructions at the end of this LPC.

**DBS cards** should be dried for a minimum of 4 hours on Whatman DBS card rack (Cat. No: 10537173).and then put in gas impermeable bag with dessicant packet. Bag should be sealed and placed in clean, dry and insect and rodent free area of the lab. Cards prepared using venous blood methods should be logged into the LDMS.

**LDMS Spec. code:** BLD/EDT/DBS.

**LDMS Spec. code:** BLD/BLD/DBS.
The number of aliquots should equal the number of whole spots per card. One LDMS label should be affixed to the card itself. Bags containing DBS cards and dessicant stored for >4 weeks but less than 4 months should be stored at -20°C, and storage >4 months should be at -70°C. DBS cards stored at -20 or -70°C and shipped at ambient temperature: remove bag and allow to equilibrate to room temperature prior to shipping. Check dessicant to be sure it is still functional (note color) and replace dessicant if necessary. DBS shipped on dry ice: Place bag in the shipping box containing dry ice directly from the freezer. All DBS cards should be shipped to NYU.

NOTE: This DBS card preparation is in addition to the pre-required Whatman Protein Saver cards for P1060.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Description</th>
<th>Storage Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1068s</td>
<td>2.0 mL EDTA tubes- Invert 10-15 times gently. Send to qualified processing lab.</td>
<td>Plasma and PBMC pellet storage for future immunology studies; mRNA transcripts of chemokines in PBMC's. Test Code: IMM STOR</td>
</tr>
<tr>
<td></td>
<td>Submit CRF SPW0273 P1068S</td>
<td>Remove 0.3mL blood to 2 mL tube containing 1.3mL RNALater (Ambion, Cat.# AM7020) Mix thoroughly by inverting the tube several times, store at 4°C for up to one month or at -20°C for &gt;30 days. LDMS spec code: BLD/EDT/BLD/RNL</td>
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<td></td>
<td></td>
<td>Spin 1.7 mL blood at 800xg for 10 mins, remove plasma, respin plasma at 800xg for 10mins. Freeze 2x0.4mL aliquots at -70°C. LDMS spec. code: BLD/EDT/PL2</td>
</tr>
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<td></td>
<td>To cell pellet add phosphate buffered saline (PBS) at volume equal to plasma that was removed. Divide into two aliquots and store at -20°C. LDMS spec. code: BLD/EDT/PEL/PBS.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ship samples monthly to NYU.</td>
</tr>
</tbody>
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<tr>
<th>Weeks 2, 4, 8, 12, 16, 24, and 36</th>
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<th>50 ul for preparation of thick and thin blood smears directly from a heel or finger stick or from an EDTA anticoagulated venous drawn specimen. <strong>NOTE:</strong> Actual amount is approximately 2 μL for thin smear and 4-6 μL for thick smear. Submit CRF SPW0273 P1068S</th>
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<td></td>
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soon as possible (no greater than 15 minutes of the blood draw) to preserve the integrity of the slide for parasite identification.

leaving slide to dry. Do NOT re-use the edge of slides to prepare another thin smear as this may result in cross-contamination of smears.

**Thick film:** On a separate slide, using the corner of a clean and new slide spreader, quickly join 2-3 larger drops of blood and spread to make an even thick film NOTE: Thick smears must be dried overnight prior to staining.

Giemsa staining technique – regular method (See step by step instructions at the end of the LPC) - will be used for staining the **thick** and **thin** smears. Thick smears are not fixed with methanol prior to Giemsa staining.

Each slide should be labeled with patient’s PID and date using soft lead pencil. – Be extremely careful not to contaminate pencil by coming into contact with blood.

If thick smears are used for diagnosis and viewed under oil immersion, clean the thick smear before storing by blotting slide gently with a clean tissue (this must be done with great care so as not to wipe the stain off).

Slides, once dried (from either staining and/or reading) should be labeled with waterproof marker and will be sent once monthly to NYU. All slides must be shipped in light sensitive slide boxes (Fisher Scientific Catalogue Number S17528) with dessicant beads (in pack, not loose, so they do not scratch slides). See detailed shipping and storage instructions at the end of this LPC.

