

PREANALYTIC PULSE

Sample Centrifugation: Manufacturer Recommendations versus Customer Needs

Proper sample handling is critical to achieving quality laboratory results. There are several steps that can affect sample quality including equipment selection, collection technique, sample transport and processing. Centrifugation is one step in sample processing workflow that seems to generate a great deal of confusion for laboratories especially with regard to making changes to manufacturer's recommendations.

Greiner Bio-One has established recommended centrifuge settings for our tubes cleared by the FDA and these settings are published in VACUETTE® Evacuated Blood Collection System Instructions for Use. These recommendations result in optimal separation of cellular components from serum or plasma. For those tubes that have gel, they also produce optimal gel movement and barrier formation. A clean serum or plasma sample is important for most analytical instruments in order to achieve accurate results and avoid potential errors.

Tube Type	Recommended g-force relative centrifugal force (rcf)	Recommended Time (Minutes)
VACUETTE® Serum Tubes (Clot Activator, No Additive)	Minimum 1500 g	10
VACUETTE® Serum Clot Activator w/ Gel Tubes	1800 g	10
VACUETTE® K ₂ EDTA w/ Gel Tubes	1800 - 2200 g	10
VACUETTE® Plasma Tubes (Lithium Heparin, Sodium Heparin, PO/NaF)	2000 - 3000 g	15
VACUETTE® Lithium Heparin w/ Gel Tubes	1800 - 2200 g	10 - 15
VACUETTE® Coagulation Tubes (Sodium Citrate)		
Platelet tests (PRP)	150 g	5
Routine tests (PPP)	1500 - 2000 g	10
Preparation for deep freeze plasma (PFP)	2500 - 3000 g	20

Most recommendations are given as relative centrifugal force (RCF) or g-force. Conversion between RCF and revolutions per minute (RPM), necessary in order to program most centrifuges, is achieved using the following formula:

$$RCF = 1.118 \times 10^{-5} \times r \times rpm^2$$

where r = the rotor radius measured from the rotor axis to the bottom of the fluid inside the tube at the greatest horizontal distance from the rotor axis. This conversion can also be accomplished using a nomograph, which is usually included in the centrifuge manufacturer's manual for users or is easily found in literature including CLSI Standards for sample handling.

It should be noted that Greiner Bio-One recommends using a centrifuge with a horizontal or swing-bucket rotor for gel tubes, which provides for more stable barrier formation following centrifugation (fig.1). We also recommend using a temperature controlled centrifuge that is able to maintain a 15-25°C internal temperature to avoid the negative effects of high temperatures on analyte stability as well as the physical properties of the gel.

If a customer would like to spin for a shorter time at a higher force or is using a centrifuge that is incapable of achieving the necessary g-force and must determine what time is required to achieve adequate separation, it is considered an off-label use because it is outside manufacturer recommendations. Under these circumstances, the customer must perform a validation to establish that the changes they are making do not affect testing outcomes.

Though it is the responsibility of the customer site to determine what is required for validation, there are several regulations, standards and guidelines to assist them in developing a validation protocol. Some of these are cited in the Greiner Bio-One Custom Conversion Program, Validation Study guidelines. As the manufacturer, we can provide general guidelines but must be careful not provide specific tools that could be mistaken as recommendations for off-label centrifuge settings. In general, a well designed validation study to compare methods, in this case centrifugation conditions, should incorporate the following parameters:

1. Design the study to meet accreditation and/or regulatory requirements, such as JCAHO, CLIA, CAP, etc., applicable to the customer's laboratory.
2. Follow facility policies and procedures including requirements of existing boards or committees responsible for laboratory oversight.
3. Allow time for study participants to familiarize themselves with protocol and study procedures such as sample collection and handling, processing requirements, manufacturer's requirements, etc.
4. Collect and handle specimens according to the facility's policies and procedures and manufacturer's recommendations.
5. Include an adequate number of samples representative of the patient population and distributed over the clinical range extending below and above the site reference range. This should include samples known to affect the analytes tested as well as samples that do not. Good distribution of samples will help ensure a quality study.
6. A minimum of forty samples is recommended for comparison. However, the customer site must make the final determination on sample number to achieve the desired statistical significance. Number of replicates (at minimum duplicate measurements should be performed) and the number of days for testing must also be established.
7. Randomization of sample collection, processing and testing order should be built into the study.
8. Storage of samples can introduce error and should be avoided unless included as a study parameter.
9. Data should be accurately recorded and reviewed for preliminary acceptability. If acceptability is questionable, the source of error should be investigated. Examine outliers and document exclusion of any results.
10. Analyze data utilizing statistical tools accepted by CLIA and other relevant accreditation agencies. Data should be graphed and examined for linearity and final acceptability.

A detailed study protocol that is developed with these guidelines in mind should allow the end user to adequately establish the impact of changes in centrifugation conditions on test results. The study protocol and accompanying data should be kept on file for inspection purposes.

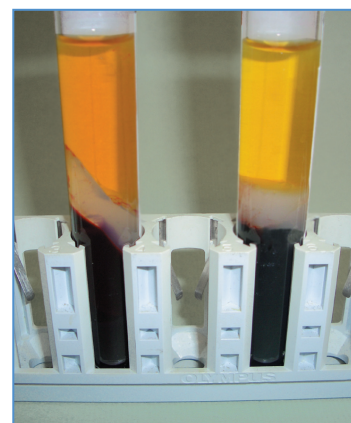


Fig. 1: Left: Separator gel tubes centrifuged in a fixed-angle centrifuge. Right: Separator gel tubes centrifuged in a swing-bucket centrifuge.

CAP Laboratory Accreditation Program Inspection Checklists are available at www.cap.org.

CLSI standards, guidelines, and reports are available from CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087; 610.688.0100, www.clsi.org.

JCAHO Standards are available in the 2005-2006 JCAHO Laboratory Accreditation Standards (LAS) Book and at www.jointcommission.org.