Estimation for amount of *M. kansasii::Rv3377-78c* required to produce enough 1-TbAd to detectably raise the pH of 1 ml of 7H9:

## Assumptions:

- negligible buffering from 7H9
- pH change from 5.2 to 5.3
- Every 1-TbAd molecule can capture one proton (no intermediate equilibrium)
- *M. tuberculosis* contains up to 7 x 10^-17 g of 1-TbAd in one cell (22); assume *M. kansasii::Rv3377-78c* contains the same amount and that all of it is available for neutralization.
- 1-TbAd is 540 g/mol. Therefore, one cell contains  $1.30 \times 10^{-19} \text{ mol } 1\text{-TbAd}$ . (alternatively put,  $1.30 \times 10^{-19} \text{ mol } \times 6.02 \times 10^{23} \text{ molecules/mol} = 78,300 \text{ molecules of } 1\text{-TbAd per cell}$ ). For a pH change of 5.2 to 5.3, the difference in number of H+ ions is  $(10^{-5.3} \text{ M}) (10^{-5.2} \text{ M}) = 1.30 \times 10^{-6} \text{ M}$  of H+. For 1 ml of solution, that is  $1.30 \times 10^{-9} \text{ moles}$  of H+. Therefore, the number of bacteria needed to change the pH from 5.2 to 5.3 in 1 ml is  $(1.30 \times 10^{-9} \text{mol}) / (1.30 \times 10^{-19} \text{ mol/cell}) = 10^{10} \text{ bacteria}$ . This is an  $OD_{600}$  of roughly 100.