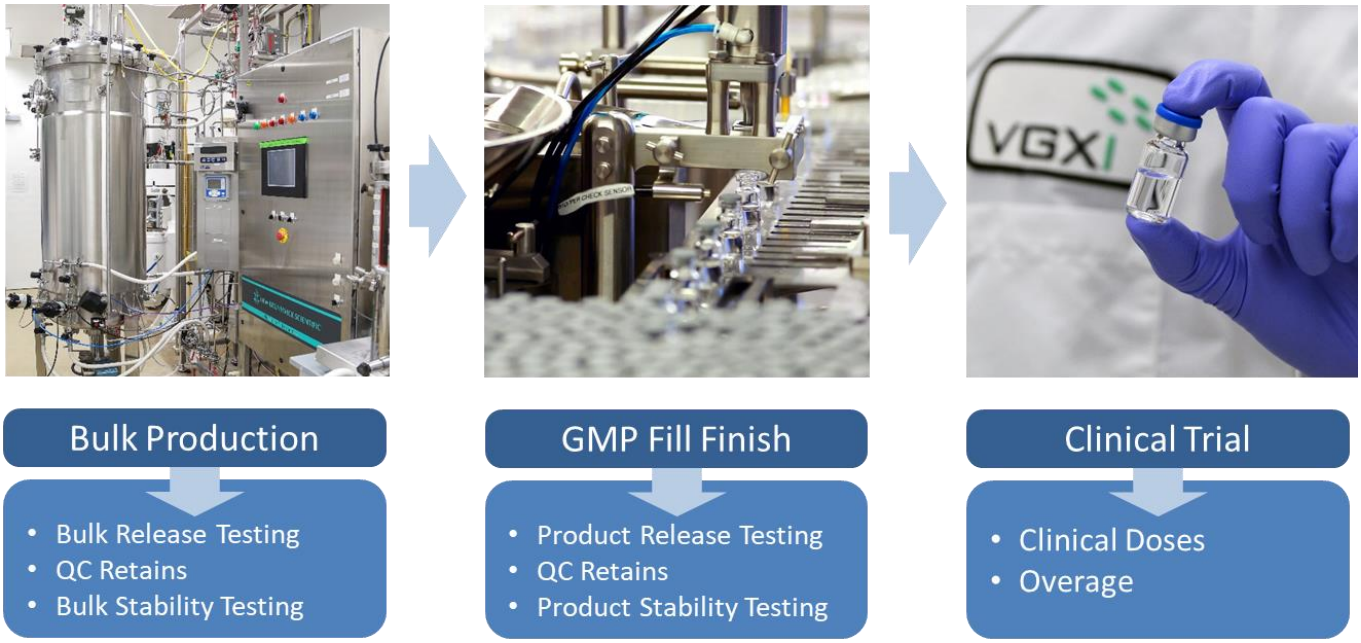
How Much Plasmid DNA is Needed for Your Clinical Trial?

Calculating the amount of plasmid DNA needed to perform a clinical trial may seem straightforward - simply multiply the dose by the number of doses to be administered. However, there are a number of additional factors that must be considered. In some cases, the production scale required can be several times the amount needed for clinical dosing. Production requirement calculations should account for each of three main phases in the overall process:



By walking through one case study, this white paper will review the key parameters that influence the overall batch size needed for bulk production. Calculations will work backwards starting from clinical doses to determine the bulk production scale as follows:

1. Clinical Trial Requirements
2. GMP Fill / Finish Requirements
    - Release testing
    - Stability testing
3. Bulk Production Requirements
    - Release testing
    - Stability testing

Case Study: Clinical Trial for a Therapeutic DNA Vaccine

1. Clinical Trial Requirements

For this case study, the trial consists of 50 patients who each receive two, 10 mg dose injections. In total, 1 gram of plasmid is required to treat all 50 patients.

$$50\ patients * 10\ mg/dose * 2\ doses = 1\ gram\ plasmid$$

The concentration and fill specifications determine how much plasmid is needed for the trial doses. In this study, the drug product will be filled into 5 mL glass vials at a final concentration of 5 mg/mL. A target fill volume of 2.3 mL (11.5 mg) is chosen to support the label claim and clinical dose of 2.0 mL (10 mg). This target fill volume is greater than the amount printed on the vial label to take into account two factors:

- Fill Tolerance - The allowed variability around the target fill volume. For example the actual fill volumes may be 2.3 +/- 0.1 mL.

- Minimum Extractable Volume - Some liquid will remain in the vial after withdrawal when dosing a patient.

The client in this case study also requests some overage above the 100 doses needed for the trial.

$$120\ vials\ * 2.3\ mL/vial * 5\ mg/mL = \mathbf{1.38\ grams\ plasmid\ delivered\ to\ the\ client}$$

For all products that will be used in humans, testing is required by regulatory agencies such as the FDA at each step in production to ensure safety and efficacy. This includes in-process testing, Quality Control (QC) release testing and stability testing. The amount of product required for these requirements are calculated for each phase below.

