Following the order of draw

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The correct order of draw is a subject that has been extensively researched. The outcome of this research? It is not only a necessity, but it is the responsibility of the phlebotomy technician to adhere to the correct order of draw at all times and to immediately correct those who do not adhere to this rule.
The main reason for adherence to this rule is to avoid the additive from the different tubes to be carried over to other tubes.
Additive carryover happens when the needle filling the tube comes in contact with the blood and additive mixture in the tube as it fills. This small amount of blood and additive is then carried over to the next tube.

The additive carryover also occurs when the tube is filled from top to bottom and not from bottom to top as is the ideal. Filling a tube from bottom to top will occur when a patient is positioned in such a way that his/her arm is hanging down slightly. This will result in the bottom of the tube being lower than the stopper of the tube and will prevent the needle from coming into contact with the additive.

Positioning a patient in this manner is unfortunately not always possible and this is why the correct order of draw is necessary and important.
Phlebotomist should remember that this transfer can occur with both the open (vacutainer) and closed (needle and syringe) systems.

Glass tubes have been mostly replaced by plastic tubes. Different additives are added to the tubes to improve the quality of the specimen. A plastic tube will not activate a clot; therefore al clot activator must be added to each tube during the manufacturing process.

Additives found in blood collection tubes and their mode of action:

Sodium Citrate tubes: Blue stopper:
The additive found in citrate tubes is Aqueous Trisodium Citrate dehydrate or Trisodium Citrate Pent hydrate. This additive prevents coagulation by binding calcium and preserves the unstable coagulation factors.

SST tubes: Yellow stopper:
The inside of the tubes are coated with silicone and micronized silica particles to accelerate clotting.

Heparin tubes: Green stopper:
This tube contains either the lithium or sodium salt of the heparin anticoagulant and it prevents coagulation by inhibiting thrombin in the coagulation process.

EDTA tubes: Purple stopper:
EDTA or Ethylenediaminetetracetic acid inhibits platelet clumping. Calcium binds to the EDTA molecules and prevents it to participate in the coagulation process.

Fluoride tubes: Grey stopper:
Different types of fluoride are found in grey top tubes. It can be Sodium Fluoride, Sodium Fluoride with Potassium Oxalate or Sodium Fluoride with Sodium Heparin.
Sodium fluoride acts as the glycolytic inhibitor and prevents the cells in the blood from utilizing the glucose. It acts as a glucose preservative, but not as an anticoagulant.
Potassium oxalate is an anticoagulant which binds the calcium enabling the glucose determination to be performed on plasma.

When additives carry over into a different tube, test results may be dramatically affected. For example:

- EDTA is rich in potassium. If it is carried over into a tube to be tested for potassium (yellow SST tube), the level of potassium may be falsely elevated leading to life threatening medical mistakes. EDTA is also a calcium chelating agent and can give false decreased magnesium and calcium results if drawn before the SST tube.

- If blood from a clot activator (yellow SST tube) is carried over into a tube to be tested for coagulation studies (blue Citrate tube), the prothrombin time (PT) or activated partial thromboplastin time (aPTT) may be falsely shortened.

- When blood cultures are collected after the same time as other lab work and not filled first, bacteria from the non-sterile stoppers of the tubes can contaminate the bottles used for blood cultures.

- When the fluoride tube (grey stopper) is drawn before an EDTA tube (purple stopper), the oxalate in the fluoride tube can interfere with the cell membranes and the sodium fluoride can alter the cell morphology which will lead to inaccurate haematology results. Flouride specifically inhibits Enzymes thus all tests that are enzyme study related in both EDTA and SST tubes will be reduced.

Common tests affected by Additive Contamination:

Additive:	Tests affected by additive:	How is test affected:
Citrate (Blue stopper)	Alkaline phosphatase Calcium	Inhibits and decreases ALP Binds to calcium
Silica (Clot activator in SST tube – yellow stopper)	Prothrombin time Partial thromboplastin time	Activates clotting of the blood. Therefore not suitable for any coagulation studies.
Heparin (Green stopper)	Activated clotting time Partial thromboplastin Acid phosphatase Calcium Sodium Lithium Prothrombin time	Accelerates the action of antithrombin and prevents clot formation This anticoagulant is not suitable for haematology test, calcium, sodium or lithium as the heparin alters the cell morphology.