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<tr>
<th>Weeks 2, 4, 8, 12, 16, 24, and 36</th>
<th>P1068S</th>
<th>250uL for Preparation of Dried Blood Spots. Directly from a heel or finger stick or from an EDTA-anticoagulated venous drawn specimen.</th>
<th>Collect 250 uL of blood to fill five spots on 1 Whatman Protein Saver Card (Cat. No. 10534612) (50 uL per spot, 5 spots per card) NOTE: Be careful to fill spots completely by placing blood drops in the center of the circle on the card and do not let blood from one spot touch next spot. See Dried blood spot SOP at the end of the LPC for details. DBS cards should be dried for a minimum of 4 hours on Whatman DBS card rack (Cat. No.: 10537173). and then put in gas impermeable bag with dessicant packet. Bag should be sealed and placed in clean, dry and insect and rodent free area of the lab. Cards prepared using venous blood methods should be logged into the LDMS. LDMS Spec. code: BLD/EDT/DBS. Cards prepared using the heelstick method LDMS spec. code:BLD/NON/DBS. The number of aliquots should equal the number of whole spots per card. One LDMS label should be affixed to the card itself. Bags containing DBS cards and dessicant stored for &gt;4 weeks but less than 4 months should be stored at -20°C, and storage &gt;4 months should be at -70°C. DBS cards stored at -20 or -70°C and shipped at ambient temperature: remove</th>
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<tr>
<td></td>
<td></td>
<td>Submit CRF SPW0273 P1068S</td>
<td>Test Code: DNAMALRT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RTQ-PCR malaria parasitemia evaluation; Genotyping - host and parasite DNA isolation</td>
<td></td>
</tr>
</tbody>
</table>
| Week 48 | P1068s | 50 ul for preparation of thick and thin blood smears directly from a heel or finger stick or from an EDTA anticoagulated venous drawn specimen. **NOTE:** Actual amount is approximately 2 μL for thin smear and 4-6 μL for thick smear. Submit CRF SPW0273 P1068S  
**Note:** If smears are made from EDTA anticoagulated venous drawn specimen, gently invert the source of blood in the tube 3 times and then aliquot 5 μL onto each of two slides for thick and thin smear preparation. Also, this MUST be done as soon as possible (no greater than 15 minutes of the blood draw) to preserve the integrity of the slide for parasite identification. |
|---|---|---|
| | | **Diagnosis of Malaria parasitemia**  
**Test Code:** PARBLDSM  
**Note**—**Thin smears** and **Thick smears** will be made separately. **Thin** smears will be fixed with methanol prior to Giemsa staining and **Thick** smears will be stained without methanol. 2 Thin and 2 Thick film smears should be made. PLEASE REFER TO THICK AND THIN SMEARS – CHECKLIST at the end of this LPC for very detailed instructions to follow. Always make smears with frosted side of slide up: Please note that heel or finger stick is the preferred method for preparation of thick and thin smears, wherever possible. **Thick and thin smears must be made on separate slides.** **Thin Smears:** Place one small drop of blood on slide and using a second clean slide as a “spreader” touch small drop with spreader and allow blood to run along edge. Firmly push the spreader along slide, keeping at 45° angle. After **drying**, (fixing before drying will cause the slide to be unreadable), fix Thin Film with methanol by taking methanol from stock aliquot (kept sterile), covering slide with methanol, and leaving slide to dry. Do NOT re-use the edge of slides to prepare another thin smear as this may result in cross-contamination of smears. **Thick film:** On a separate slide, using the corner of a clean and new slide spreader, quickly join 2-3 larger drops of blood and spread to make an even thick film **NOTE:** Thick smears must be dried overnight prior to staining. Giemsa staining technique – regular method (See step by step instructions at the end of the LPC) - will be used for staining the **thick and thin** smears. Thick smears are not fixed with methanol prior to Giemsa staining. Each slide should be labeled with patient’s PID and date using soft lead pencil. – Be extremely careful not to contaminate pencil by coming into contact with blood. If thick smears are used for diagnosis and viewed under oil immersion, clean the thick smear before storing by blotting slide gently with a clean tissue (this must be done with great care so as not to wipe the stain off). Slides, once dried (from either staining and/or reading) should be labeled with waterproof marker and will be sent once monthly to NYU., All slides must be shipped in light sensitive slide boxes. |
(Fisher Scientific Catalogue Number S17528) with dessicant beads (in pack, not loose, so they do not scratch slides). See detailed shipping and storage instructions at the end of this LPC. 

