

# *In vitro* characterisation of a nasal spray comprising an extremely potent human monoclonal antibody

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## Key Message

A formulation suitable for nasal delivery was prepared for MAD0004J08, a highly potent human antibody against SARS-CoV-2, employing a chitosan platform already applied to other mAb. MAD0004J08 maintained the same binding and neutralisation activity before and after spraying, highlighting the suitability of the combination device/formulation developed for nasal delivery of monoclonal antibodies.

## Introduction

The fragment crystallisable of the antibody (IgG 1) named J08 (or MAD0004J08), isolated from COVID-19 recovered patients [1], was engineered to reduce the risk of antibody-dependent enhancement of disease and to prolong half-life. This antibody showed to neutralise the authentic wild-type virus and all emerging variants of concern, including alpha, beta, gamma, delta and omicron even though with much less potency for this latter variant [1-2]. In addition, in a phase 1 clinical study, a single dose of MAD0004J08 administered intramuscularly (i.m.) showed to induce extremely high serum neutralisation titres against SARS-CoV-2 and its variants of concern [3].

The aim of this study was to redesign MAD0004J08 formulation used for i.m. administration to exploit nasal delivery for local and systemic action. A formulation platform already applied to another mAb was employed in combination with Aptar CPS preservative-free nasal pump [4]. The final product was then characterised *in vitro* for performance, binding and neutralisation activity.

## Experimental Methods

0.5% w/v of chitosan lactate, 0.1% w/v of polysorbate 80 were mixed with MAD0004J08 i.m. formulation (8.04 mg/mL) in phosphate buffer at pH 7.4. Hydrodynamic diameter and Zeta potential of the mAb in the nasal formulation was determined with Malvern Zetasizer Nano. pH and osmolality were measured as well. Stability of the mAb in the nasal formulation was assessed visually and by size exclusion chromatography (SEC) analysis. The formulation prepared was then loaded into the Aptar CPS pump, 100 µL (Aptar Pharma, DE). Formulation-device combination was characterised in terms of droplet size distribution (DSD), spray pattern (SP) and plume geometry (PG) and spray content uniformity (SCU) for ten consecutive shots. MAD0004J08 concentration and stability (area percentage of aggregates and fragments) were determined by SEC coupled with Diode Array Detector.

Affinity was evaluated before and after spraying from Aptar CPS employing different concentrations of SARS-CoV-2 spike protein. Following the capture of mAb, different concentrations of spike protein were injected. Kinetic rates and affinity constant of spike protein binding to each mAb sample were calculated applying a 1:1 binding as fitting model using the Bia T200 evaluation software 3.1. Finally, the neutralisation activity of MAD0004J08 produced as recombinant protein and both nasal formulation and sample sprayed, was evaluated by cytopathic effect-based microneutralisation (CPE-MN) assay using the live viruses. Plates were incubated for 3-4 days at 37°C, then examined for CPE by means of an inverted optical microscope by two independent operators. Antibodies were tested at a starting concentration of 1 µg/mL and diluted step 1:2. Technical triplicates were performed to evaluate the 100% inhibitory dilution (IC100) of tested antibodies.

## Results and Discussion

- ◇ No protein aggregation was observed after manufacturing and spraying (97.97% of mAb monomer in the formulation vs 97.78% i.m. formulation), confirmed also by hydrodynamic diameter ( $15.12 \pm 0.25$  nm for nasal formulation vs  $14.45 \pm 0.21$  nm of i.m. formulation).
- ◇ Dv50 (Table 1) was greater than 50 µm, which has been reported to increase the likelihood of deposition in the anterior and middle region of the nose.
- ◇ As previously reported [4], SP and PG were lower than the placebo nasal formulation (Figure 1), consistent with a more viscous formulation when MAD0004J08 was present (3.3 cP versus 1.1 cP).

