

# Get low with Honeybun: 15 microliter rapid viscosity measurements

## Introduction

Biologics screening is all about keeping antibody and formulation candidates moving through the development pipeline efficiently. The measurement of viscosity, a critical quality attribute and a key factor in determining injectability, can often clog up that pipeline. In addition to being difficult for subcutaneous injections, high viscosity candidates cause havoc to manufacturing steps like filtration and fill/finish. Spotting problematic candidates early and accurately tracking viscosity throughout the process helps keep the development pipeline flowing smoothly.

Despite this importance, no one gets as much viscosity data as they need. Classic techniques require hours of hands-on time and consume a lot of volume in super slow, one-sample-at-a-time instruments. Even modern tech creates a one-by-one bottleneck and needs lots of cleaning and calibration.

Honeybun (Figure 1A) is the only rapid microvolume viscometer and rheometer that combines low sample volume requirements with the ability to run up to 10 samples in parallel, in under 10 minutes. Simply pipette your analyte into the Bun consumable (Figure 1B), insert into Honeybun, and get your accurate and precise viscosity results. Measurements don't need complicated add-ons that require lengthy maintenance between runs and are prone to clogging.

## Methods

Medical Grade Viscosity Standards (MGVSs) of 1.2, 2, 4, 6, and 10 cP (Paragon Scientific, UK) were stored at 4 °C and used as directed. Glycerol was prepared to 88% w/w concentration in deionized water. 200 mg/mL monoclonal antibody (mAb) solutions in 10 mM histidine, pH 7.4 were prepared from 10 mg/mL stock solutions using Unagi and the

A



B



Figure 1: Honeybun (A) is the only rapid microvolume viscometer and rheometer for proteins, vaccines, viral vectors and injectables. Honeybun and its consumable Bun (B) read up to ten samples in minutes with just 35  $\mu$ L (Channels 1–3) or 15  $\mu$ L (Channels 4–6) of sample.

concentration, hydrodynamic diameter, and polydispersity were verified using Stunner.

MGVS and 88% glycerol viscosities were measured in quadruplicate at 25 and 22 °C, respectively, on Honeybun in low volume (15  $\mu$ L) and default (35  $\mu$ L) modes. High concentration mAb solution viscosities were measured in triplicate in low volume and default modes at the indicated temperatures. All results are depicted as means plus or minus one standard deviation.

## Results

Keeping viscosity low for easy syringeability and injectability is a key component of a positive developability profile of a biologic.<sup>1</sup> Reliable low volume methods make it easier to collect the required data throughout the development process.

Honeybun delivers reliable viscosity results from 35  $\mu\text{L}$  of sample in default mode and from 15  $\mu\text{L}$  in low volume mode across its entire dynamic range from 0.5–150 cP (Figure 2). The average viscosities measured in low volume mode for each of the 5 MGVSs and the 88% aqueous glycerol were indistinguishable from those measured using default mode.

Honeybun viscosity results were within 3% of the specified viscosity value of the standards in default mode and had CVs less than or equal to 1.8% (Table 1). In low volume mode the CVs were less than or equal to 4% and the average values were within 5% of the target value of the MGVSs. This slight increase in variability in low volume mode is ascribed to the narrower range of shear rates applied as an adaptation to the lower available sample volume.

Checking the viscosity of a biologic in a range of temperatures gives clues to how it will behave under other conditions, for example straight from the fridge or at body temperature. Honeybun is equipped with finely tuned temperature controls, allowing viscosity measurements from 10–45 °C.

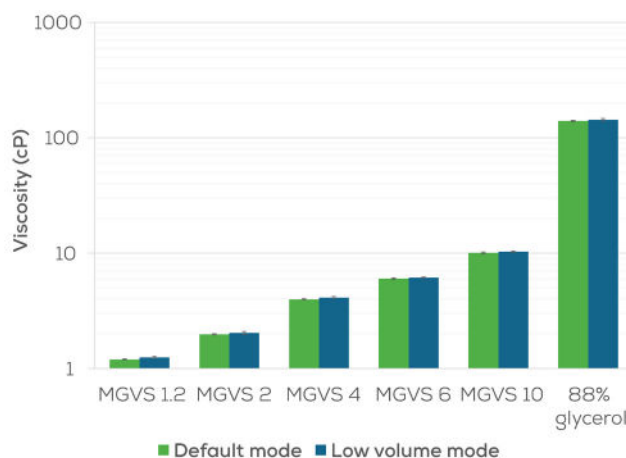


Figure 2: The viscosity of Medical Grade Viscosity Standards (MGVSs) and 88% glycerol were measured in quadruplicate in default and low volume modes on Honeybun at 25 and 22 °C, respectively. No significant differences between the measurement modes were observed. Note the graph is on log scale.

