

Measuring Colloidal Immunogold Conjugate Release from Lateral Flow Conjugate Release Matrixes

Nina Lukinova, Ph.D., Galina Fomovska, Ph.D. and Andrew Dubitsky, Pall Life Sciences, Port Washington, NY

ABSTRACT

Objectives — Establish a protocol for evaluation of colloidal immunogold release from a conjugate pad matrix as a tool for lateral flow assay development and optimization. Compare different commonly used materials (cellulose-based media, treated and untreated borosilicate glass fiber) in terms of kinetics and percentage of colloidal immunogold release. Correlate total conjugate release with performance in a model immunoassay.

Background — In most lateral flow (LF) point-of-care (POC) assays, a conjugate release pad is harboring a dry detection reagent. Conjugate contains detection particles coupled with antibodies specific to a target analyte. To ensure consistent performance of the LF assay, the conjugate pad matrix should be able to hold the conjugate stable over a defined shelf life and quickly release it when a sample flows into the pad. Industry expectation is over 90% release of the conjugate in 180 seconds; however, there are no published methods for monitoring conjugate release kinetics or evaluation of conjugate pads for POC assays.

Methods — Ten 12 mm discs per pad type were used for applying hCG-specific immunogold at a volume of 10 µL. Discs were dried at 37 °C for 2 hours and the color of each disc was measured with a handheld densitometer. Color intensity of the media before applying conjugate immunogold was used as a control. Release of conjugate gold was performed using a 12 mm holder, chase buffer and a syringe pump set for a flow rate 0.05 mL/min. Fractions were collected every 15 seconds, and OD at 530 nm was measured in a 96-well plate. After collecting 12 fractions (180 seconds of conjugate release), discs were placed on a nitrocellulose strip to dry and color intensity was measured again to calculate total conjugate release.

Results — A method has been established to monitor the release of conjugate reagent during the first 180 seconds after initiation of an immunoassay and to measure the total amount of released conjugate at endpoint. It was shown that under tested conditions, glass fiber-based conjugate pads resulted in 99-100% release of conjugate immunogold in less than 120 seconds. Cellulose-based media demonstrated similar early kinetics of conjugate release, with a slightly lower amount of released gold for each time point and 92% total conjugate immunogold release. A model hCG immunoassay demonstrated that cellulose-based conjugate pad with an average 92% conjugate release resulted in a slightly lower test line intensity compared to a glass fiber pad providing 99-100% conjugate release.

Conclusions

1. A procedure for the evaluation of conjugate pads for POC assays has been developed, including the monitoring of early kinetics of conjugate reagent release from a conjugate pad matrix.
2. All tested conjugate release pads performed within industry expectations providing > 90% of conjugate release in 180 seconds.
3. Lower total conjugate release resulted in lower test line intensity in an hCG immunoassay.

INTRODUCTION

Conjugate release pads are one of the most critical components in lateral flow assays. Conjugate pads are used to immobilize and release the colloidal or latex detector reagent once the sample has passed over the pad, uniformly delivering the sample and detector reagent to the reaction membrane. The ability of the conjugate pad to uniformly wet in order to accept the conjugate into the matrix is critical to LF assays. The intrinsically hydrophilic nature of Pall's conjugate pad materials ensure that the pads wet quickly, resulting in efficient capture and release of the conjugate reagent. The properties of these materials facilitate development of a strong, sharp capture line, ensuring optimal assay performance and increased sensitivity in diagnostic assays. Pall's family of conjugate release pads (see Table 1) are available in a variety of base materials. Pall conjugate pads exhibit low non-specific binding to minimize concerns of binding the analyte of interest or detection reagent.

Table 1
Characteristics of Conjugate Pads Available from Pall Life Sciences

Grade	Base Material	Typical Thickness (µm)	Typical Base Weight (g/m ²)	Water Absorption Capacity (µL/cm ²)
8301	Cellulose with PVA binder	355.6-444.5	50	28
8964	Borosilicate glass fiber with PVA binder	355.6-508.0	75.1	54
8975	Borosilicate glass fiber with PVA binder	22.6-330.2	49.1	19

MATERIALS AND REAGENTS

- Conjugate Pads – three grades of glass fiber pads and one grade of cellulose-based pad
- Conjugate – hCG-specific conjugate immunogold (British Biocell International, Inc.)
- Conjugate Release Buffer – 1x PBS + 3% Sucrose + 0.5% Triton X100 + 1% BSA
- Conjugate Dilution Buffers 1-7 (see Results for list)

