Development of Lateral Flow Assays for in Process Testing of Biopharmaceutical Products

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Abstract

There is continued interest in developing simple, easy to transfer and use methods for in process testing in biopharmaceutical manufacturing. These methods need to be specific and selective for the analytes of interest, but need not necessarily be automated or instrumented. Immunochromatographic assays, or lateral flow assays, offer these properties and provide ease of use, ease of transfer, and minimal operator-to-operator variability. Additionally, new developments in materials and reading technology allow for improved reproducibility and quantification of these rapid test formats where required. This paper will present the progress to date on the application of lateral flow assay technology for the development of quality control tests for biopharmaceutical manufacturing.

Introduction

Lateral flow assays represent an established technology that has for many years been used in simple point of sampling applications, primarily to generate qualitative "yes/no" results. **The advantages of this technology include:**

- **Ease of use – can be run by minimally or untrained users**
- **Low cost**
- **Relative ease of manufacture**
- **Relative ease of scale-up**
- **Known technology**
- **Robustness – typically don't require refrigeration**
- **Shelf life – typically very stable**
- **Low technology – typically don't require readers or complex instrumentation to run**
- **Manufacturing instrumentation is available off the shelf – little customization required**

This technology has not, in the past, been widely applied to analyses that require high reproducibility from test to test or a quantitative result, due to inherent manufacturing and measurement issues that lead to relatively high test-test variability.

In recent years, however, lateral flow assays have undergone a renaissance with the advent of a variety of new measurement methodologies and manufacturing technologies that allow for the manufacture of highly reproducible, very sensitive lateral flow assays which utilize measurement systems that allow for full quantitation and complete, traceable documentation of results.

The application of appropriate manufacturing and measurement technologies combined with the inherent advantages of lateral flow assays make lateral flow assays ideal candidates for the measurement of a variety of analytes in the biopharmaceutical industry.

This poster illustrates some of the development, manufacturing and measurement techniques required to produce highly reproducible, quantitative lateral flow systems, and presents experimental results that demonstrate the potential results achievable with a variety of analytes from biopharmaceutical and industrial biotechnology quality control and environmental testing applications.

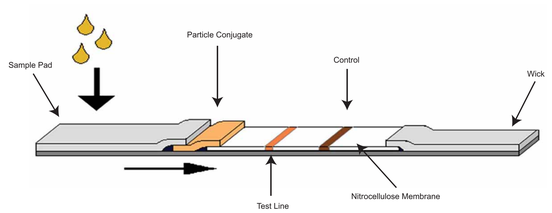


Figure 1: Typical Lateral Flow Assay Configuration

Methods

Manufacture of highly reproducible lateral flow assays

Typical manufacturing processes for lateral flow assays

1. High precision dispensing of proteins and particulate conjugates onto heterogeneous matrices including nitrocellulose and pad materials including glass fibers and polyesters
2. Immersion of pad materials into heterogeneous protein and detergent – containing buffers
3. Drying of pads and membranes
4. Lamination of pads onto backing materials
5. Cutting of cards into individual strips
6. Insertion of strips into cassettes
7. Pouching of cassettes with desiccants and sealing of pouches.

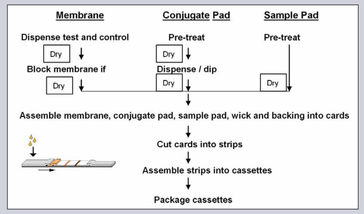


Figure 2: Typical Lateral Flow Assay Manufacturing Process Flow

In order to minimize assay – assay variation caused by typically used manufacturing processes and the inherent variability of the materials and reagents used in these assays, certain manufacturing procedures need to be adopted:

In line (continuous) processing of parts is critical to minimizing variation

Traditionally, the majority of lateral flow manufacturing processes are batch processes. Equivalent processing of parts is not possible in this scenario, resulting in sometimes extreme test-test variation in results. For highly reproducible assays, in – line processes should be used. These processes include dispensing of protein lines using non-contact, quantitative dispensers, immersion of pads using dip-tanks and drying of materials in in-line drying towers, followed by continuous lamination and cutting operations.