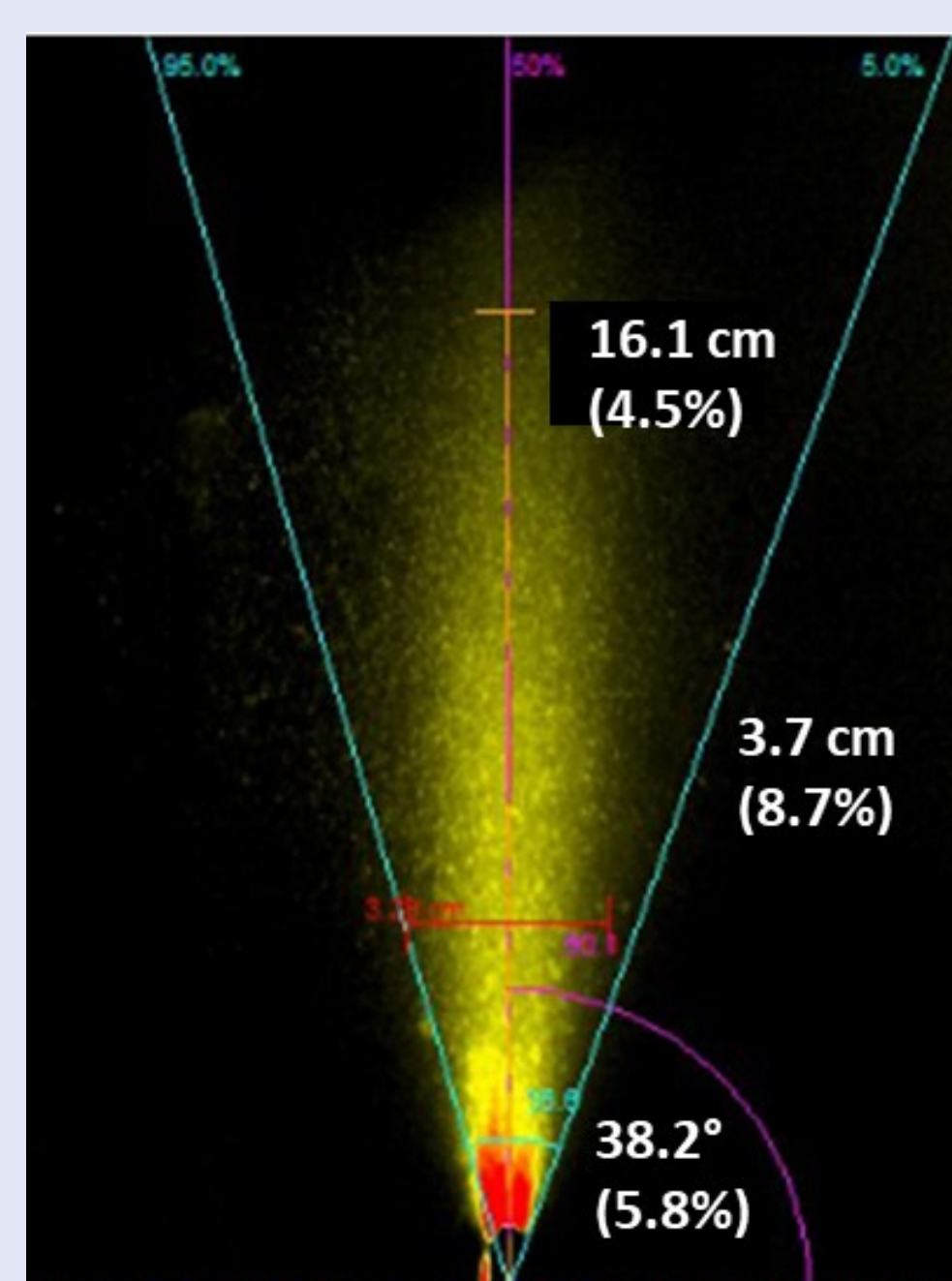


Figure 1. PG with Aptar CPS

Table 1. DSD and SCU results with Aptar CPS pump.

