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Research paper

A rapid lateral flow immunoassay for identity testing of biotherapeutics

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ABSTRACT

Identity testing of biopharmaceutical products is conducted at multiple steps in the manufacturing process, for drug product lot release, and often for product importation. Because of the chemical and structural similarities of antibody-based products, they present a unique challenge for the development of a QC friendly identity assay where specificity is the critical attribute. Here we report on the development of a novel, rapid and highly specific assay designed to simplify identity testing of antibody-based biopharmaceutical products. A lateral flow immunoassay platform (LFIA) was optimized and used to develop seven identity-specific tests against therapeutic monoclonal antibodies. The specificity of each assay was verified against 10–40 antibody products. An average linear range of antibody detection from 50 to 10,000 ng/ml was observed, allowing minimal sample dilution to be performed. The optimized LFIA platform consistently produced a strong visual signal and showed no false positive results. Three of the seven LFIA-based identity assays have been successfully validated for product release, in accordance with ICH validation guidelines. Additional tests will be validated as products reach the commercial phase. We demonstrate that a lateral flow-based identity assay is an ideal analytical tool for identity testing of antibody therapeutics. The assay platform can easily be adapted for new antibody products and it can be quickly transferred and validated for product testing.

1. Introduction

Identity testing of pharmaceutical products in their final container is required by the US Food and Drug Administration (FDA), per CFR Part 21. Beyond this regulatory requirement, there is often a need to establish product identity at various points in the manufacturing and product supply process. The chemical and physical properties of the product are typically utilized to determine identity using a variety of analytical assays. Unfortunately, for identity testing, the characteristics of some pharmaceutical products, such as monoclonal antibodies, are very similar; therefore, establishing identity between them can be difficult (Berkowitz et al., 2012). More than twenty monoclonal antibody therapeutics have been approved by the FDA and hundreds more are in various stages of clinical development (Reichert, 2012; Scott et al., 2012; Adams and Weiner, 2005). A rapid, simple, and specific analytical identity test is needed to support this growing class of therapeutics.

Current methods used for identity testing have significant drawbacks. Bioactivity based methods such as target specific ELISAs and cell-based bioassays require a skilled analyst, various critical reagents, and are not rapid (de Wolf et al., 2017). Physical property-based methods such as high-pressure liquid chromatography (HPLC) coupled with mass spectroscopy can be used to identify molecules based on mass (Wei et al., 2013; Zhang et al., 2014; Beck et al., 2012). While this method is sensitive, it requires expensive equipment, trained personnel, and a substantial amount of time to execute. Chemical property-based methods such as capillary isoelectric focusing (cIEF) separate molecules based on their charges. The cIEF method is faster and less expensive to execute than most other physicochemical methods, but cIEF profiles of some antibodies can be quite similar which makes establishing specificity challenging (He et al., 2010; Suba et al., 2015). Additional methods such as label free Raman spectroscopy, FTIR and NIR use the unique intrinsic spectral properties of the antibody to determine identity. While Raman spectroscopy is potentially highly specific and inexpensive, it still requires additional technology development before it is ready to be used in a validated environment (Paidi et al., 2016; Carey, 1999; Puppels et al., 1990). FTIR and NIR could be used for ID testing of

Abbreviations: BAW, BioAssay Works LLC; cIEF, Capillary Isoelectric Focusing; ID, Identity; LFIA, Lateral Flow Immunoassay; mAb, Monoclonal Antibody; O.D., Optical Density

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raw materials, however it is unlikely they have the specificity or sensitivity to discriminate between highly similar biological molecules.

An ideal identity test for antibody-based therapeutics would be rapid, specific, and easy to perform and transfer. The assay would have a wide linear dilution range to enable testing of products from ~2–200 mg/ml and have no buffer matrix interference. The assay would require no additional reagents or instruments to execute, removing the need for annual qualification and validation of additional critical reagents or instruments. The test would be portable, read visually and would not require a skilled analyst to execute. Herein, we introduce a novel, rapid and highly specific identity assay designed explicitly for antibody therapeutics. This lateral flow immunoassay (LFIA) platform was engineered specifically for Medimmune's identity testing requirements by Bioassay Works, LLC (BAW). The strategy utilizes a simple immunometric (sandwich) capture composed of a gold-labeled anti-product detection antibody and a mouse anti-human IgG Fcγ capture antibody. The optimized test meets all the desired properties of an identity assay for antibody-based products.

