cytiva

Top 10 tips

For blood separation in point-of-care tests

Blood separation is an important step for point-of-care testing that uses whole blood. However, blood separation has challenges that can be addressed by selecting appropriate membranes and using suitable techniques.

Blood is the most commonly used biological fluid in diagnostic testing. But blood is a complex matrix with many components. Whole blood contains red blood cells (RBCs), white blood cells (WBCs), platelets, and plasma. Biomarkers detected during diagnostic testing is found in plasma, which is the liquid part of blood. The other components, particularly RBC and WBC can interfere with the detection of the biomarkers.

Red blood cells are (unsurprisingly) red. Because many point-of-care diagnostic tests rely on observing a color change, the presence of RBCs can make this observation difficult and lead to false results. RBCs are also active in oxidation-reduction systems, which can interfere with the assay reaction.

White blood cells contain nucleic acids, which will influence the sensitivity of detecting target nucleic acids.

Read on to learn how to achieve successful blood separation in your point-of-care diagnostic test.

1 Depth filtration for blood separation in point-of-care tests

Traditionally, blood is separated by centrifugation, but that method is impractical for point-of-care diagnostics. Centrifugation is an inconvenient sample preparation step and equipment to do it can be costly. Moreover, the volumes of blood collected for point-of-care tests are very small and not suited for spinning down.

Therefore, membranes are often used to separate whole blood in point-of care tests. With membranes, this separation is done by depth filtration (Fig 1).

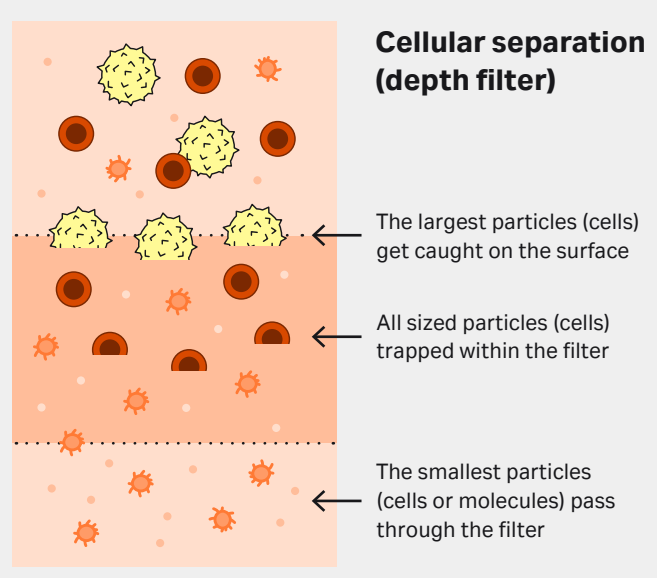


Fig 1. During depth filtration, larger particles (such as WBCs) are trapped on the surface of the membrane while medium-sized particles (such as RBCs) are trapped within the membrane. Smaller particles such as plasma molecules and biomarkers pass through the membrane.

2 Choose the way that blood can flow through a membrane

Blood can flow through a membrane vertically, laterally, or by a composite flow (Fig 2). Composite flow combines a vertical flow of blood followed by a lateral flow. The type of diagnostic test you are building will determine the type of flow that will be used.

Vertical flow is used in flow-through assays. It is suitable for high-volume applications that require fast separation. However, vertical flow can result in low filtration efficiency and low plasma recovery.

Lateral flow is used in dipstick assays and is suited for small sample volumes. Its filtration efficiency is high as is its plasma recovery. However, lateral-flow tests are prone to clogging when large volumes of sample are used, and the filtration process is slow compared to vertical flow filtration.

Composite flow is used in cassette-based, lateral-flow assays. This type of flow has the benefits of vertical and lateral flow: it has high filtration efficiency and plasma recovery and can handle larger volumes of blood.

