Performance of Whatman FF120HP and Millipore Hi-Flow Plus HF135 nitrocellulose membranes in a colloidal gold myoglobin assay

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Performance of Whatman™ FF120HP and Millipore™ Hi-Flow™ Plus HF135 nitrocellulose membranes in a colloidal gold myoglobin assay

The purpose of this study was to evaluate the performance of two brands of nitrocellulose membrane—Whatman FF120HP from GE Healthcare and Hi-Flow Plus HF135 from EMD Millipore—in a colloidal gold myoglobin assay developed by DCN Diagnostics. Intra- and inter-lot variability of the FF120 HP membrane was also measured. In these limited studies both membrane types produced similar peak heights, % coefficient of variation (% CV), and reproducibility when assayed at a constant antibody concentration. Whatman FF120HP nitrocellulose membrane showed low intra- and inter-lot variability within the dynamic range of this assay. Taken together, these data suggest that Whatman FF120HP membrane is a viable option for use in lateral flow immunoassays.

Introduction

There is an increasing demand within the diagnostic industry for the development of test kits that produce rapid and reliable results, especially in the detection of target molecules in liquids such as water, urine, blood, saliva, etc. One type of assay for point-of-care immunodiagnostics is the lateral flow immunoassay, which includes a nitrocellulose membrane as a key functional part. The membrane must provide sufficient protein binding to produce a sharp and intense capture line, but at the same time the level of nonspecific background must be low enough for easy interpretation of the results. The FF High Performance (HP) nitrocellulose membranes from GE Healthcare Life Sciences are optimized for use in lateral flow assays. These membranes are manufactured using improved casting procedures that yield a uniform, powder-free surface that delivers razor-sharp lines and highly reproducible results.

Methods

Manufacture of the test components, assembly of tests, performance studies, including all data and conclusions, were provided by DCN Diagnostics, 6354 Corte del Abeto, Suite B, Carlsbad, CA USA 92011.

The colloidal gold myoglobin assay was previously developed at DCN for internal use and demonstration purposes using Millipore Hi-Flow Plus HF135 membrane. The assay, utilizing the same conjugate pads, antibodies, colloidal gold conjugate, and processes, was transferred to GE Healthcare's Whatman FF120HP membrane.

Anti-human myoglobin colloidal gold conjugate was sprayed using an Airjet dispenser (BioDot, Inc) on G041 Glass Fiber Conjugate Pad Sheets (EMD Millipore) blocked with 10 mM borate, 3% BSA, 1% polyvinylpyrrolidone (M.W. = 40 000), 0.25% Triton™ X-100, pH 8.0 at the rate of 10 µl/cm. The conjugate pads were used to prepare test strips with either the FF120HP or the HF135 membrane.

All tests were performed in DCN's laboratories using standards with various concentrations of myoglobin prepared in myoglobin-free serum. Tests were done by pipetting 100 µl of the myoglobin standard into a test tube then adding a test strip and waiting for 15 min. After 15 min the strip was removed and graded visually using DCN's 0–10 scale, or peak heights were determined using an ESEQuant Lateral Flow Immunoassay Reader (Qiagen).

Results and discussion

Performance of FF120HP and HF135 membranes using conditions optimized for HF135

The Whatman FF120HP membrane (casting lot G1471608, roll 7A/Q) and the Hi-Flow Plus HF135 membrane (lot R1KA07368, roll 11CR D) were striped with a test line of 0.2 mg/ml mouse anti-human myoglobin antibody and a control line of 0.15 mg/ml goat anti-mouse antibody. Dispensing was performed at 1 µl/cm and 50 mm/s, which were the conditions optimized by DCN for the HF135 membrane. Tests were performed using the procedure described in the Methods section.