2. Materials and methods

2.1. Test samples and detection antibodies

The test samples were humanized monoclonal antibodies which are highly purified, clinical grade preparations of various subtypes including IgG 1, 2 and 4. The mouse monoclonal anti-identity (ID) antibodies used in the LFIA production were produced by Medimmune (Gaithersburg, MD, USA), using standard hybridoma technology. All experimental procedures were performed in accordance with the ethical business standards for human and animal rights set by Medimmune and AstraZeneca.

2.2. LFIA components (optimized configuration)

Lateral flow strips consist of a sample pad, a blocking ribbon (BAW Conjugate Ribbon, P/N: TTRK-120-002, BAW, MD, USA), a gold-conjugate pad, a membrane and an absorbent pad. The strip components were sequentially layered onto an adhesive backing card, as depicted in Fig. 1A. **Conjugate Pad**: The mouse monoclonal ID antibodies were obtained from and individually coated on NakedGold® 40 nm gold nanoparticles (BAW) at 2 µg/O.D. in a final 20 O.D./ml solution. A pH slightly above the antibody isoelectric point was selected for each reaction. **Membrane**: A cellulose ester membrane was sprayed with a test line solution containing 0.8 mg/ml of affinity purified mouse anti-human IgG Fcγ fragment specific antibody and a proprietary control line solution. The test and control line solutions were dispensed at a rate of 0.1 µl/mm. Membranes were allowed to dry at 37 ± 2 °C in a roll-in incubator (Bellco Glass Inc., NJ, USA) from 16 to 24 h. A capillary flow rate of 30 s/cm was selected to fulfill the 5–10 min reading time requirements, without compromising the line signal intensity. **Manufacturing.** Lateral flow cards were cut into 5.1 mm strips using a guillotine cutter (Index-Cut II, A-Point Technologies Inc., NJ, USA). The first and last strip of each card was systematically tested (positive and negative samples) to evaluate the assay performances, as per BAW internal "in-process" quality control procedures. Once approved, the strips were manually inserted into lateral flow plastic cassettes and pressed using a friction feeder apparatus (Closure-I, A-Point Technologies Inc., NJ, USA). Cassette top information was engraved on a custom photopolymer/steel printing plate (ColorComp Inc., NJ, USA) and printed with a G2–60 pad-printer (Printex®, CA, USA). Each test was individually packaged in a labeled foil pouch containing a 1 g desiccant pouch and sealed in an upright position with a rotary band sealer (TBS-3/8, APM Corp., NY, USA), as shown in Fig. 1B. All lateral flow components were kept in a low humidity environment (% RH ≤ 20%) until completion of the sealing procedure. A final functional testing was performed on randomly selected pouches in a lot-size dependent manner (minimum of three assays per testing condition) to ensure conformance to manufacturing specifications.

2.3. LFIA components (prototype configuration)

The same components, development techniques, and manufacturing practices as listed for the optimized configuration except for the following: **Conjugate Pad:** The mouse anti-human conjugate, prepared according to BAW proprietary gold conjugation protocol. **Membrane**: The anti-identity antibody at 1.0 mg/ml.

2.4. LFIA test procedure

Test samples were diluted in a phosphate buffered solution (PBS) pH 7.4 to a pre-determined optimal concentration, typically obtained using a 1:1000 to 2000 dilution. Each LFIA test was allowed to reach room temperature, removed from its pouch and placed on a flat, horizontal surface. One hundred (100) microliters of negative or positive control was deposited in the sample well of each lateral flow cassette using an analytical or disposable pipette. The sample solution subsequently flowed across the different zones of the strip, from the sample pad to the absorbent pad. Excess sample, buffer and gold particles were collected in the absorbent pad, allowing a constant flow and the reading window clearance. Results were read at 10 min but strong positive could be observed after five minutes. Positive results were characterized by clear visual red lines in the test (T) and control (C) regions, while negatives only showed a control line.

