Automation of mAbs stress testing with visual inspection on Junior

Introduction

Visual inspection of biologics is a critical step in evaluating the stability of a formulation. Color change, particle formation, or increased turbidity are evidence of biologic instability. Manual inspection of biologic drug products is the common technique to determine the color of a sample. Manual inspection is a limited qualitative analysis with subjective results, is prone to error, and does not retain images for data retrieval to verify results. Turbidity analysis and particle counting methods often consume sample, and require multiple duplicate samples to monitor the formulation over time.

The Visual Inspection Station (VIS), integrated into a Big Kahuna or Junior, automates and quantifies color, particle count, and turbidity of a formulation in minutes without ever opening the vial (**Figure 1**). VIS reduces hands-on time, eliminates human error, and increases throughput for non-destructive visual inspection. VIS images and data are stored in a report for users to refer to later for verification.

In this application note, Junior with VIS was used to analyze the color, visible particle count, and turbidity of monoclonal antibodies (mAbs) of various concentrations and formulations. The LEA software suite paired with the LEA Analysis Add-in for Excel were used to analyze results.

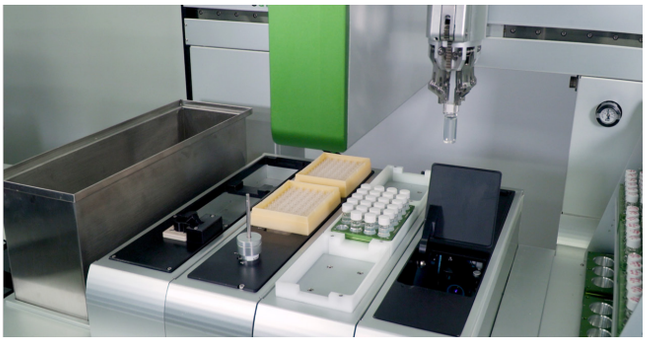

Figure 1: Big Kahuna or Junior (pictured) with VIS automates sample analysis, and quantifies color, visible particle count, and turbidity of biologics non-destructively.

Visual inspection station

Color analysis compares the color of a sample to that of color calibration standards to find a match based on the relevance score and match distance. The relevance score is a percentage calculation that predicts the relevance of the match; that is, how likely that the sample matches the calibration standards. The match distance is the Euclidean distance between the average pixel values of the color standard and the sample. First, a high relevance score (typically above 95%) is necessary to be sure the calibration standards are appropriate for the sample set, then the lowest match distance is used to determine the color match of the sample.

Visual particle analysis first uses a motion profile based on the vial size and type to suspend particles throughout the sample and induce motion. The motion profile spins the vial, then stops the vial to cause particles to move for imaging (**Figure 2**). After the vial is stopped, 30 successive images are captured and subtracted to locate only moving particles within the image (**Figure 3**). By subtracting successive images, a resulting binary image contains only moving particles and eliminates any stationary vial defects. Analysis of the resultant

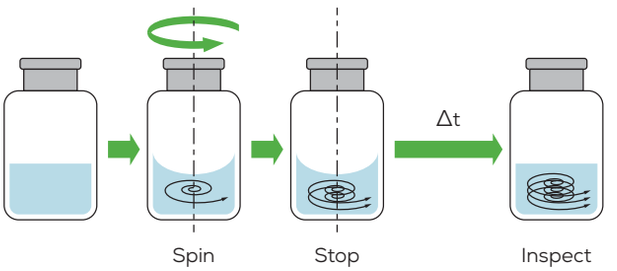

Figure 2: Particle count on VIS. A vial is loaded, spun, and stopped to suspend particles and induce motion before image capture.

