Application Highlight

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Surfactants Selection Guide for Lateral Flow Assays

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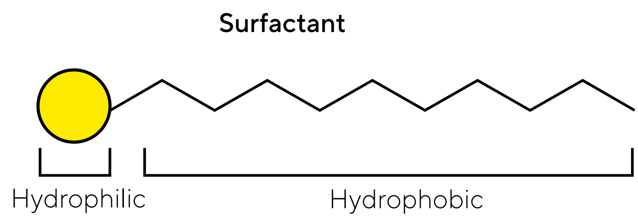
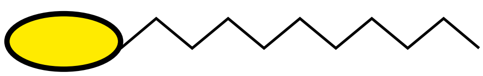
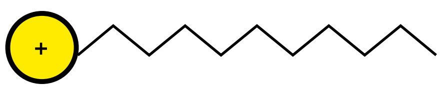
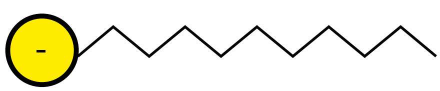
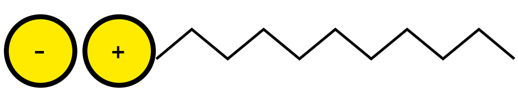
Introduction

Lateral flow assays (LFA) are a type of immunographic assay widely used for the rapid detection of pathogens, toxins, environmental contaminants, etc. Their ease of use and accuracy make these assays well suited for the point-of-care market. For purposes of this study, three lateral flow tests were selected. The effects of surfactants on test performance were demonstrated by adding different surfactants (surface active agent) during the pre-treatment of sample pads.

1. **Classification of surfactants and their function in lateral flow assay:**

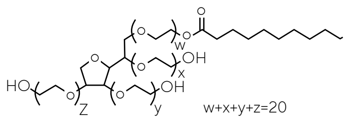
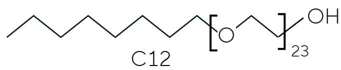
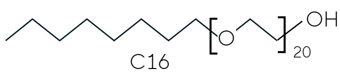
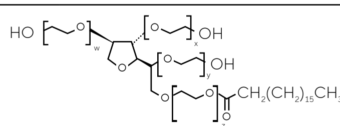
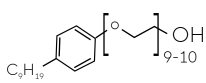
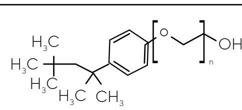
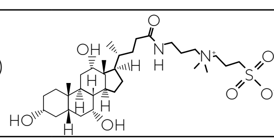
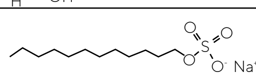
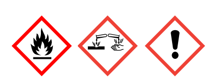
Figure 1: *Different forms of surfactants (Surface Active Agent)*



Classification		Function in Lateral Flow Assay
Non-ionic		▪ Enhance or decrease the test line intensity
Cationic		▪ Accelerate or decelerate the background clearance
		▪ Reduce the unspecific binding
Anionic		▪ Increase wettability of the pad material
Zwitterionic		▪ Support the blocking

As listed in Table 1 the following surfactants were selected. All surfactants were used as additives in pre-treatment of samples pads of the lateral flow tests.

Table 1. *Overview of surfactants used for this study*

Commercial Name	Structure	Type	GHS	HLB	Application
Tween 20 (Polysorbate 20)		Non-ionic		16.7	Gentle surfactant and commonly used in LFA
Brij 35		Non-ionic		16.9	Performance optimization[1]
Brij 58		Non-ionic		15.7	Performance optimization
Tween 60 (Polysorbate 60)		Non-ionic		14.9	Gentle surfactant
Tergitol™ type NP-40		Non-ionic		17.8	Background clearance and conjugate pad release
IGEPAL®CA-630		Non-ionic		13.4	Background clearance and conjugate pad release
CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate)		Zwitterionic		/	Protein solubilization
SDS (sodium dodecyl sulfate)		Anionic		40.0	Commonly used in protein chemistry

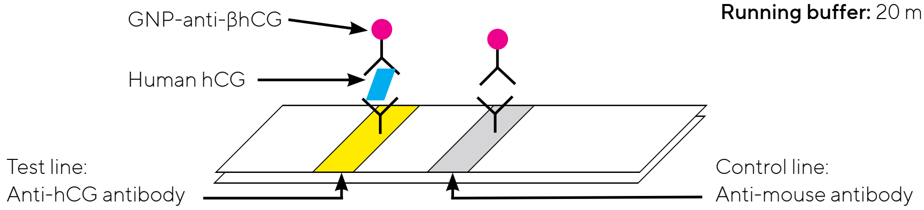
HLB: hydrophilic-lipophilic balance, high value hydrophilic (water soluble), low value hydrophobic (oil soluble)

