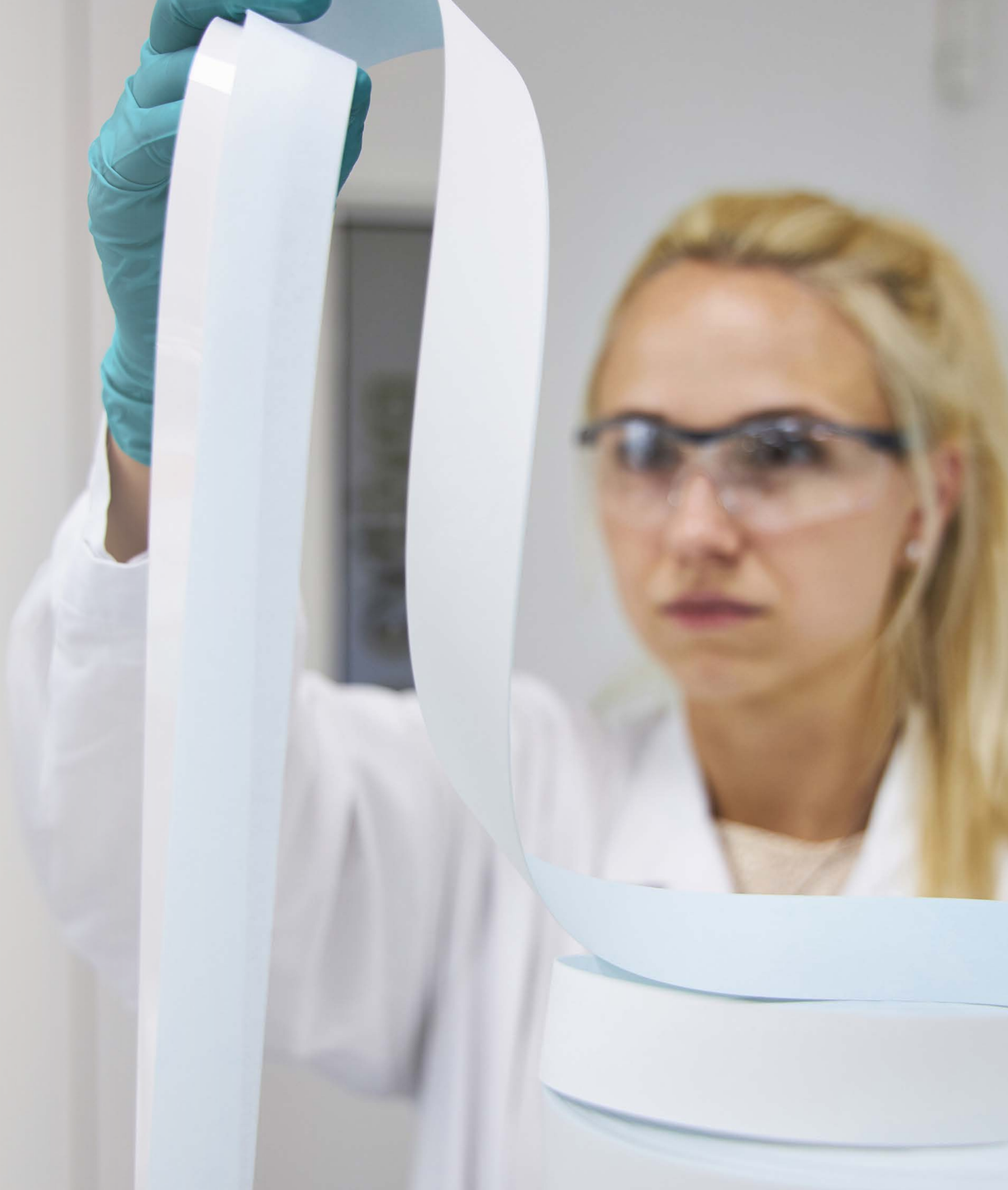
A step-by-step guide

Creating a lateral-flow assay



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A step-by-step guide for creating a lateral-flow assay

If you're curious about the practical aspects of the assembly and striping process for a lateral-flow assay, this guide is for you. Please note that this information is a guide for creating a generic hCG test format. Different ways of assembling the system and/or applying reagents exist. You should thoroughly investigate all patents and methods that could be used in the creation of your diagnostic test to optimize your process.

