Nucleic Acid and Protein Concentration and Content: How to Measure & Why

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Quantitative methods for determination of RNA, DNA, and protein concentrations in tissues, organs, cell cultures and biochemical assay systems have many applications. Protein concentration estimates are required to determine yield and extent of purification in enzyme isolations and assays, and in protein purification protocols. Protein concentrations must be known to standardize loading of electrophoresis gels, to standardize enzyme activities, to characterize ligand binding data in receptor assays, and to assess changes or differences in growth of individual skeletal muscles, other tissues, and organs. Protein concentration estimates are also required to estimate carcass composition and composition of gain responses to experimental treatments or manipulations, and in measurement of tissue-specific fractional protein synthesis rates. Protein concentration measurements are employed as part of FSIS standards of identity compliance in the manufacture of processed meat products (i.e. PFF, moisture-protein ratio and water-added specifications), as well as in least cost formulation of products from several sources of raw materials. Some applications require quick, less sensitive methods for process management, while others must conform to "official" methods requirements.

Similarly, several applications of quantitative measures of RNA and DNA have evolved along with our improved understanding of the regulation of the cell cycle and rates of transcription and translation which lead to native or altered forms of important gene products. The emergence of molecular techniques used to study the regulation of gene expression, enumeration of specific microorganisms, and in investigations of mechanisms by which muscle and tissue growth are regulated require increased sensitivity, specificity, and use in liquid or solid phase formats.

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A single source of names, brief descriptions, and characteristics of these methods is lacking. Therefore, the objective of this reciprocation session was to share this information in concise form with AMSA members and meeting attendees, and to promote discussion on use of these methods. This summary of methods is produced with the objective of providing a practical list of commonly used techniques for total protein, RNA, and DNA concentration estimates in most mammalian tissues or combinations thereof. Methods described can be adopted with relative ease and confidence of good accuracy and precision being achieved if procedures are faithfully followed. Important considerations are mentioned to assist the reader in selection and application of the techniques. The summary does not provide protocol details for each method, nor is the list exhaustive; but it is intended to be used as a quick reference in a research or certified laboratory setting.

Protein Determination

Protein determination methods fall into two general classes: solution-based assays or immobilized or solid-support systems. These are listed and described as follows.

Solution-based Methods

1. **Spectrophotometric analysis**
   Description: Involves measurement of absorbance at 260 nm (corresponds to concentration of phenylalanine) and 280 nm (corresponds to concentrations of tryptophan and tyrosine) in comparison to some protein standard (i.e., bovine serum albumin [BSA]). Background correction (A_{280}-A_{320}) for particle scattering in solution can be employed. Values for A_{320} should be less than 0.02; otherwise presence of significant amounts of impurities in the sample is indicated. Protein mass required for this test: 0.05 to 2 mg.
   Advantages:
   a. Quick.
   b. Nondestructive.
   c. Less sensitive to protein structure variations than other methods.

Disadvantages:

a. Is protein specific, strictly depends on amino acid composition of the protein(s).
b. Only gives an estimate of the amount of aromatic groups in protein.
c. Interfered by UV-absorbing materials.

Reference: van Iersel, J., Jzn, J. F., and Duine, J. A. (1985) Anal. Biochem. 151. 196-204

2. **Kjeldahl digestion, distillation and titration method (macro or micro)**

Description: Involves measurement of total nitrogen present. Protein is digested, distilled, and borate ion titrated with standardized HCl. Total nitrogen can be converted to crude protein through a general or protein-specific factor (6.25 unless otherwise stated).

Advantages: Is the legal basis for determination of protein content of food products.

Disadvantages:

a. Is slow, involves use of hot and hazardous chemicals and equipment.
b. Requires very good analytical technique.
c. Requires conversion of nitrogen to protein and the conversion factor depends on amino acid composition of the protein or protein mixture (varies from protein to protein).
d. Complete digestion is difficult to achieve; recovery of tryptophan difficult.

Reference: AOAC Official Methods of Analysis (1995), 16th Ed., AOAC International, Arlington, VA.

