# INO-4800, a DNA Plasmid Vaccine Encoding the Ancestral SARS-CoV-2 Spike Protein, Is Stable Over a Range of Temperatures Not Requiring Ultra-Cold Chain Storage

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## BACKGROUND

- Since being initially reported in December 2019 as a pneumonia of unknown cause in Wuhan, China, COVID-19, caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has resulted in 615 million cases of illness and 6.5 million deaths globally, as of October 5, 2022[1–4]
- Although the 12 billion doses of COVID-19 vaccines administered to date have effectively reduced disease incidence, hospitalization, and mortality worldwide, only 68% of the global population and only 22.7% of the population in low-income countries have received at least 1 dose of a COVID-19 vaccine, as of October 5, 2022[5,6]
- These low vaccination rates may be partly attributable to the fact that some COVID-19 vaccine types require storage at ultra-low temperatures for stability and longevity, hindering their deployment in resource-constrained settings that lack ultra-cold chain infrastructure[7,8]
  - By contrast, DNA vaccines are temperature stable and consequently have an extended shelf life,[9–11] permitting their use in global regions without ultra-cold chain capabilities
- INO-4800, a DNA vaccine encoding the spike (S) protein of the ancestral (Wuhan) strain of SARS-CoV-2, has demonstrated safety and immunogenicity in Phase 1[12,13] and Phase 2[14] clinical trials
- We assessed the stability and potency of multiple INO-4800 lots used in Phase 3 clinical trials over a range of storage temperatures for durations of up to 18 months

## METHODS

- Production lots of INO-4800 were stored at a range of test temperatures:
  - Storage temperature (2–8°C)
  - Accelerated temperature (25 ± 2°C)
  - Stress temperature (40 ± 2°C)
- Samples were obtained at the end of the first month and subsequently at 3-month intervals beginning in the third month for assessment of stability and potency
  - Stability was assessed through DNA isoform homogeneity by capillary gel electrophoresis (CGE) to quantify relative proportions of different plasmid topologies (supercoiled, open circular, and linear) at different timepoints at each of the test temperatures
  - Potency was measured using a cell-based assay that involved flow cytometric detection of fluorescently labeled S protein encoded by the plasmid

**Stability: Determination of homogeneity of plasmid forms by CGE**

- The CGE assay helps quantify the relative percentage of different plasmid DNA topologies, using a 100% peak area method based on the portion of corrected peak areas of the DNA isoforms (supercoiled, open circular, and linear) in relation to the overall corrected peak area
- Corrected peak areas were used instead of mere signal strengths because peaks pass through the detector with different velocities, owing to different retention times (earlier peaks being faster than later peaks), making later peaks appear to be larger. Corrected peak areas normalize peak areas to migration times and allow for the correct quantitative comparison of relative peak areas of the different DNA forms

**Potency: In vitro quantitative cell-based protein expression bioassay**

- 96-well cell culture plates were seeded with specific cells, followed by serial dilutions of the sample and the reference standard (spanning a validated concentration range), as well as a cationic lipid transfection reagent
  - Transfection reagent without DNA added to the cells served as a negative control
- Transfected cells were collected within a validated time post transfection and stained with an antibody against the S1 portion of the S protein
- The fluorescently labeled expression products were measured using flow cytometry, and percentages of positive cells were recorded for each dilution
- The resulting dose-response curves were analyzed using a 4-parameter, nonlinear regression analysis, and a percentage relative potency result was obtained by comparing the dose-response curve of the sample with that of the reference standard

## RESULTS

**Stability**

- At the regular storage temperature (2–8°C), all 4 tested lots of INO-4800 retained structural homogeneity above the minimum regulatory authority–approved specifications (≥85% for circular and ≥80% for supercoiled isoforms) for ≥12 months, with 1 lot retaining homogeneity for up to 18 months (**Figure 1A**)
- At the accelerated temperature (25 ± 2°C), all 3 tested lots retained structural homogeneity above target specifications for ≥6 months (**Figure 2A**), with 1 lot retaining homogeneity above target specifications for up to 9 months
- At the stressed temperature (40 ± 2°C), neither of the 2 tested lots were homogeneous above target specifications at the 1-month timepoint (**Figure 3A**)