| Week 48 | P1068s | 250uL for Preparation of Dried Blood Spots. Directly from a heel or finger stick or from an EDTA-anticoagulated venous drawn specimen. | RTQ-PCR malaria parasitemia evaluation; Genotyping - host and parasite DNA isolation | Collect 250 uL of blood to fill five spots on 1 Whatman Protein Saver Card (Cat. No. 10534612) (50 uL per spot, 5 spots per card) NOTE: Be careful to fill spots completely by placing blood drops in the center of the circle on the card and do not let blood from one spot touch next spot. See Dried blood spot SOP at the end of the LPC for details. 
DBS cards should be dried for a minimum of 4 hours on Whatman DBS card rack (Cat. No.: 10537173).and then put in gas impermeable bag with dessicant packet. Bag should be sealed and placed in clean, dry and insect and rodent free area of the lab. Cards prepared using venous blood methods should be logged into the LDMS. 
LDMS Spec. code: BLD/EDT/DBS. 
Cards prepared using the heelstick method LDMS spec. code: BLD/NON/DBS. 
The number of aliquots should equal the number of whole spots per card. One LDMS label should be affixed to the card itself. 
**Bags containing DBS cards and dessicant stored for >4 weeks but less than 4 months should be stored at -20°C, and storage >4 months should be at -70°C.** DBS cards stored at -20 or -70°C and shipped at ambient temperature: remove bag and allow to equilibrate to room temperature prior to shipping. Check dessicant to be sure it is still functional (note color) and replace dessicant if necessary. DBS shipped on dry ice: Place bag in the shipping box containing dry ice directly from the freezer. All DBS cards should be shipped to NYU. 
NOTE: This DBS card preparation is in addition to the pre-required Whatman Protein Saver cards for P1060.

| Week 48 | P1068s | 2.0_mL EDTA tubes- Invert 10-15 times gently. Send to qualified processing lab ambient. | Plasma and PBMC pellet storage for future immunology studies | Remove 0.3mL blood to 2 mL tube containing 1.3mL RNAlater (Ambion, Cat.# AM7020) Mix thoroughly by inverting the tube several times, store at 4°C for up to one month or at -20°C for >30 days. 
LDMS spec code: BLD/EDT/BLD/RNL 
Spin 1.7 mL blood at 800xg for 10 mins, remove plasma, respin plasma at 800xg for 10mins. Freeze 2x0.4mL aliquots at -70°C. 
LDMS spec. code: BLD/EDT/PL2 
To cell pellet add phosphate buffered saline (PBS) at volume equal to plasma that was removed. Divide into two aliquots and store at ambient.

Submit CRF SPW0273 P1068S 
Test Code: DNAMALRT 
Submit CRF SPW0273 P1068S 
Test Code: IMM STOR
| Every 12 Weeks (+/-6 weeks) | P1068s | 50 μL for preparation of thick and thin blood smears directly from a heel or finger stick or from an EDTA anticoagulated venous drawn specimen. **NOTE:** Actual amount is approximately 2 μL for thin smear and 4-6 μL for thick smear. Submit CRF SPW0273 P1068S

Note: If smears are made from EDTA anticoagulated venous drawn specimen, gently invert the source of blood in the tube 3 times and then aliquot 5 μL onto each of two slides for thick and thin smear preparation. Also, this MUST be done as soon as possible (no greater than 15 minutes of the blood draw) to preserve the integrity of the slide for parasite identification. | Diagnosis of Malaria parasitemia