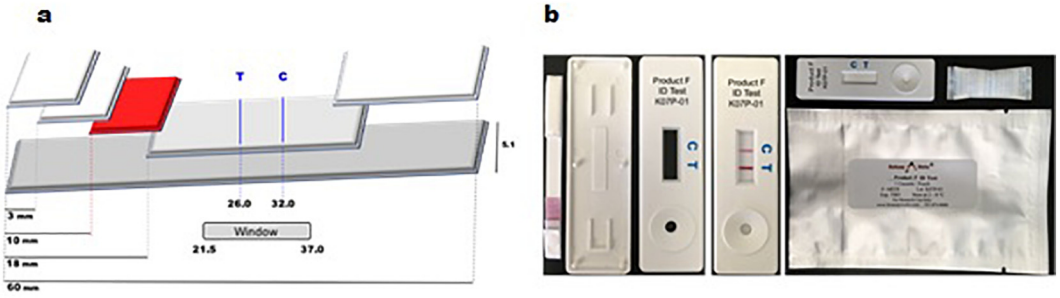

Fig. 1. LFIA Configuration A- Schematic representation of strips, consisting of a sample pad (Berkowitz et al., 2012), a blocking ribbon (Reichert, 2012), a gold conjugate pad (Scott et al., 2012), a membrane (Adams and Weiner, 2005) and an absorbent pad (de Wolf et al., 2017). All components were fixed on a polystyrene backing card (6, 60 × 300 mm) and cut into strip (5.1 × 60 mm). The blue "C" and "T" symbolize the control and test line positions, respectively. All measurements are in millimeters (mm). B- Assembly of "Product F" LFIA. The strips are manually placed into the channel of the lateral flow cassette bottom and covered with a labeled cassette top. Assembled cassettes were tested to ensure the line alignment and conformance to manufacturing specifications. The final packaging includes a cassette, a desiccant and a labeled foiled pouch. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.5. Accelerated stability study

Ninety-six (96) pouched assays were randomly selected from an initial production lot (Range: 1500–3000 units) and submitted to an accelerated stability study. Briefly, sixteen (16) pouches were incubated at −20 °C, forty (40) pouches at 20–25 °C (RT) and forty (40) pouches at 37 °C (day 0). Two units were tested daily for one week (−20 °C, RT and 37 °C), followed by a weekly (RT and 37 °C) or a monthly (−20 °C) assessment over a four-month period (starting on day 1). Selected assays were removed from incubation and allowed to reach RT before use. After sample application, the tests were allowed to run for 10 min and read with a lateral flow assay reader (Optricon, opTrilyzer® Lateral Flow Reader, Berlin, Germany). Data analysis was performed with Microsoft® Excel® v15.29.1. Data entry, formulas and calculations were double checked manually. Values were expressed as ratio (Raw test line intensity value/ Raw control line intensity value * 100) and considered positive for ratio ≥ 80 and negative for ratio ≤ 10. An initial one-year shelf life expectancy was projected with Arrhenius stability modeling (Predicted stability (52 weeks) = Accelerated Stability (16 weeks) * $2^{\Delta T/10}$, where $^{\Delta T}$ = RT - Storage temperature; 37 °C, −20 °C), but accumulation of real-time data is ongoing.

