# Characterization of Important Raw Materials Used in the Transient Transfection Process for rAAV Production

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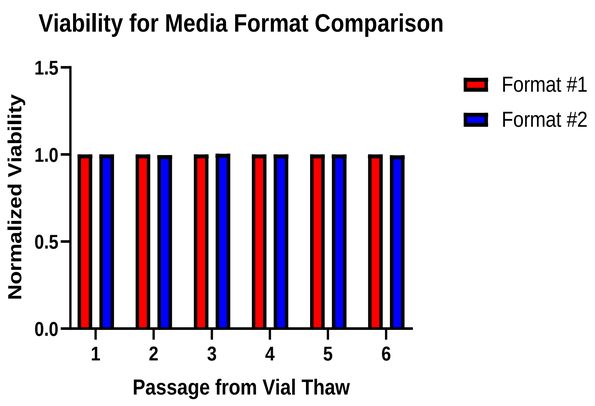
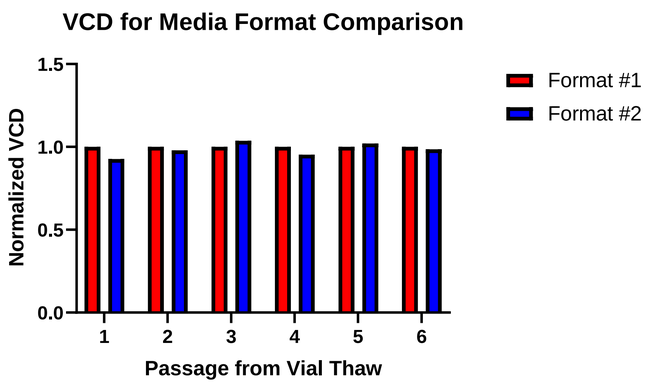
## Abstract

Recombinant adeno-associated viral vectors (rAAV) are the vehicle of choice for therapeutic gene delivery in the gene therapy field. The transient transfection method of production remains one of the most attractive processes for the manufacture of rAAV due to its fast turnaround and versatility towards the production of a wide range of rAAV constructs. However, raw materials such as plasmids, media and the transfection reagent can possess some level of lot-to-lot variation in their properties and qualities. In this work, we have extensively studied and characterized the raw materials used in AAV manufacturing by transient transfection, including the different lots of plasmids, media, and the transfection reagent, as well as the polymer material of the complexation container. We have identified the raw materials that would have a high level of variability as well as a significant impact on the process. This enables us to implement a data driven testing and in-process controlling strategy on raw materials to ensure the success of AAV production from the lab scale to manufacturing scales at 1000L and beyond.

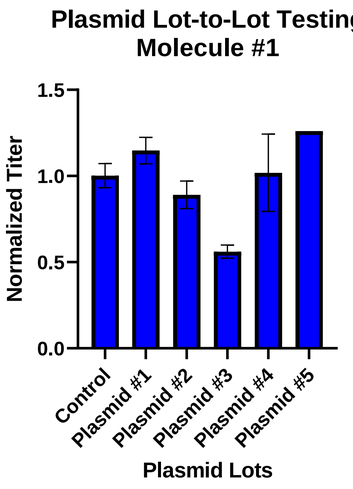
## Media Format

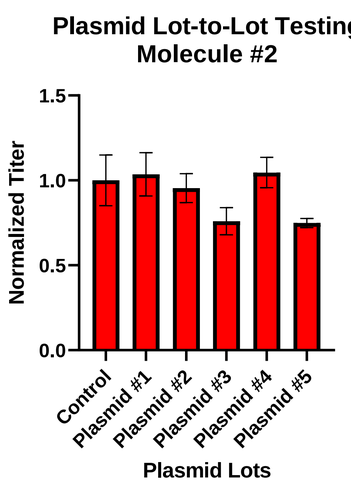


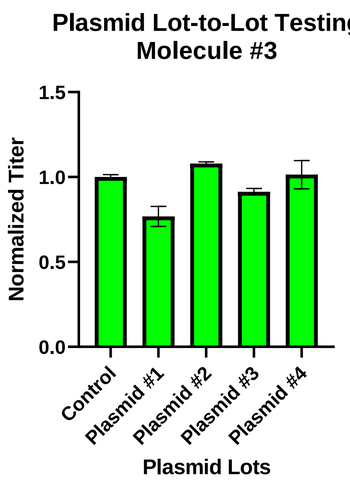




There were two different formats that the media could be developed in. A comparison of those two formats in terms of viability and viable cell density (VCD) showed no significant difference between the two formats. The format of the media does not appear to be impactful.

