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**SAMPLE LAYOUT OF AN EXPERIMENT:
PROTEIN CONCENTRATION IN UNKNOWN BY MICROBIURET**

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From DBF's Hopkins Notebooks, III, p. 102 & VI, p. 75.

1. PLAN YOUR EXPERIMENT, WRITE OUT YOUR EXPERIMENT TABLE:

Calculate the dilutions of unknowns needed to bring their protein concentrations down to approximately 1-5 mg/mL. Plan two tubes per each diluted sample, one with 0.1 mL, the other with 1.0 mL. Calculate and record the amount of water required for each tube to *q.s.* to 2.0 mL. **Create a table** similar to one in the standardization with these nine columns:

_____	dilution	mL	sample	mL	mg protein	mg protein
_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____

Include a blank, as in the standardization procedure, and **standardization tubes** with 0.5 and 1.0 mg standard protein each.

2. PREPARE SAMPLE: DILUTE, SUSPEND OR DISSOLVE:

The final concentration of protein in the diluted samples should be between 1 to 5 mg/mL.

Solids: For a 1% suspension: weigh out 300-500 mg. Grind very fine in mortar and pestle. Add a few drops dH₂O, grind to paste, add few more drops, make slurry, wash grindings into graduated cylinder, *q.s.* to 100x weight with dH₂O (i.e., 30 mL for 300 mg solid).

Liquids: For concentrated fluids (egg white or yolk, blood, milk, etc) make a 1:50 dilution: add 0.1 mL to 4.9 dH₂O. Collect saliva in 10 mL beaker, dilute 1:5 (0.4 mL + 1.6 mL dH₂O). For dilute biological fluids like urine, use undiluted as a first approximation. Dilute protein-rich materials 200x, saliva 5x. Vortex thoroughly after the diluting.

3. SET UP TUBES, ADD dH₂O TO TUBES AS IN YOUR TABLE:

Set up the appropriate number of labeled, clean 13 x 100 mm test tubes in a rack (2 tubes/sample). Add the dH₂O first [*then* the protein sample, *finally* the microbiuret reagent].

4. ADD PROTEIN ALIQUOTS TO THE SET OF TUBES:

Carefully following your protocol table, add the prescribed amounts of standard protein to the standardization tubes, and 0.1 and 1.0 mL aliquots of diluted specimens to their tubes. Samples should always be added just below the surface of the water.

5. ADD 1 mL OF MICROBIURET TO EACH TUBE: Make a visual check to see that all tubes appear to have a identical final volume of 2 mL in them (water + sample). *Then* add 1.0 mL of microbiuret reagent by repipet, mix well by vortex, let sit for 15 min.

6. READ ABSORBENCY AT 325 nm: Use the B tube (containing no protein) as the blank, determine the A₃₂₅ of each tube in succession. You may not need to wash the cuvette between samples, but drain it thoroughly, touching off the last drop from the cuvette on a paper towel prior to adding the next specimen to minimize cross contamination.

7. CALCULATE THE PERCENT PROTEIN IN THE ORIGINAL SPECIMEN:

Calculate how much protein is in each tube using the conversion factor from the previous lab (A₃₂₅ of the specimen x conversion factor (C.F.)). Do the two standard tubes agree with the standardization? Calculate the concentration in the original sample:

$$\% \text{ protein} = (A_{325}) \times (\text{C.F. mg}/A_{325}) \times (1/\text{mL aliquot used}) \times (\text{dilution factor}) \times (\text{suspension factor})$$

examples:
$$\frac{A_{325}}{\text{conversion factor}} \times \text{aliquot fact} \times \text{Dil.F.} \times \text{Sus.Fac.}$$

$$\% \text{protein in saliva} = 0.052 \times (2.80 \text{ mg protein}/A_{325}) \times (1/0.3 \text{ mL}) \times 5 \times 1 = 2.43 \text{ mg/ml} = 0.24\%$$

$$\% \text{protein in dog food} = 0.144 \times (2.80 \text{ mg protein}/A_{325}) \times (1/0.1 \text{ mL}) \times 1 \times 100 = 400 \text{ mg/g} = 40\%$$

Prepare a table of data of the results from the entire class.