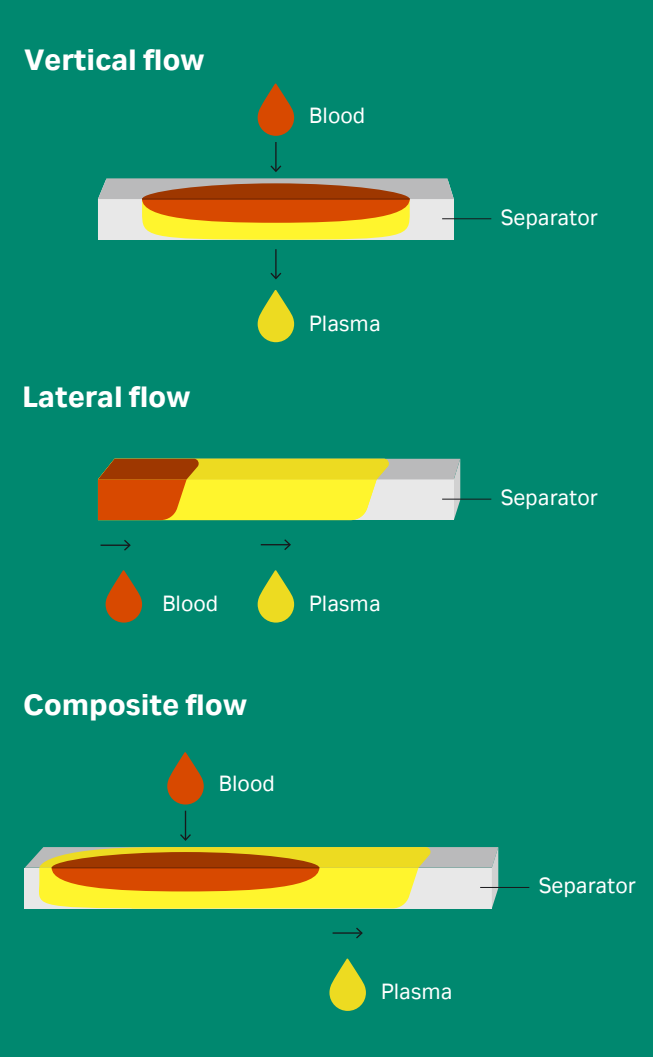


Fig 2. Different types of flow in membranes used to separate blood in point-of-care diagnostic tests.

3 Decide on the most suitable membrane to separate blood

Glass fiber membranes or asymmetric polysulfone membranes are both good choices for separating RBCs from plasma, but they work via different mechanisms. Glass fiber membranes trap RBCs due to a cell-fiber interaction, and polysulfone membranes filter RBCs by size exclusion (Fig 3).

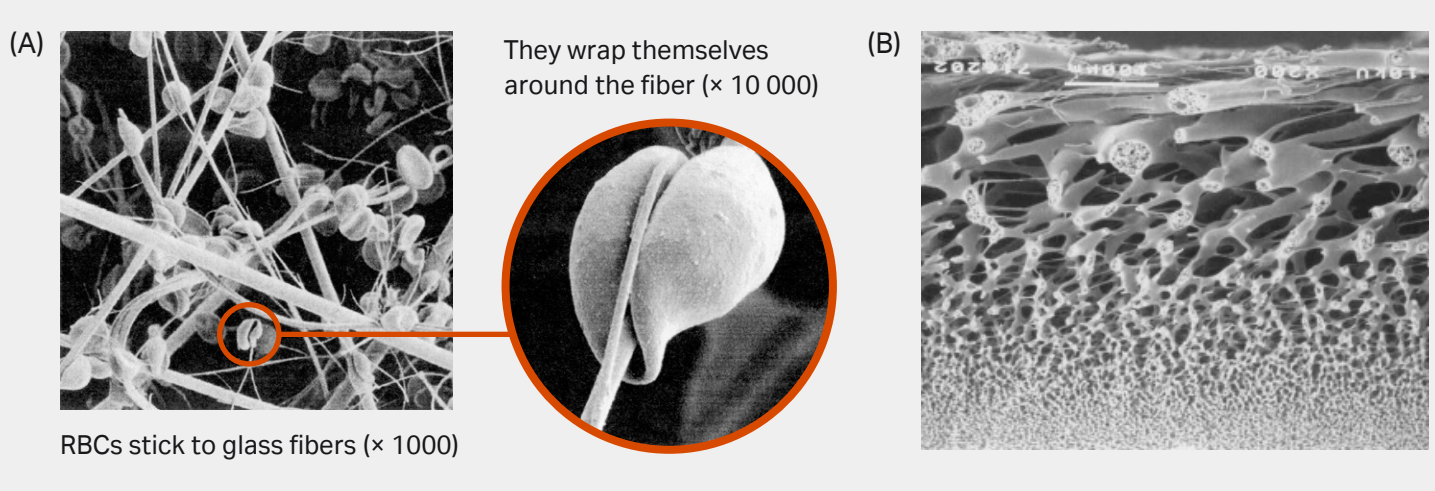


Fig 3. (A) RBCs wrap around the fibers in a glass fiber membrane as blood flows vertically or laterally through the membrane. (B) The spaces within an asymmetric polysulfone membrane gradually get smaller. As blood flows vertically through the membrane, the RBCs get trapped when they are too large to pass through the spaces.

