**TG colorimetric assays** on samples (plasma/serum) from exp. 43 from Raghav

Samples were thawed on ice. I pulled samples from two test tubes together, pipetted up and down and then spun down in the cold room.

Before they were added to well of the plate, they were set to RT for around 4-5 min.

**TG COLORIMETRIC ASSAY**

1. Pipette amount of sample and standard into assigned wells.   
   30 ul of serum/plasma

|  |  |  |
| --- | --- | --- |
| SAMPLE/CALIBRANT | VOLUME ADDED TO THE WELL/uL | EXPECTED CONCENTRATION mg/dl |
| water | 4 | 0 |
| calibrant | 0.5 |  |
| calibrant | 1 |  |
| calibrant | 2 | 48 |
| calibrant | 4 | 96 |
| calibrant | 6 | 144 |
| calibrant | 8 | 192 (186.4) |
| calibrant | 12 | 288 (270) |
| calibrant | 16 | 384 (351) |
| Calibrant | 32 |  |

**The following samples’ volumes were used: 30, 30, 30 uL**

W - water

8 points + 22 samples = 30 x 3 = 90 wells

12 columns

Sample layout



1. Pipette 90ul **of reagent 1 (**Color A) into each well.  
   1080 uL  
   90 uL x 12  
   total: 8,7 = 9 mL

Mix the contents of the wells by gentle rotation

1. Incubate 5min at 37°C (room 353).
2. Measure absorbance at 600nm. This will serve as the blank.
3. Pipette 30ul of **reagent 2 (**Color B) into each well.  
   360 uL  
   30 uL x 12  
   total:2,8 = 3 mL

Mix the contents of the wells by gentle rotation

1. Incubate 5min at 37°C (room 353). Samples with high TG amounts will turn blue.
2. Measure absorbance at 600nm.
3. Calculate the final absorbance by subtracting the first (blank) measurement from the second (final).
4. Plot the final absorbance vs. concentration for the standard curve.
5. Determine sample TG measurements by plotting along the standard curve.

Results are in the Excel file:TG\_plasma\_from\_Raghav\_30\_30\_30uL\_raw

**Problem!**

**When a pipette samples in some wells I had bubbles.**

**Obraz zawierający w pomieszczeniu, prysznic, zasłona, wanna

Opis wygenerowany automatycznie**

**Some of the disappeared after I added reagent 2 to wells.**

**Obraz zawierający tekst

Opis wygenerowany automatycznie**

**In the results file I marked red those wells that had significant amount of bubbles while taking blank readings.**

**I marked yellow those well, in which bubbles seemed to disappear after adding reagent 2, so they were not present while taking the actual readings.**

In earlier experiments

Calibration curve absorbance range:

0.045 – 0.34

For 12 uL plasma the range of absorbance I got was: 0.01 -0.037

For 16 uL plasma the range was: 0.013 – 0.05