3. **Colormetric analyses**

Biuret method

Description: Involves use of a copper complex in strong alkaline solution to measure number of peptide bonds present. The number of peptide bond is then converted to grams of protein by a conversion factor which can be determined by the amino acid composition or assumed to be 115 (the average amino acid residue weight). To avoid conversion of number of peptide bonds to weight of protein, a sample can be compared against some protein standard (i.e., BSA). Protein required for this test: 0.05 to 5 mg.

Advantages:

a. Low protein-to-protein variation.
b. Rapid color formation.

Disadvantages:

a. Interfered by lactose (binds to copper) or some other commonly used reagents that also produce colored products.
b. Destructive.
c. Low sensitivity.

Reference: Gornall, A. G., Bardawill, G. J., and David, M.M. (1949) J. Biol. Chem. 177,751-766.

Lowry method

Description: Involves protein quantitation from reaction of Folin-Ciocalteu phenol reagent with peptide bonds, tyrosine, and tryptophan by comparison to some standard protein. Requires 0.05 to 0.5 mg protein in the assay, is the most cited method in scientific literature.

Advantages: Is more sensitive than the Biuret method.

Disadvantages:

a. Is subject to much interference: detergents, denaturants, organic buffer, and thiols.
b. Destructive.
c. Slow color formation.

Reference: Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275.

Bicinchroninic acid method (BCA)

Description: A modification of the Lowry procedure that replaces the Folin-Ciocalteu phenol reagent with BCA. Reduced copper is chelated to two molecules of BCA, yielding a purple product with strong absorbance at 562 nm. Protein concentration is determined by comparison to some standard protein.

Advantages: More tolerant to interfering agents than Lowry.

Disadvantages: Remains sensitive to reducing agents and chelating agents.

Reference: Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, E. K., Fujimoto, E. K., Goeke, N. M., Olson, B. J., and Klenk, D.C. (1985) Anal. Biochem.150, 76-84.

Bradford method

Description: Uses Coomassie Brilliant Blue to form a dye-protein complex. Measurement of A_{595} is compares to the A_{595} of a standard protein. Requires 0.01 to 0.05 mg protein.

Advantages:

a. Faster than Lowry, rapid color formation.
b. More tolerant to chemical reagents in sample.
c. Very sensitive.

Disadvantages:

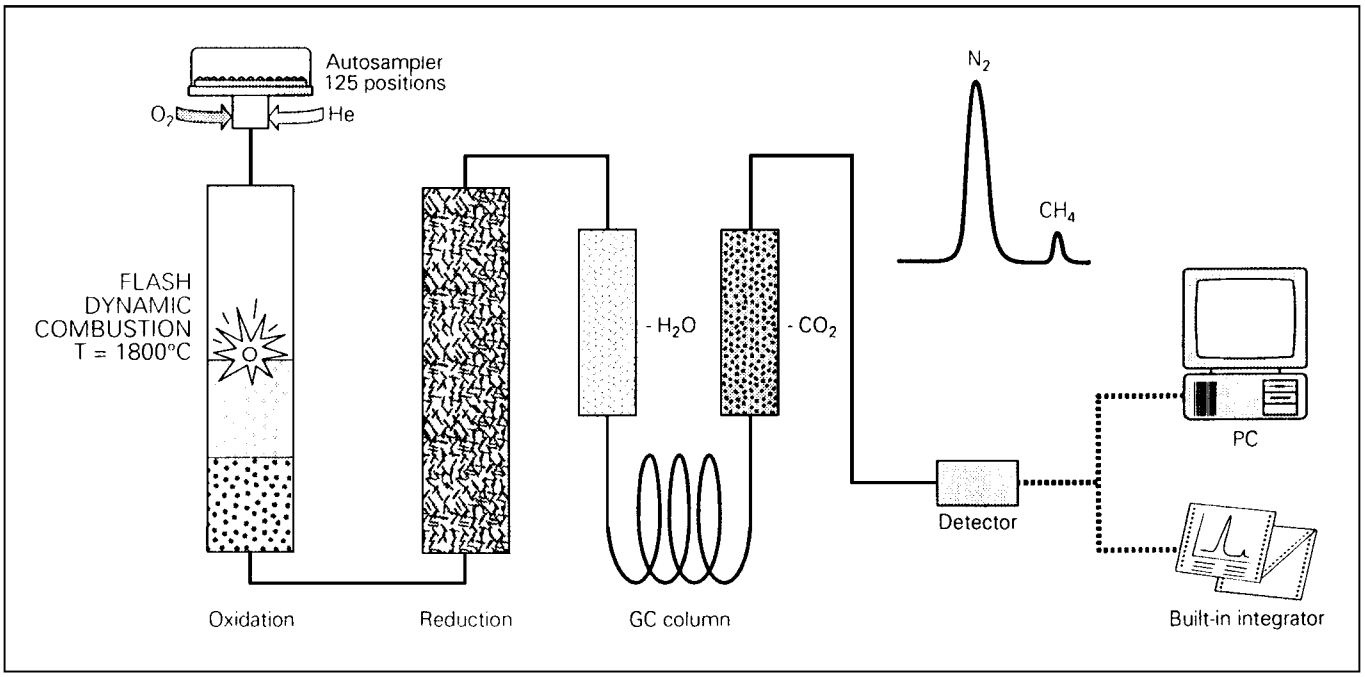
a. Is still interfered by glycerol, detergent, 2-mercaptoethanol, acetic acid, ammonium sulfate, Tris, and certain alkaline buffer.
b. Is more subject to protein-to-protein variation (dye binds to α- and ϵ-amino side-chains) than the Biuret and Lowry.
c. Destructive.
d. Dye adsorbs to glassware and to cuvettes.