4 Compare glass fiber and polysulfone membranes

Glass fiber and polysulfone membranes differ in various ways.

	Glass fiber	Polysulfone
Handling	• Difficult due to mechanical properties	• Easy due to high mechanical stability
Design	• Vertical flow • Lateral flow • Composite flow	• Vertical flow
Stacking	• Can stack to increase blood capacity	• Cannot stack with additional polysulfone membranes • Can be used as a bottom layer in a glass fiber stack

5 Be cautious about the blood volume used

Whether you use glass fiber or polysulfone membranes, you must avoid overloading the membrane with blood. If glass fiber membranes are overloaded, you'll observe RBC breakthrough. If polysulfone membranes are overloaded, the membrane will become clogged, and the plasma flow will stop.

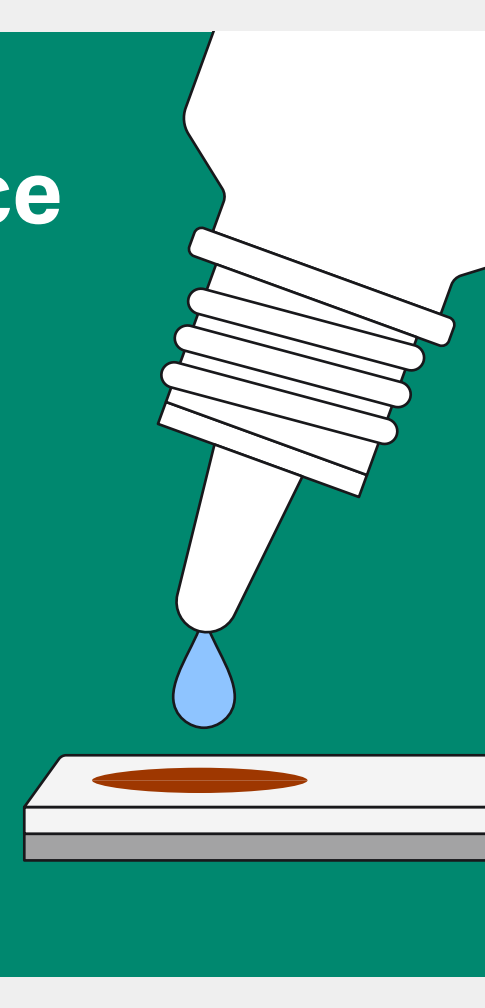
Cytiva offers a variety of membranes to handle a wide range of blood volumes.

Blood volume per 1 cm^2 to be separated	Glass fiber membrane recommended
10–15 µL	LF1
15–50 µL	MF1, Fusion 5
> 50 µL	VF2, GF/DVA

Blood volume per 1 cm^2 to be separated	Polysulfone membrane recommended
20–25 µL	Vivid™ GF
30–40 µL	Vivid GX
40–50 µL	Vivid GR

You can stack multiple glass fiber membranes to increase blood volume, but you cannot stack multiple polysulfone membranes. You can, however, add a polysulfone membrane to the bottom of a glass fiber stack.

6 Recognize the importance of a chase buffer



A chase buffer is a solution added to a membrane after the blood sample has been absorbed by the membrane. A chase buffer also helps move plasma through the test to increase plasma recovery.

Care must be taken when choosing and using a chase buffer. Hypotonic chase buffers can cause RBCs to lyse releasing hemoglobin, which can interfere with reading the results of the assay. Conversely, hypertonic chase buffers cause RBCs to shrink, which allows them to pass through the separation membrane. Furthermore, adding a chase buffer too early to glass fiber pads can cause RBC retention to fail.