Table 1. Visual grades (0–10) of colloidal gold myoglobin assay using conditions optimized for the HF135 membrane

Membrane type	[Myoglobin] (ng/ml)	Test line grade	Control line grade
HF135	100	9	8
	50	9	8
	25	9	8
	10	7	8
	5	6	8
	2.5	4	8
	1.25	3	8
	0.75	2	8
	0.5	2	8
	0.25	1	8
	0	0	8
FF120HP	100	9	7
	50	9	7
	25	8	7
	10	7	8
	5	7	9
	2.5	6	9
	1.25	4	9
	0.75	3	9
	0.5	2	9
	0.25	1	9
	0	1	9

The two membranes produced similar visual test line grades (Table 1). However, the control and test lines for the FF120HP membrane had very dark leading edges as shown in Figure 1.

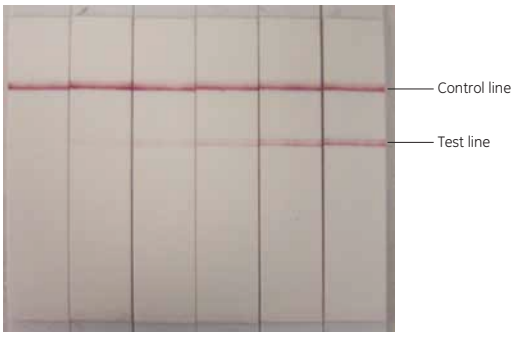

Fig 1. FF120HP test strips from the colloidal gold myoglobin assay using conditions optimized for the HF135 membrane. Fluid flow is from bottom to top.

Lines with this type of appearance are difficult to interpret properly visually or using a reflectance reader.

Performance of FF120HP and HF135 membranes using equalized antibody concentrations

In an attempt to overcome this issue the amount of antibody per test was equalized by striping the FF120HP membrane at 0.6 µl/cm and 50 mm/s with 0.33 mg/ml of anti-human myoglobin antibody, which provides 0.099 µg of antibody/test. The lines on the FF120HP membrane did not have a dark leading edge (Fig 2), and the lines on both the FF120HP and the HF135 membranes had a similar optical appearance (data not shown).

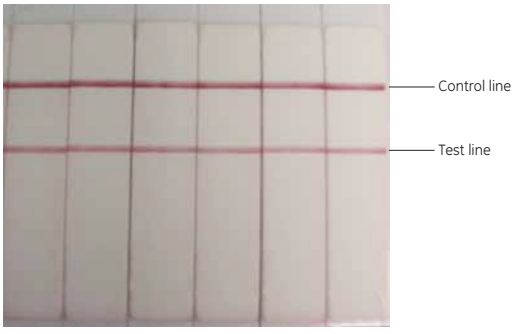

Fig 2. FF120HP test strips from a colloidal gold myoglobin assay in which the amount of anti-human myoglobin antibody was equalized (0.099 µg/test). Fluid flow is from bottom to top..

Reproducibility of the FF120 HP and HF135 membranes was tested using the conditions described for Figure 2. Concentrations of myoglobin in myoglobin-free serum were 0, 0.25, 2.5, and 10 ng/ml. Twenty test strips were prepared for each membrane type and concentration of myoglobin. Test strips were read using a reflectance reader (Table 2), and peak heights were obtained. The % CV was determined by dividing the standard deviation (SD) by the mean.

Table 2. ESEquant reader results of colloidal gold myoglobin assay using a constant amount of anti-human myoglobin antibody (0.099 µg/test).*

Membrane	[Myoglobin] (ng/ml)	Average peak height (mV)	SD	%CV
HF135 (roll 11CR D)	0	0	0	N/A
	0.25	127	15	12.2%
	2.5	720	43	5.9%
	10	974	47	4.9%
FF120HP (roll 7A/Q)	0	0	0	N/A
	0.25	107	11	10.5%
	2.5	579	28	4.9%
	10	822	33	4.1%

