The strength of a solution is amount of a substance dissolved in a diluent, or the CONCENTRATION.

Absorbance measurements are used to determine concentration. Beer's Law shows how:

Absorbance = abc

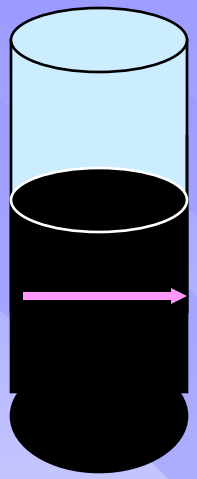
where a is an absorptivity constant unique to each material (this can be looked up in a table)
b is the distance that light travels through a sample (PATHLENGTH).
and c is the concentration.

When a and b are both fixed, absorbance is directly proportional to concentration.

Abs = abc
A is always fixed so Abs is proportional to bc.
When b is fixed, (horizontal photo-meter or flow cell) then Abs is proportional to c.

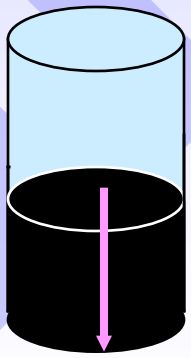
In vertical photo-Meters, b is related to volume and is Not fixed.

Pathlength Related to Vessel is Fixed.



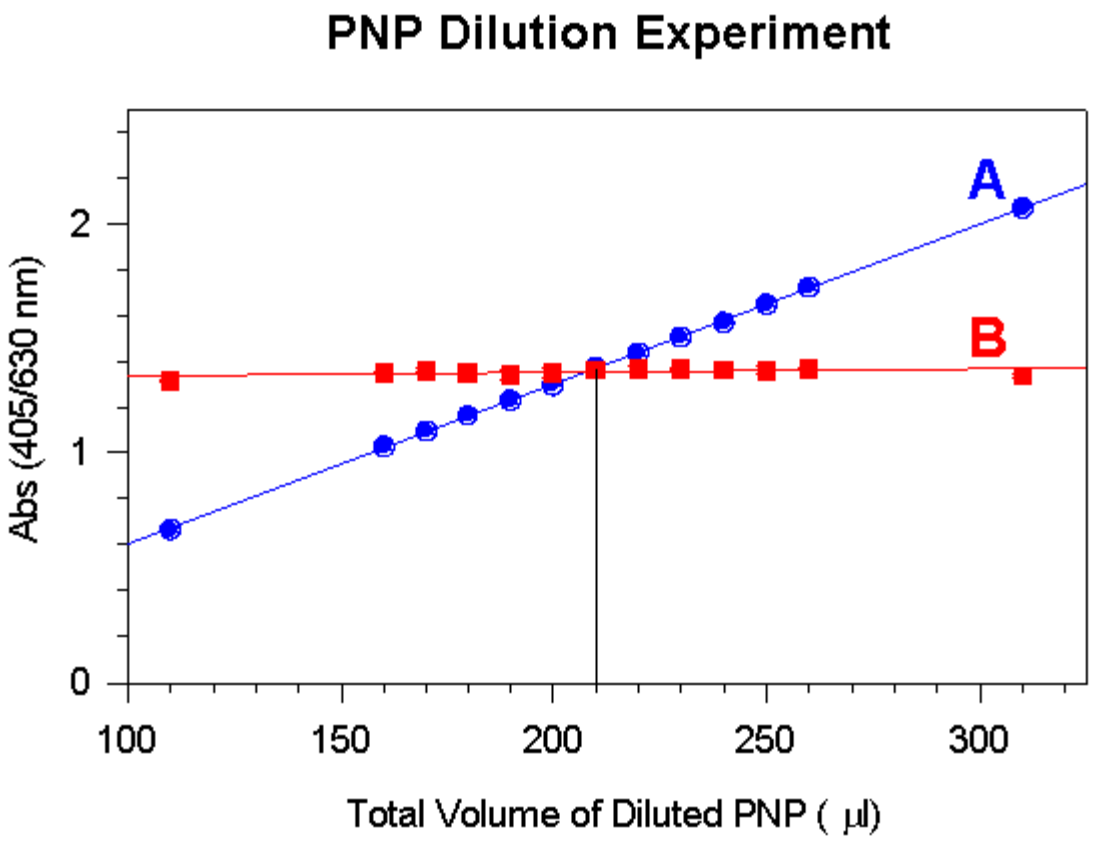
If concentration Changes, then Absorbance also Changes. (Evaporation, for example.)

Pathlength related to volume



In this case, Absorbance Readings are Volume-Dependent.

