CANCELLATION COMMENTS

As the study progresses and visits and samples are being returned to Q² Solutions, your site may receive a test cancellation notice. This notice is intended to advise your site if any testing has been cancelled for a particular patient visit. The notification will detail the specific parameters that have been cancelled, in addition to the reason for the test cancellation.

The following table provides additional information on the most commonly encountered cancellation comments that you may see from Q² Solutions and possible ways to avoid cancelled tests.

Cancellation Comment	**Possible Cause**	**How to prevent in future**
No Sample Received	No sample or wrong sample received by the lab.	Follow instructions in laboratory manual and on requisition forms carefully. Collect all required samples for visit. It may be that the investigator never intended for this testing to be done. This needs to be clear on the requisition form.
Quantity Not Sufficient - *Insufficient sample to perform testing*	Blood draw tubes not filled. Serum/plasma not dispensed accurately into multiple tubes.	Follow sampling instructions carefully. For EDTA tubes, fill contents to the mark. This is important as insufficient blood with the fixed amount of coagulant in the tube will affect the sample quality. Ensure the tubes are capped tightly to avoid leaking during transport.
Unable To Obtain Valid Results	Unknown interference with one or more analytical tests: • drugs ? • IV fluids? • wrong tube? EDTA?	Wherever possible samples should be taken from a venipuncture. Be careful if using a cannula. Ensure that samples are properly centrifuged.
Haemolysis - *Leakage of haemoglobin from red blood cells*	Difficult blood draw. Rough handling, e.g., vigorous mixing. Samples taken by syringe and squirted into EDTA tube. Sample has frozen and thawed.	Always draw directly into EDTA tube. Mix promptly by gentle inversion. Do not mix vigorously. Minimise the use of syringes for blood collection. If you do use a syringe, inject the blood gently into the draw tube. Follow packaging instructions carefully.

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Sample Not Centrifuged Or Inadequately Centrifuged At Site	Sample has been centrifuged too slowly, for too short a time, or not at all. This applies to gel tubes where the draw tube should be centrifuged and sent to Q² Solutions without transferring the serum to a secondary tube.	Spin gel tubes for the recommended time, allowing time for the centrifuge to get up to speed. The gel barrier in the tube needs time to move and form a solid barrier between the red cells and the serum. If the gel has not moved to the middle of the tube, it must be centrifuged again, faster, or for longer. Make sure the centrifugation speed is adequate. The gel will move at speeds well below what we recommend. This may be enough to sediment the red cells but not enough to make the gel form a barrier between the red cells and the serum.
Sample Clotted - *EDTA Sample Has Clotted* Fibrin Clots - *Microclots that result from partial coagulation of the blood*	Sample starting to clot before anti-coagulant is mixed, i.e., sample laid aside before being mixed with the anti-coagulant. Difficult venipuncture.	Always draw directly into EDTA tube. Mix the EDTA tube promptly by gentle inversion. If syringe is used, arrange to transfer the blood to vacutainer and mix promptly. Do not store samples in the refrigerator. Samples should be maintained at approximately 25 degrees Celsius. Ensure the EDTA sample is fully covered in the gel wrap. The gel protects the sample from freezing in very low ambient temperatures. Ensure tubes are warmed before blood draw. Example: Use both hands/palms to warm the tubes. Good venipuncture technique.
Platelet Clumping **	Same causes as clotting.	The blood of some patients is predisposed towards platelet clumping in EDTA tubes. In these patients, it may be best to collect the blood into a sodium citrate tube as well as the EDTA tube; please speak with your Q² Solutions Project Manager regarding this.

** Platelets have a tendency to clump and, whilst the EDTA in the haematology tube should prevent this, sometimes it is not completely successful.

When platelets clump, it is impossible to count them accurately. If we do not see a shortage of platelets, a comment will be added to the laboratory report indicating them as “adequate.”
Platelet clumps may incorporate white blood cells, making it impossible to count these accurately either.

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Degenerated Cells	White blood cells deteriorating in the collection tube more rapidly than normal. High ambient temperatures will contribute to this.	White blood cells in some individuals are prone to rapid degeneration. Aim for the shortest possible delivery time. Ship samples as per the flowchart and requisition forms, and adhere to last call by times provided by courier. Follow packaging instructions carefully.

Cancelled tests are always reported to the site on a separate test cancellation notification. This notification will be delivered via the Site Portal. Any cancelled tests will also indicated on the laboratory reports provided for the associated visit.

