Dimension® clinical chemistry system
Dimension Vista® System

The Effects of Sample Integrity on Accuracy and Precision

Prepared by
Helen Bouchard BA, BS, MT(ASCP)

Technical Bulletin

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Contents

Abstract

This technical bulletin discusses the effects of sample integrity on accuracy and precision of all assays including Dimension Vista® CA (K1023), GLU (K1039) and electrolytes. Further discussion shows how sample quality issues may be minimized by abidance of the recommended guidelines for sample mixing encompassing physical and mechanical aspects. Examination of pre-analytical sources of error should include short sampling events and centrifugation processes. The troubleshooting process should begin with the most common source of error: sample integrity. Factors to consider before processing specimens include centrifugation conditions appropriate to tube type, clot formation, post centrifugation handling, and adequate sample/aliquot volume.

Introduction

Siemens recognizes that laboratories are under constant pressure to reduce turnaround-time (TAT) for reporting test results. When appropriate, using plasma instead of serum can reduce TAT by eliminating the need to wait for complete clot formation in a serum tube. However, plasma specimens also have unique characteristics concerning specimen quality and integrity. Centrifugation time will also affect the TAT; consequently, many laboratories consider shortening the time for sample preparation in an effort to reduce testing time. Haphazardly, inadequate centrifugation conditions may result in incomplete separation of plasma or serum from the cells, as well as incomplete gel barrier formation. Both of these conditions may adversely affect the accuracy of test results and increase TAT by necessitating repeat analysis. Sample preparation is one of the most critical operations in the laboratory. Failure to observe manufacturer's recommendations could result in excessive maintenance to the analyzer and non-compliance to these procedures can compromise patient results. Careful handling of samples in serum and plasma tubes, both gel and non-gel, will help prevent instrument problems and ensure sample integrity. The brand of specimen collection material is not important, however, it is important to adhere to the IFU provided by the tube manufacturer regarding specimen processing and centrifugation.

Specimen Handling Guidelines

If your laboratory is experiencing an increased occurrence of CA, GLU or electrolyte outliers, an evaluation of your specimen handling practices should be completed before pursuing steps to evaluate instrument malfunction. Adhering to the following specimen handling and processing steps will help to ensure acceptable serum and plasma specimen quality and overall performance.

1. Ensure correct phlebotomy technique is utilized to minimize hemolysis and platelet activation.
2. Fill evacuated blood collection tubes to the stated draw volume. This will ensure the proper blood-to-additive ratio in the tube.
3. Invert tubes 8 to 10 times immediately after collection to ensure that the blood and clot activator or anticoagulant are mixed thoroughly.
4. Ensure centrifuge g-force (RCF), spin time, and brake time are sufficient to obtain adequate sedimentation of cells, platelets, and other debris. Follow sample tube manufacturers recommendations.
5. Centrifuge tubes within 2 hours of collection to separate serum or plasma from cells.
6. Carefully aliquot serum or plasma from non-gel tubes after centrifugation. Refrain from utilizing the "pouring off" technique.

7. Avoid mixing/agitation of plasma gel tubes between centrifugation and testing.
8. Evaluate and enforce "add-on testing" policies that your facility has validated for analyte stability in heparin plasma, including the effects of specimens containing latent fibrin.
9. Do not re-centrifuge gel barrier tubes. Instead, remove serum from gel barrier tubes and re-centrifuge the removed serum.
10. Do not apply brake or stop centrifuge manually.

PRE-CENTRIFUGATION

Tube Storage

Always store collection tubes per the manufacturer's recommendations. Storage at inappropriate temperatures may compromise the performance of the gel barrier, tube draw volume, or additives.

Mixing and Clotting

Tubes with Clot Activator

Many serum tubes contain a clot activator, therefore, it is extremely critical to thoroughly mix tubes at the time of blood collection. If clot activator is aspirated, it can build up in the instrument tubing and probes. Because it is an inert substance, cleaning solutions may not flush the clot activator out. Aspiration of smaller fibrin "microclots" do not cause probe blockage but can result in gradual deposition of fibrin in reaction pathways or chambers, and/or interference with measurement systems or reagents. Latent fibrin formation may also continue within the analyzer. These various effects of fibrin can lead to instrument downtime, failure to provide test results, or erroneous test results. Instrument fibrin issues are often incorrectly attributed to gel contamination. While issues associated with fibrin formation can be reduced, they can be difficult to eliminate, especially in settings with patients on anticoagulant therapy and/or where specimens are ready for processing well before recommended clotting times have elapsed. Inadequate mixing may result in incomplete clot formation in serum tubes. Formation of latent fibrin can range from thin strands to large cloud-like masses and after aspiration can impact patient results. Complete clotting of serum samples normally occurs within 30 to 60 minutes at room temperature. Serum from patients with coagulation disorders and those receiving anticoagulant therapy may require longer clotting times.