	Dv10 (µm)	Dv50 (µm)	Dv90 (µm)	Span	SCU (µg)
MAD0004J08	26.14 (7.22%)	56.62 (8.86%)	135.70 (13.46%)	1.93 (9.52%)	816.25 (1.88%)
Nasal Formulation					

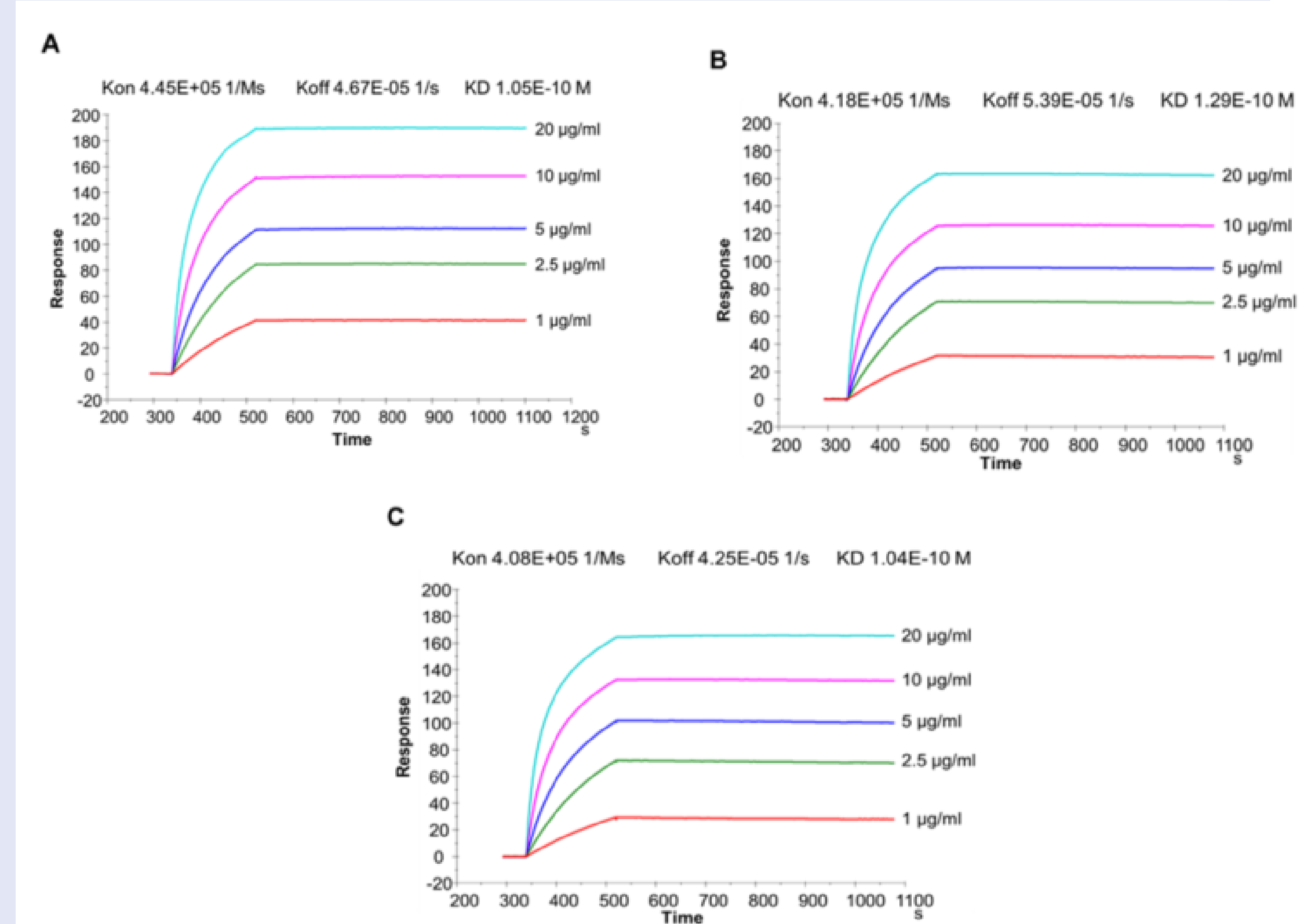


Figure 2. Binding kinetics of MAD0004J08 original (A), in nasal formulation (B) and post spray from Aptar CPS (C) to the spike protein antigen.