Test Code: PARBLDSM

**Note—** **Thin smear**s and **Thick smear**s will be made separately. **Thin smear**s will be fixed with methanol prior to Giemsa staining and **Thick smear**s will be stained without methanol. 2 **Thin** and 2 **Thick** film smears should be made. PLEASE REFER TO THICK AND THIN SMEARS – CHECKLIST at the end of this LPC for very detailed instructions to follow. Always make smears with frosted side of slide up: **Please note that heel or finger stick is the preferred method for preparation of thick and thin smears, wherever possible.** **Thick and thin smears must be made on separate slides.** **Thin smears:** Place one small drop of blood on slide and using a second clean slide as a “spreader” touch small drop with spreader and allow blood to run along edge. Firmly push the spreader along slide, keeping at 45° angle. After drying, (fixing before drying will cause the slide to be unreadable), fix Thin Film with methanol by taking methanol from stock aliquot (kept sterile), covering slide with methanol, and leaving slide to dry. Do NOT re-use the edge of slides to prepare another thin smear as this may result in cross-contamination of smears. **Thick film:** On a separate slide, using the corner of a clean and new slide spreader, quickly join 2-3 larger drops of blood and spread to make an even thick film **NOTE:** Thick smears must be dried overnight prior to staining. Giemsa staining technique – regular method (See step by step instructions at the end of the LPC) - will be used for staining the **thick** and **thin** smears. Thick smears are not fixed with methanol prior to Giemsa staining. Each slide should be labeled with patient’s PID and date using soft lead pencil. – Be extremely careful not to contaminate pencil by coming into contact with blood. If thick smears are used for diagnosis and viewed under oil immersion, clean the thick smear before storing by blotting slide gently with a clean tissue (this must be done with great care so as not to wipe the stain off). Slides, once dried (from either staining and/or reading) should be labeled with waterproof marker and will be sent once monthly to NYU. All slides must be shipped in light sensitive slide boxes (Fisher Scientific Catalogue Number S17528) with dessicant beads (in pack, not loose, so they do not scratch slides). See detailed shipping and storage instructions at the end of this LPC. |
| Every 12 Weeks (+/-6 weeks) | P1068S | 250uL for Preparation of Dried Blood Spots. Directly from a heel or finger stick or from an EDTA-anticoagulated venous drawn specimen. Submit CRF SPW0273 P1068S | RTQ-PCR malaria parasitemia evaluation; Genotyping - host and parasite DNA isolation Test Code: DNAMALRT | Collect 250 uL of blood to fill five spots on 1 Whatman Protein Saver Card (Cat. No. 10534612) (50 uL per spot, 5 spots per card) NOTE: Be careful to fill spots completely by placing blood drops in the center of the circle on the card and do not let blood from one spot touch next spot. See Dried blood spot SOP at the end of the LPC for details. DBS cards should be dried for a minimum of 4 hours on Whatman DBS card rack (Cat. No.: 10537173).and then put in gas impermeable bag with dessicant packet. Bag should be sealed and placed in clean, dry and insect and rodent free area of the lab. Cards prepared using venous blood methods should be logged into the LDMS. LDMS Spec. code: BLD/EDT/DBS. Cards prepared using the heelstick method LDMS spec. code: BLD/NON/DBS. The number of aliquots should equal the number of whole spots per card. One LDMS label should be affixed to the card itself. Bags containing DBS cards and dessicant stored for >4 weeks but less than 4 months should be stored at -20 °C, and storage >4 months should be at -70 °C. DBS cards stored at -20 or -70 °C and shipped at ambient temperature: remove bag and allow to equilibrate to room temperature prior to shipping. Check dessicant to be sure it is still functional (note color) and replace dessicant if necessary. DBS shipped on dry ice: Place bag in the shipping box containing dry ice directly from the freezer. All DBS cards should be shipped to NYU. NOTE: This DBS card preparation is in addition to the pre-required Whatman Protein Saver cards for P1060. |