But if concentration changes are proportional to volume changes, such as in our example of evaporation, then absorbance remains unchanged.



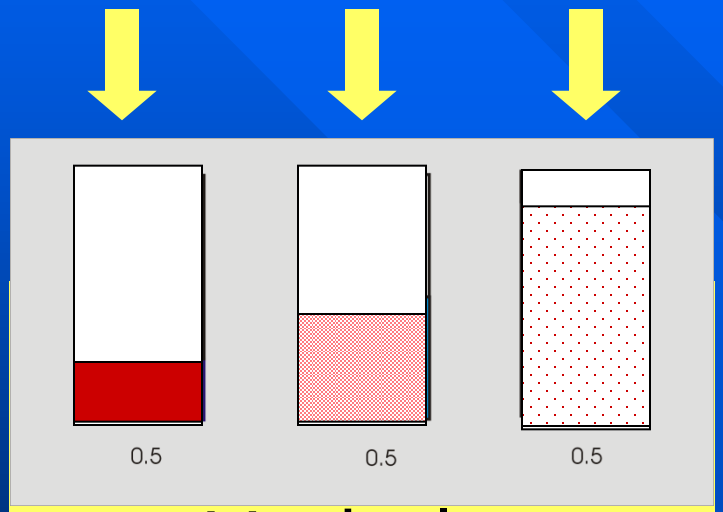
A) Diluted stock PNP solution 1:21 prior to dispensing. Variable volumes pipetted into wells.

B) 10 µl PNP stock solution pipetted into each well. Variable volumes of diluent pipetted into wells.

Absorbance depends on Pathlength and concentration

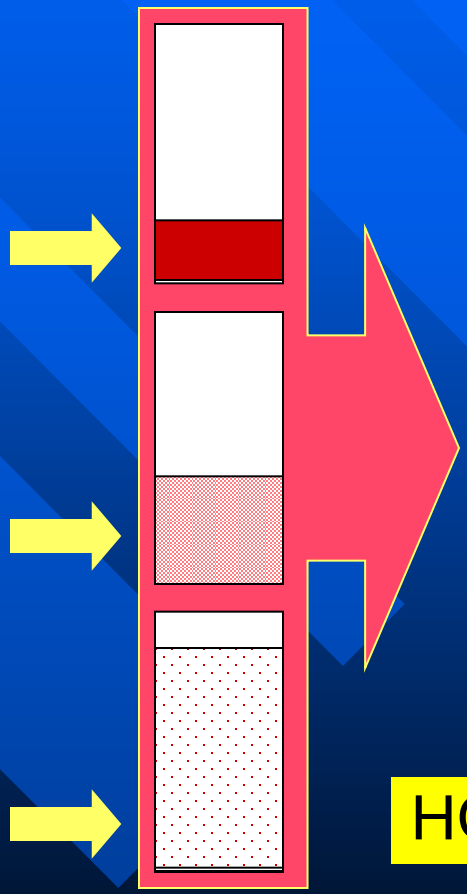
Color development depends on the analyte in the patient serum.