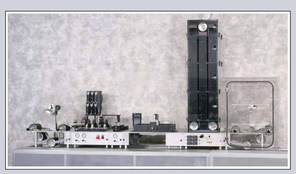


Figure 3: BioDot RTR 4500 In-line dispensing, dipping and drying machine

Figure 4: BioDot LM 6000 Continuous lamination and cutting machine

Quantitative non-contact dispensing is critical to minimizing variability in results.

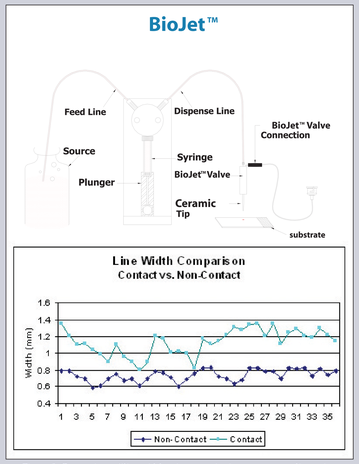


Figure 5: Comparison of line width using quantitative non-contact dispensing (BioJet dispensing) with a contact tip dispenser. Reproducibility of line width is critical to achieving good test-test reproducibility, especially when automated readers are in use.

Quantitative dispensing of conjugates and use of appropriate labels are critical

The reporters used in lateral flow assays are particulate conjugates, typically colloidal gold, colored or fluorescent latex, or paramagnetic particles. These conjugates must be quantitatively delivered to the assay and the appropriate particle used for the assay in question.

- Conjugates must be quantitatively dispensed and carefully dried and stored
- Dispensing method must be quantitative & reproducible
- Conjugate pad material and pretreatment should allow for:
  - Conjugate evenly distributed throughout material
  - Conjugate is readily mobilized on addition of sample
- Depending upon test design, a rapid release of conjugate, or a sustained release may be desirable. Either way consistency is a must!

Quite commonly, application of conjugates to conjugate pad materials is performed by immersing the pads or pipetting large volumes of conjugates onto sheets followed by spreading by wicking of the fluids. These processes result in high variability in the final product. A far more efficient and reproducible process involves the dispensing of the conjugates using a quantitative non contact dispenser, such as BioDot's AirJet dispenser.

	Colloidal gold	Colored or fluorescent latex	Paramagnetic particles
Positive	Small particles	Choice of colors	Ideal for quantitative assays
	Easy to work with	Easy to work with	Signal is non-visible
	Conjugation process well documented and simple	Conjugation process well documented and simple	Very high sensitivities can be achieved
		Conjugation can be covalent	Conjugation can be covalent
		Available as fluorescent or colored particles	Same conjugation process as latex particles
		Ideal for use in reader based systems	
Negative	Only one color available	Difficult to reproduce lot-lot	Difficult to reproduce lot-lot
	Conjugations are by passive adsorption only	Aggregation can be an issue	Aggregation can be an issue
	Scale up is difficult	Typically require higher antibody concentrations than gold	Typically require higher antibody concentrations than gold
		Must use a reader for fluorescent particles	Must use a reader for fluorescent particles

Criteria for choice of reporting system

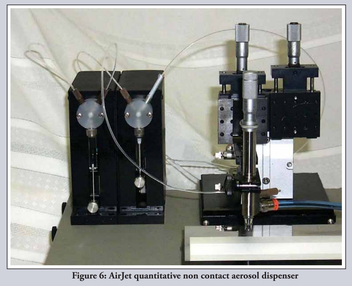


Figure 6: AirJet quantitative non contact aerosol dispenser

Experiment

- Magnetic particles were applied to pads in normalized amounts by dipping/dispensing
- Pads were build into strips and chromatographed normally, then dried
- Total Fe analysis performed to determine the amount of Fe recovered from the pads.

	Dipped	Dispensed
S&S Polyester 16s	**65.6**	**92.7**
S&S Glass Fiber 32	**41.4**	**81.9**
Accuflow G	**N/A**	**93.3**
Accuflow P	**N/A**	**94.3**

% Recovery Dipped vs Dispensed Particles on a variety of pad materials. Dispensed materials released more completely and reproducibly than dipped materials.

