Estimation for amount of $M$. kansasii::Rv3377-78c required to produce enough 1-TbAd to detectably raise the pH of 1 ml of 7 H 9 :

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 $7 \times 10^{\wedge}-17 \mathrm{~g}$ 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 $540 \mathrm{~g} / \mathrm{mol}$. Therefore, one cell contains $1.30 \times 10^{\wedge}-19 \mathrm{~mol} 1$-TbAd. (alternatively put, 1.30 x $10^{\wedge}-19 \mathrm{~mol} \times 6.02 \times 10^{\wedge} 23$ molecules $/ \mathrm{mol}=78,300$ molecules of 1 -TbAd per cell). For a pH change of 5.2 to 5.3 , the difference in number of $\mathrm{H}+$ ions is $\left(10^{\wedge}-5.3 \mathrm{M}\right)-\left(10^{\wedge}-5.2 \mathrm{M}\right)=1.30 \times 10^{\wedge}-6 \mathrm{M}$ of $\mathrm{H}+$. For 1 ml of solution, that is $1.30 \times 10^{\wedge}-9$ moles of $\mathrm{H}+$. Therefore, the number of bacteria needed to change the pH from 5.2 to 5.3 in 1 ml is $\left(1.30 \times 10^{\wedge}-9 \mathrm{~mol}\right) /\left(1.30 \times 10^{\wedge}-19 \mathrm{~mol} / \mathrm{cell}\right)=10^{\wedge} 10$ bacteria. This is an $\mathrm{OD}_{600}$ of roughly 100.