Tubes with anticoagulant

All plasma tubes contain an anticoagulant. Inadequate mixing will cause platelet clumping or clotting. Both can impact test results. The ideal plasma specimen would thus be one that is cell/platelet-free where the anticoagulant functions (clotting and fibrin formation during collection and continuing over extended periods of time) have been inhibited. In practice, this ideal is often not attained with heparin-plasma specimens. Platelets are typically the most abundant of these components in heparin plasma, followed by white blood cells (mainly lymphocytes and monocytes). Heparin plasma can also contain thin fibrin strands which are formed due to incomplete anticoagulation. Incomplete dispersion of heparin (inadequate specimen mixing/tube inversions immediately after collection) and patient factors such as disease state and medication can both diminish the efficacy of heparin activity and lead to increased fibrin formation and "microclots". In addition, platelet aggregates can form which may also contain fibrin and/or white blood cells. These aggregates can be large enough to be visible to the unaided eye and have been termed "white particulate matter" (WPM) due to their typical white color. WPM has also been observed in blood bags used in transfusion medicine, where it was

determined to consist predominantly of platelets; white blood cells and cellular debris including fragments and granules from white blood cells; and occasional fibrin strands. As a result of the potential for variable amounts of cells, platelets, fibrin, and WPM, heparin plasma is generally a more complicated matrix to manage than serum.

When blood is anticoagulated, the cellular components compact downward in a relatively uniform manner during centrifugation; the yield of plasma above the cells is generally greater than the yield of serum above the clot in serum tubes. Plasma yield is generally near 50% of blood draw but depends on patient factors such as hematocrit. The clot also has a significant influence on the movement of gel in gel-containing serum blood collection tubes. When a clot is present, the gel is forced to move upward around the clot and against the tube wall during centrifugation. With anticoagulated blood, however, there is no obstruction to upward gel movement and the gel moves up in pieces similar to a "lava lamp". Hence, the time required for the gel to complete its upward movement to form a thick barrier is generally shorter with plasma tubes than with serum tubes (under the same centrifugation conditions). Depending on the centrifugation conditions used, this can result in more reproducible gel barrier formation in plasma tubes. Increasing speed of centrifugation beyond manufacturer's recommendations may cause rapid gel movement in plasma tubes which can have an adverse effect on plasma purity by trapping cells and platelets in the plasma compartment before their separation is complete. The "lava lamp" mechanism of gel movement in plasma tubes also entraps more cells in the formed gel barrier after centrifugation as compared with serum gel tubes.

Tubes with separator gel

Separator gels are designed with a specific density that falls between those of the plasma/serum and the cells. This characteristic makes it an ideal material for separating two phases of different densities by centrifugation. The accuracy and precision of Calcium and Glucose may be adversely affected if tubes are inadequately mixed. The table below outlines the mixing recommendations of a popular industry tube manufacturer, Becton-Dickinson Vacutainer Collection Tubes (BD).

BD Vacutainer® Tube Type	Cap/Closure Color	Number of Inversions
EDTA	Lavender	8 - 10
Citrate	Light Blue	3 – 4
SST with Polyester gel Tiger	(Red Gray) or Gold	5
SST II with Acrylic Gel	Gold	5
Serum	Red	5
Sodium Fluoride	Gray	8 – 10
Heparin	Green	8 – 10
PST with Polyester Gel	Light Green	8 – 10
PST II with Acrylic Gel	Light Green	8 – 10

Vacutainer® is a registered trademark and SST™ and PST™ are trademarks of Becton-Dickinson Preanalytical Systems, Franklin Lakes, NJ 07417

These best practices must be communicated to those who collect patient samples to ensure sample integrity.