**Potency**

- At the regular storage temperature (2–8°C), all 4 tested lots of INO-4800 remained potent above the regulatory authority–specified minimums (potency being 77%–130% of that of the reference standard) for ≥12 months, with 1 lot retaining potency for 18 months (**Figure 1B**)
- At the accelerated temperature (25 ± 2°C), all 3 tested lots retained potency above target specifications for 9 months, with 2 lots and 1 lot retaining potency for up to 12 and 18 months, respectively (**Figure 2B**)
- At the stressed temperature (40 ± 2°C), both tested lots retained potency above target specifications for 1 month, with 1 lot retaining potency for 3 months (**Figure 3B**)

**Figure 1. Stability (A) and potency (B) of INO-4800 at 2–8°C***

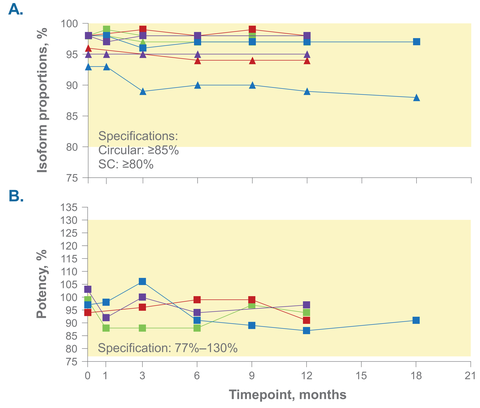



**Figure 2. Stability (A) and potency (B) of INO-4800 at 25 ± 2°C***

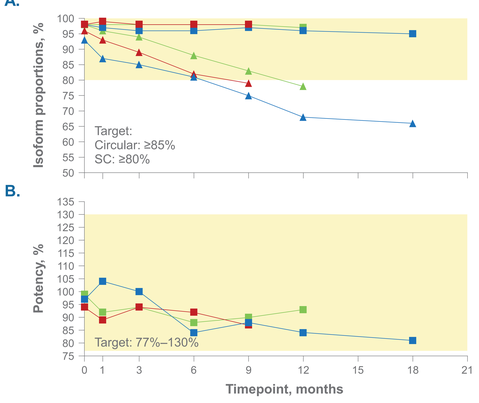
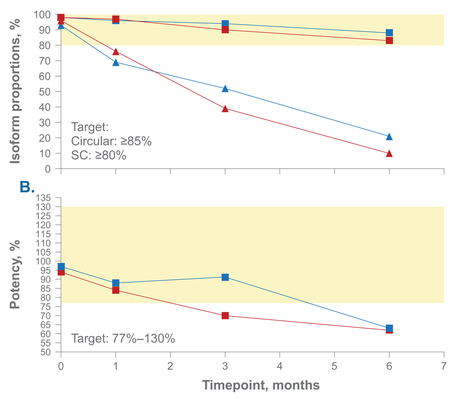



**Figure 3. Stability (A) and potency (B) of INO-4800 at 40 ± 2°C***




Production lot # — P-batch #1 — P-batch #1 SC — P-batch #2 — P-batch #2 SC — P-batch #6 — P-batch #6 SC — GR-batch #2 — GR-batch #2 SC

SC, supercoiled. * Shaded areas represent regulatory authority–approved specifications.

## CONCLUSIONS

- All tested lots of INO-4800 retained both stability and potency exceeding minimum regulatory authority specifications for at least 12 months at 2–8°C and above target specifications for at least 6 months at 25 ± 2°C, with 1 lot remaining stable and potent for up to 18 months at 2–8°C
- At the accelerated and stressed temperatures, the potency of INO-4800 was independent of its stability; INO-4800 retained potency for durations beyond other stability-indicating assays falling below specification
- The demonstrated stability and potency of INO-4800 for durations of up to 1 year at 2–8°C, a temperature range achievable with common household refrigerators, permit its deployment globally, even in settings without adequate cold chain infrastructure
- INO-4800 has the potential to help vaccinate a greater proportion of the global population, especially in low-income countries, thereby contributing to COVID-19 pandemic control

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**Disclosures**

Robert J. Juba, Jr, Anthony Samawova, Katherine Seals, and EJ Brandreth are employees of Inovio Pharmaceuticals, Inc. Peggy Kinney was an employee of Inovio Pharmaceuticals, Inc. at the time of study.

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