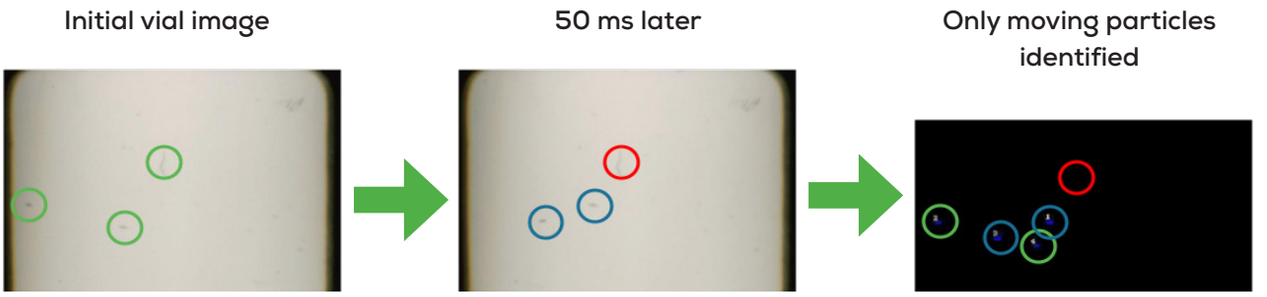

Figure 3: The visible particle count application on VIS identifies potential particles in the initial image captured (green) and monitors their movement between successive images. Potential particles are determined to be either moving particles (blue) or stationary vial defects (red).

image is carried out based on an analysis profile determined by the vial type and size.

Turbidity analysis uses a light source and camera at 180° for a more linear turbidity analysis over a traditional turbidimeter with a detector at 90°. Images of samples are compared to a standard curve based on calibration standards to determine the turbidity of the sample.

Methods

Color and particle analysis

A concentration series of 1-150 mg/mL was prepared with a stock mAb in 10 mM histidine pH 6.0 with 0.1% PS80. A 4 mL glass vial was filled with 3.5 mL of each concentration. Vials were prepared outside of a clean room, and the outside surface was wiped with a lint-free cloth prior to inspection.

The VIS color match application was calibrated using Color Reference Solutions according to Ph Eur BY1 through BY6 (Sigma-Aldrich) and a blank of water. The particle motion profile and analysis profile were predetermined based on the vial used. The total time to automatically analyze the color and visible particle count of these eight vials in triplicate was 15 minutes.

Turbidity analysis

Twenty-one 4 mL vials were filled with 3.5 mL of mAb A at 10 mg/mL in 10 mM histidine, pH 6.0 and 21 vials were filled with 3.5 mL of mAb A in the same formulation with 0.1% PS80 added. An additional 21 vials of mAb B in a proprietary formulation were prepared as a control. The vials were manually vortexed with a benchtop analog vortex mixer for varying amounts of time and stored at 4 °C until analysis.

The VIS turbidity application was calibrated with AMCO Clear turbidity standards from 0-800 NTU (GFS Chemicals). Each of the vials were automatically loaded into VIS, and triplicate turbidity measurements were collected. The total time to automatically analyze the turbidity of these 63 vials in triplicate was 66 minutes.

Experimental design and execution

The LEA software suite was used for experimental design, execution, and analysis of results. The experimental designs were created in Library Studio with Design Creator and were executed in Automation Studio. Average particle count, turbidity, and standard deviation for each sample were calculated in Excel using the LEA Analysis Add-in. Hands-on time to design each experiment in Library Studio was less than 5 minutes, and experiment execution in Automation Studio was less than 2 minutes.

Results

Color analysis

Each concentration of mAb A was compared to BY1 through BY6 standards through automated image collection and analysis by VIS. Images of the samples captured by VIS show an increase in yellow coloration with increased concentration and are visibly similar to the matching color standard (Figure 4).

Each sample of mAb A was successfully matched to a color standard by VIS with high match relevance