Sample Volume

It is pertinent to check the sample volume before placing vial onto the Dimension Vista® because CA is the last test from a limited, surplus or low sensitivity aliquot of a SADD dispense the outlier may be due to insufficient sample volume. For aliquots from limited and surplus cups the user must ensure adequate volume is available for testing.

Mechanism of gel movement in gel barrier tubes

In the gel barrier tube, an inert gel moves into position to provide a mechanical barrier between cells and serum or plasma. The gel's thixotropic properties (it exhibits solid-like properties in static conditions and flows when force is applied) enables it to flow during centrifugation. The density of the gel material is between that of serum or plasma and cellular material allowing for the formation of the gel barrier between the two layers. Under centrifugal force, the gel material becomes flowable allowing for movement of the gel. The direction of gel movement is governed by the "state" of the blood. In a clotted sample, the gel must move around the cross linked network of clotted cells. Therefore, in a serum tube the cellular portion of the blood centrifuges down and the gel moves up the sides of the tube around the clotted network. In an anticoagulated sample, there is no clot to impede the gel movement, so as the cellular material settles, the gel is able to move up through the center of the tube. In either case, the time at which the gel begins to move and the speed at which it moves to the interface is dependent on the speed of centrifugation. The centrifugation recommendations of the tube manufacturer are based on the gel properties and are designed to allow for optimum gel performance, barrier formation, and sample quality.

Centrifugation

It is essential to centrifuge specimens according to the tube manufacturer's recommendations for speed and length of time. In general, sedimentation increases with increasing relative centrifugal force (RCF) and spin time. Plasma platelet counts in lithium-heparin tubes with gel decreased from 321 to 5 x 109/L when centrifugation was increased from 500 g/5 min to 3000 g/10 min. Tube manufacturers and Clinical and Laboratory Standards Institute (CLSI), formerly known as NCCLS, provide information for determining the correct RPM setting for a centrifuge based on its rotating radius to obtain the desired centrifugal force. For gel barrier tubes, the time and centrifugation g force are set according to the properties of the gel in order to ensure separation, barrier integrity, and a high quality sample. Due to the properties of the gel material, optimum separation of the sample above the gel can be compromised by centrifugation at RCFs less than or greater than recommended by the tube manufacturer. After tubes have been centrifuged, do not apply the brake or stop the centrifuge manually. Abrupt interruption of rotation may agitate centrifuged material. Serum and plasma should be physically separated from the cells within 2 hours of collection and gel barrier tubes should not be re-centrifuged.

If repeat centrifugation is required, the serum/plasma specimen should be transferred to a clean tube and then centrifuged according to the tube manufacturer's recommendations. This also applies to collection tubes that may have been centrifuged at one location and then transferred to another for processing. Serum or plasma that has been stored in the refrigerator or freezer should be centrifuged prior to testing on the analyzer. Frozen samples should be thawed and mixed well prior to centrifugation.

In the attempt to increase TAT, laboratory personnel may try increasing the speed of centrifugation beyond the manufacturer's recommendations which may cause rapid gel movement in plasma tubes having an adverse effect on plasma purity by trapping cells and platelets in the plasma compartment before their separation is complete. The "lava lamp" mechanism of gel movement in plasma tubes also entraps more cells in the formed gel barrier after centrifugation compared with serum gel tubes.

Centrifugation recommendations

CLSI recommends centrifugation of collection tubes for 10 minutes (+/- 5 minutes) at 1000 to 1200 x g and also suggests following the tube manufacturer's recommendations. BD provides the following recommendations for centrifugation of BD Vacutainer® collection tubes:

Tube Type	Recommended g-force (RCF*)	Recommended Time (minutes**)
BD SST™ and BD PST™ Tubes (glass)	1000 – 1300	10
BD SST™ Plus and BD PST™ Plus Tubes – 13mm	1100 – 1300	10
BD SST™ and BD PST™ Plus Tubes – 16mm	1000 – 1300	10
BD SST™ Transport Tubes	1100 – 1300	15
All BD Non-gel Tubes	<1300	10
BD Coagulation (Sodium Citrate) Tubes***	1500	15

Griener provides the following recommendations for centrifugation of Greiner Vacuette® collection tubes.