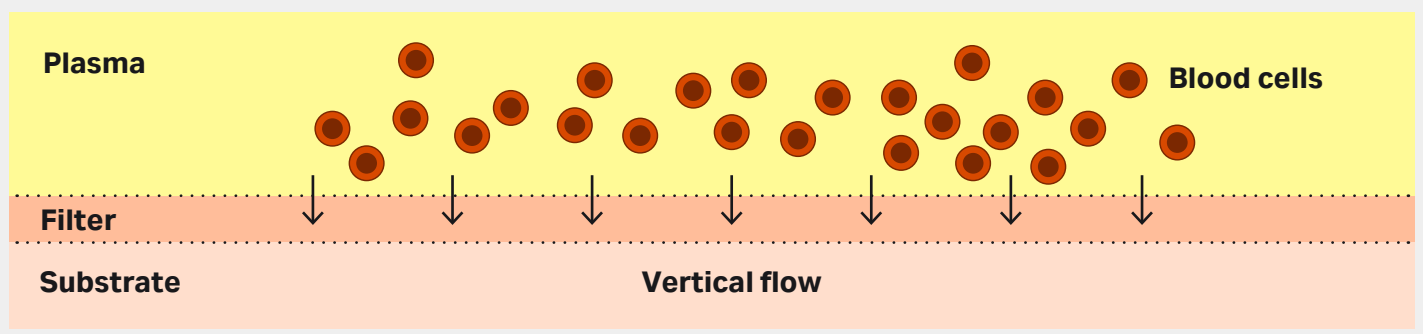
7 Increase the plasma yield

Plasma makes up about 60% of whole blood's volume, so you will naturally lose a large portion of the whole blood that is applied to a test. One way to increase your plasma yield is to use lateral flow or composite flow for your separation.

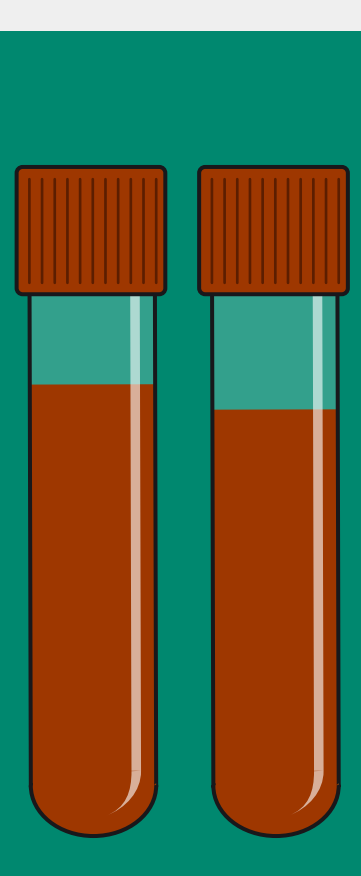
You can also increase the amount of plasma by increasing the amount of whole blood applied to the separation membrane, but of course, you cannot exceed the upper limit of blood volume for the membrane or stack. Conversely, you can reduce the thickness of the membrane or membrane stack while maintaining the original whole blood volume. But again, you must not exceed the volume limits.

Another possible cause of low plasma yields is insufficient capillary forces in an assay's nitrocellulose membrane, conjugate release pad, or microfluidic channel. Adjust these sources of capillary action to increase your plasma yield.

Finally, proper use of a chase buffer will help increase your plasma yield. Optimize the chase buffer volume to increase your yield.



8 Avoid hemolysis



Hemolysis is the rupturing of RBCs. A good way to avoid hemolysis is to use only fresh blood in your test. Ideally, blood from male donors should be used within 48 h, and blood from female donors should be used within 72 h. If blood must be stored between collection and testing, it should be stored at 4°C.

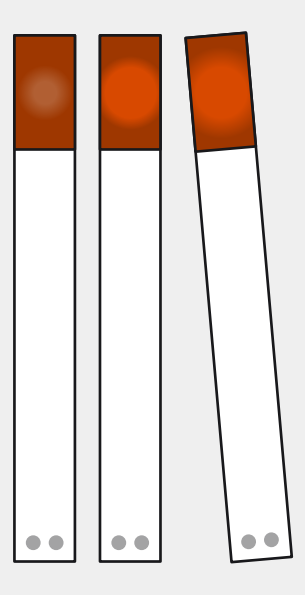
Buffers used to pretreat a separator membrane or to chase a sample can also damage RBCs. Adjust your buffers accordingly to eliminate lysis.

Finally, RBCs can be damaged if external pressure is used to push plasma out of your separator. Instead of pressure, use a chase buffer or adjust the capillary forces used to pull the plasma out of the separator.