ABSORBANCE = (Є x CONCENTRATION) x PATHLENGTH



Vertical

Absorbances are All equal

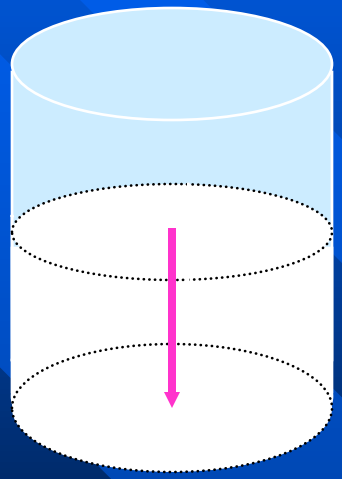


Absorbances are Not equal

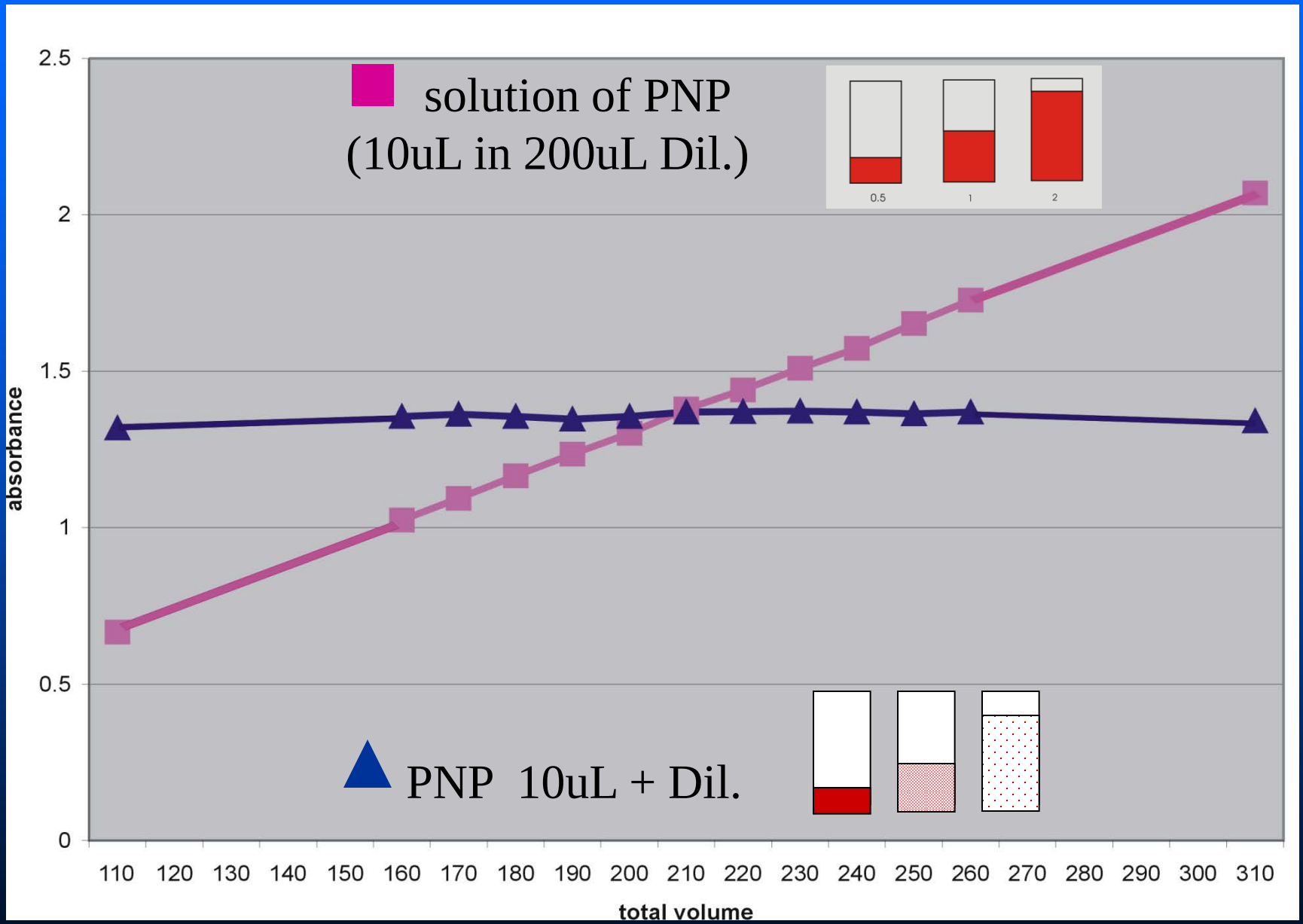
HORIZONTAL

Chem Well uses vertical photometry to remove the effects of reagent pipeting error and evaporation, thus enhancing precision despite low total volumes.