Getting started

Bring all your components and laminate cards together so that you can begin to assemble your assay in an orderly and organized manner.

Step 1

Lay out your laminate. Some device manufacturers create small jigs that allow you to align all the components of your test uniformly and prevent the cards from slipping. You can mount pins on the jig to keep the laminate securely in place and to mark specific sites where material will be placed or overlapped.

Top tip: A jig is not always necessary but is of great assistance in building a uniform test.

Step 2

Once the laminate is laid out, uncover the adhesive section of the plastic so that it is facing up and not in contact with anything else.

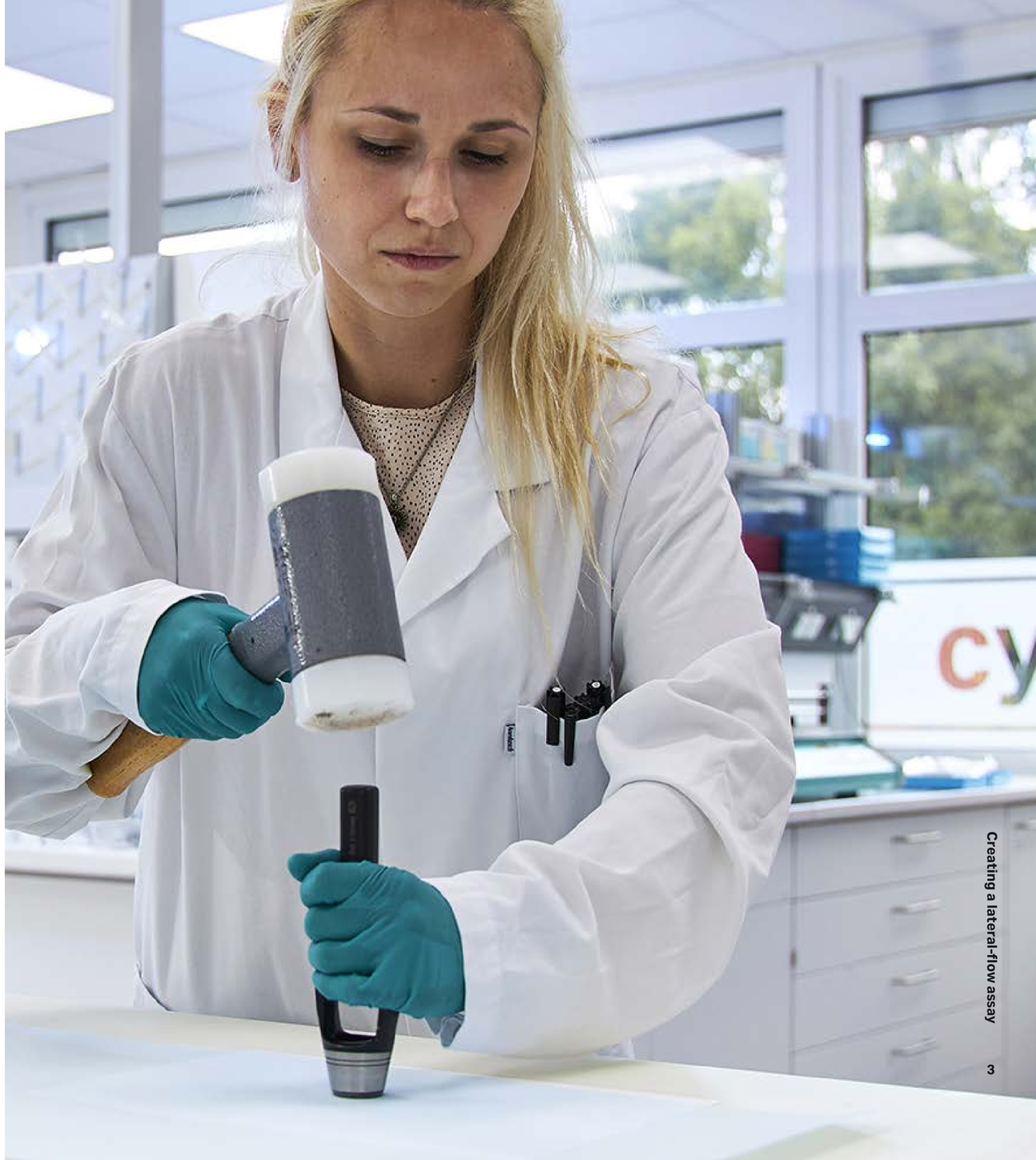
You'll lay down the diagnostic membrane of your choice in the next step, but first you should examine the membrane to be certain that it's free from scratches, particles, or other defects that may alter your test results.