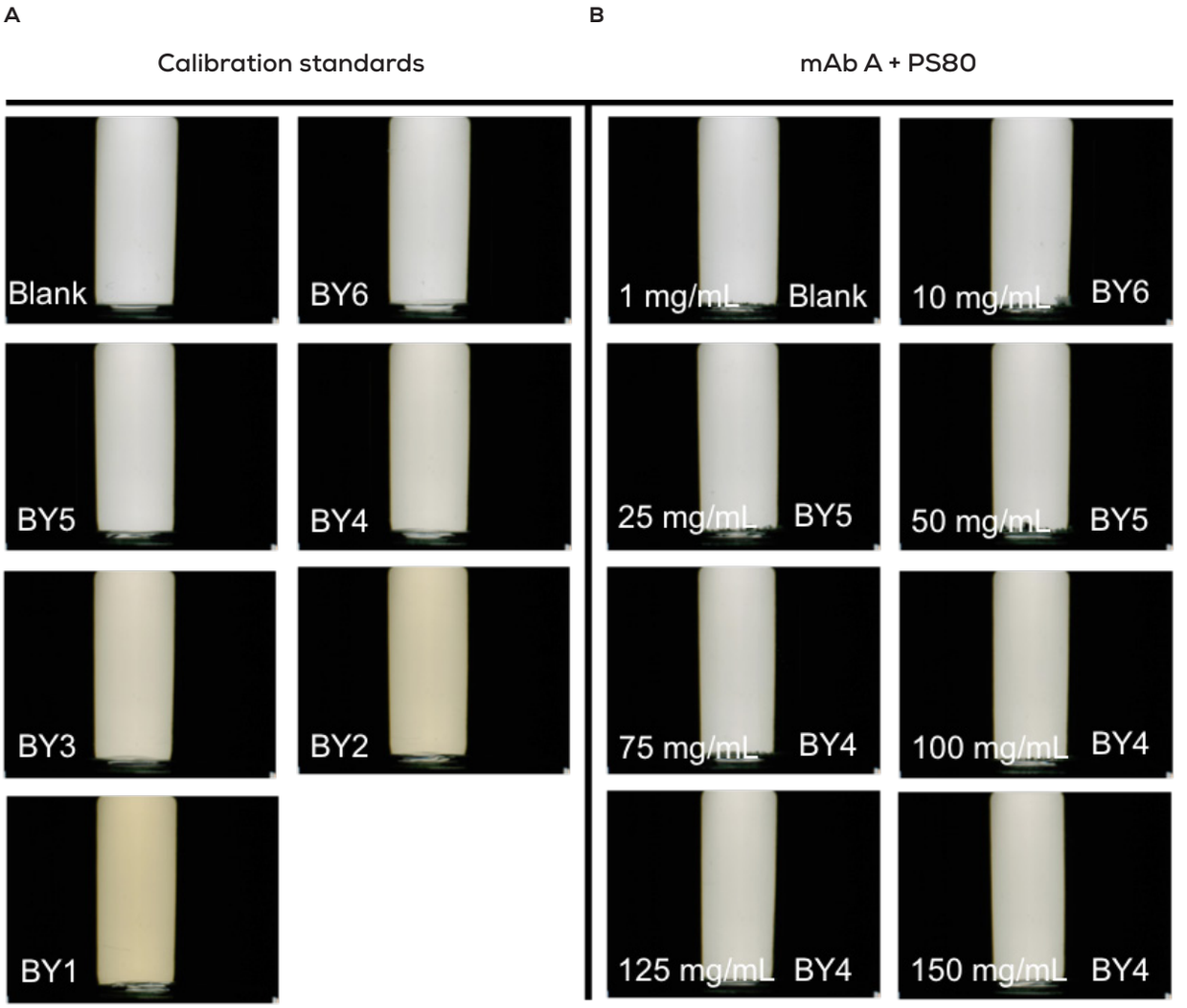

Figure 4: **(A)** Images of BY1 through BY6 color standards captured, analyzed, and stored by VIS. **(B)** mAb samples of increasing concentration and their determined color matches.

mAb conc. (mg/mL)	Color match	Relevance Score (%)	Match distance
1	Blank	99.9	0.70
10	BY6	99.9	1.17
25	BY5	100.0	1.53
50	BY5	100.0	1.79
75	BY4	99.7	2.02
100	BY4	99.8	1.90
125	BY4	99.9	1.85
150	BY4	99.8	2.16

Table 1: Quantitative analysis by VIS determined the best color match for each mAb sample based on the shortest match distance with a relevance score of at least 95%.

scores (99.7 – 100%) and low match distances (Table 1). The quantitative analysis by VIS followed the expected trend with 1 mg/mL matching the blank standard and yellow coloration increasing to match BY4 as concentration increased to 150 mg/mL (Table 1). The quantitative analysis is verifiable and matches a qualitative analysis of images stored by VIS (Figure 4).

Visible particle count

Visible particle count varied with protein concentration, with higher concentration proteins having fewer particles (Table 2). Results from VIS were obtained without ever opening the vial, preserving the sample for further analysis or particle identification. The images captured by VIS show identified particles and vial defects (Figure 3), enabling a user to verify

mAb conc. (mg/mL)	Average visible particle count
1	32 ± 5.3
10	39 ± 2.5
25	31 ± 1.2
50	40 ± 5.2
75	45 ± 8.1
100	40 ± 2.8
125	21 ± 3.7
150	8 ± 0.9

Table 2: Average visible particle count of a concentration series of mAb A calculated by automated analysis with VIS.

the results, further analyze them, and have a stored image of the particle(s) in question.

