STANDARDIZED TESTING / OPERATING PROTOCOL REQUEST/ANNOUNCEMENT

Beckman Coulter Procedural Update: Specimen Handling Instructions

Description:	The Cone Health Laboratories will add specimen handling instructions for Beckman Coulter testing to their analyzer procedures.
Implementation Date:	April 11, 2017
Performing Locations:	**Click on the boxes that apply:** ☒Annie Penn Hospital ☒Moses Cone Hospital ☒Med Center at High Point ☒Wesley Long Hospital ☒Women's Hospital ☒Alamance Regional
Affected Locations:	**Click on the boxes that apply:** ☒Annie Penn Hospital ☒Moses Cone Hospital ☒Med Center at High Point ☒Wesley Long Hospital ☒Women's Hospital ☒Alamance Regional
Affected Departments:	**Click on the boxes that apply:** ☐Blood Bank ☐Cytology ☐Flow Cytometry ☐Histology ☐Microbiology ☒Phlebotomy ☐Point of Care ☒Rapid Response Lab ☐Respiratory Therapy ☒Specimen Processing
Specimen Type:	See CHEM-0369L-CH Clinical Chemistry Information Sheet for specimen types

Updated Clinical Lab Procedures:	CHEM-0365-MC Beckman DxC 880i Operating Procedure CHEM-0366-CH Beckman DxC 600i Operating Procedure CHEM-0367-CH Beckman DxC 600 Basic Operating Procedure CHEM-0371-CH Basic Operating Procedure for Beckman Access2
Retired Clinical Lab Procedures:	N/A
Notification to Client:	**Click on the boxes that apply:** ☒Section Not Applicable ☐Memo Needed **Distribution of Memo:** ☐Medical Staff ☐ Allied Health Professionals (PA, Nurse Practioners) ☐ Anesthesia ☐ Annie Penn (Primary Source Physicians) ☐ Dentist ☐ Emergency Department/Urgent Care Centers ☐ Family Practice ☐ Infectious Docs #ID Docs (John Campbell, Robert Comer, Jeffrey Hatcher, Cynthia Snider, Kees Van Dam) ☐ OB/GYN ☐ Pathology ☐ Pediatricians ☐ Psych ☐ Radiology ☐ Surgery ☐ #Nursing Leadership (Directors, Asst. Directors, Clinical Nurse Manager) ☐ Pharmacy - Send to DeAnne Brooks & Jim Hasspacher ☐ #IM Residents ☐ Kim Helsabeck ☐ Phlebotomy Managers and Supervisors ☐ Point of Care: Sheila, Kim & Marty
Accreditation Section:	**Click on the boxes that apply:** ☒Section Not Applicable ☐CAP Test menu change needed ☐CMS Analyte form change needed ☐Proficiency Testing surveys changes needed or ordered
Laboratory IT section:	**Click box and type needed changes/additions:** ☒Section Not Applicable ☐LIS changes

	☐Reference range change/addition ☐Technical Failure change/addition ☐Critical Value change/add ☐Text comments needed ☐Specimen collection instructions ☐Need to monitor TAT ☐CPT code for tests(s)
Technical Staff Update:	**Technical Staff Update:** Please read and review the attached documentation from Beckman Coulter: Sampling Handling Makes a Difference The Role of Preanalytical Factors in Chemistry and Immunoassay Testing Please note the following points and implement them into your work processes: 1. Keep serum tubes **vertical** when allowing them to clot. 2. **Aspirate, don't pour!** If you need to transfer sample to a secondary container or insert cup, always make sure to pipette your sample and leave a small amount of serum/plasma on top of the separator or packed cells. Never pour over the specimen – this disturbs cellular debris/fibrin at the gel/plasma/serum interface. 3. If debris or fibrin is noted in sample, aliquot specimen into a secondary container and recentrifuge. Do **NOT** respin a gel tube. Upon completion of secondary spin, pipette serum/plasma into appropriate container for testing. **Why?** Failure to follow the above instructions may lead to cellular debris and/or fibrin in the specimen. This is known interference for Troponin I testing and **may cause false positive results.** **Phlebotomy Staff Update:** Please read and review the attached documentation from Beckman Coulter: Sampling Handling Makes a Difference The Role of Preanalytical Factors in Chemistry and Immunoassay Testing Please note the following points and implement them into your work processes: 1. **Draw the correct volume.** Fill evacuated tubes until the vacuum is exhausted and blood flow ceases. 2. **Adequately mix** any tube containing additives per manufacturer's recommendations: a. Light Green/Green: 8-10 times b. Lavender: 8-10 times c. Gray: 8-10 times d. Red/Gold: 5 times 3. Keep serum tubes **vertical** when allowing them to clot. **Why?**