2. Effect of different surfactants on the performance of UniSart® diagnostic membranes

2.1 hCG pregnancy test: an example for impacts of surfactants on signal intensity and background clearance

The hCG test is known to be robust and highly reproducible. Thus, it was selected to demonstrate the effects of surfactants on signal intensity and background clearance. The surfactants listed in Table 1 were applied in three categories of concentration: Low concentration: ≤ 0.1 % (w/w) or (v/w), moderate concentration: 0.2-0.5 % (w/w) or (v/w), and high concentration: ≥ 0.5 % (w/w) or (v/w). The half-strips assay was used to simplify the test process. This method is commonly used for the initial screening of buffer conditions (Table 2).

Construction of hCG test



Printing buffer: 5 mM Borate, 150 mM NaCl, 1 % sucrose, pH 8.2
Running buffer: 20 mM Tris-buffer, 150 mM NaCl, 0.05 % BSA, pH 8.2

Table 2 . *Summary of test results of hCG test with different surfactants*

Criteria	Color	Description
Bad	Dark grey	No signal/strong background
Neutral	Light grey	Low signal
Good	Yellow	Strong signal/clear background

UniSart® Membrane	Surfactant Concentration	Tween 20	Brij 35	Brij 58	Tween 60	Tergitol™ type NP-40	IGEPAL® CA-630	CHAPS	SDS
		Test line and control line signal (naked eye detection)							
CN95 backed	Low	Good	Good	Good	Bad	Good	Good	Bad	Bad
	Moderate	Good	Good	Good	Good	Good	Good	Bad	Bad
	High	Bad	Good	Good	Bad	Good	Good	Good	Bad
CN110 backed	Low	Good	Good	Good	Good	Good	Good	Bad	Bad
	Moderate	Good	Good	Good	Good	Good	Good	Good	Bad
	High	Good	Good	Good	Good	Good	Good	Neutral	Bad
CN140 unbacked	Low	Good	Good	Good	Good	Good	Good	Bad	Bad
	Moderate	Good	Good	Good	Bad	Good	Good	Bad	Bad
	High	Bad	Good	Good	Bad	Good	Good	Good	Bad
CN140 backed	Low	Good	Good	Good	Neutral	Good	Good	Bad	Bad
	Moderate	Good	Good	Good	Good	Good	Good	Bad	Bad
	High	Bad	Good	Good	Bad	Good	Good	Good	Bad
CN150 backed white	Low	Good	Good	Good	Good	Good	Good	Bad	Bad
	Moderate	Good	Good	Good	Good	Good	Good	Good	Bad
	High	Bad	Good	Good	Bad	Good	Good	Good	Bad
CN150 backed clear	Low	Good	Good	Good	Good	Good	Good	Bad	Bad
	Moderate	Good	Good	Good	Good	Good	Good	Good	Bad
	High	Bad	Good	Good	Bad	Good	Good	Good	Bad
CN180 backed	Low	Good	Good	Good	Neutral	Good	Good	Bad	Bad
	Moderate	Good	Good	Good	Good	Good	Good	Bad	Bad
	High	Bad	Good	Good	Bad	Good	Good	Good	Bad

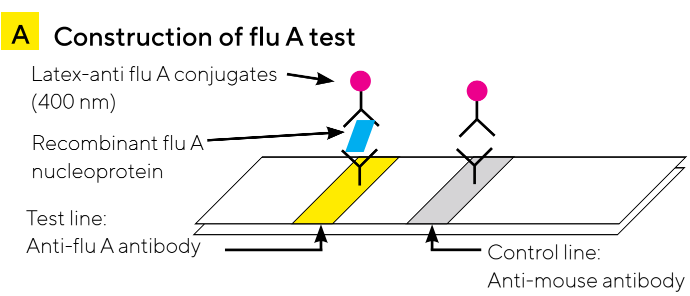
Low concentration: ≤ 0.1 %; Moderate concentration: 0.2-0.5 %; High concentration: ≥ 0.5 %

For non-ionic surfactants, the application of Brij 35, Brij 58, Tergitol™ type NP-40, and IGEPAL® CA-630 generated good test performance at all ranges of concentration tested. Tween 20 could be used at low and moderate concentration, whereas Tween 60 is only recommended at moderate concentration. At high concentration, both Tween surfactants resulted in false positive signals. A possible explanation would be that the high concentration of Tween surfactants adsorbed on the surface of gold-nanoparticles and disrupts the adsorption of conjugate-antibodies[2]. The zwitterionic surfactant CHAPS showed positive effects on performance, but only at higher concentration. The anionic surfactant SDS did not contribute to signal intensity or background clearance like other surfactants. The denaturing nature of proteins by SDS could explain these observations. Tergitol™ type NP-40 and IGEPAL® CA-630 were selected as replacement of Triton X-100.