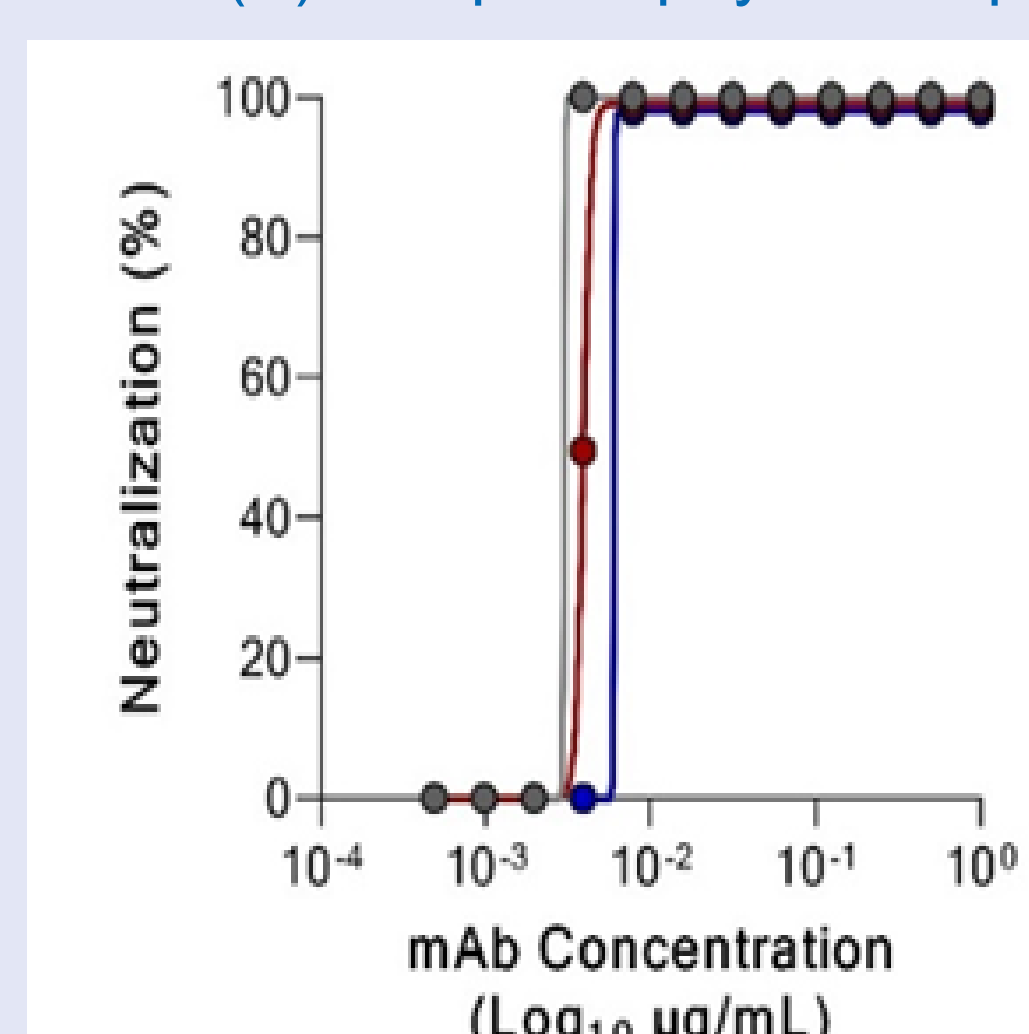


Figure 3. Neutralization activity of original mAb, in nasal formulation and post spray.

## Conclusion

This study reports another example of application of the chitosan/polysorbate formulation platform for nasal delivery of mAbs intended for infectious diseases prophylaxis.

The formulation prepared was suitable for nasal delivery (pH and osmolality). Mixing did not impact the size or percentage of the dimeric fraction of the mAb. DSD reported a likely deposition of the formulation in the anterior-middle region of the nose, whereas the chitosan positive charge ensures mucoadhesion and, therefore, will help to obtain a prolonged presence of MAD0004J08. Viscosity of the final nasal formulation showed to influence spray geometry. Binding and neutralization activity before and after spraying was maintained, highlighting the suitability of our platform for nasal delivery of monoclonal antibodies.

## References

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