Drying

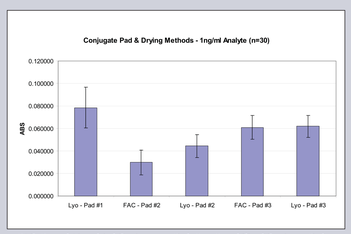
Appropriate and controlled drying conditions are critical to ensuring reproducibility in results in these assays. Three methods are commonly used:

1. Forced air at elevated temperature
2. Lyophilization
3. Desiccation

While lyophilization can be an extremely efficient way of drying materials, it can be a difficult process to scale up and validate. Desiccation is a slow and uncontrolled process. Forced air at elevated temperatures is the method of choice for this reason.

Effects of conjugate dispensing and drying

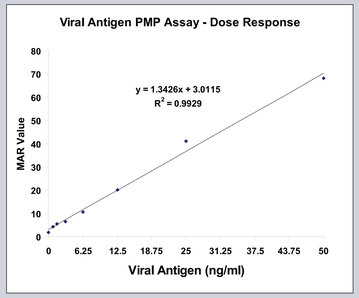
This graph demonstrates the comparison between signals developed on a lateral flow assay system when conjugates dried on different types of pads using different methods are used. Forced air using pad #3 would be the method of choice due to the small CV and efficient release as evidenced by high signals.



FAC: Forced Air – dried Conjugate Pad Lyo: Lyophilized ConjugatePad

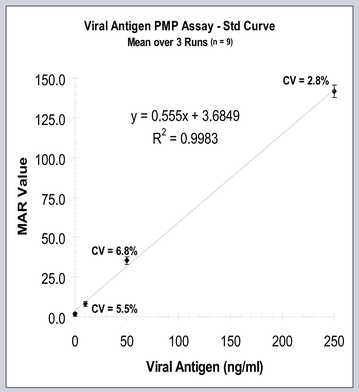
Representative Data

A. Assay for product identification and determination of concentration (in-process QC): Sensitivity and dose response curve in a biotechnology-derived viral antigen prep
This assay utilized paramagnetic particles as labels.



Results are expressed as MAR (Magnetic Assay Reader) units, essentially a millivolt measurement

B. Lot-lot reproducibility in a biotechnology-derived viral antigen assay
This data was generated over three manufacturing lots of a lateral flow assay. Manufacturing process CV's were extremely low and sensitivity extremely high.



2. Reproducibility and sensitivity of an assay for environmental testing for contamination during manufacturing of an industrial enzyme. Note that the level of significance for this enzyme was 10 ng/mL.

MAR 5 Readings (rounded to whole number)

	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
10 ng/mL	1809	1729	1576	1599	1667
1 ng/mL	407	493	444	421	501
0.1 ng/mL	87	95	112	88	103
0.01 ng/mL	32	37	47	40	42
0 ng/mL	32	37	20	33	29

	Avg	StDev	% CV
10 ng/mL	1676.4	95.0	**5.7**
1 ng/mL	453.2	42.2	**9.3**
0.1 ng/mL	97.0	10.6	**10.9**
0.01 ng/mL	39.6	5.6	**14.1**
0 ng/mL	30.2	6.4	**21.1**

Assay performance, now optimized, appears to be very reproducible. The coefficient of variation at 0.1 ng/mL and above was at 10% or better. This level of reproducibility would allow for quantitative values to be obtained.

Overall Results

The results obtained to date indicate that lateral flow technology can be employed as a suitable method for quality control in biopharmaceutical manufacturing. As long as appropriate manufacturing technologies are utilized, assays can be developed that are highly reproducible, fully quantitative and can be validated and fully documented. Assay results are comparable, if not better, than current methodologies in terms of precision and analysis time.

Conclusion

Lateral flow technology can play an increasingly important role in the quality control environment for biopharmaceutical and industrial / medical biotechnology manufacturing. **Appropriate applications would include:**

- **In process testing of analytes for purity or cross contamination**
- **In process testing for removal of contaminants(e.g. protein A)**
- **In process verification of identity**
- **Environmental contamination testing**

The ease of use, ease of transfer, and overall lower cost of development and use make these assays worth considering as alternatives to the standard assays in use today. The speed at which these tests can be performed, coupled with their ease of use and the fact that testing can be performed at the point of sampling can also result in significant improvements in workflow and productivity. As other rapid test formats come into maturity, there will be even greater opportunity to innovate in the development of new quality control tests.