

Dear AAV Reference Standard Working Group,

Mycoplasma testing was performed as part of the AAV Reference Standard characterization and, so, Introgen (filling the vector into vials) would know the mycoplasma status of the material prior to accepting it into their GMP fill facility.

Mycoplasma testing occurs on the cell harvest (ie when the transfected cells were collected from the cell factories) and includes cells mixed with the spent culture supernatant that the cells were grown in. 1×10^7 cells in 15ml of spent culture supernatant is tested by the "Points to Consider" assay using direct and indirect tests.

Since 18 batches of 10 Cell factories each were transfected, the harvests were pooled prior to testing to reduce cost (this is an expensive test). The sample submitted (1×10^7 cells) was equal to $6.7 \times 10^{-3} \%$ of the total number of cells from the 18 batches ($=1.5 \times 10^{11}$ cells). The harvest was positive for mycoplasma and the test was valid (ie all controls performed). I asked that the test be repeated to be sure, and the second test was also positive.

The Powell gene Therapy Center Vector Core (a Non-GMP facility where the vector was produced and purified) contacted HyClone (they donated the serum used in cell culturing) to see if they had recalled any of the serum lots used/donated. None of the serum lots was positive for mycoplasma. At this time, it is not known what batch(es) were contaminated or when the contamination occurred during the 13 months of production, because the harvests were pooled.

I wanted to determine if the AAV Reference Standard Filtered Purified Bulk was positive for mycoplasma (this is what Introgen currently has in their possession for the vial fill). I requested that a modified mycoplasma test be developed and used by Apptec. The Filtered Purified Bulk (3ml, equal to 0.12% of the total 2570ml, so this is actually 18X more sample on a percentage basis than was tested for the harvest) was inoculated onto Vero cells and then the Vero cells were harvested (by scraping) and used to infect a second set of Vero's, followed by a third round. These cycles were designed to allow growth and amplify any myco that could be present in the Bulk. The final cycle cell harvest was tested by the "Points to Consider" assay using direct and indirect tests. The AAV Reference Standard Filtered Purified Bulk tested **NEGATIVE** for mycoplasma and the test was valid (this result was received on Friday August 10, 2007 from Apptec).

In addition, the harvest and the filtered purified bulk were tested by PCR (Apptec) for mycoplasma DNA. The harvest was confirmed positive and the purified bulk is negative.

I believe the AAV Reference Standard Filtered Purified Bulk is negative for myco because during purification the cells were lysed using microfluidization (which is highly effective at disintegrating cells, including other organisms like bacteria) in the presence of deoxycholate (a detergent, which may have inactivated the myco), followed by 3 chromatographic steps which may have purified myco from the AAV, followed by a

series of filtration steps which may have removed myco. No data supports this other than the negative result following these combined steps.

The AAV Reference Standard was made in a RESEARCH environment, not in a GMP environment, however reagent and consumables lots were recorded. Making the AAV RSS under GMP would have been cost prohibitive. If this lot of AAV was intended for humans, it would not be acceptable to use a harvest that was Myco positive, and the entire lot would need to be discarded. However, since this is a reference standard to be used in research and QC labs, **I recommend that we continue forward and fill, bank, characterize, and distribute the AAV2 Reference Standard for the following reasons:**

1. We have spent almost 2 years generating the RSS
2. An investment worth over \$400,000 in donations and grants was made to fund the effort
3. The Filtered Purified Bulk is negative for myco

I believe that if we include a summary on the Product Information Sheet that states that the harvest was myco positive, but the purified bulk was negative, then labs requesting the RSS will be fully informed and can decide if they want to bring it into their facilities. (Something like “the RSS has been exposed to myco, but is myco free”).

Please let me know if you agree or disagree with my recommendation, or have any comments/suggestions.

Thank you, Richard