Tube Type	Recommended g-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® K2EDTA w/Gel Tubes	1800 – 2200 g	10
VACUETTE® Plasma Tubes (Lithium Heparin, Sodium Heparin, NaF/KO)	2000 – 3000 g	15
VACUETTE® Lithium Heparin w/Gel Tubes	2200 g	15
VACUETTE® Coagulation and CTAD Tubes (Sodium Citrate)***	1500 – 2000 g	10

*RCF = Relative Centrifugal Force. Units are expressed as number of times greater than gravity (g).
**Minutes in swing bucket centrifuge. Adjust to 15 minutes for all gel tubes in a fixed angle centrifuge.
***Citrate tubes should be centrifuged at a consistent speed and time to produce platelet-poor plasma (platelet count <10,000/uL) per CLSI Guidelines.

Different brands of tubes require different durations of centrifugation, it is imperative to follow the proper guidelines indicated by the tube manufacturer your facility supplies.

The following tables illustrate guidelines for conversion from RCF to RPMs in both the CLSI document and the BD Vacutainer® literature. These tables have been adapted from conversion documents that are based on BD recommendations. The minimum RPM is equivalent to RCF of 1000xg and maximum RPM is equivalent to RCF of 1300xg.

Table 1. Conversion of RCF to RPM (radius in inches)

Radius* (inches)	Speed–RPM Minimum	Speed–RPM Maximum
3	3500	4000
4	3000	3500
5	2700	3100
6	2500	2800
7	2300	2600
8	2100	2400
9	2000	2300
10	1900	2200

Table 2. Conversion of RCF to RPM (radius in centimeters)

Radius* (cm)	Speed–RPM Minimum	Speed–RPM Maximum
8	3300	4000
10	3000	3400
12	2700	3100
14	2500	2900
16	2300	2600
18	2200	2500
20	2100	2400
25	1900	2100

*Radius is the rotational radius of the centrifuge as measured from the center of the rotor head to the outside bottom of the rotor bucket (when the rotor bucket is held at 180 degrees).

Post-centrifugation

An important factor that influences specimen quality is how the specimen is handled after centrifugation. In practice, this is often a function of whether the blood collection tube used contains a gel barrier or not. In both heparin-plasma tubes with and without gel, the packing of cells with concurrent expression of plasma above the cells continues during centrifugation. In the tube with gel, however, the gel begins to form a barrier between the cells and the plasma within one minute; barrier formation is almost complete within 2–3 minutes. The quick formation of the gel barrier traps some cells (mostly white blood cells) and platelets in the plasma compartment before their sedimentation is complete. After centrifugation, these cells and platelets are concentrated on or near the gel surface, versus being concentrated at the red blood cell-plasma interface in a tube without gel. These cells in plasma tubes are sometimes referred to as the "buffy coat". When tubes are kept upright and not mixed/agitated after centrifugation, plasma sampled or aliquoted from the top of the plasma column will be relatively "clean " with only small amounts of cells and platelets. This applies to both tubes with and without

gel. Gel tubes offer the benefit of not having to aliquot the plasma. However, gel tubes may be held in a variety of orientations after centrifugation, especially when transported to reference laboratories or other off-site testing facilities. This can lead to mixing and re-suspension of components that were previously on or near the gel surface which can affect sample aspiration by sampling probes and can lead to physical obstruction of the probe and/or insufficient sampled volume.

Centrifuged tubes, including non-gel and gel barrier tubes, should be kept upright during transport from the centrifuge to the analyzer. There may be cellular material adhering to the stopper or "buffy coat" material on the surface of the gel barrier and failure to keep the tube upright could resuspend this particulate matter. Never "pour off" serum or plasma from the primary collection tube; instead, pipette sample from the original tube into clean tube. Take care to avoid contact with the gel in barrier tubes and also the very surface of the sample where uncentrifuged material may remain in the meniscus.

Check for efficiency of centrifugation

It is good laboratory practice to inspect samples prior to processing on the analyzer. Serum and plasma samples should be examined for the presence of clots, fibrin, particulate matter, or other debris. When using gel barrier tubes, the sample should also be examined for gel globules, which are similar in appearance to oil droplets. Any of these substances may impact test results. If these substances are present, it may be necessary to re-centrifuge according to the manufacturer's recommendations or recollect the sample.

Follow the manufacturer's recommendations for maximum g-force (RCF) and spin time. After centrifugation, check to see if a button (aggregate of cells/ fibrin) is formed in the bottom of the tube. If present this collection of material may be evidence of cellular debris and/ or fibrin left behind in the original sample due to inadequate centrifugation of the sample tube. Reassess centrifuge settings vs. manufacturer's recommendations.