The viscosities of two different monoclonal antibodies (mAb1 and mAb2) were determined in triplicate at 10, 25, and 40 °C (Figure 3). Both antibody solutions were at a concentration of 200 mg/mL in 10 mM histidine, pH 7.4. The viscosities measured in low volume and default mode were indistinguishable for the same mAb at each temperature. Increasing temperatures caused the viscosity of both mAbs to decrease non-linearly.

Despite being in the same formulation, mAb1 was consistently lower viscosity than mAb2, indicating weaker protein-protein interactions.<sup>3</sup> A commonly cited upper limit for comfortable subcutaneous injection is 20 cP, which mAb2 exceeded at 10 and 25 °C in this formulation.<sup>4</sup>

Sample	Target (cP)	Default mode viscosity	Low volume mode viscosity (cP)
MGVS 1.2	1.201	1.199 ± 0.015	1.252 ± 0.021
MGVS 2	2.004	1.978 ± 0.023	2.035 ± 0.062
MGVS 4	4.009	3.981 ± 0.064	4.111 ± 0.087
MGVS 6	6.027	6.018 ± 0.088	6.122 ± 0.069
MGVS 10	9.99	10.08 ± 0.175	10.31 ± 0.146
88% (w/w) glycerol	144.72	139.65 ± 1.98	142.85 ± 4.086

Table 1: Manufacturer-supplied viscosities of the MGVSs at 25 °C and expected viscosity of 88% glycerol in water at 22 °C ("Target").<sup>2</sup> Mean and standard deviation of Honeybun viscosity measurements in default and low volume modes.

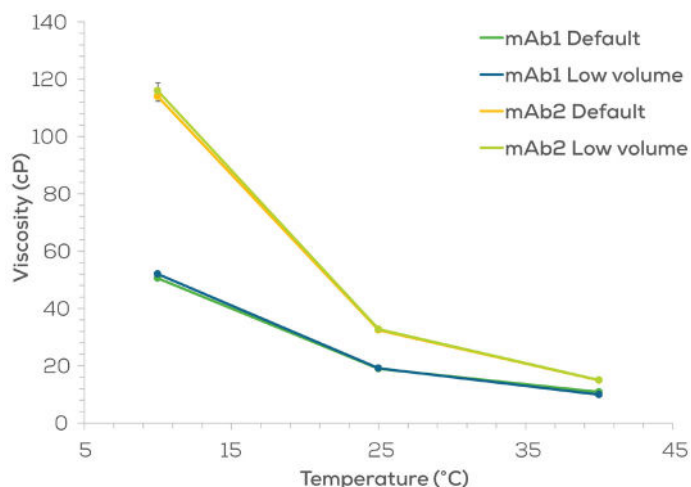


Figure 3: 2 mAbs were measured in triplicate at 200 mg/mL in 10 mM histidine, pH 7.4, at 10, 25, and 40 °C in default and low volume modes on Honeybun. Viscosity of both antibodies decreased with increasing temperature. The results of the two instrument modes were indistinguishable.

## Conclusion

Honeybun keeps biologics flowing freely through the development pipeline by delivering straight-forward, fast, and low volume viscosity measurements. It can process up to 10 samples simultaneously while using as little as 15  $\mu$ L per sample. Accuracy, reliability, and precision are no problem at all for Honeybun with CVs less than 2% in default mode and under 4% in low volume mode. In just a few minutes you can get viscosity data across a range of temperatures to check and see how your protein will perform at low temperature or body temperature. Fast to set-up and run, low volume, hands-free, cleaning-free, and hassle-free – Honeybun checks all the boxes to deliver as much viscosity data as you need.

## References

- 1 Strategies to deal with challenges of developing high-concentration subcutaneous (SC) formulations for monoclonal antibodies (mAbs). SJ Shire. *Monoclonal Antibodies*. 2015:139–152.
- 2 Formula for the Viscosity of a Glycerol-Water Mixture. NS Cheng. *Industrial & Engineering Chemistry Research*. 2008; 47(9):3285–3288.
- 3 Viscosity Control of Protein Solution by Small Solutes: A Review. T Hong, et al. *Current Protein & Peptide Science*. 2017; 19(8):746–758.
- 4 Evaluation of the impact of viscosity, injection volume, and injection flow rate on subcutaneous injection tolerance. C Berteau, et al. *Medical Devices (Auckland, N.Z.)*. 2015; 8:473–484.



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