3. Results

3.1. Optimization of antibody-gold conjugates

Lateral flow identity assays (LFIAs) are intended as rapid, qualitative and easy to use tools for identity testing of drug products. The initial assay development focused on optimizing the test line signal intensity from a one-step dilution of the raw and vialed sample material (humanized monoclonal IgG, approximately 10–150 mg/ml). The prototype design included a drug-specific capture antibody for the solid phase and a general mouse anti-human antibody-gold conjugate for detection. A variety of suitable detection antibodies were screened, including a goat anti-human IgG (H + L), goat anti-human IgG (H + L; min X Bov, Hrs, Ms. Sr Prot), goat anti-human IgG Fc, goat anti-human IgG Fcγ (min X Bov, Hrs, Ms. Sr Prot), mouse anti-human IgG Fc and mouse anti-human IgG Fcγ (min X Bov, Hrs, Ms. Sr Prot). BAW proprietary gold conjugation procedure was carried out for each antibody. The optimal gold conjugation pH, antibody loading, gold concentration and volume of conjugate dried per cm of ribbons were addressed in the LFIA stability and suitability evaluation for manufacturing. Our data revealed an unusual non-specific affinity between goat antibodies and the mouse anti-ID monoclonal antibodies (Supplementary Fig. 1A). This cross-reactivity was observed independently to the presence of a blocking reagent, such as normal goat or mouse serums. Interestingly, the mouse antibodies showed some cross-reactivity only in presence of normal goat serum, as opposed to normal mouse serum or omission of blocking strategy (Supplementary Fig. 1B). Consequently, a mouse anti-human IgG was selected as the detection antibody for the prototype gold-conjugation procedures. More precisely, we selected an anti-IgG Fcγ to limit the steric hindrance and to generate a directional capture of the drug targets, where the detection antibody binds the Fcγ region and the capture antibody binds the antigen-binding site.

3.2. Accelerated stability studies

The accelerated study data provided a good indication of the assay configuration performance. As an example, the initial project study, using the prototype configuration revealed a substantial false-positive detection rate amongst the negative tests (17%, 8/48), independent from the storage temperature (Fig. 2). In contrast, no false positives were observed (0/48) using the optimized assay configuration, consistent with an increased assay specificity (Fig. 3). The mean positive signal intensity was significantly increased ($p \leq 0.005$) by ~1.5 fold for each temperature group using the inverted configuration (146 ± 10, n = 8, −20 °C; 144 ± 6, n = 20, 20 °C; 141 ± 5, n = 20, 37 °C), as opposed to the prototype (112 ± 12, n = 8, −20 °C; 104 ± 8, n = 20, 20 °C; 96 ± 9, n = 20, 37 °C). While the signal intensity was increased, the coefficients of variation were systematically lowered (7 vs 11%, −20 °C; 4 vs 8%, 20 °C; and 4 vs 10%, 37 °C), indicative of a better assay reproducibility. These observations were consistently repeated in all assays developed with the optimized assay configuration (Table 1). Thus far, we have real time stability data showing that the tests are stable for 57 months.

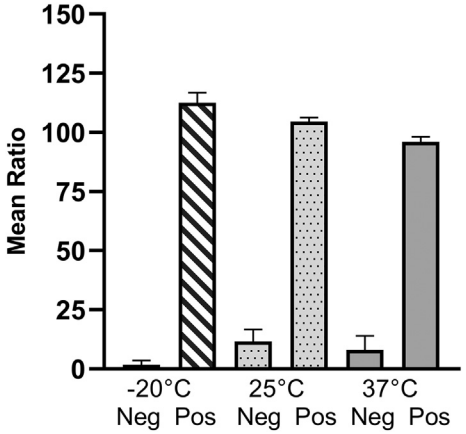

Fig. 2. Product C Study Data Obtained with Prototype Assay Configuration. A high rate of false-positive assays was observed within the negative samples, in 8 of 48 assays. Values are the mean ratio of the test line signal/control line signal*100.

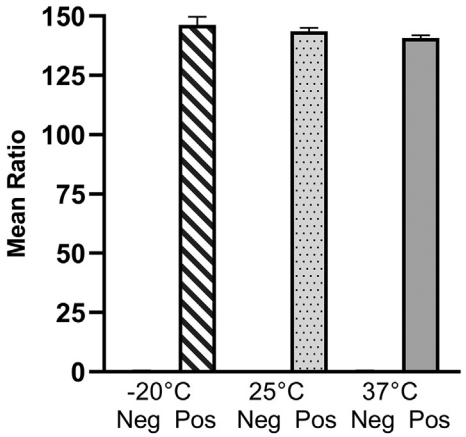

Fig. 3. Product C Stability Study Data Obtained with Optimized BAW Assay Configuration. No false-positives were detected throughout the entire study. Values are the ratio of the test line signal/control line signal*100. Notably, the overall signal intensity was significantly increased for each temperature group compared to the prototype assay configuration.