Conclusion

The preparation of blood samples is a critical first step in the testing process. Blood collection tube manufacturers have done extensive studies to determine the centrifugation conditions required for optimal separation of blood samples. Although many laboratories make adjustments to pre-analytical conditions in an effort to improve turnaround-time for test results, Calcium and Glucose results are impacted by such preanalytical adjustments. While Siemens recognizes the pressures and demands laboratories face, the accuracy of these test results can be impacted when testing is done on inadequately prepared samples from which particulate matter has not been completely cleared (e.g. white and red blood cells, fibrin, platelets, cellular fragments, flocculates, micro aggregates, cold agglutinins, gel material, etc.). It is the responsibility of the laboratory to ensure that samples are collected and processed according to good laboratory practices, tube manufacturer's recommendations, and in accordance with CLSI guidelines.

References

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Trademark Information

Vacuette is a trademark of Greiner Bio-one.
Vacutainer is a trademark of Becton Dickerson.

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Siemens Global Headquarters

Siemens AG
Wittelsbacherplatz 2
80333 Muenchen
Germany

Global Siemens Healthcare Headquarters

Siemens AG
Healthcare Sector
Henkestrasse 127
91052 Erlangen
Germany
Telephone: +49 9131 84 - 0
www.siemens.com/healthcare

www.siemens.com/diagnostics

Global Division

Siemens Healthcare Diagnostics Inc.
1717 Deerfield Road
Deerfield, IL 60015-0778
USA
www.siemens.com/diagnostics

