HPV ELISA Statistical Analysis

Introduction:
An HPV ELISA assay was conducted with the goal of being able to predict the amount of Antigen protein from Spectrophotometer(A450) readings. The hypothesis is that the A450 values varies linearly with the amount of antigen protein present in the sample. This would imply that changing the dilution by a factor of 2 would lead to a change in A450 readings by a factor of 2. This claim was validated by fitting a linear curve to the data. The linear curve would then be used for back predicting the values of input protein by observing the A450 value.

In the next step a lysate solution was made to undergo the HPV ELISA assay and the A450 readings were taken. Several readings were taken at different levels of dilution. It was observed that causing a dilution of factor 2 did not lead to a corresponding drop in back-predicted values by a factor of 2. It was suggested that perhaps a correction factor of some sort would lead to the desired behavior. The data was statistically investigated and the following conclusions were drawn :

(1) The A450 values returned by the protein mixture samples are very noisy. Possibly beyond the acceptable tolerance.
(2) The A450 values still follow a linear curve. This implies that a change in factor 2 of dilution will lead to a corresponding change in back predicted values by a factor 2.
(3) It was initially conjectured that some interesting biochemistry might be the cause for the errant results. However, it looks like the assay is proceeding as expected. The only cause for concern is that there is significant noise in the observations. This can be ameliorated to a certain extent by repeating an observation several times and taking the average of the observations.
(4) Every 1 microgram/ml of lysate contains 0.6668 ng/ml of antigen protein
(5) Use the same back prediction method as before. Take multiple observations and average the results in order to get a cleaner result.

Methods :
Step 1:
The Standard curve was plotted using pure anitgen protein at various levels of dilution. A Linear curve(straight line) was chosen for fitting this curve. A Linear curve has the property that a drop in factor 2 of input leads to a drop in factor 2 of output.
Consider
f(x) = mx (Linear curve normalized so that intercept = 0)
=> f(x/2) = mx/2 = f(x)/2

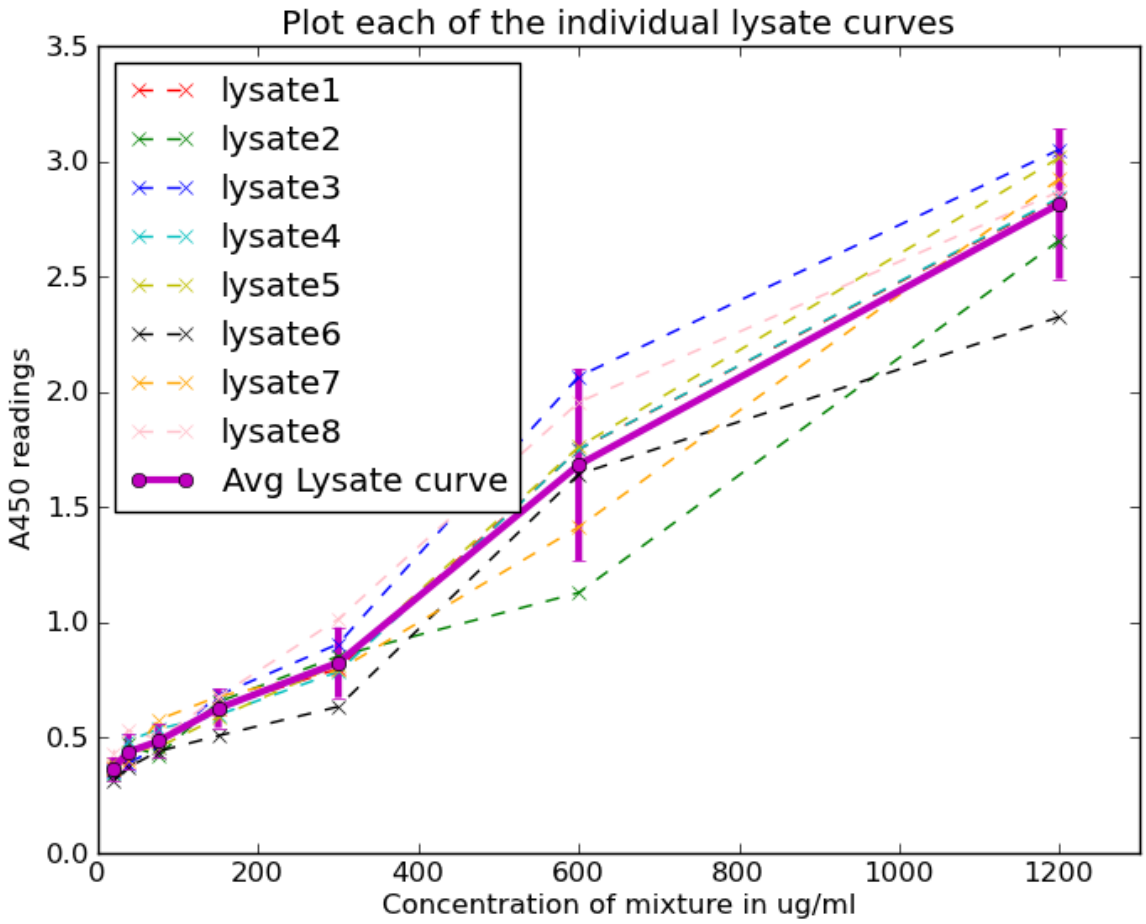
The following linear fit was obtained :

$$A450_{standard} = 0.003144 X_{stanard} + 0.28$$

This can be used to back predict the values of X_standard using the following equation:

$$X_{standard} = \frac{A450_{standard} - 0.28}{0.003144}$$

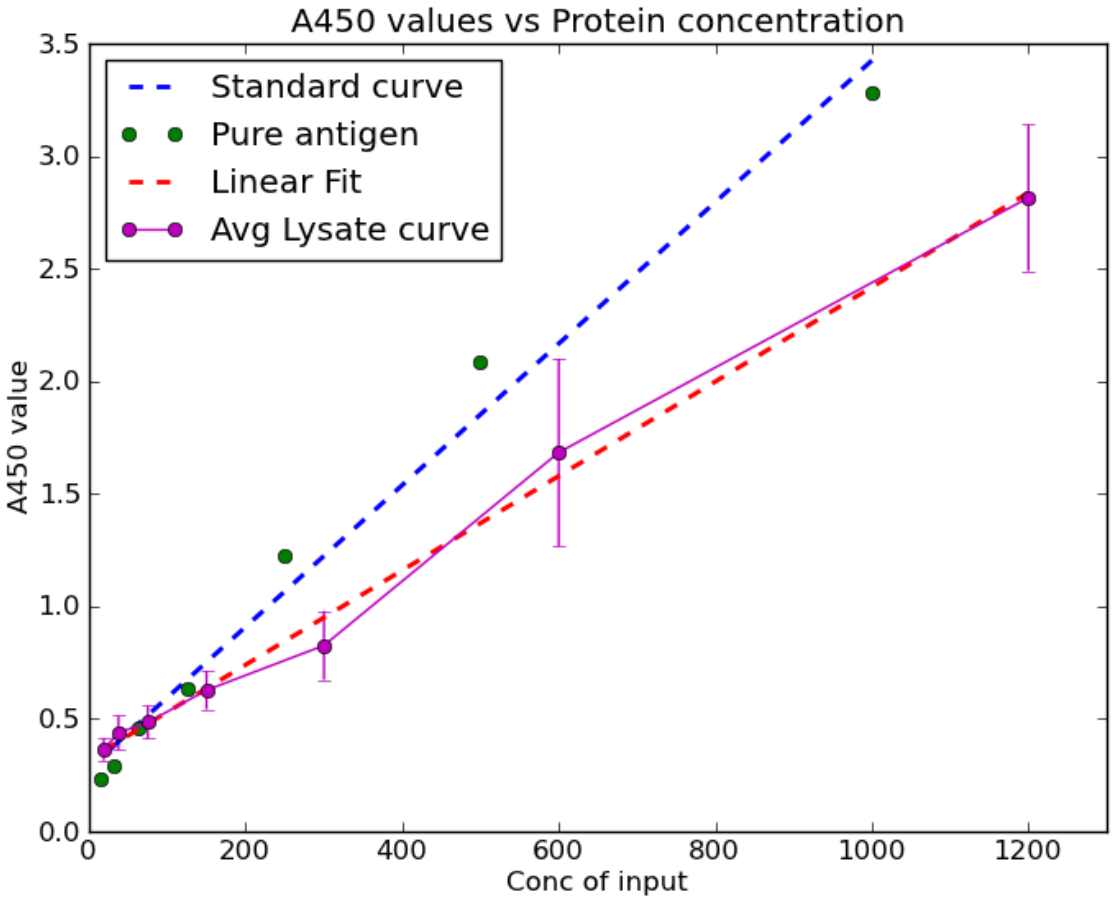
Step 2 :
The A450 values obtained for the samples was plotted and their trend was observed. It looks the curves might be slightly sigmoidal, but overall their effect looked linear when averaged.



Step 3 :
A Linear curve was fitted for the average of the lysate sample curves. The fit looks reasonable with an R2 value of 0.959.
The equation of this curve was found to be :

$$A450_{mixture} = 0.002096 X_{mixture} + 0.32$$

where X_mixture is the concentration of mixture in ug/ml



Step 4 :
The average values obtained from each of the samples was backpredicted using the following formula.

Average A450 value -
[2.815125 1.683625 0.82625   0.627625 0.485   0.438375 0.365125]

Back predicted values using linear curve :
[806.3374681933841, 446.44561068702285, 173.7436386768448, 110.56774809160304, 65.203562340966911, 50.373727735368952, 27.075381679389295]

$$X_{sample} = \frac{A450_{sample} - 0.28}{0.003144}$$

Back Predicted values using Non-Linear curve as computed by Gokhale:
[867.57525519843091, 438.98202192892319, 209.09826894955194, 161.54916428740262, 127.96196225065353, 117.01350777577919, 99.778189531322894]

$$X_{sample} = 971(\frac{-0.014 - 6.05}{A450_{sample} - 6.05} - 1)^{\frac{1}{1.19}}$$

Step 5 :
It is possible to determine what fraction of sample contains Anitgen protein by looking at the slope of the Linear curves

$$A450_{standard} = 0.003144X_{stanard} + 0.28$$
$$A450_{mixture} = 0.002096X_{mixture} + 0.32$$
$$Fraction_{antigen} = slope(A450_{standard}) \ / \ slope(A450_{mixture})/1000$$
$$= 0.6668^{*} \text{ 10e-3}$$

So in every 1000 ug/ml of sample there is 666 ng/ml of anitgen protein on an average.

Final Suggestions
(1) Average the results of multiple lysate experiments
(2) Use either the linear or the non-linear back prediction methods
(3) Operate at higher concentration range if possible since the Assay seems to have high relative error but lower absolute errors.
(4) Deliberate on the desired accuracy, this assay may be unsuitable if accuracy and sensitivity needs are stringent.