3.3. Sensitivity

A serial dilution study was systematically performed for each newly developed test to determine the analytical range of detection. As shown in Fig. 4, a typical linear range of detection was observed from ~50 to 10,000 ng/ml of sample, supporting that the application of sample at 1: 1000 to a 1: 128,000 dilution would deliver results within the linear range of detection of the assay. Importantly, the intensity of the test line signal was significantly reduced using a ≤ 1: 100 dilution, most likely due to a hook effect. This interference phenomenon is commonly observed when the sample concentration surpasses the detection and capture antibody concentrations.

Table 1
Summary of accelerated stability studies.[a]

Test	Configuration	−20 °C					20–25 °C						37 °C					
		Negative		Positive			Negative			Positive			Negative			Positive		
		Mean	StDev	n	Mean	StDev	n	Mean	StDev	n	Mean	StDev	n	Mean	StDev	n	Mean	StDev	n
Product A	Prototype	0	± 1	8	117	± 9	8	7	± 16	20	117	± 10	20	1	± 3	20	123	± 7	20
Product B	Prototype	1	± 1	8	111	± 40	8	2	± 2	20	124	± 13	20	3	± 2	20	116	± 21	20
Product C	Prototype	3	± 4	8	112	± 12	8	12	± 23	20	104	± 8	20	8	± 26	20	96	± 9	20
Product C	Inverted	0	± 0	8	146	± 10	8	0	± 0	20	144	± 6	20	1	± 1	20	141	± 5	20
Product D	Inverted	0	± 0	8	98	± 10	8	0	± 0	20	91	± 11	20	0	± 1	20	90	± 8	20
Product E	Inverted	0	± 0	8	171	± 9	8	0	± 0	20	169	± 9	20	0	± 0	20	161	± 6	20
Product F	Inverted	0	± 0	8	185	± 9	8	0	± 0	20	180	± 9	20	1	± 2	20	172	± 9	20
Product G	Prototype	1	± 1	8	74	± 3	8	1	± 1	20	81	± 5	20	1	± 0	20	88	± 8	20

[a] The mean values are expressed as a ratio (test line intensity/control line intensity*100). Noticeably, the BAW optimized assay configuration showed lower mean values for negative samples and an overall reduction of standard deviation values, suggesting an enhanced specificity and improved reproducibility.

3.4. Specificity testing

Specificity is the only required evaluation parameter for validation of an identity assay according to ICH guidelines. Fig. 5 shows the typical results for specificity testing. Six antibodies were diluted in PBS to the same final dilution that was pre-determined to be optimal for the test sample. The results show that only the “Product B" LFIA cassette shows a red band at the test line which indicates a positive ID result. The six non-specific products and the buffer only sample (negative control) show no band at the test line which indicates a negative ID result. All eight LFIA cassettes show a solid red band at the control line which confirms that the test performed properly and passes system suitability. A validation test set is larger and typically includes all the products which could be present at a particular manufacturing site. Thus far, we have demonstrated specificity of seven unique LFIA cassettes each against ~11–40 antibodies with no false positive results. In addition, three of the seven tests have been successfully validated. The remaining tests will be validated when the product reaches the commercial phase.

4. Discussion

Here we demonstrate that the optimal design for this lateral flow-based identity assay is with the anti-Fc detection antibody on the solid phase and the gold-labeled anti-ID antibody in the mobile phase. By inverting the original test design, the false positive rate decreased from 16% to 0%. Using the prototype format, any sample with an Fc is labeled with gold conjugate which increases the risk of a false positive test result. Using the inverted assay format, only sample is labeled with gold conjugate from the anti-ID antibody so the labeling is more specific.

The prototype configuration was developed previously and used as a model for initial ID test development by BAW. This configuration, with the anti-ID antibody in the solid phase facilitates developing assays with multiple product detection on one test strip. Multiple product ID tests are of limited utility. Single product ID tests are more desirable for products at the commercial stage due to their less complex lifecycle management. The preference for a single product ID test led to the development of the inverted design which was shown to be optimal. This assay design has been successfully implemented for five products and is currently being used for development of additional ID tests.