Should you require additional information to that provided, please do not hesitate to contact your Q^2 Solutions team.

NOTE: Q^2 Solutions are unable to advise whether or not a test should be repeated following cancellation. Please contact the medical monitor for your study to determine whether a retest is required or not.

CANCELLATION COMMENTS - FURTHER DETAIL

Platelet Clumping

Platelet clumping can occur when there has been a difficult venepuncture or inadequate mixing of the sample, there are occasions where the patients' platelets react with the EDTA in the tubes, when we see a patient has clumped platelets on more than one visit we ask that a sodium citrate is sent with the EDTA on the next visit. Sodium citrate has a greater diluting effect on the whole blood so we can only provide the platelet count after a calculation has been applied.

The most common cause of platelet clumping in an EDTA anticoagulated specimen is improper mixing of the tube. It is recommended that the EDTA tube be inverted eight to 10 times immediately after the specimen is collected. Fewer inversions may result in incomplete mixing of the additive in the blood and therefore, platelet clumping. Improper collection of the blood sample may cause thrombin release and a falsely low platelet count due to platelet aggregation. This may be due to an excessively traumatic venipuncture or inadequate anticoagulation. When performing the skin puncture, it is best to avoid scraping the skin with the collection tube, as this may result in platelet clumping and/or clotting of the specimen
All blood collection tubes should be filled to their stated draw volume. Overfilling an EDTA tube can result in an improper blood to additive ratio. Insufficient EDTA in the sample may contribute to platelet clumping and/or clotting of the blood.

EDTA Contamination

EDTA contamination can occur when the citrate or EDTA tubes are drawn before the serum tube. EDTA is an anticoagulant which is added to prevent whole blood from clotting, it does this by binding to Calcium which inhibits the proteins used to clot blood. EDTA contamination can give inaccurate results of a high potassium and UIBC and a low Calcium, ALP and Magnesium, hence why these testing are cancelled when EDTA is present.

Degenerated cells and partial panel resulting

Haematology parameters are counted and determined using different methods because they have different methods they have different stability times.
Haematology parameters tolerate transport, temperature and aging differently.

Degeneration has a number of causes such as aging of sample, extremes of temperature, longer transit time, inadequate protection/packaging of sample or the patient’s medical condition. In colder weather there is an increase in the number of cancellations seen. The sites should ensure samples are passed to labs in a timely manner and all packing instructions are followed to protect samples. Degenerated cells look the same no matter the root cause.

CANCELLATION COMMENTS - FURTHER DETAIL

Hemolysis

Hemolysis can also occur by improper handling of the sample (such as if the sample accidently fall or is shaken).

Evacuated Tubes

· An improper choice in the venipuncture site, such as drawing from a distal site to the antecubital region of the arm rather than drawing from an antecubital site, has been shown to result in more hemolysis.

· Prolonged tourniquet time causes the interstitial fluid to leak into the tissue and cause hemolysis.

· Cleansing the venipuncture site with alcohol and not allowing the site to dry may cause hemolysis.

· An improper venipuncture, indicated by a slow blood flow, may indicate occlusion due to the lumen of the needle being too close to the inner wall of the vein, causing hemolysis.

· The use of a small-bore needle, resulting in a large vacuum force applied to the blood, may cause shear stress on the red blood cells, causing them to rupture.

· The use of a large bore needle may result in a much faster and more forceful flow of blood through the needle, resulting in hemolysis.

Syringe Draws

· Pulling the plunger of a syringe back too far while using a large bore needle, may cause enough pressure for hemolysis to result during collection. The pressure may be greater than a standardized evacuated tube.

· Transferring into a tube by pushing down on the syringe plunger in order to force blood into a tube may cause hemolysis, as well as create a positive pressure in the tube which may cause the stopper to come off.

IV Catheters

· Several studies have noted that when blood is drawn from a peripheral IV catheter, a higher incidence of hemolysis occurs due to frothing of the blood from a loose connection of the blood collection assemblies.

Specimen Processing:

· Vigorous mixing or shaking of a specimen may cause hemolysis.

· Not allowing the serum specimen to clot for the recommended amount of time can result in fibrin formation in the serum. The use of applicator sticks to dislodge the fibrin may cause rupture of RBCs, resulting in hemolysis.

· Prolonged contact of serum or plasma with cells may result in hemolysis.

· Exposure to excessive heat or cold can cause RBC rupture and hemolysis.