RESULTS

Conjugate Release Method Development

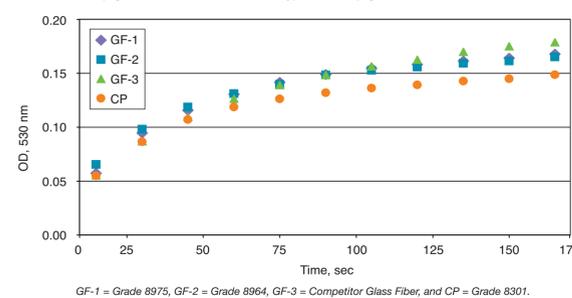
The following procedure was developed to monitor conjugate release from the pads (Figure 1):

- Prepare 12 mm discs of every type of media (~15 discs per type).
- Prepare conjugate immunogold in a specified buffer and apply at a volume of 10 µL/disc (10 discs per media type), dry 2 hours at 37 °C.
- Release conjugate immunogold from the discs (3-5 discs per media type) using 12 mm holder, buffer and syringe pump with 0.05 mL/min. flow rate; collect fractions (~105 µL) every 15 seconds.
- Place 100 µL of each fraction into a 96-well plate, read at 530 nm.
- Place discs after conjugate release on Vivid™ 170 nitrocellulose membrane strip, dry for 1 hour at room temperature.
- Measure color before and after the test in the center of each disc using an X-Rite® densitometer. Use color of media discs without conjugate immunogold as control.
- Percent of conjugate immunogold release was calculated as the following: background color (i.e. average of X-Rite measurements for discs before applying conjugate immunogold) was subtracted from the highest and lowest values for discs with dried immunogold ("Color Before") and from average value after conjugate release ("Color After"); percent release was calculated as % = "Color After"/"Color Before" x 100.

Performance of Different Types of Conjugate Pads

In order to compare the performance of different conjugate pads, three grades of glass fiber and one grade of cellulose were tested for conjugate release kinetics (see Figure 2) and total conjugate release (see Table 2).

Figure 2
Kinetics of Conjugate Release From Different Types of Conjugate Pads. N = 5, CV < 3.5%.



Glass Fiber Conjugate Pads (GF) – All tested glass fibers demonstrated 99-100% release of conjugate immunogold. Kinetics of conjugate immunogold release demonstrated that a practically full release of the conjugate immunogold has been achieved in less than 2 minutes, i.e. between 105 and 120 seconds.

Cellulose Conjugate Pad (CP) – Demonstrated over 90% of conjugate immunogold release in less than 2 minutes; however, the total release was slightly lower compared to glass fibers. Discs of cellulose media were visibly pinkish after the test (see Figure 1, arrow). 90-93% conjugate release compared to practically full release from glass fiber may be due to sub-optimal testing conditions for the cellulose-based pad.

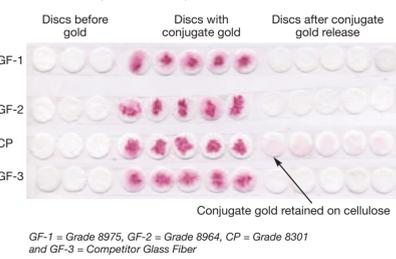
Model hCG Immunoassay Method

The performance in hCG immunoassay was assessed by quantification of test line intensity (see Figure 3).

- Vivid 170 nitrocellulose membrane was striped with Test (hCG-specific) and Control (IgG-specific) antibodies at 1 mg/mL with density 1 µL/cm.
- Test strips were assembled from striped nitrocellulose, absorbent pad and conjugate pad with dried conjugate immunogold.
- Test was performed by applying 30 µL of hCG-positive sample followed by 100 µL of conjugate release buffer on conjugate pad.
- Test strips were air-dried and scanned; test line intensity was calculated using ImageTool® software.

Maximum test line intensity was observed with glass fiber conjugate pads. Cellulose pads resulted in slightly lower test line intensity in hCG immunoassay.

Figure 1
Images of Media Discs Before Treating With Conjugate Immunogold, Discs With Applied Conjugate, and Discs After Conjugate Immunogold Release



RESULTS (continued)

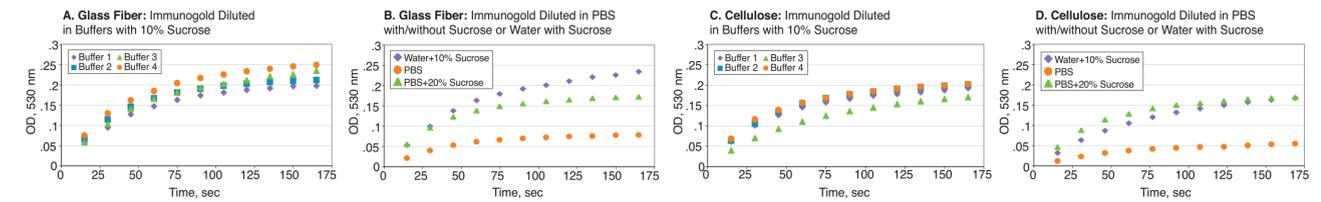
Applicability of Conjugate Release Kinetics for Conjugate Dilution Buffer Optimization

In order to determine applicability of the suggested procedure for conjugate buffer optimization, discs of two types of conjugate pads (Glass Fiber Grade 8975 and Cellulose Grade 8301) were dried with immunogold conjugate diluted in seven different buffers. Testing of conjugate release kinetics (see Figure 4) and measurement of total conjugate release (see Figure 5) was performed.

