# Absorbance values >2, theoretically possible?

I've been boggled by this question in the topic ever since I got absorbance readings on an ELISA test kit >2, up to Absorbance units of
3.5 and so on.

I speculated how it could be possible, and with George's explanation came up with all sorts of theories that plate readers probably
correct for the light path etc.

In my mind, since the formula for absorbance is the following: Abs = 2 – log (%T), my thoughts were that it is impossible to have absorbance values more than 2.  Hence I thought Abs. should be discarded above 2.

I have however seen on this page,
which explained it quite well with a table, that it is indeed possible to
obtain absorbance values >2 if the light source is strong enough and the
spectrophotometer is sensitive enough to obtain accurate readings in this
range.

The theory is however, when the transmittance of <1% happens, log part of the formula (log%T), becomes a negative value. One thus *subtracts* a *minus,* theoretically making absorbances possible to indefinite values.

| Absorbance (optical density) | Transmittance % |
|---|---|
| 0 | 100 |
| 1 | 10 |

| | |
|---|---|
| 2 | 1 |
| 3 | 0.1 |
| 4 | 0.01 |
| 5 | 0.001 |
| 6 | 0.0001 |

*"At an absorbance of 6, only one 10,000$^{th}$ of one percent of a particular wavelength is being transmitted through the filter (lens). Absorbance is measured with a spectrophotometer, which establishes the light transmission and calculates the absorbance. However, the spectrophotometer can only measure absorbance up to 4.5 directly. Beyond this level all values must be extrapolated. For example, if a 2 mm thick filter is measured to have an absorbance of 3, then it is assumed that a 4 mm thick filter should have an absorbance of 6."*

Obviously there are still limitations to this and the general principle remains that absorbance units should be sought to be <1.8 (actually ideally now that I think of it <1.0) to make the standards and measurements more in the linear range (i.e. %Transmittance less than (100-10^10)=**<90**), for Abs. <1. I do think however that spectrophotometers (and plate readers in particular) these days are probably more sensitive than historically and hence one could go up a bit with the absorbance, given the understanding of the limitations regarding imprecision at these Absorbance levels.

One should understand that the absorbance >2 units does measure light intensity at a %Transmittance value between 99% and 100%, hence the room for error becomes exponentially bigger if the spectrophotometer's CV is not precise enough at these %T values.

Still to be revealed to me is the fact that absorbance values I obtained in Spectrophotometers and plate readers often did not correlate well, even when correcting for the light path

length, and I would
probably just need to read more to get proper clarity, or the path length
through the well in the plate reader was not accurately measured by me.
One way to correct for path length of water could be to measure the absorbance
of the water / solvent at 977nm (infrared; IR) and correct therefor, but most
specs we use don’t have IR measuring capabilities.

---

# Albumin Assay – Bromocresol Green method

Practical assay for albumin measurement

---

# Total Protein assay – Bradford

A practical experiment to illustrate the measurement of total protein in serum using the Bradford assay