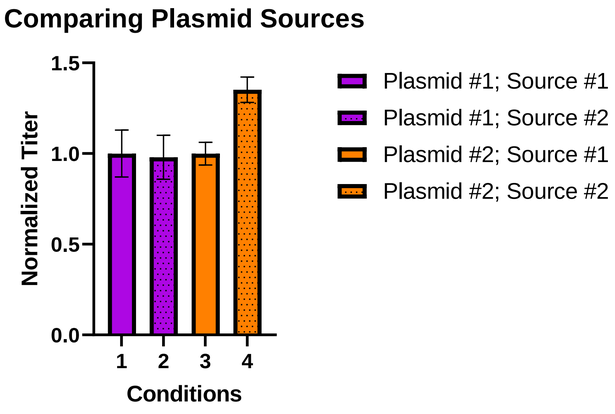
## Plasmids



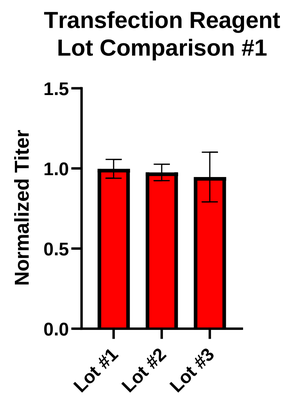



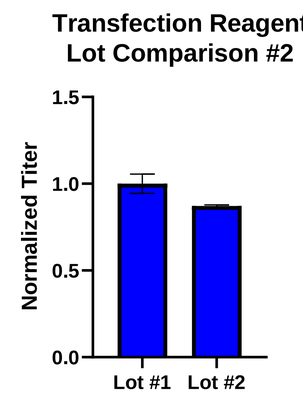



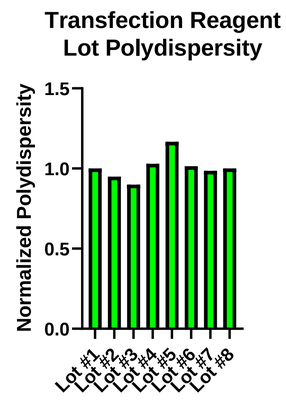

Significant differences between plasmid lots were observed when comparing the titer between lots. This was shown across three different molecules, and it implies that the plasmid lot is very impactful to the titer results. It was also noted that the source of the plasmid can be impactful as well.




## Transfection Reagent

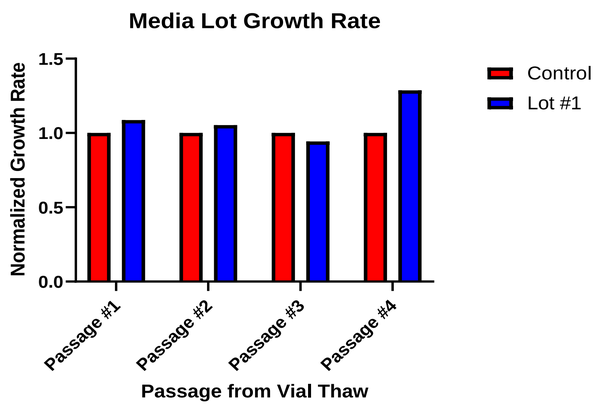


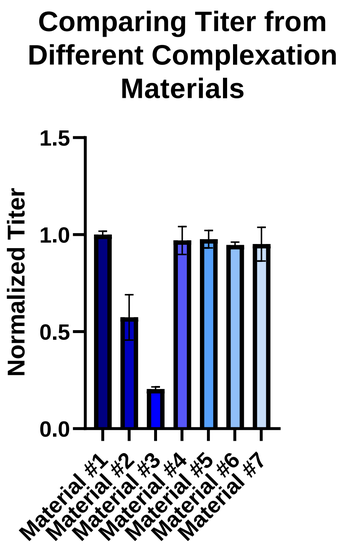

Two experiments comparing transfection reagent lots have been completed and neither experiment showed a significant difference in the titer between the lots. Additionally, the polydispersity was calculated for eight different lots and there was minimal difference seen. So, transfection reagent lot is minimally variable and not impactful.

## Media Lot

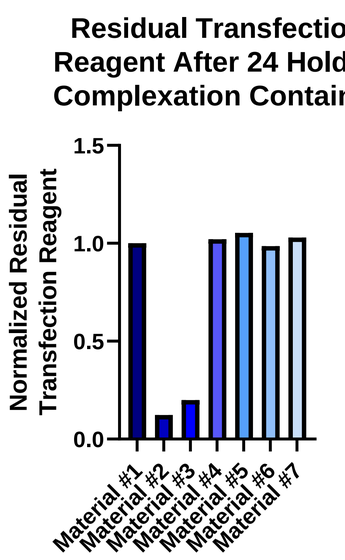



Comparing the growth rate from the control to another lot of the same format showed no significant difference in the media lot growth rates.

## Complexation Container




*Error bars are technical replicates, not biological replicates*




Seven different complexation containers with different polymer materials were tested. The transfection reagent was held in the containers for five hours prior to complexing. It can be observed that some materials can negatively impact titer. Complexation container is a controllable, but impactful raw material.

## Conclusion

Raw materials can have a large impact on titer, and some are very variable. In these studies, the raw materials of the plasmid lot and complexation container were shown to be very impactful in the productivity of AAV. Transfection reagent lot was shown to have little variation and not a large impact. The medium lot and format of media were shown to have little variation, and both showed little impact. From these studies, it is important to note that the complexation container is controllable, but impactful, so care should be taken when choosing a complexation container for complexation. It is also important to screen plasmid lots prior to implementation since they are very variable and are also impactful.

## Acknowledgements

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