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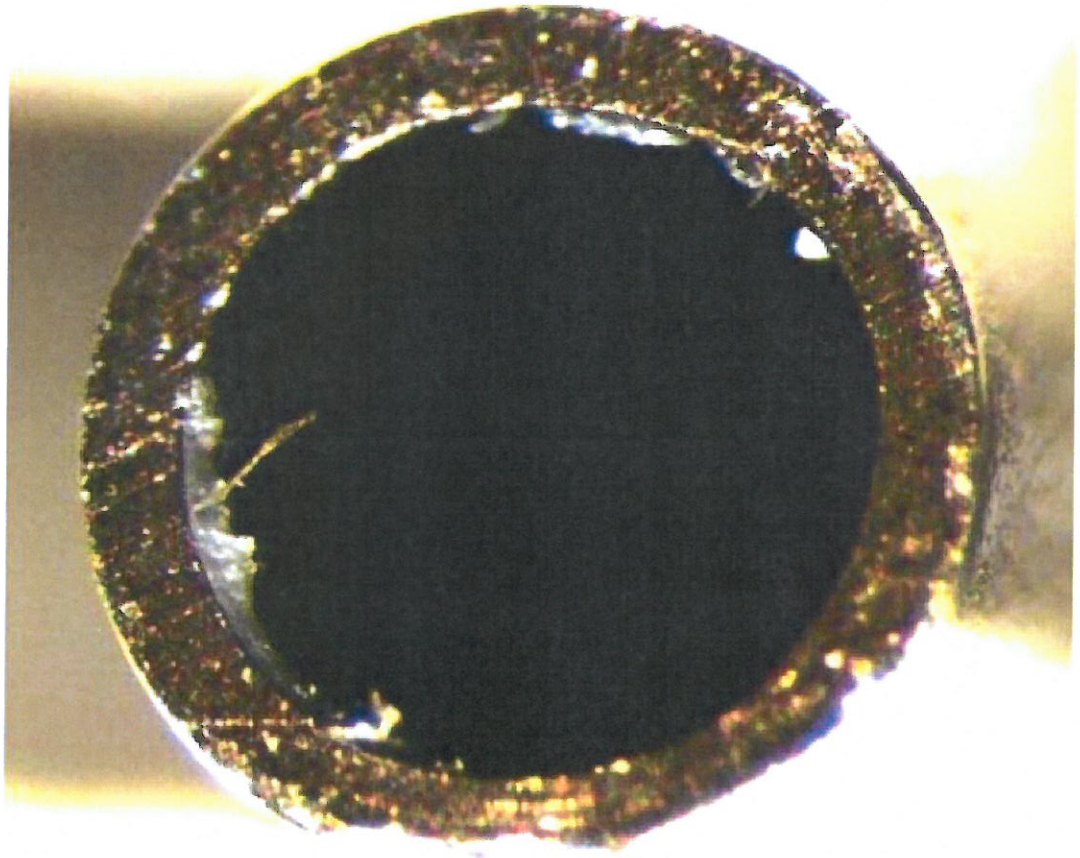
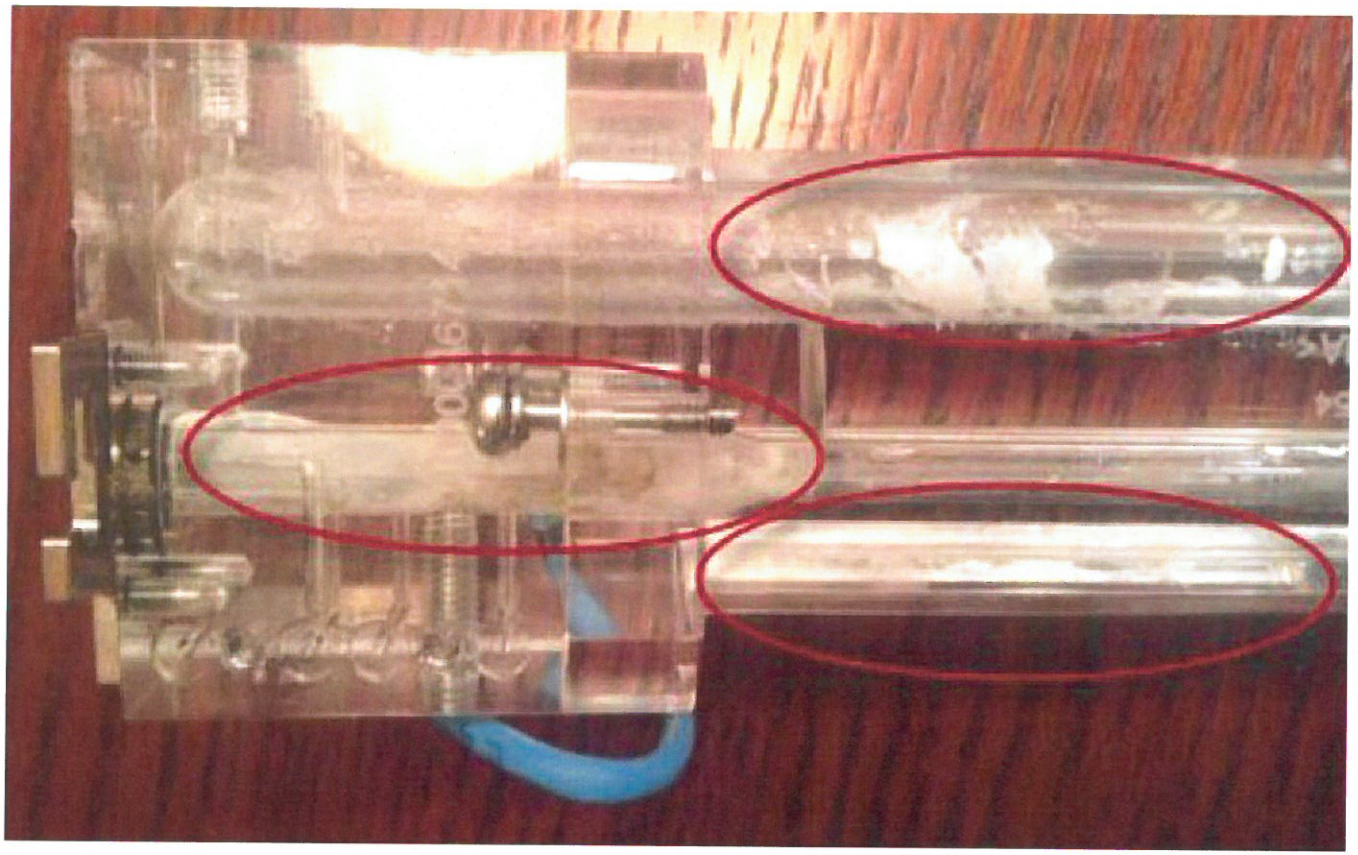
Probe with gel



drain with gel



Cellular Debris in Aliquot plate

- It is not always obvious when sample quality is an issue, the inspection of used Vista aliquot plates co