Reference: Bradford, M. M. (1976) Anal. Biochem. 72, 255-260.

4. **Solid-phase protein assays**

Description: The protein is specifically bound to mem-

FIGURE 1.



Flow diagram of the nitrogen/protein configuration.

brane and contaminants are washed away. Protein is quantified by densitometry (protein bands in polyacrylamide gel) or spectrophotometry (protein eluted from membrane).

Advantages: Is less susceptible to interfering agents commonly found in samples than solution-based assays.

Example: Double-metal chelate stain (DMC assay, uses ferrozine and ferrous as chelating agents).

Reference: Patton, W., L. Lam, Q. Su, M. Lui, H. Erdjument-Bromage and P. Tempst. 1994. Metal chelates as reversible stains for detection of electroblotted proteins: application to protein microsequencing and immunoblotting. Anal. Biochem. 220:324-335.

5. **Gas combustion method**

Description: Uses 50 to 200 mg of finely ground (1.0 mm or smaller) sample that is combusted with oxygen at high temperature (≥ 950°C) followed by a reduction to liberate nitrogen. Excess oxygen is removed. The isolated nitrogen gas is measured by thermal conductivity (see figure on last page). Percentage of nitrogen or percent protein is displayed if a protein conversion factor has been supplied. Newer instruments (Leco FP 2000) can handle up to 4 g samples.

Advantages:

a. Commercial automatic analyzers are available (i.e., LECO FP-428, Nitrogen Determinator, LECO; NA2000 Nitrogen/Protein Analyzer, Fison—Figure 1).
b. Saves time; more efficient than Kjeldahl.
c. Safe, no caustic chemicals.
d. Minimal sample preparation required.
e. AOAC official method 992.15.

Disadvantages: Some units are limited to a small sample size (50 to 200 mg), but not all; requires very fine grind of non-homogenous samples to achieve a "good" representative sample.

Reference: AOAC Official Methods of Analysis (1995), 16th Ed., AOAC International, Arlington, VA.

Total DNA Estimation

1. **Perchloric acid (PCA) method**

Description: Pulverized frozen tissue or fresh tissue is homogenized in buffer on ice. Cold 0.2 N PCA is used to precipitate tissue protein, phospholipids, and nucleic acids. RNA is solubilized without degrading DNA by addition of hot 0.3 N NaOH and the DNA and protein is precipitated by addition of 2.3 N PCA. RNA is collected after centrifugation and DNA is then extracted from the DNA/protein pellet with hot 0.4 N PCA, cooled, and collected after centrifugation.

References: Schmidt, G. and Thannhauser, S.J. 1945. A method for determination of deoxyribonucleic acid and phosphoproteins in animal tissues. J. Biol. Chem. 161:83-89.

Fleck, A. and Begg, D., 1965. The estimation of ribonucleic acid using ultraviolet absorption measurements. Biochem. Biophysica. Acta 108:333.

