|  |
| --- |
| **Hettich Mikro 200 Operating Procedure** |
| **Purpose** | This procedure provides instructions for operation of the Hettich Mikro 200 high speed centrifuge for the clarification of serum and plasma. |
| **Principle** | For the clarification of serum and plasma the use of high speed centrifugation (>10,000 RCF) has been shown to be equivalent to ultrafugation when performed for the appropriate period of time.1 RCF (Relative Centrifugal Force) is a unit free value given as a multiple of the acceleration due to gravity (g). Centrifugation utilizes the relative density of parts of a blood specimen (for example lipids, serum, and blood cells) to separate the individual components. RCF is related to RPM (Revolutions per minute) by the equation $$RCF=\left(\frac{RPM}{1000}\right)^{2}x r x 1.12$$RCF = relative centrifugal forceRPM = rotational speed (revolutions per minute)R = centrifugal radius in mm |
| **Clinical Significance** | For specific assays, sample preparation may require the removal of interfering substances such as lipids, platelets, cellular components, etc.  |
|  |  |
| **Materials** | **Equipment** | **Supplies** |
|  | * Hettich Mikro 200
* 24 Position Angle Rotor #2434
 | * Disposable Glass pipettes
* 1.5 mL SP Microtubes - Cat. C1300-29 – Cardinal
* Rotor lubricant – Included
 |
| **Sample** | Serum or plasma samples up to 1.5 mL. |
| **Special Safety Precautions**  | The centrifuge must be loaded symmetrically. Verify the rotor is firmly seated and that the biosafety cover is secured in place before operation. Observe standard precautions as the device can become contaminated. Disconnect from power before cleaning or servicing.  |
| **Maintenance** | Perform user maintenance weekly. Annual inspections or repair performed by Biomed. Document actions on the CH 5.63.f1 Hettich Mikro 200 Maintenance Log as they are performed.

|  |  |
| --- | --- |
| **Task** | **Instructions** |
| Surface cleaning/disinfection | Inspect outside surface, accessories, rotor, and centrifuge chamber. Wipe up condensation or liquids. Clean lightly with warm soapy water or disinfect with alcohol pads as needed. Ensure centrifuge is completely dried before use. |
| Rotor maintenance | Check seating and normal-use wear of rotor. To clean, disassemble by removing rotor from motor shaft with 5 mm hex key. Lightly grease motor shaft before re-assembly. |

 |
| **Procedure** |  |
|  | **Step** | **Action** |
|  | 1 | Verify centrifuge rotor is securely in place, that there is no liquid or sample residue in the centrifuge, and that the centrifuge gasket is not corroded or cracked. Verify routine maintenance has been documented and is up to date before use. |
|  | **If** | **Then** |
|  | Rotor is loose/unbalanced | Remove and replace using 5 mm hex key, take care to seat the rotor aligned with the guide.  |
|  | Liquid/residue in the centrifuge | Clean with a small amount of warm soapy water. Wipe away soap residue and dry before use. |
|  | Gasket is corroded/cracked | Do not use centrifuge. Contact biomed for repair.  |
|  | 2 | Aliquot patient sample into appropriately labeled 1.5 mL microcentrifuge tube. |
|  | 3 | Find or create appropriate balance tube. To create a balance tube, fill microcentrifuge tube with DI water, label as “balance”.  |
|  | 4  | Load centrifuge in a symmetrical manner. Ensure biosafety cover is in place by turning knob in a clockwise manner, taking care to not cross thread. |
|  | 5  | Close cover and push the start button. |
|  | 6 | After centrifugation (10 minutes at 15,000 RPM) the centrifuge will come to a halt. Push the STOP/Open button. Remove biosafety cover and remove sample. |
|  | 7 | Remove clarified sample from the microcontainer by use of glass pipette. Insert pipette tip beneath the lipid layer into the clarified sample, taking care to avoid any cellular pellet if present, and aspirate. Dispense clarified sample into container labeled with appropriate patient identifiers. Clearly mark with sharpie as **clarified**, **ultracentrifuged**, or **airfuged** (these terms are synonymous for this purpose).  |
|  | 8 | Reassess sample quality by visual inspection or by instrumentation (ex: Abbott HIL). Perform patient testing.  |
| **Calculations** | The default program is set to 10 minutes at 15,000 RPM (21,420 RCF). If this program is accidentally deleted or modified, see section 15 of the manufacturer’s operating manual for instructions to reprogram.  |
| **Limitations** | Take care to follow assay specific instructions for removal of interfering substances. Some analytes do not allow for clarification by high speed centrifugation (ex: ammonia, triglyceride). |
| **References** | 1. “Removing Lipemia in Serum/Plasma Samples: A Multicenter Study” Ann Lab Med. 2018 Nov; 38(6): 518–523
2. Hettich Mikro 200 Operating Instructions. 2020 April, Rev. 09. 30-51, 92
 |
| **Appendices** | CH 5.63.f1 Hettich Mikro 200 Maintenance Log |
|  |  |  |  |
| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Matt Johnson | 8/16/2021 | Initial Version |