2. GMP Fill / Finish Requirements

Initial testing for the drug product (DP) occurs at release, or when the plasmid DNA is filled into vials. DP release testing includes a comprehensive list of assays to evaluate the quality, purity, and sterility of the drug product. In the example being presented, the following quantities must be filled for this release testing.

Bacteriostasis / Fungistasis Testing	*120 vials*
Sterility Testing	*20 vials*
Other Release Tests and Retains	*20 vials*
Total for DP Release:	*160 vials*

$$160\ vials\ * 2.3\ mL/vial * 5\ mg/mL = \mathbf{1.84\ grams\ plasmid\ for\ DP\ release}$$

In addition to initial, or time = 0 testing, vials should be prepared for use in evaluating the drug product's long term stability. A typical ICH (International Conference on Harmonization) conforming stability study will include timepoints every 3 months during the first year, every 6 months during the second year, and annually thereafter throughout the proposed shelf-life of the product.

Additional stability study variables to be considered include:

- Number of storage temperatures to be evaluated (real-time or accelerated)
- Vial orientations to be evaluated (upright or inverted)
- Testing required at each timepoint (impacts vial quantity needed per timepoint)
- Any additional testing at the stability endpoint (e.g. sterility)
- Required regulatory retains

In this case study, stability testing has been requested on upright vials stored at -20˚C and 4˚C over a period of 2 years. Testing will be at 3, 6, 9, 12, 18, and 24 months. Based on the fill volume for product in this study, only 1 vial will be required at each timepoint.

*6 timepoints * 1 vial each*	*6 vials*
Final timepoint sterility testing	*20 vials*
QC Retains	*4 vials*
Total per condition tested:	*30 vials*
Total for two conditions:	*60 vials*

$$60\ vials\ * 2.3\,mL/vial * 5\,mg/mL = \mathbf{0.690\ grams\ plasmid\ for\ DP\ stability\ testing}$$

To summarize, the GMP fill must be of sufficient scale to meet all of the above requirements, plus some overage. In the given case study, the fill will target a total of 350 vials and require 4.03 grams of plasmid DNA based on the following:

	Vial Quantity	***Plasmid DNA***
Clinical Trial	*120 vials*	*1.38 grams*
DP Release Testing	*160 vials*	*1.84 grams*
DP Stability Testing	*60 vials*	*0.69 grams*
Minimum Quantity Needed:	*340 vials*	*3.91 grams*
Target Fill Quantity:	***350 vials***	***4.03 grams***

3. Bulk Production Requirements

In addition to plasmid DNA needed for the GMP fill, material must also be available for bulk release testing, required QC retains, and bulk drug substance (DS) stability testing (if requested). In the current example, stability testing has been requested on the bulk material stored at -80°C for 2 years. The following table outlines each of these requirements:

*DS Stability Testing (6 timepoints * 10mL each)*	*60 mL*
*DS Stability Retains (4 samples * 10mL each)*	*40 mL*
Bioburden Testing at Release	*50 mL*
Other DS Release Tests and Retains	*20 mL*
Total	*170 mL*

$$170\ mL\ * 5\ mg/mL = \mathbf{0.85\ grams\ plasmid\ for\ DS\ release\ and\ stability}$$

Bulk DS for GMP Fill/Finish	*4.03 grams*
Bulk DS for Release and Stability	*0.85 grams*
Total Plasmid Needed	***4.88 grams***

Taking all of the above into consideration, the final bulk plasmid production scale for the case study presented is 4.88 grams. Surprised? In order to conduct the given trial requiring 1.38 grams of plasmid, over three times as much plasmid DNA must be manufactured to supply all release testing, stability studies, and QC retains.

Final Considerations and Planning

It is generally recommended to manufacture even more plasmid than specified strictly by the calculations, because it is more cost effective to manufacture one large batch than two smaller batches. In addition, the excess product acts as an insurance policy in case changes need to be made to the trial, such as adding another study arm or additional dosing. Producing a single larger batch also saves the time—about six months—that it would take to make a new batch of product. This can be especially crucial when facing clinical deadlines.

Fortunately, plasmid DNA manufacturing is primarily a fixed-cost activity so the cost does not increase proportionally with the amount of plasmid produced.

Getting Help

The best approach when determining how much plasmid DNA to manufacture is to talk to an experienced CMO like VGXI, while still in the planning stages of the clinical program. Early discussions will provide a realistic assessment of how much plasmid DNA is needed, how long it will take to produce, and how much it will cost. VGXI is happy to work with you to develop an appropriate manufacturing plan for your project. Contact us today to get started!

VGXI®

QUALITY….IT'S IN OUR DNA™

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About VGXI

VGXI is a contract manufacturing organization that specializes in the production of plasmid DNA under cGMP for use in human clinical trials. With over 15 years experience, the company has produced products for numerous clinical trials in the U.S., Europe, Asia and Australia. VGXI's patented AIRMIX® purification technology produces the highest quality plasmids in the industry with supercoil percentages over 90% and final product concentrations as high as 15 mg/mL. Our highly experienced team can work with each project's unique requirements to create a manufacturing solution that ensures the success of your clinical program.