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This Week's Citation Classic®

Mans R J & Novelli G D. Measurement of the incorporation of radioactive amino acids into protein by a filter-paper disk method.
Arch. Biochem. Biophys. **94**:48-53, 1961.
[Biology Division, Oak Ridge National Laboratory, TN]

A rapid and sensitive method was described for the measurement of amino acid incorporation in cell-free systems. [The *SCI*® indicates that this paper has been cited in over 1,865 publications since 1961.]

Rusty J. Mans
Department of Biochemistry and Molecular Biology
J. Hillis Miller Health Center
University of Florida
Gainesville, FL 32610

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It was a surprise to learn that our article on the filter-paper disk method for measuring incorporation of amino acids into protein has been highly cited. Perhaps like many techniques this one arose as a consequence of both impatience and laziness. As a postdoctoral fellow in the Biology Division of Oak Ridge National Laboratory, I was put to the task of constructing a cell-free amino-acid-incorporating system from corn tissues by my mentor, G. David Novelli. What with scouring the east Tennessee countryside for mature ears of corn in late fall, long centrifugation schedules for the preparation of soluble and particulate components, and elaborate multicomponent reaction mixtures designed for assay of amino acid incorporation, I became distraught with using Phil Siekevitz's procedure for washing trichloroacetic acid precipitated proteins in a test tube.[1] Extensive loss of product upon repeated reprecipitation of the protein, inherent low efficiency of the incorporating system, limited availability of labeled amino acid of high specific activity, and my wife's evermounting complaints about too little time devoted to pressing family affairs drove us to seek shortcuts.

Harry Peck, as a member of the enzymology group at that time, was chasing sulfur atoms in the ADP sulfurylase system from *Clostridium* sp. and had an assay whereby he cut up paper chromatograms and dropped the pieces into vials bearing scintillation fluid to determine the level of either ^{32}P or ^{35}S directly in our newly acquired "tube, switch gear"-model Packard scintillation spectrometer. It was really at Harry's insistence that I attempted to modify the washup procedure by first precipitating the products of the amino-acid-incorporating system onto filter paper and then carrying the pieces of filter paper through the successive extraction, washing, and drying reagents.

Finding the method suitable for all the amino-acid-incorporating systems from an array of tissues being worked on in our laboratory as well as those of others, the method was then extended to other assays by simple modifications. We utilized the filter-paper disk method to follow the transfer of labeled amino acid from amino acyl tRNA into a polypeptide linkage in a ribosomal preparation.[2] I recall journeying to James Bonner's laboratory at the California Institute of Technology to show Max Bernstiel and RuChi Huang the filter-paper disk method if they in turn would show me how to harvest lots of plant seedling material and how to make active preparations of RNA polymerase. Seeing their assay involving the conversion of acid-soluble ribonucleotides into acid-insoluble material, it was immediately obvious that the procedure could be used for RNA polymerase assays.[3] Fred Bollum had for many years been assaying the incorporation of ^{32}P-labeled deoxynucleotides into acid-insoluble material by mammalian DNA polymerases on strips of filter paper, subsequently counting with a Geiger-Mueller tube.[4] We have utilized the filter-paper disk assay to monitor poly-A polymerase activities derived from eukaryotic tissues.[5]

Over the years we have learned a few "tricks" not usually mentioned in published accounts. It occurred to us one day to insert the pin off to the side of the filter-paper disk rather than in its center and thus reserve the center of the disk for sample application. When aliquots greater than 0.1 ml are required for sufficient counts, we simply stack two to five disks on one pin, apply the larger sample, and process the disks together through the washup. The lengthy extraction procedure enumerated for amino acid incorporation can be shortened considerably, depending, of course, upon the heterogeneity and composition of the extracts assayed. An abbreviated washup for the RNA polymerase assay is detailed in a more recent publication.[6]

I trust my colleagues have dreamed up more modifications of this procedure than I could even comprehend. It is gratifying to know that the method has been of some help to others in their efforts to resolve biochemical riddles in life's processes. However, I cannot let this opportunity pass without indicating that the greatest contribution of the filter-paper disk method probably befell the manufacturers of scintillation spectrometers, filter-paper disks, and stainless steel straight pins. (I don't think manufacturers of vials are too happy with the reuse of their product.) Oh yes, my wife and I have just celebrated our 33rd wedding anniversary—owing in some measure to the filter-paper disk method.

1. **Siekevitz P.** Uptake of radioactive alanine in vitro into the proteins of rat liver fractions. *J. Biol. Chem.* **195**:549-65, 1952. (Cited 730 times since 1955.)
2. **Mans R J, Purcell C M & Novelli G D.** The involvement of soluble ribonucleic acid in amino acid incorporation by a maize system. *J. Biol. Chem.* **239**:1762-8, 1964.
3. **Mans R J & Novelli G D.** Ribonucleotide incorporation by a soluble enzyme from maize. *Biochim. Biophys. Acta* **91**:186-8, 1964.
4. **Bollum F J.** Thermal conversion of nonpriming deoxyribonucleic acid to primer. *J. Biol. Chem.* **234**:2733-4, 1959. (Cited 510 times.)
5. **Mans R J & Huff N J.** Utilization of ribonucleic acid and deoxyoligomer primers for polyadenylic acid synthesis by adenosine triphosphate:polynucleotidylexotransferase from maize. *J. Biol. Chem.* **250**:367-8, 1975.
6. **Mans R J.** RNA polymerase in higher plants. (Laskin A I & Last J A, eds.) *Nucleic acid biosynthesis.* New York: Dekker, 1973. p. 93-125.