When pathlength is related to total volume,

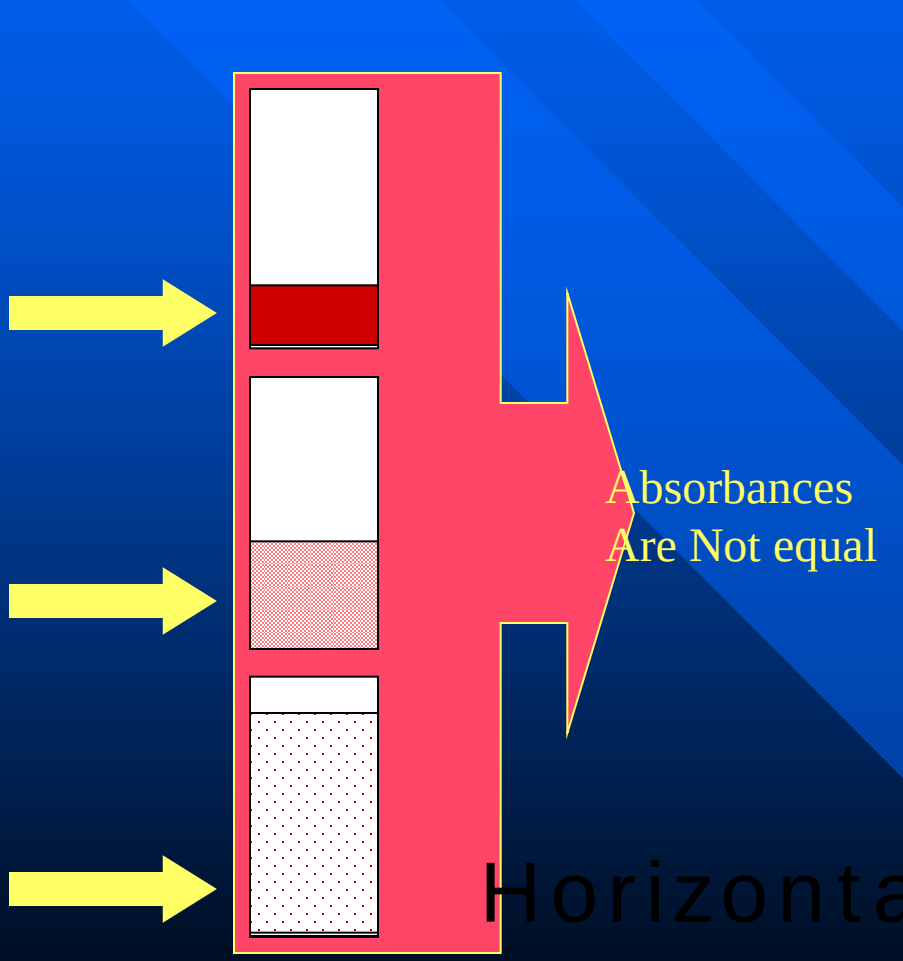


the system is self-correcting for evaporation and reagent pipeting variations.


solution of PNP
(10uL in 200uL Dil.)
0.5
1
2
PNP 10uL + Dil.
absorbance
2.5
2
1.5
1
0.5
0
110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310
total volume

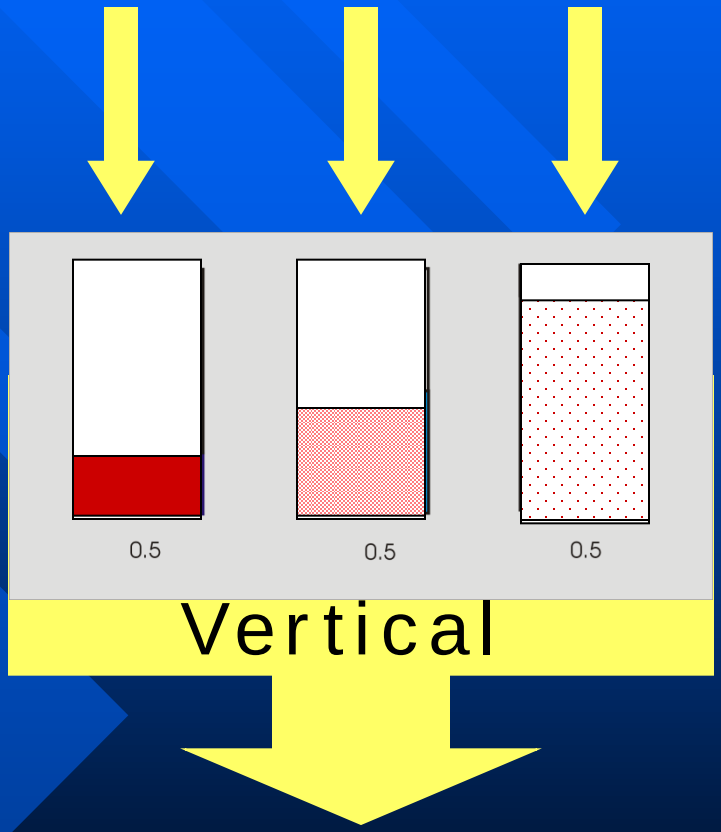
In clinical chemistry, concentration (color) is not based on reagent. Color development depends on the analyte in the patient serum.

For this case, vertical photometry is better because it is volume independent.



Absorbances
Are Not equal

Horizontal



Absorbances
Are All equal

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Absorbance depends on pathlength and concentration.

If the concentration (color development) depended on reagents,
then horizontal photometry would be better because its pathlength is fixed
and therefore absorbance is independent of reagent volume.

Here are 3 tubes.

Each contains a different volume of the same red-colored reagent.

Absorbance is measured horizontally and vertically.