no signal in any cell types either in the absence (Panel C) or the presence (Panel D) of 0.3 M NaCl.

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Use of UV Methods for Measurement of Protein and Nucleic Acid Concentrations
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Measurement of absorbance at 260 nm is a well-established method for estimation of concentration of nucleic acids and oligonucleotides in solution. Measurement at more than one wavelength, e.g., 260 and 280 nm, from which a ratio can be determined, is a check of the validity of the A260 reading. It is also a means of estimating whether the nucleic acid is contaminated with protein or other materials (6).

Several colorimetric methods are commonly used for estimation of protein, but it is often as practical to estimate concentration simply by absorbance at 280 nm (1,4,8). Measurement also of A260, which for a pure protein should be lower than A280, helps validate the 280-nm reading. The A280/A260 ratio can, moreover, be used to check whether, if suspected, a protein solution contains nucleic acid, as originally proposed by Warburg and Christian (12). Based on their data and others¹, a formula for the estimation of protein in the possible presence of RNA was proposed by Layne (5) as mg protein/mL = 1.55A280 - 0.76A260.

One of the limitations of using this equation is that the specific absorption coefficient (ε280) for proteins varies in proportion to their contents of tyrosine and tryptophan (1,2,4). Harlow and Lane (4) suggest ε280 values for IgG and IgM of 1.35 and 1.2, respectively, as against 0.7 for bovine serum albumin (BSA) (1 mg/mL solutions), but also quote the equation without comment as to its accuracy. This communication describes how the coefficients in the above equation are derived and how, therefore, new coefficients can be calculated that may be more appropriate for specific situations.

Let the coefficient for the 280-nm reading equal P and that for 260 nm equal Q. If the A260/A280 ratio for RNA = 2.0, then a similar ratio for P/Q will result in any absorbance, due to RNA contained in the A280 and A260 readings, making no contribution to the value derived for mg protein/mL. However, the value of P has to be greater than the reciprocal of the specific absorption coefficient of the protein in order to compensate for the negative term in the equation. The general expressions determining P and Q are P = 1/(a280 - a260/r) and Q = 1/(a280 + r - a260), where r is the A260/A280 ratio for RNA and a260 and a280 are the specific absorption coefficients for the protein at the wavelengths specified. The general A280/A260 ratio for proteins is given as 1.75 (12), so the a260 can be replaced by a280/1.75. For a protein where a280 = 1.0, P is 1.40 and Q is 0.70. If serum albumin were the protein of interest, a280 = 0.70, P = 2.0 and Q = 1.0; and if IgG were the protein of interest, then P = 1.04 and Q = 0.52. The coefficients of 1.55 and 0.76 in the equation of Layne (5) envisage the case where ε280 is about 0.90. If one is dealing with an unusual protein, e.g., a mucopolysaccharide, then the A280/A260 ratio for the protein will be lower (5), and the values of P and Q are modified accordingly.

Protein concentrations can also be measured from their absorbance at 205 nm (9). Again, the a205 for different proteins varies in proportion to their tyrosine and tryptophan contents, but this problem is taken care of in the equation (incorrectly stated in Reference 4) mg protein/mL = A205/(27 + 120A205/A205). Another two-wavelength procedure has been suggested by Waddell (11) in which mg protein/mL = 0.144 (A215 - A225). Both of these methods and that of Groves et al. (3) make use of the much greater intensity of absorbance of proteins in the 205–225 nm range. This means that absorbance by contaminants is in principle likely to be less serious. The methods require high performance spectrophotometers and use of buffers that do not absorb at these wavelengths. For these reasons the methods are not commonly used.

Clearly there are potential complications in the use of absorbance readings, but the procedure is rapid and non-destructive. The Bradford, Lowry and bicinchoninic acid methods are more sensitive, but colorimetric methods are subject to interference by numerous compounds (10), and BSA that is widely used as a standard is no more suitable as a reference protein than for absorbance data (1,4,8,10).

Two-wavelength methods are also available for measurement of RNA and DNA contents of tissues after extraction, and they depend on the same principles as described above (7). One procedure for RNA, measuring absorbance at 260 and 232 nm, involves measurement at a wavelength (232 nm) where the rate of change of absorbance of protein and peptides with change in wavelength (dA/dλ) is quite exceptionally large (0.67/nm at 232 nm for an A280 of 1.0; Reference 7). This condition again puts a premium on the reliability and accuracy of the spectrophotometer.
When estimating the purity of RNA and DNA from the $A_{260}/A_{280}$ ratio, where there is also a high absorbance at 280 nm, the performance of the spectrophotometer can be checked by use of standards (6). For estimation of tissue contents of RNA and DNA by the two-wavelength methods, a check of the accuracy of the instrument to be used is essential.

All methods have their individual limitations and should only be used with understanding.

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Reusing the Same Bloodstained Punch for Sequential DNA Amplifications and Typing

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Bloodstain cards, which are commonly known as Guthrie cards, are routinely utilized as a solid medium for the long-term storage of blood (3). The Department of Defense has established a repository to store bloodstain cards as a reference source for DNA to aid in the identification of casualty remains. Collected blood is placed on a card, dried, sealed with a desiccant and stored at -20°C. When a DNA reference is required for a polymerase chain reaction (PCR)-based DNA typing, a 0.5-cm punch is taken from the card, and the DNA is removed from the punch by either an organic extraction (i.e., phenol) or a chelating resin (i.e., Chelex®). These extractions have traditionally been time-consuming and tedious since the techniques require either long incubation times, vortex mixing and/or centrifugation.

Recently, we have described a fast, inexpensive protocol for the purification of DNA from the bloodstain card (2). This modified organic method removes inhibitors of PCR from the punch, but the DNA remains associated with the punch for direct amplification. Since there are no vortex mixing and centrifugation steps, this method can be automated using a robotic workstation (2). One concern about this method, however, was that if multiple tests were desired from a particular sample, then multiple punches would have to be taken from the card, and the purification procedure would have to be performed again. In this paper, we have demonstrated that this is not the case, since the same extracted bloodstain punch can be reused for consecutive amplifications.

Blood samples were collected from the laboratory staff. FTA923™ bloodstain cards (Fitzco, Mapleplain, MN, USA) were spotted with whole blood and allowed to dry. All samples were previously typed by validated methods using the AmpliType® HLA-DQα, AmpliType Polymarker and AmpFLP™ D1S80 amplification and typing kits, all from Perkin-Elmer (Norwalk, CT, USA). Schleicher & Schuell 903™ bloodstain cards (Schleicher & Schuell, Keene, NH, USA) can be substituted.

For sample processing, a 0.5-cm punch was taken from the bloodstain card and placed in either a 96-well microplate for automated purification (2) using a Biomek™ 1000 Robotic Workstation (Beckman Instruments, Fullerton, CA, USA) or a 1.7-mL microcentrifuge tube for performing the purification manually. For the manual method, the samples were incubated for 30 min in 500 µL of buffered phenol (Life Technologies, Gaithersburg, MD, USA), washed 3 times with 500 µL