| Every 48 Weeks (+/-6 weeks) | P1068s | 2.0_mL EDTA tubes- Invert 10-15 times gently. Send to qualified processing lab ambient. Submit CRF SPW0273 P1068S | Plasma and PBMC pellet storage for future immunology studies Test Code: IMM STOR | Remove 0.3mL blood to 2 mL tube containing 1.3mL RNAlater (Ambion, Cat.# AM7020) Mix thoroughly by inverting the tube several times, store at 4°C for up to one month or at -20°C for >30 days. LDMS spec code: BLD/EDT/LBL/RNL Spin 1.7 mL blood at 800xg for 10 mins, remove plasma, respin plasma at 800xg for 10mins. Freeze 2x0.4mL aliquots at −70°C. LDMS spec. code: BLD/EDT/PL2 To cell pellet add phosphate buffered saline (PBS) at volume equal to plasma that was removed. Divide into two aliquots and store at -20°C. LDMS spec. code: BLD/EDT/PEL/PBS. Ship samples monthly to NYU. |
| Inter- | P1068S | 50 ul for preparation of thick and thin blood | Diagnosis of Malaria Note—Thin smears and Thick smears will be made separately. |
| **Current Illness Visits** | smears directly from a heel or finger stick or from an EDTA anticoagulated venous drawn specimen. 
**NOTE:** Actual amount is approximately 2 µL for thin smear and 4-6 µL for thick smear. 
Submit CRF SPW0273  P1068S | parasitemia  
Test Code: PARBLDSM  
**Thin smears** will be fixed with methanol prior to Giemsa staining and **Thick** smears will be stained without methanol. 2 Thin and 2 Thick film smears should be made. PLEASE REFER TO THICK AND THIN SMEARS – CHECKLIST at the end of this LPC for very detailed instructions to follow. Always make smears with frosted side of slide up: **Please note that heel or finger stick is the preferred method for preparation of thick and thin smears, wherever possible.** Thick and thin smears must be made on separate slides. 
**Thin Smears:** Place one small drop of blood on slide and using a second clean slide as a “spreader” touch small drop with spreader and allow blood to run along edge. Firmly push the spreader along slide, keeping at 45° angle. **After drying,** (fixing before drying will cause the slide to be unreadable), fix Thin Film with methanol by taking methanol from stock aliquot (kept sterile), covering slide with methanol, and leaving slide to dry. **Do NOT** re-use the edge of slides to prepare another thin smear as this may result in cross-contamination of smears. 
**Thick film:** On a separate slide, using the corner of a clean and new slide spreader, quickly join 2-3 larger drops of blood and spread to make an even thick film 
**NOTE:** Thick smears must be dried overnight prior to staining. 
Giemsa staining technique – regular method (See step by step instructions at the end of the LPC) - will be used for staining the **thick and thin** smears. Thick smears are not fixed with methanol prior to Giemsa staining. 
Each slide should be labeled with patient’s PID and date using soft lead pencil. – Be extremely careful not to contaminate pencil by coming into contact with blood. 
If thick smears are used for diagnosis and viewed under oil immersion, clean the thick smear before storing by blotting slide gently with a clean tissue (this must be done with great care so as not to wipe the stain off). 
Slides, once dried (from either staining and/or reading) should be labeled with waterproof marker and will be sent once monthly to NYU., All slides must be shipped in light sensitive slide boxes (Fisher Scientific Catalogue Number S17528) with dessicant beads (in pack, not loose, so they do not scratch slides). See detailed shipping and storage instructions at the end of this LPC. |
| **Inter-Current Illness Visits** | 250uL for Preparation of Dried Blood Spots. Directly from a heel or finger stick or from an EDTA-anticoagulated venous drawn specimen. 
Submit CRF SPW0273 P1068S | RTQ-PCR malaria parasitemia evaluation; 
Genotyping - host and parasite DNA isolation  
Test Code: DNAMALRT  
**Collect 250 uL of blood to fill five spots on 1 Whatman Protein Saver Card (Cat. No. 10534612) (50 uL per spot, 5 spots per card)** 
**NOTE:** Be careful to fill spots completely by placing blood drops in the **center** of the circle on the card and do not let blood from one spot touch next spot. See Dried blood spot SOP at the end of the LPC for details. |
DBS cards should be dried for a minimum of 4 hours on Whatman DBS card rack (Cat. No.: 10537173). and then put in gas impermeable bag with dessicant packet. Bag should be sealed and placed in clean, dry and insect and rodent free area of the lab. Cards prepared using venous blood methods should be logged into the LDMS.