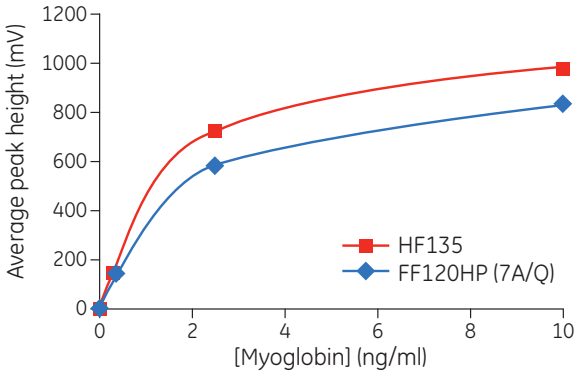

Fig 3. Average peak heights of HF134 (roll 11CR D) and FF120HP (roll 7A/Q).*

The results of this limited testing of a single roll of each membrane type showed that the HF135 membrane produced slightly higher peak heights (Fig 3) but that the FF120HP had lower variability (Table 2). The signal generated on the FF120HP membrane saturated at a lower analyte concentration than on the HF135 membrane. It is expected that increasing the concentration of the antibody solution used to stripe the FF120HP membrane would produce peak heights similar to those produced by the HF135 membrane and allow for the saturation point to be equalized between the two membrane types. However, additional testing would be required to confirm this expectation and to determine whether the differences observed here are applicable to other lots of both membranes.

Intra-lot variability of Whatman FF120HP membrane

The intra-lot variability of the FF120HP membrane was evaluated by striping membranes from three different rolls (7A/Q, 7A/R, and 7A/S) from a single casting lot (G1471608). A test line of 0.2 mg/ml of anti-human myoglobin antibody was dispensed at 0.6 µl/cm and 50 mm/s. Twenty tests were performed for each roll using 0, 0.25, 2.5, and 10 ng/ml of myoglobin in myoglobin-free serum.

The results show that the three rolls within the same casting lot produced similar peak heights when striped with the same antibody solution.

* Results represent 20 replicates per data point

Table 3. Intra-lot variability of Whatman FF120HP membrane (same casting lot.*

FF120HP roll number	[Myoglobin] (ng/ml)	Average peak height (mV)	SD	%CV
7A/R	0	0	0	N/A
	0.25	62	10	16.3%
	2.5	367	32	8.7%
	10	656	31	4.7%
7A/S	0	0	0	N/A
	0.25	61	5	8.5%
	2.5	419	23	5.4%
	10	664	29	4.3%
7A/Q	0	0	0	N/A
	0.25	59	13	21.6%
	2.5	380	26	6.7%
	10	657	35	5.3%

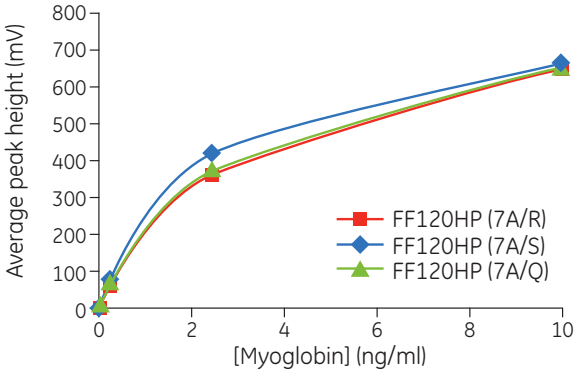

Fig 4. Intra-lot variability of FF120HP based on testing of three rolls from the same casting lot.*

Inter-lot variability of Whatman FF120HP membrane

The inter-lot variability of the FF120HP membrane was evaluated by striping membranes from three different casting lots (G1471608, roll 7A/Q; G1471610, roll 1A/T; and D013024, roll 15A/T) with a test line of 0.2 mg/ml of anti-human myoglobin antibody dispensed at 0.6 µl/cm and 50 mm/s. Twenty tests were performed for each roll using 0, 0.25, 2.5, and 10 ng/ml of myoglobin in myoglobin-free serum.