	Failure to follow the above instructions may lead to cellular debris and/or fibrin in the specimen. This is a known interference for Troponin I testing and **may cause false positive results.**
STOP Initiator:	Jackie Hobbins
GSO/Reidsville Medical Director Signature/Date:	Quality Department will obtain signature: John Patterson, MD 4/5/17
Alamance Medical Director Signature/Date:	Quality Department will obtain signature: Sara C. Rubinas MD 4/6/17

SAMPLE HANDLING MAKES A DIFFERENCE

In clinical laboratory testing, sample preparation processes must be followed carefully to ensure accurate results. Improperly handled samples can give misleading results and compromise the function of diagnostic instruments. The following guidelines are recommended for the proper handling of serum and plasma samples. Always be sure to follow your laboratory's official procedures for collecting, processing and handling samples.*

Draw the Correct Volume

Fill evacuated tubes until the vacuum is exhausted and blood flow ceases. Too little blood means too much of the anticoagulant or other additives, which can affect sample quality and interfere with lab tests.

Mix: It's Essential

- Mix any tube containing additives immediately after collection. Gently invert plasma tubes as many times as are directed by the tube manufacturer
- Insufficient mixing of tubes with anticoagulants allows microclots to form
- Insufficient mixing of tubes with separator gel can interfere with barrier formation, causing gel material to remain in the serum or plasma layer

Allow Time to Clot

- Most serum tubes need a minimum of 30 minutes to clot. Inadequate clotting time can lead to clot formation later
- Tubes are available with clot activators/accelerators that decrease the clot time to as little as 2-5 minutes
- Keep tubes vertical while clotting

Spin Under the Correct Conditions

- Centrifuge according to the tube manufacturer's recommendations
- Do not re-spin primary tubes; cells can rupture and leak, contaminating the sample. Transfer sample layer to another container first

Aspirate, Don't Pour

- If you need to transfer the sample to a secondary container, always make sure to aspirate your sample and leave a small amount on top of the separator or packed cells. Processed plasma tubes often contain a layer of cellular debris on top of the gel or packed cells

For More Information

- Refer to the product insert or package labeling supplied by the manufacturer of any blood collection product for complete recommendations on sample collection processing
- The Clinical Laboratory Standards Institute (CLSI) has approved two guidelines for specimen collection and processing:
  - Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard – Sixth Edition (GP41-A6) 2007
  - Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline – Fourth Edition (GP44-A4) 2010

*Refer to product insert

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INFORMATION BULLETIN

THE ROLE OF PRE-ANALYTICAL FACTORS IN CHEMISTRY AND IMMUNOASSAY TESTING

Introduction

Measurement of biochemical markers is an important aid to clinicians in the early detection, diagnosis, monitoring and prognosis of disease. Specimen quality plays a key role in ensuring the accuracy of those measurements in clinical laboratory testing. To gain efficiencies in workflow and decrease turnaround time (TAT), many laboratories have adopted new strategies and practices, including transitioning from:

- **Glass to plastic specimen collection tubes**
- **Serum to anticoagulated plasma samples**
- **Manual processing to laboratory automation**
- **Sample collection by laboratory staff to non-laboratory personnel**

As laboratories automate more processes, less time is dedicated to sample inspection steps and, consequently, monitoring specimen quality. Pre-analytical factors can be magnified by sensitive immunoassays and present an increasing challenge to quality clinical care.

Pre-analytical variables that could affect results

As much as 84% of laboratory errors can be attributed to the pre-analytical phase of clinical laboratory testing. This phase comprises an evaluation of a patient's condition as well as specimen collection, transport, processing and placement on the analyzer.[1-5] Patient samples with circulating protein interferants, such as human anti-animal antibodies and rheumatoid factor, may affect the results of certain assays. These are also examples of potential sources of errors outside the control of the laboratory.[6,7] Knowledge of such factors is important when determining the appropriate interpretation of results.

The large majority of pre-analytical errors are due to compromised sample quality,[1-5] which can occur during specimen collection, storage, transport and processing. Common factors contributing to errors include: incorrect labeling of tubes, insufficient blood draw volume, insufficient mixing, cellular contamination in plasma specimens and inadequate clotting of serum specimens.

To maintain sample quality, each stage of sample preparation is important. It is critical that personnel performing blood collection adhere to all recommendations specified by blood collection tube manufacturers. Deviations from the manufacturer's recommendations must be validated in individual laboratories.

Factors affecting plasma samples

While serum may provide the cleanest sample from an interference perspective, certain factors can affect processing the sample in a timely manner. Because urgent, critical decisions are based on STAT results, heparinized plasma samples have become the preferred sample type. Laboratory Medicine Practice Guidelines, published by the National Academy of Clinical Biochemistry (NACB), recommend plasma for STAT analysis of cardiac markers.[8] Plasma provides the best opportunity for achieving the desired rapid turnaround time. However, there are variables that must be controlled to obtain the best possible sample for analysis.