While the general format of the LFIA is platform, assay parameters can be optimized to meet the unique properties of the product which is necessary because of the diversity of products in development. Products with mutations in the Fc, bispecific molecules, antibody-drug conjugates (ADC), and fusion proteins are common. Using the methods described here, single product ID tests have been developed for wild-type IgG1, Fc mutated IgG1, IgG2 and IgG4 monoclonal antibodies. Development of a test for an ADC molecule mostly complete with the goal to ID both the ADC-labeled and unlabeled molecule. ADC products require additional ID testing compared to a standard product because there are more manufacturing steps. Parameters of the LFIA such as gold conjugation levels, detection antibodies, and test line saturation can be tuned to produce a test optimized for specific molecules. While new molecular formats may require additional development work, this work should not exceed that needed for other methods chosen for ID testing.

The ease of use, portability, and stability of the LFIA is a great advantage compared to other ID testing methods. Only simple dilution of the sample and application to the test is required. Result interpretation is straightforward, as the test produces a yes or no answer that is easily read with the naked eye. This ease of use and interpretation also leads

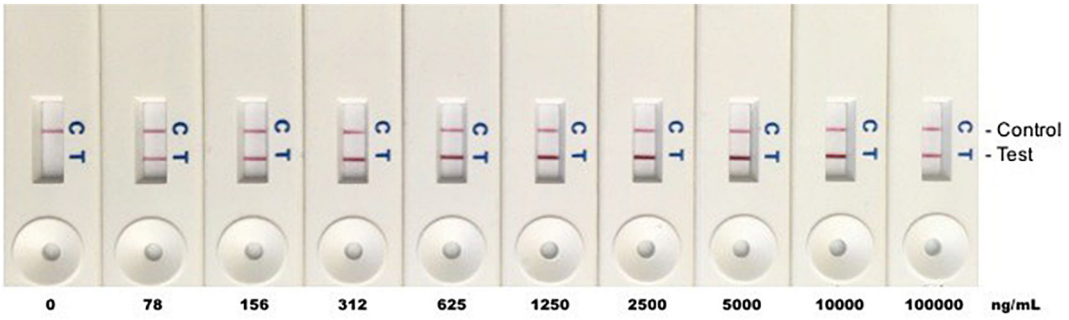

Fig. 4. Product G Range of Sensitivity. Serial dilutions of product G drug were applied onto the product G lateral flow immunoassay to determine the analytical sensitivity. A linear phase of detection was observed between 78 and 10,000 ng/ml. The decreased test line intensity at 100,000 ng/ml suggest a hook effect, characterized by an ongoing competition between gold-labeled and unlabeled product G antibodies at the test line location. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

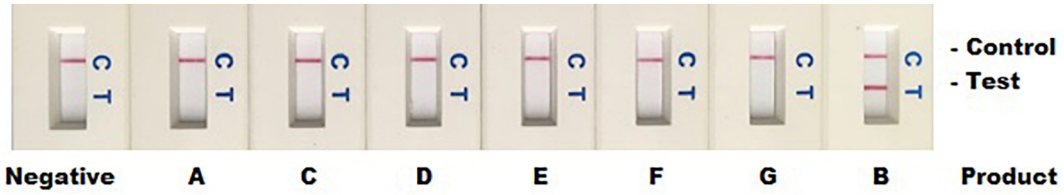

Fig. 5. Specificity Testing. Each product was diluted 1:2000 from its drug product concentration and added to the lateral flow cassette. Only the product-specific LFIA cassette resulted in a red band at the test line which indicates a positive ID result for the product. During assay validation, each product at the manufacture's fill finish site would be tested for specificity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to straightforward validation of the test. Pipettes are the only required equipment, therefore there are no computers or instruments to validate. Three ID tests have been successfully validated to date and the experimental portion of the validation can be performed in one day. In addition, the small footprint of the test makes it very portable.

Given that the ID assay is essentially an immunoassay some logistical hurdles may be encountered with lifecycle management of the test. For example, stocks of the anti-ID antibody must be maintained for creation of new lots of the test as supplies are exhausted or they reach end of shelf life. The anti-ID antibodies described here were used for PK/PD studies therefore they were readily available, if not they would need to be produced for test development. The test itself is essentially a critical reagent therefore, its qualification status must be maintained as well. This requires a qualification and requalification strategy to demonstrate that the test remains fit for purpose over the shelf life. Traditional physiochemical tests such as peptide mapping or cIEF are less reagent dependent.