Turbidity analysis

The images collected by VIS show an obvious increase in turbidity with increased mixing time for mAb A when surfactant was not present to stabilize the formulation (Figure 5). Stored images are useful for a qualitative representation of turbidity and verification of results, but the non-destructive, quantitative analysis of VIS is key to analyzing the turbidity of biologics while preserving sample.

mAb A was compared to mAb B as a control, since mAb B is known to be stable under vortex mixing stress. After 180 minutes of vortex mixing, the turbidity of mAb B remained stable (Figure 6). Conversely,

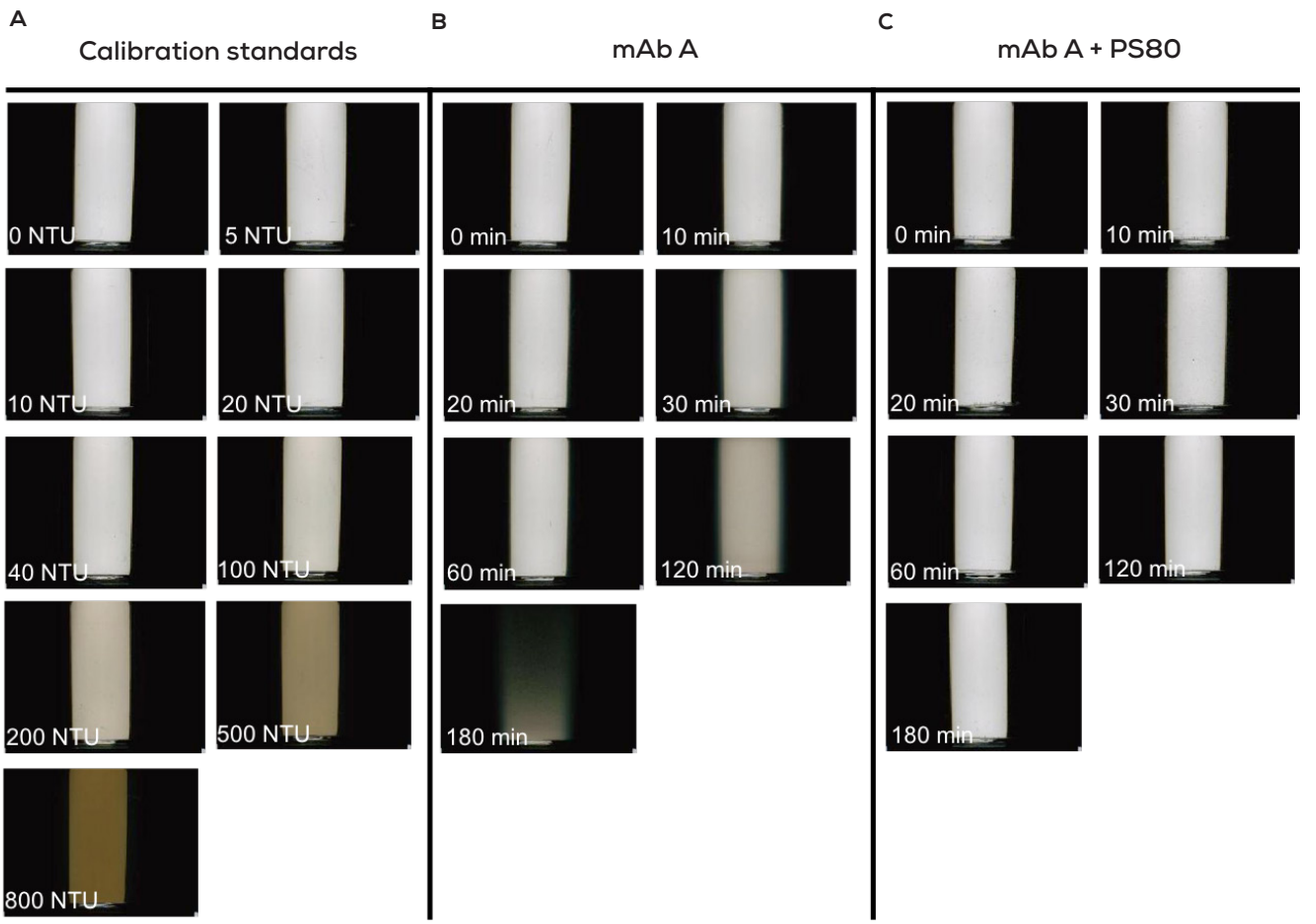

Figure 5: **(A)** Images of turbidity standards captured, analyzed, and stored by VIS for calibration. **(B)** mAb samples show an increase in turbidity as a function of vortex time. **(C)** The mAb in a formulation supplemented with PS80 shows reduced turbidity with vortex mixing.