Given that plasma samples contain anticoagulants, the cellular components (i.e., white blood cells, red blood cells and platelets) are not trapped in a clot as they would be during the normal coagulation process of a serum sample. Following centrifugation, plasma samples may still contain trace amounts of cellular material, as well as latent fibrin. Gel separator tubes reduce the incidence of resuspension of these formed elements. However, some materials (e.g., platelets) may remain above the plasma gel interface barrier. These factors can cause non-specific binding to the solid phase—the microparticles—leading to erroneous results.

Heparin as an anticoagulant

Heparin, a negatively charged molecule used to inhibit clotting, can bind to some analytes, antibodies and cellular material. This can interfere with the antigen-antibody interaction in the test method.[9,10]

If a tube has insufficient blood volume, there is an excess of heparin. Maintaining an optimum sample-to-additive ratio is important for effective anticoagulation and accurate laboratory tests.[11-13]

A key step in the sample-handling process is ensuring that the blood-draw sample volume is at least 90% of the stated volume on the collection tube.[11] The Clinical and Laboratory Standards Institute (CLSI) has published guidelines for blood specimen handling. Heparin is also a commonly used pharmaceutical agent for inhibiting clotting in critical care patients. Inadequate cleaning of an intravenous line prior to blood collection can also create an excess of heparin in the sample.

Possible mechanisms that could interfere with heparin anticoagulant activity

Certain mechanisms might interfere with heparin anticoagulant action, resulting in fibrin formation in a plasma sample.[12] Heparin has the ability to bind cell membranes and proteins, such as platelets, making its pharmacological action unpredictable. The presence of cellular proteins and membranes could result in the binding of heparin, therefore competing and interfering with anticoagulation. Upon re-exposure to heparin, some patients will exhibit heparin-induced thrombocytopenia (HIT). This condition can cause heparin-induced or heparin-facilitated platelet aggregation and result in low platelet counts. These activated platelets release platelet factor 4 (PF4), which promotes clotting by neutralizing heparin.

Effect of fibrin in plasma and serum samples

Chemistry and immunoassays are susceptible to interference by fibrin. Small amounts of fibrin (and other membrane fragments or cell stroma) may affect sensitive immunoassays and chemistry tests. The presence of significant amounts of fibrin in the specimen (serum or plasma) may cause blockage of the instrument's sample aspiration probes. Such a blockage could lead to erroneous assay results.

Plasma samples

Inadequate collection tube mixing may result in uneven distribution of the heparin additive throughout the specimen. This could lead to localized areas within the specimen where the anti-thrombin effect of the heparin is insufficient to prevent the formation of fibrin. Thus, it is essential to ensure thorough mixing by gentle inversion immediately after blood is drawn in the tube (following manufacturer's guidelines for each tube type). In the past, a liquid anticoagulant was used in many glass tubes, facilitating easy mixing. Today, the walls of the tube are coated with a powdered anticoagulant. This is not as easily mixed in the sample, unless the required mixing occurs immediately after collection.

Since the heparin additive in specimens typically degrades over time, residual thrombin in the specimen can convert soluble fibrinogen to insoluble fibrin. Flocculent matter can frequently be observed in stored samples. Care should be taken to recentrifuge such samples prior to analysis.

Serum samples

Inadequate clotting time, improper mixing and failure to place the tube in an upright position can lead to incomplete clot formation. Following centrifugation, the resulting sample may appear satisfactory, with a defined layer of cells at the base of the tube and a clear layer of serum above. Despite this appearance, the clotting process may not have been completed prior to transportation, centrifugation and placement of the specimen on the analyzer. Further coagulation in the serum may subsequently occur, leading to the production of "latent" fibrin, which can interfere with the quality of a result.

For plastic tubes, thorough mixing by gentle inversion is essential to ensure even distribution of the clot activator throughout the specimen. This will also allow completion of the clotting process. Some cardiac patients will have therapeutic levels of anticoagulant in their blood. This could increase clotting time in the tube and thus increase the potential for the formation of "latent" fibrin in the pre-analytical phase.

Conclusion

Considering all of the factors above, serum appears to be the superior sample for immunoassays. Many laboratories use heparinized plasma for faster test TATs and to avoid prolonged clotting times in patients with high circulating levels of heparin. Regardless of which sample type is used, following the blood collection tube manufacturer's specimen collection and handling recommendations will help to reduce pre-analytical laboratory errors. In order to minimize laboratory errors due to specimen quality, the key pre-analytical actions are:

- Adequately fill the collection tube to the full volume
- Ensure proper mixing immediately after collection
- Allow adequate clotting time (minimum of 30 minutes) for serum specimens
- Ensure proper centrifugation
- Avoid resuspension of separated samples, including tubes with a gel barrier

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