The results showed that the three rolls of FF120HP membrane produced very similar peak heights when striped with the same antibody solution.

The % CV for the intra- and inter-lot rolls of FF120HP membrane was below 10% at myoglobin concentrations of 2.5 and 10 ng/ml. The higher % CV values at the assay limit of detection (LOD; 0.25 ng/ml) can be attributed to very low values for peak height. Even small variances in total numbers will lead to high CVs; if the mean is a small number, even a small SD will lead to a double-digit CV. It is possible that electronic background noise of the reader system may also contribute to signal variability, although there are no data describing this noise.

The low variability of intra- and inter-lot rolls of FF120HP could simplify the manufacture of lateral flow assays by reducing the time and material needed to adjust antibody concentrations to give the correct test line intensity.

Table 4. Inter-lot variability of Whatman FF120HP membrane (different casting lots)*

FF120HP casting lot number	[Myoglobin] (ng/ml)	Average peak height (mV)	SD	%CV
G1471610	0	0	0	N/A
	0.25	75	8	10.4%
	2.5	464	31	6.6%
	10	760	35	4.6%
D013024	0	0	0	N/A
	0.25	68	13	19.2%
	2.5	454	29	6.4%
	10	741	34	4.6%
G1471608†	0	0	0	N/A
	0.25	68	13	19.4%
	2.5	446	41	9.1%
	10	747	36	4.8%

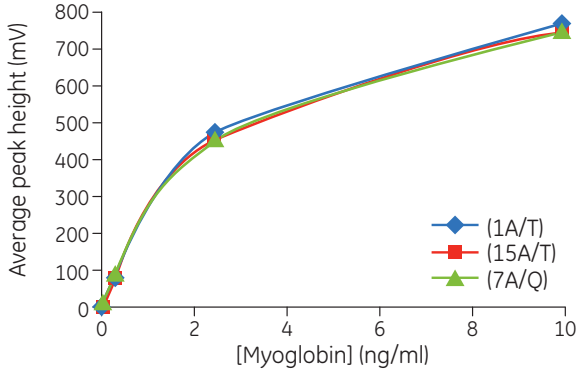

Fig 5. Inter-lot variability of FF120HP based on testing of three rolls from three different casting lots.*

* Results represent 20 replicates per data point
† Roll number 7A/Q

Conclusions

The following conclusions can be made from the data presented here:

1. Based on experiments using one roll of each membrane type, the FF120HP membrane required that the antibody be striped at a lower dispense rate than did the HF135 membrane. Using dispensing rates above 0.6 µl/cm and 50 mm/s resulted in test lines with dark leading edges, which could result in poor interpretation both visually and with a reader.
2. Striping the same mass of antibody on one roll of each membrane type resulted in slightly lower peak heights with the FF120HP membrane than those seen with the HF135 membrane. Signal strength saturated at a lower analyte concentration with the FF120HP membrane, which could potentially have an impact on dynamic range in the assay. However, it is anticipated that this can be easily overcome with optimization of the striped antibody mass.
3. Importantly, particularly for quantitative assays, the FF120HP membrane showed very low variability (CV < 10%) within the dynamic range of the colloidal gold myoglobin assay. Control of assay CV is critical to the ability to quantify in lateral flow assays.
4. The intra- and inter-lot variability of the FF120HP, as measured by assay signal strength, was very low. These results indicate very high reproducibility of membrane characteristics within and between lots, as the signal strength CV includes multiple sources of process, reagent, and operator variability as well as membrane variation. The other sources of variability were largely controlled for in the direct comparison between the HF 135 and FF120HP membranes. However, the fact that the absolute CVs were very low with multiple rolls and multiple lots of the Whatman FF120HP membranes points to highly reproducible membrane performance. This level of reproducibility could translate into better control of manufacturing processes, reduced QC burden, and lower scrap rates in assay manufacturing.

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