Don't handle the membrane with your bare hands!
Hold the membrane up to the light using forceps, tweezers, or gloved hands so that you can look at it at an angle along the surface.

Step 3

Hold each end of the membrane so that the membrane is taut. Your gloved fingertips (or forceps) should touch only the last centimeter of each end of the membrane. Lay the membrane down in a straight line on the adhesive.

Use a wooden brayer to carefully press the membrane down onto the adhesive so that there are no air bubbles. A brayer is a small tool with rotating head similar to a wallpaper roller. Before using, thoroughly wash the brayer's roller in alcohol and allow it to dry. Then gently use the brayer to press the membrane onto the adhesive.



Step 4

Add the absorbent pad. The absorbent pad is placed at the far end of the test in the direction of flow, after the test area, and should overlap the membrane by a few millimeters. The exact amount for overlap depends upon your assay chemistry, but a typical amount is 1.8 to 2.0 mm.

The absorbent pad should be handled in the same manner as the membrane. It can be rolled with the brayer to ensure evenness and to remove air bubbles.

Step 5

The next component to add to the laminate is the conjugate pad. This material is added to the opposite end of the test as the absorbent pad (i.e., at the origin of sample flow) and should overlap the membrane by 1 to 2 mm. Again, handle the conjugate pad the same way as the other components and roll to secure.

Normally, the conjugate release pad is pretreated with a combination of reagents to create a stable environment for the protein and ensure good resolubilization by the sample. Another option is using pretreated conjugate release media.

Step 6

Next, place the sample wick on top of the conjugate release pad. The sample wick is the beginning point for the test. Ensure a small overlap between the sample wick and conjugate pad. The sample wick may also be pretreated. Sample wicks often contain a cocktail of detergent and buffer to condition the sample prior to entering the experimental zones.

Step 7

The next step is to stripe the test. Normally, you stripe 3 lines.

- The first line in a one-step hCG system is a gold coupled mouse anti-hCG antibody. The conjugate should be striped down in a buffer that allows rapid resolubilization by the sample. Rapid resolubilization is necessary to allow the antibody and analyte to bind to one another to create a complex that will then attach at the readout (test) line.
- The next line is the test line, which is where the antibody-analyte complex binds if present in the sample. This line is located on the membrane. In our generic hCG system, this line is another mouse-anti-hCG antibody.
- The final line is also located on the membrane and is the control line. At this line, a goat-anti-mouse antibody is bound irreversibly to the membrane. Any conjugate material not bound to the original test line will bind here to signal that the sample front has passed the test line and an accurate test reading can be made.

Step 8

Select the reagents to make up the various buffers and impregnating agents in the test. Buffers suitable to optimize are:

- Buffer for striping capture antibodies on the reaction membrane: 10 mM phosphate, pH 7.2
- Buffer for conjugate application: 20 mM phosphate, 10% sucrose, 0.2% Tween 20, 1% PVP K-30

Step 9

Once you've completed the processes of assembly and striping, you can cut the laminated cards into appropriately sized strips. You can cut the strips by hand or use an automated "guillotine" device that cuts the completed cards into strips of uniform width. The strips are then ready to be placed into a cassette that will protect the test and provide ease of handling for the end user.

Useful content

Lateral-flow assay development

- Lateral flow assay troubleshooting guide & how to switch diagnostic membranes
- Exploring the future of Lateral Flow Assays Development
- 10 top tips for LFA development
- Troubleshooting lateral-flow tests
- Top 5 tips for testing stability in lateral-flow assays
- Interactive brochure: Every step of the assay
- Blog: Custom lateral flow assay projects
- Blog: How depth filters work in blood separation
- Blog: Blood separation challenges (and how to solve them!)
- Infographic: Considerations for lateral flow membrane selection
- Sample pads
- Blood separators
- Conjugate release pads
- Nitrocellulose membrane selection
- Unbacked membranes for lateral flow immunoassays
- Absorption pads

Other point-of-care testing

- Blog: Membranes 101: A guide to membranes for POC diagnostics
- Infographic: Navigating flow-through immunoassays
- Infographic: Did you know...track-etched membranes
- Flow-through immunoassays
- Dipstick colorimetric assays

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