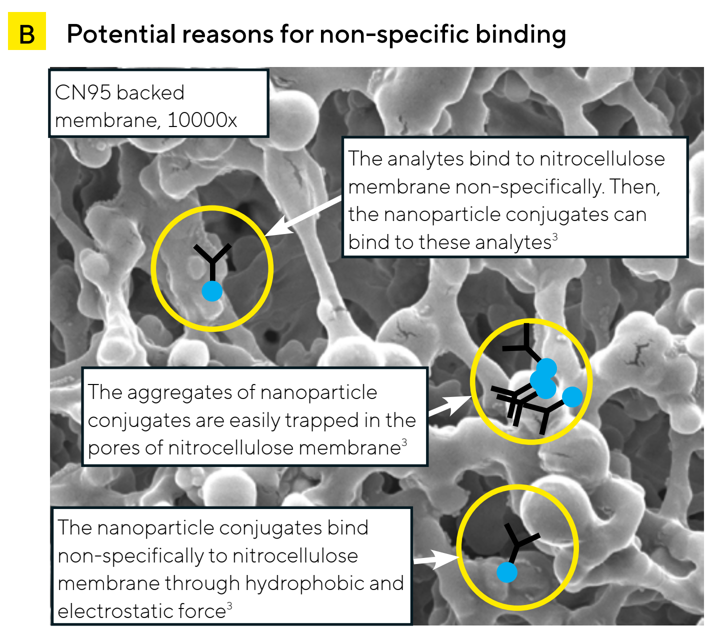
2.2 The flu A test was chosen to demonstrate the effect of surfactants on non-specific binding

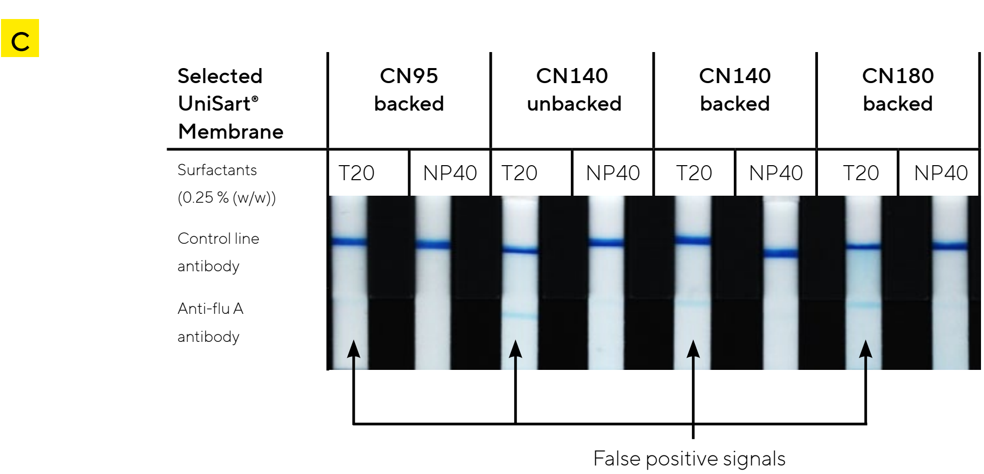
The application of moderate concentration (0.25 % (w/w)) of Tween 20 revealed a good test performance in the hCG test. However, using the same concentration of Tween 20 in the flu A assay resulted in a false positive signal. In the flu A test system, the latex beads with a 400 nm diameter was conjugated by manufacturer's protocol. The latex beads with such large diameter might cause aggregation or non-specific binding during the test running. Potential reasons for non-specific binding (false positive) are illustrated in Figure 2B. Utilizing Tergitol™ type NP-40 at moderate concentration, could solve the problem of non-specific binding and improve the test readout (Figure 2C).