LDMS Spec. code: BLD/EDT/DBS.
Cards prepared using the heelstick method LDMS spec. code: BLD/NON/DBS.

The number of aliquots should equal the number of whole spots per card. One LDMS label should be affixed to the card itself. Bags containing DBS cards and dessicant stored for >4 weeks but less than 4 months should be stored at -20°C, and storage >4 months should be at -70°C. DBS cards stored at -20 or -70°C and shipped at ambient temperature: remove bag and allow to equilibrate to room temperature prior to shipping. Check dessicant to be sure it is still functional (note color) and replace dessicant if necessary. DBS shipped on dry ice: Place bag in the shipping box containing dry ice directly from the freezer. All DBS cards should be shipped to NYU.

NOTE: This DBS card preparation is in addition to the pre-required Whatman Protein Saver cards for P1060.

| Early Disc./End of Study | P1068S | 50 ul for preparation of thick and thin blood smears directly from a heel or finger stick or from an EDTA anticoagulated venous drawn specimen. 
NOTE: Actual amount is approximately 2 μL for thin smear and 4-6 μL for thick smear. | Diagnosis of Malaria parasitemia 
Test Code: PARBLDSM | Diagnosis of Malaria parasitemia 
Test Code: PARBLDSM | Note—Thin smears and Thick smears will be made separately. Thin smears will be fixed with methanol prior to Giemsa staining and Thick smears will be stained without methanol. 2 Thin and 2 Thick film smears should be made. PLEASE REFER TO THICK AND THIN SMEARS – CHECKLIST at the end of this LPC for very detailed instructions to follow. Always make smears with frosted side of slide up. 
Thin Smears: Place one small drop of blood on slide and using a second clean slide as a “spreader” touch small drop with spreader and allow blood to run along edge. Firmly push the spreader along slide, keeping at 45° angle. After drying, (fixing before drying will cause the slide to be unreadable), fix Thin Film with methanol by taking methanol from stock aliquot (kept sterile), covering slide with methanol, and leaving slide to dry. Do NOT re-use the edge of slides to prepare another thin smear as this may result in cross-contamination of smears. Thick film: On a separate slide, using the corner of a clean and new slide spreader, quickly join 2-3 larger drops of blood and spread to make an even thick film.Giemsa staining technique – regular method (See step by step instructions at the end of the LPC) - will be used for staining the thick and thin smears. Thick smears are not fixed with methanol.

Submit CRF SPW0273 P1068S 
Note: If smears are made from EDTA anticoagulated venous drawn specimen, gently invert the source of blood in the tube 3 times and then aliquot 5 μL onto each of two slides for thick and thin smear preparation. Also, this MUST be done as soon as possible (no greater than 15 minutes of the blood draw) to preserve the integrity of the slide for parasite identification.
prior to Giemsa staining. Each slide should be labeled with patient’s PID and date using soft lead pencil. – Be extremely careful not to contaminate pencil by coming into contact with blood. If thick smears are used for diagnosis and viewed under oil immersion, clean the thick smear before storing by blotting slide gently with a clean tissue (this must be done with great care so as not to wipe the stain off). Slides, once dried (from either staining and/or reading) should be labeled with waterproof marker and will be sent once monthly to NYU., All slides must be shipped in light sensitive slide boxes (Fisher Scientific Catalogue Number S17528) with dessicant beads (in pack, not loose, so they do not scratch slides).

| Early Disc./ End of Study | P1068S | 250uL for Preparation of Dried Blood Spots. Directly from a heel or finger stick or from an EDTA-anticoagulated venous drawn specimen. Submit CRF SPW0273 P1068S | RTQ-PCR malaria parasitemia evaluation; Genotyping - host and parasite DNA isolation Test Code: DNAMALRT | Collect 250 uL of blood to fill five spots on 1 Whatman Protein Saver Card (Cat. No. 10534612) (50 uL per spot, 5 spots per card) NOTE: Be careful to fill spots completely by placing blood drops in the center of the circle on the card and do not let blood from one spot touch next spot. See Dried blood spot SOP at the end of LPC for details. DBS cards should be dried for a minimum of 4 hours on Whatman DBS card rack (Cat. No.: 10537173).and then put in gas impermeable bag with dessicant packet. Bag should be sealed and placed in clean, dry and insect and rodent free area of the lab. Cards prepared using venous blood methods should be logged into the LDMS. LDMS Spec. code: BLD/EDT/DBS. Cards prepared using the heelstick method LDMS spec. code:BLD/NON/DBS. The number of aliquots should equal the number of whole spots per card. One LDMS label should be affixed to the card itself. Bags containing DBS cards and dessicant stored for >4 weeks but less than 4 months should be stored at -20°C, and storage >4 months should be at -70°C. DBS cards stored at -20 or -70°C and shipped at ambient temperature: remove bag and allow to equilibrate to room temperature prior to shipping. Check dessicant to be sure it is still functional (note color) and replace dessicant if necessary. DBS shipped on dry ice: Place bag in the shipping box containing dry ice directly from the freezer. All DBS cards should be shipped to NYU. NOTE: This DBS card preparation is in addition to the pre-required Whatman Protein Saver cards for P1060. |

| Early Disc./ End of Study | P1068S | 2.0 mL EDTA tubes- Invert 10-15 times gently. Send to qualified processing lab ambient. | Plasma and PBMC pellet storage for future immunology studies; m | Remove 0.3mL blood to 2 mL tube containing 1.3mL RNAlater (Ambion, Cat.# AM7020) Mix thoroughly by inverting the tube several times, store at 4°C for up to one month or at -20°C for |
Submit CRF SPW0273 P1068S