Diphenylamine assay

Description: DNA is treated with strong hot acid and diphenylamines to yield products with absorption maximum near 600 nm. DNA standards (range from 0 to 120 ug/ml) are read at 260 nm. The concentration of the DNA standard is determined using the extinction coefficient in the following formula: mg DNA/ml = Absorbance (260)/26.0 x 3.0/ 0.1. Color development is enhanced with the addition of acetaldehyde.

Reference: Burton, K. 1956. A study of conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J. 62:315-323.

2. **Rapid preparation of genomic DNA from tissue**

Description: Use polytron to homogenize tissue; SDS, potassium acetate are used to denature protein. DNA is extracted with phenol/chloroform and chloroform alone, then precipitated by ethanol.

Advantages:

a. Requires minimum time and effort.
b. DNA produced is adequate for use as template for PCR or for restriction enzyme digest and DNA blot.

Disadvantages:

a. DNA is not for use in genomic library construction because the polytron shears large genomic DNA fragments.
b. OD_{260} will overestimate concentration of DNA due to substantial amount of RNA contamination. One should determine DNA concentration by fluorescence spectroscopy method.

Reference: Basic Methods in Molecular Biology. 1994. Leonard Davis, Michael Kuehl, and James Battey. 2nd Ed., Appleton and Lange, East Norwalk, CT.

3. **Preparation of high molecular weight genomic DNA from tissue**

Description: Frozen tissue is pulverized to powder and digested and solubilized by SDS and proteinase K. DNA is extracted with phenol/ chloroform and recovered by ethanol precipitation.

Advantages: Produces high molecular weight genomic DNA for genomic library formation, restriction enzyme digest, DNA blotting and use for PCR template.

Reference: Current Protocols in Molecular Biology. 1987. John Wiley and Sons, Inc.

DNA Quantitation

1. **Spectrophotometric analyses**

UV absorption

Description: Measurement of A260, A280, and A320 of purified sample.

DNA concentration (ug/ml) = OD_{260} reading x 50 x dilution factor.

A_{260}/A_{280} > 1.8: highly pure DNA.

A_{260}/A_{280} ~ 1.5: 50% protein/ 50% DNA.

A_{320} should be < 0.02, otherwise presence of contamination is indicated.

Advantages: Fast, simple procedure.

Disadvantages: More sensitive to contamination.

Reference: Current Protocols in Molecular Biology. 1987. John Wiley and Sons, Inc.

Fluorescence

Description: Involves use of fluorometry to measure DNA concentration through binding of DNA with a fluorescent dye (i.e., ethidium bromide). Read A_{458} and compare to A_{458} of DNA standards. Linear range: 1 to 15 ug/ml.

An improved rapid microfluorometric method using 33258 Hoechst fluorochrome was published in 1979 (Cesarone, C.F., C. Bolognesi and L. Santi, Analytical Biochemistry 100:188-197). This method has only very little interference by routinely used preparation reagents or cellular components, and can be used without purification steps in some cases.

Advantages:

a. Dye binds minimally to single stranded nucleic acids (especially RNA). Allows minor protein or RNA contamination in samples.
b. Useful when only small quantities of nucleic acid are available.

Reference: Current Protocols in Molecular Biology. 1987. John Wiley and Sons, Inc.

Total RNA Estimation

RNA Extraction

1. **Perchloric acid (PCA) method**

Description: Pulverized frozen tissue is homogenized. Cold 0.2 N PCA is used to precipitate tissue protein, phospholipids, and nucleic acids. RNA is solubilized by addition of hot NaOH; DNA and protein are precipitated by addition of 2.3 N PCA . Solubilized RNA is collected in the supernatant following centrifugation.

Reference: Schmidt, G. and Thannhauser, S.J. 1945. A method for determination of deoxyribonucleic acid and phosphoproteins in animal tissues. J. Biol. Chem. 161:83-89.

Orcinol assay

Description: Heating of RNA sample mixed with orcinol reagent produces a blue-green color. Absorbance is read at 600 nm. RNA standards (range from 0 to 2400 ug/ml) are read at 260 nm. The concentration of the RNA standard is determined using the extinction coefficient in the following formula: mg RNA/ml = Absorbance (260)/30.4 x 3.0/ 0.1.