Figure 2 . *Effect of surfactants on flu A test*



Printing buffer: 5 mM Borate, 150 mM, NaCl, 1 % sucrose, pH 8.2
Sample pad pre-treatment buffer: 100 mM Tris-buffer, 0.5 % BSA, 0.25 % surfactants, pH 8.0
Running buffer: 20 mM Tris-buffer, 150 mM NaCl, 0.05 % BSA, pH 8.2



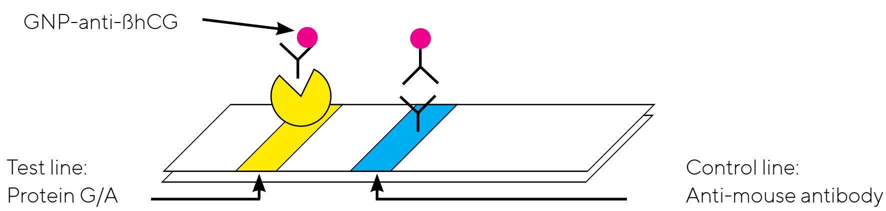


T20: Tween 20; NP-40: Tergitol™ type NP-40

2.3 Protein G and protein A test: an example for surfactants' effect on the interaction of carrier proteins and antibodies

Protein G and protein A are commonly used in lateral flow assays to enhance the sensitivity. Both proteins interact with the Fc fragment of the antibody. It allows for better binding of the detection protein to the membrane surface. In such test, protein G/A-antibody complex is immobilized on the nitrocellulose membrane via protein G/A. Therefore, analysis of protein G/A on membrane immobilization and their interaction with antibodies are important for the optimization of the assay. Here, the effects of surfactants on protein G/A and their antibody interaction were evaluated (Table 3). The concentration of surfactants used 0.25 % (w/w) or (v/w) was adapted from the hCG test due to the good performance.

Construction of protein G and protein A test



- High test line intensity = strong interaction between protein G/A and antibody
- Low test line intesnity = weak interaction between protein G/A and antibody

Printing buffer for protein G: 100 mM Citrate, pH 5
Printing buffer for protein A: 5 mM Borate, 150 mM NaCl, 1 % sucrose, pH 8.2
Sample pad pre-treatment buffer: 100 mM Tris-buffer, 0.5 % BSA, 0.25 % surfactants, pH 8.0
Running buffer: 20 mM Tris-buffer, 150 mM NaCl, 0.05 % BSA, pH 8.2

Table 3. *Summary of test results for protein G/A and antibody-interaction with different surfactants*

	Quantitative Signal Intensity (a.u.)											
Unisart® Membrane	Tween 20		Brij 35		Brij 58		Tween 60		Tergitol™ type NP-40		IGEPAL® CA-630	
	Protein G	Protein A	Protein G	Protein A	Protein G	Protein A	Protein G	Protein A	Protein G	Protein A	Protein G	Protein A
CN95 backed	+++++	++++++	++	+++++	++++	+++	++++++	++++	+	+	+++	++
CN110 backed	+++++	+++++	+++	++++	++++	++	++++++	++++++	++	+	+	+++
CN140 unbacked	++++++	++++++	++++	++++++	+++	++++	+++++	+++	+	+	++	++
CN140 backed	++++++	++++++	+++	++++++	+++++	++++	++++	+++	+	++	++	+
CN150 backed white	++++++	+++	+++	+++	++++	++++	+++++	++++++	+	++	++	+
CN150 backed clear	++++++	++++	++++	++++	+++	+++	+++++	++++++	+	+	++	++
CN180 backed	+++++	++++++	++++++	++++++	+++	++	++++	+++	+	+	++	++++

++++++ : strongest interaction, + : weakest interaction

Conclusion

The effect of surfactants on test performance varied depending on the assay and the membrane, but also the type and concentration of surfactant used. Production costs must be considered if using a surfactant at high concentrations. It is therefore recommended to screen a variety of surfactants and concentrations to ensure the best performance.

Additional reading:

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2. Y. Zhao et al., Adsorbed Tween 80 is unique in its ability to improve the stability of gold nanoparticles in solutions of biomolecules, Nanoscale (2010) 2, 2114-2119.
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4. Global Lateral Flow Assays Market By type, By Technique, By Sample Type, By Application, By End User, By Regional Outlook, Industry Analysis Report and Forecast, 2021-2027.
5. G.A. Posthuma-Trumpie et al., Lateral flow (immune)assay: its strengths, weaknesses, opportunities, and threats. A literature survey, Anal Bioanal Chem (2009) 393:569–582
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7. F. Di Nardo et al., Ten Years of Lateral Flow Immunoassay Technique Applications: Trends, Challenges and Future Perspectives. Sensor (2021) 2921, 21, 5185

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