RNA transcripts of chemokines in PBMC’s. Test Code: IMM STOR

>30 days.
LDMS spec code: BLD/EDT/BLD/RNL
Spin 1.7 mL blood at 800xg for 10 mins, remove plasma, respin plasma at 800xg for 10 mins. Freeze 2x0.4mL aliquots at −70°C.
LDMS spec. code: BLD/EDT/PL2
To cell pellet add phosphate buffered saline (PBS) at volume equal to plasma that was removed. Divide into two aliquots and store at -20°C.
LDMS spec. code: BLD/EDT/PEL/PBS.
Ship samples monthly to NYU.

Clinicians: Specimen Labels must include the following: PID, SID, date of collection, VID, time of collection Lab Techs: Aliquot Labels must include the following: LDMS Specimen number, PID, protocol, date of collection, VID), and LDMS Specimen code.

LABORATORY CONTACT AND SHIPPING INSTRUCTIONS:
ALL dried blood spots, ALL thick and thin smears, and ALL cell pellet and plasma specimen shipments should be addressed to:

Charlotte Hobbs, M.D. or William Borkowsky, M.D.
New York University School of Medicine
Department of Pediatrics
Division of Infectious Diseases
Pediatric Infectious Disease Laboratory
8N16
462 First Avenue
New York, New York 10016
United States
Phone: (212) 562-3612, (212) 263-8971, (212) 263-6513
FAX: (212) 263-7806
Shipping notification should be sent PRIOR TO SHIPMENT to charlotte.hobbs@nyumc.org, with a copy to andre.fidelia@nyumc.org, borkow01@med.nyu.edu and charlottehobbs@gmail.com. Please ensure shipment is during the week so as not to arrive on a weekend or holiday.

NOTE: CHECKLISTS FOR MAKING THIN AND THICK SMEARS AND FOR DRIED BLOOD SPOT PREPARATION, STORAGE AND SHIPPING ARE ALSO POSTED ON THE LABORATORY SECTION OF THE P1068S WEBSITE.
Note regarding Thick and Thin Smear Storage and Shipment:
Regarding SLIDE storage: please ensure slides are stored in a cool, dry place, and if thick or thin smears are used for diagnosis and viewed under oil immersion clean the thick smear before storing by blotting slide gently with a clean tissue (this must be done with great care so as not to wipe the stain off). This can be done with a Kimwipe or GENTLY blotting with a paper towel. After slides are completely dry and labeled, they can be stored in light sensitive slide boxes (Fisher Scientific Catalogue Number S17528) with desiccant beads (in pack, secured in box (e.g., with tape) and NOT loose, so they do not scratch slides. Slides must be stored in a cool, dry place away from insects and light until ready for shipment. If slides will be stored for <6 months, 4 degrees is appropriate. For longer storage, we recommend -70 or colder (frost-free). When ready to ship, pack the paired thick and thin smears pairs from the light sensitive storage box into a Fisherbrand Two-Place Plastic Microscope Slide Mailer and Transporter (Fisher Scientific, Catalogue Number 04-335-45). For each time period, paired thin smears and paired thick smears will be placed in each Slide Mailer (each Mailer holds two slides). Insert the Slide Mailer into the sealable plastic bag containing a desiccant pack and seal the bag, wrap the Slide Mailer in bubble wrap, confirm that the appropriate documentation is filled out prior to shipment.

Helpful Links
- Preparation of thick and thin smears: http://www.dpd.cdc.gov/dpdx/HTML/PDF_Files/Malaria_procedures_benchaid.pdf
- Basic Malaria Microscopy: http://nzdl.sadl.uleth.ca/cgi-bin/library?e=d-00000-00-000----off-0efeller--00-0--0-10-0----0---0prompt-10---4-------0-11--11- en-50---20-about---00-0-1-00-0-0-11-1-0utfZz-8-00&a=d&cl=CL1.1&d=HASH01a1641e9e04f6e6c9f5acf7.15