9 Avoid RBC breakthrough

RBC breakthrough has several causes. Incorrectly using a chase buffer or using the wrong chase buffer will result in RBCs in the plasma. Do not mix buffer with your blood sample or apply a chase buffer to your test before all the blood is absorbed. Also, be sure your chase buffer is not too hypertonic.

Overloading glass fiber pads can also result in RBC breakthrough, so be sure to follow the volume guidance for the membranes. Also, if you are using a stack of membranes, be sure that edges are properly sealed so that whole blood doesn't leak around the edges.

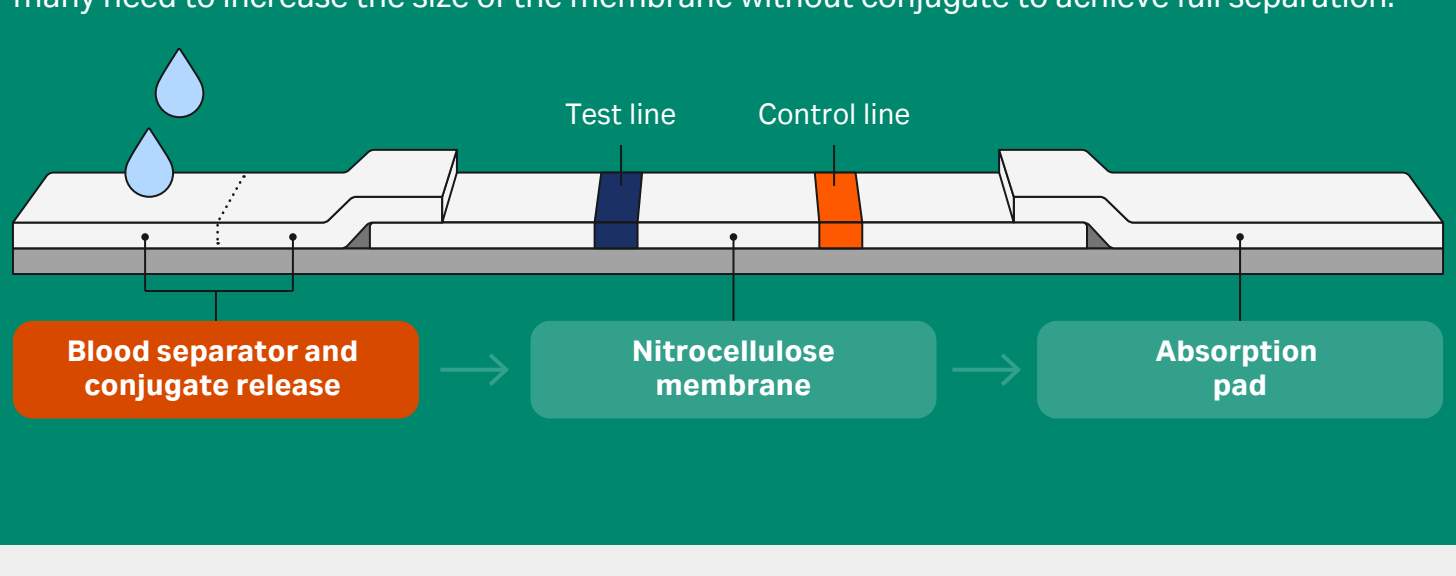


10 Consider combining the blood separation membrane with the conjugate release pad

Combining your blood separation membrane with the conjugate release pad eliminates one material overlap, which will make manufacturing easier. However, this combination is only possible with glass fiber pads.

During manufacturing, the conjugate must be applied to the membrane via dispensing, and any pretreatment buffer should be compatible with RBC stability.

Also keep in mind that area of the membrane that contains the conjugate is not available for blood separation. The plasma must be fully separated before it reaches the conjugate area. You many need to increase the size of the membrane without conjugate to achieve full separation.



Want to learn more about blood separation membranes?

- **Contact a specialist for information and samples**
- **Watch a webinar**
  Blood as a sample in rapid tests: challenges and solutions
- **Read blogs**
  Diagnostic assays: blood separation challenges (and how to solve them!)
  Diagnostic assays: how depth filters work in blood separation
  Learn how to choose between Cytiva blood separation membranes
- **Get personalized help from Immunoassay Development Services**

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