Additive:	Tests affected by additive:	How is test affected:
EDTA (Purple stopper)	Alkaline phosphatase Calcium Partial thromboplastin Prothrombin time Potassium Serum Iron Sodium	This anticoagulant can be used for some biochemistry but is not suitable for: • Alkaline phosphatase – falsely decreases alkaline phosphatase by binding magnesium. • Potassium and sodium - due to the addition of potassium to the sample – falsely increases potassium levels • Calcium and magnesium - these are chelated (binded) by the EDTA.
Oxalates (Grey stopper)	Acid and Alkali phosphatase Amylase Calcium Potassium Partial thromboplastin Red cell morphology Prothrombin time	Fluoride/oxalate samples are used for glucose (and lactate) determination only. Fluoride is a potent inhibitor of many enzymes and the inhibition of glycolysis tends to cause fluid shifts.
Sodium Fluoride (Grey stopper)	Sodium Urea nitrogen	

EDTA in tubes has been the source of more carryover problems than any other additive. Heparin causes the least interference in tests other than coagulation tests because it also occurs in blood naturally but may affect tests done on a clotted tube (SST) if blood is poured over.

Since we know which additives adversely affect which tests, we can arrange the blood culture bottles and tubes in such a way that any additive carryover becomes irrelevant. This arrangement is called the **order of draw.** When tubes are filled according to the recommended order or draw, any additive carryover that may occur, will have no significant impact on test results.

<u>This recommended order of draw is as follows</u>:

1. Blood culture bottles
2. Blue sodium citrate tubes
3. Yellow SST tubes with clot activator
4. Green heparin tubes
5. Purple EDTA tubes
6. Gray Fluoride tubes

Memory Jogger to remember the order of draw sequence:

Because Better Specimens Expedite Goals!

Because:	**B**lood Culture Bottles
Better:	**B**lue (Citrate) tube
Specimens:	**S**ST (Yellow) tube
Hugely:	**H**eparin tube
Expedite:	**E**DTA (Purple) tube
Goals:	**G**ray (Fluoride) tube

This order is the same regardless of the equipment (for example: syringe and needle, vacutainer holder and needle or the winged butterfly set). However, it is important to note that a separate order of draw exists when collecting capillary samples. This is based on the fact that when the skin is punctured, platelets are attracted to the site to assist with the blood clotting process. These platelets can exist in the blood specimen being collected in quantities that does not accurately reflect what's really circulating.
Because platelets adhere to damaged capillary vessels and clump to each other in order to stop the bleeding, the potential for clumps of platelets can interfere with results. Therefore, the EDTA (purple stopper tube) used for FBC (full blood count) must be collected first.

The recommended order of draw for capillary samples is as follows

First: EDTA tubes

Second: Other additive tubes

Third: Non-additive tubes

Important point to remember:
Tubes with additives must be thoroughly mixed and inverted. Erroneous test results may occur when the blood is not thoroughly mixed with the additive. In addition, always remember to draw enough blood to ensure that the blood-additive ratio is adhered to!

Remembering which tests the various additives affect, can be difficult and confusing. The order of draw eliminates this confusion by presenting a sequence of collection that results in the least amount of interference should carryover occur.

Where the order of draw is followed without fail, patients are more likely to be treated according to results that truly and accurately reflect their physiology. On the other hand, ignoring the order of draw can have severe consequences in regards to how the patient is diagnosed, medicated and managed.

So if you are still wondering if you have to follow the order of draw, stop wondering. You absolutely have to.

Resources:

- PT STAT! Centre for Phlebotomy Education (http://www.phlebotomy.com) September 2008
- http://www.labreference.com/pdfs/Order_of _draw)
- Phlebotomy Essentials, Ruth E McCall, Fifth Edition