Reference: Dische Z and Schwartz K. 1937. Mikromethode zur Bestimmung verschiedener Pentosen

nebeneinander bei Gegenwart von Hexosen. Microchim Acta. 2:13.
(or) Use the dual wavelength estimate: (39.1 x OD260) – (15.5 x OD_{232}) x ml extract x ml homogenate ÷ muscle sample weight.

2. **Single-step RNA isolation from tissue**
Description: Involves use of selective precipitation and(or) selective partition to separate RNA from others. Frozen tissue is homogenized in a denaturing solution containing guanidine thiocyanate (denature proteins/ribonucleases). The solution is mixed sequentially with sodium acetate, phenol, and chloroform/isoamyl alcohol. The mixture is centrifuged, yielding an upper aqueous phase containing RNA. Proteins and DNA remain in the interphase and organic phase.
Advantages: 1. Quick; can do large numbers of sample simultaneously.
   a. Permits recovery of total RNA from small quantities of tissue.
   b. Adequate for Northern blots or nuclease protection analyses.
Reference: Current Protocols in Molecular Biology. 1987. John Wiley and Sons, Inc.

3. **CsCl purification of RNA from tissue**
Description: Frozen tissue is homogenized in a guanidine solution without detergent. RNA is then separated from DNA and protein in a CsCl step-gradient due to its greater density after ultracentrifugation.
Advantages:
   a. Requires very little manipulation, thus increases the probability of producing intact RNA and reduce hands-on time for the experiment.
   b. Can simultaneously prepare RNA and DNA from a limited amount of tissue.
Disadvantages:
   a. Requires an ultracentrifuge, thus limiting the number of samples that can be processed simultaneously.
   b. Requires long centrifugation time (~16 hours).
Reference: Basic Methods in Molecular Biology. 1994. Leonard Davis, Michael Kuehl, and James Battey. 2nd Ed., Appleton and Lange, East Norwalk, CT.

RNA Quantitation

1. **Spectrophotometric measurement by UV absorption**
Description: Measurement of A_{260}, A_{280}, and A_{320} of purified sample.
RNA concentration (ug/ml) = OD_{260} reading x 40 x dilution factor.
A_{260}/A_{280} > 1.9: highly pure RNA.
A_{260}/A_{280} < 1.6: partially solubilized RNA.
A_{320} should be < 0.02, otherwise presence of contamination is indicated.
Advantages: Fast, simple procedure.
Disadvantages: More sensitive to contamination.
Reference: Current Protocols in Molecular Biology. 1987. John Wiley and Sons, Inc.

SUMMARY

Methods for determining the concentration and content of protein and nucleic acids were presented and discussed. In general methods were discussed based on their merits and limitations. One fairly new method for protein determination is the gas combustion method. This method appears to have some real advantages in terms of minimal labor requirements, automation, and reductions in the production of hazardous waste products. Caution was expressed about methods utilizing standard curves, in that, it is important to ensure that the standard used is appropriate for the material being measured. This is particularly important with protein analysis methods which estimate protein based on the prevalence of particular amino acid residues. It is important with these procedures to make sure that your standard is similar in composition to the proteins being measured. It is also important, when using standard curves, to account for the solubility of your standard. This is particularly important in DNA determinations where solubilization of the standard DNA may be incomplete.

In terms of the application of these measurements, it was pointed out that the measurement of protein and nucleic acids content and concentrations can give valuable information about the potential mechanism(s) for changes in muscle growth; however, it must be remembered that these measurements are still just indicators. It was shown that accretion of nucleic acids and protein contents of a muscle mimic accretion of the whole muscle, allowing these measures to be used as indicators of muscle accretion. There was also discussion on using ratios of these measurements to make assertions about the mechanism(s) for muscle growth. Protein:DNA ratios have frequently been referred to as an indicator of cell size and caution was expressed about using that specific terminology, rather it was suggested that protein:DNA be discussed in terms of the amount of protein being regulated by a given amount of DNA. Protein:DNA may indicate a change in cell hypertrophy but may not necessarily indicate the cause. One other interesting point of discussion was that growth of most proximal hindlimb muscles can be used individually to indicate changes in whole carcass muscle mass. It was also stated that the composition of these muscles, in particular the semitendinosus muscle, is a good indicator of whole carcass composition.