mAb A became significantly more turbid with increased mixing time and even surpassed the calibration range of VIS after 180 minutes of vortex mixing. To stabilize mAb A, a surfactant (PS80) was added to the formulation. With the stabilizing surfactant the turbidity of mAb A + 0.1% PS80 remained as stable as mAb B.

Conclusion

Junior with VIS quantifies the color, visible particle count, and turbidity of biologic formulations in minutes. Quantitative, reproducible color analysis with VIS allows for an analysis of discoloration of a biologic formulation after stress or storage. Non-destructive particle count preserves samples for further analysis, enables monitoring of the same samples over time, and preserves particles for identification to determine the source of the particle. Turbidity analysis with VIS allows for rapid and non-destructive screening of biologic formulations to compare the stability of various proteins and formulations. VIS stores images for each of these applications, allowing for verification of all results. The non-destructive nature of VIS is beneficial during development of biologic drugs to screen protein candidates and formulations while minimizing sample volume requirements.

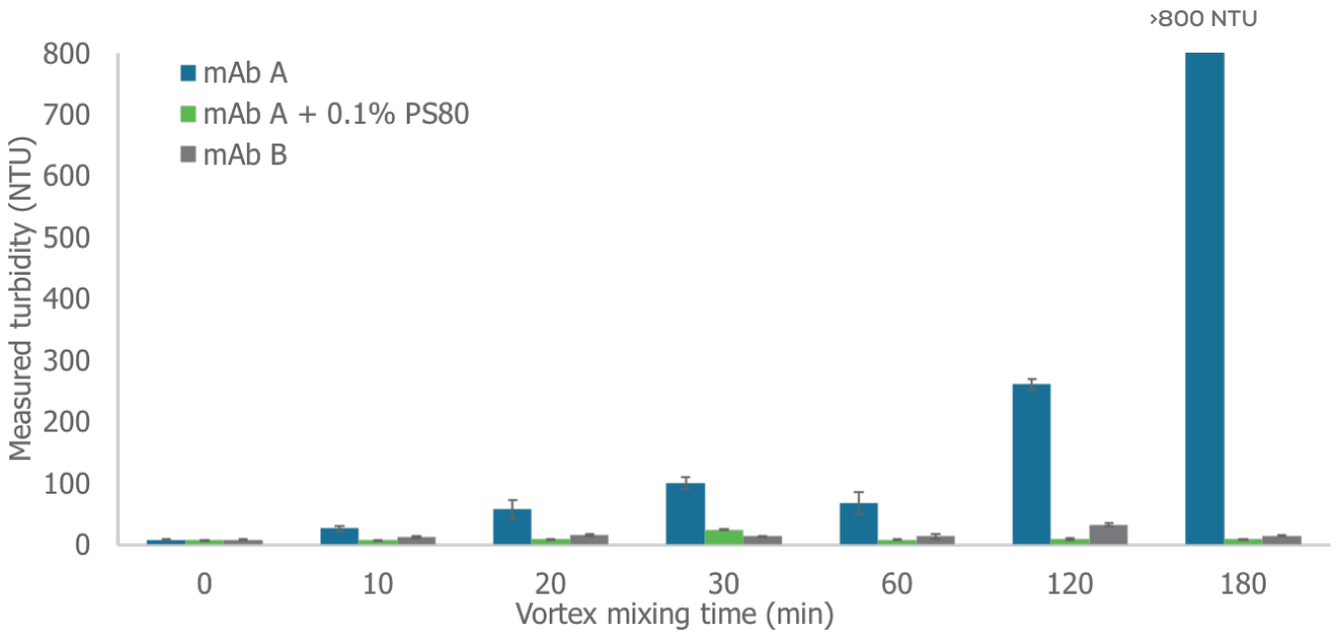

Figure 6: Quantitative turbidity analysis of mAb samples after vortex mixing show mAb B was stable with increased vortex mixing time, while mAb A was only stable when surfactant was added.

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