Buffers

- 1) 20 mM K₂HPO₄, pH 7.4 + 10% Sucrose + 0.25% Triton X100 + 5% BSA;
- 2) 20 mM K₂HPO₄, pH 7.4 + 10% Sucrose;
- 3) Water + 10% Sucrose;
- 4) 2 mM Borate Buffer, pH 7.0 + 10% Sucrose;
- 5) 2 mM Borate Buffer, pH 7.0 + 1% Sucrose;
- 6) 1x PBS;
- 7) 1x PBS + 20% Sucrose.

Figure 4
Kinetics of Immunogold Release From Glass Fiber and Cellulose Discs Containing Conjugate Diluted in Different Buffers. N = 3, CV < 3.5%.



Kinetics of Conjugate Release

- Four different solutions containing 10% Sucrose resulted in similar early conjugate release kinetics from glass fiber pad (see Figure 4A).
- Water + 10% Sucrose provided better conjugate release from glass fiber pad compared to PBS + 20% Sucrose (see Figure 4B). Low amount of conjugate was released from glass fiber pads containing conjugate diluted in PBS without Sucrose.
- Three different solutions containing 10% Sucrose resulted in similar early conjugate release kinetics from cellulose-type pad. Discs with conjugate diluted in Water + 10% Sucrose demonstrated less conjugate release from cellulose pad (see Figure 4C).
- PBS + 20% Sucrose provided slightly faster early release from cellulose pad and was similar at the end of 180 second time compared to Water + 10% Sucrose (see Figure 4D). As with glass fiber, a low amount of conjugate was released from cellulose pads containing conjugate diluted in PBS without Sucrose.

Total Conjugate Release

The results (see Figure 5) demonstrated that glass fiber pads released over 90% conjugate with most of the buffers except PBS without Sucrose and 2 mM Borate with 1% Sucrose. Cellulose pads provided over 90% release in buffers 1 and 7 only.

DISCUSSION AND CONCLUSIONS

The role of a conjugate pad in a lateral flow assay is to accept conjugate reagent, preserve it during drying and storage, and efficiently release it during the test. The experiments were performed with BBI hCG-specific conjugate immunogold (40 nm) applied to different types of conjugate pads. In tested conditions, three glass fiber-based conjugate pads demonstrated practically full release of conjugate immunogold in less than 2 minutes. Cellulose-type media demonstrated similar kinetics of conjugate release. Total conjugate release was slightly lower compared to glass fiber pads, but still within industry expectations of over 90%. The lower total conjugate release in the cellulose pad resulted in lower test line intensity in an hCG immunoassay, as compared to glass fiber pads. To show that the proposed method is applicable for optimization of conjugate dilution buffer, we tested seven different buffers with two types of media (one glass fiber grade and one cellulose) for conjugate release kinetics and total release. As expected, concentration of Sucrose was critical for full conjugate release. We found that performance of different solutions containing 10% Sucrose resulted in similar conjugate release kinetics and total release. Interestingly, performance of Water + 10% Sucrose and PBS + 20% Sucrose provided similar performance with the cellulose-based pad, but Water + 10% Sucrose was superior in the case of the glass fiber pad. This might be an indication of salts interfering with efficient conjugate release. Thus, this method can be used for tuning the performance of different types of conjugate pads with different conjugate dilution buffers.

- A procedure to monitor early conjugate immunogold release from conjugate pads for lateral flow assays has been developed to facilitate lateral flow assay development programs.
- All tested conjugate release pads available from Pall provided > 90% of conjugate immunogold release; thus performing within industry expectations.
- Lower total conjugate release resulted in lower test line intensity in a model hCG immunoassay.
- The proposed method can be applied for optimization of conjugate dilution buffer and choice of media during assay development.
- The utilization of Sucrose in the conjugate dilution buffer facilitates release of the conjugate particles from the conjugate pad.

Figure 3
Maximum Test Line Intensity in hCG Immunoassay.

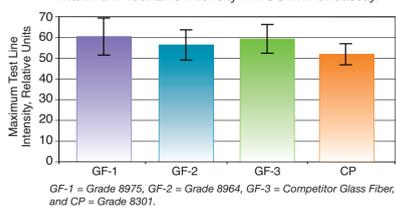


Figure 5
Percent of Total Conjugate Release From Glass Fiber and Cellulose Discs Containing Immunogold Conjugate Diluted in Different Buffers.