5. Conclusion

Identity testing of biological therapeutics is a regulatory requirement for product release and serves as an important control throughout the manufacturing process. Identity tests help ensure patient safety by making certain the correct product is vialed and labeled and/or shipped. There are many ways to establish product identity, with the preferred test being simple to develop, perform, transfer and validate. Data presented here demonstrate that use of a lateral flow-based test for identity can fulfill these requirements. This test platform has simplified previously complex release test workflows by providing a streamlined assay that is easily implemented across multiple testing sites.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jim.2019.112666.

Declaration of Competing Interest

The authors declare no conflict of interest.

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References

Adams, G.P., Weiner, L.M., 2005. Monoclonal antibody therapy of cancer. Nat. Biotechnol. 23 (9), 1147–1157. https://doi.org/10.1038/nbt1137.
Beck, A., Sanglier-Cianferani, S., Van Dorsselaer, A., 2012. Biosimilar, biobetter, and next generation antibody characterization by mass spectrometry. Anal. Chem. 84 (11), 4637–4646. https://doi.org/10.1021/ac3002885.
Berkowitz, S.A., Engen, J.R., Mazzeo, J.R., Jones, G.B., 2012. Analytical tools for characterizing biopharmaceuticals and the implications for biosimilars. Nat. Rev. Drug Discov. 11 (7), 527–540. https://doi.org/10.1038/nrd3746.
Carey, P.R., 1999. Raman spectroscopy, the sleeping Giant in structural biology, awakes. J. Biol. Chem. 274 (38), 26625–26628. https://doi.org/10.1074/jbc.274.38.26625.
de Wolf, C., Van de Bovenkamp, M., Hoefnagel, M., 2017. Regulatory perspective on in vitro potency assays for human mesenchymal stromal cells used in immunotherapy. Cytotherapy. 19 (7), 784–789. https://doi.org/10.1016/j.jcyt.2017.03.076.
He, Y., Lacher, N.A., Hou, W., Wang, Q., Isele, C., Starkey, J., Ruesch, M., 2010. Analysis of identity, charge variants, and disulfide isomers of monoclonal antibodies with capillary zone electrophoresis in an uncoated capillary column. Anal. Chem. 82 (8), 3222–3230.
Paidi, S.K., Siddhanta, S., Strouse, R., McGivney, J.B., Larkin, C., Barman, I., 2016. Rapid identification of biotherapeutics with label-free Raman spectroscopy. Anal. Chem. 88 (8), 4361–4368. https://doi.org/10.1021/acs.analchem.5b04794.
Puppels, G.J., De Mul, F.F.M., Otto, C., Greve, J., Robert-Nicoud, M., Arndt-Jovin, D.J., Jovin, T.M., 1990. Studying single living cells and chromosomes by confocal Raman microspectroscopy. Nature. 347 (6290), 301–303. https://doi.org/10.1038/347301a0.
Reichert, J.M., 2012. Marketed therapeutic antibodies compendium. mAbs. 4 (3), 413–415. https://doi.org/10.4161/mabs.19931.
Scott, A.M., Wolchok, J.D., Old, L.J., 2012. Antibody therapy of cancer. Nat. Rev. Cancer 12, 278–287.
Suba, D., Urbanyi, Z., Salgo, A., 2015. Capillary isoelectric focusing method development and validation for investigation of recombinant therapeutic monoclonal antibody. J. Pharm. Biomed. Anal. 114, 53–61. https://doi.org/10.1016/j.jpba.2015.04.037.
Wei, H., Tymiak, A.A., Chen, G., 2013. High-resolution MS for structural characterization of protein therapeutics: advances and future directions. Bioanalysis. 5 (10), 1299–1313. https://doi.org/10.4155/bio.13.80.
Zhang, H., Cui, W., Gross, M.L., 2014. Mass spectrometry for the biophysical characterization of therapeutic monoclonal antibodies. FEBS Lett. 588 (2), 308–317. https://doi.org/10.1016/j.febslet.2013.11.027.