Differential staining of neuronal and glial nuclei

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The histological differentiation of neuronal from glial nuclei may be useful in the identification of tumours and in the study of developing nervous tissues. The following method does this by staining the glial nucleus red and the neuronal nucleus green. It also stains other structures in the neural parenchyma. Formol-fixed tissues taken down to water and embedded in paraffin are used.

1 The sections (10μ) are placed for 10 minutes in 1% brilliant crystal scarlet (Ponceau R or Xyledine) solution made up with 1% acetic acid diluted in distilled water.
2 Washed in distilled water
3 Five minutes in 0·5% phosphotungstic acid
4 Washed briefly in distilled water
5 Stained 30 seconds to one minute in 1% methyl green (made with distilled water)
6 Washed in distilled water
7 Dehydrate, clear, and mount in DPX

The glial nucleus stains red, neuronal nucleus green, the nucleolus red, myelin red, axons green, red blood cells red, and connective tissue red.

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An immunological method for the detection and estimation of fetal haemoglobin—continued

Chernoff, A. I. (1953). Immunological studies of haemoglobins. II. Quantitative precipitin test using anti-fetal haemoglobin. *Blood*, **8**, 413-421.

Kohn, J. (1970). Method for the detection and identification of alpha-fetoprotein in serum. *J. clin. Path.*, **23**, 733-735.

Mancini, G., Vaerman, J. P., Carbonara, A. O., and Heremans, J. F. (1963). A single-radial-immunodiffusion method for the immunological quantitation of proteins. *Prot. biol. Fluids*, **11**, 370.

Improved sensitivity of the electrophoresis method by tannic acid for detection of Australia antigen

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For detection of Australia antigen (Au-Ag) by counter-immunoelectroosmophoresis (CIEOP) staining the agarose gel plates with certain dyes has been claimed to improve the sensitivity (Combridge and Shaw, 1971). In our experience, however, a simpler and less time-consuming procedure is that of layering the gel plates with 1% freshly made tannic for 10 minutes (Alpert, Munroe, and Schur, 1970) after the routine CIEOP procedure (Das, Hopkins, Cash, and Cumming, 1971). This has resulted in a significantly increased sensitivity by improving visualization of precipitin lines.

Serial dilutions of Au-Ag containing serum and anti-Au (human origin) were set up in the test system using a 'chessboard' design. After the electrophoresis 'run' the gel plates were observed at an angle under direct light over a dark background. The results were scored as + for sharp precipitin lines, ± for weak precipitation, and − for no reaction. Table I shows that the titre of Au-Ag against the neat antiserum was 1/4, and no significant improvement was noticed when the same plate was reviewed after overnight incubation. Tannic acid was now added and the plate read after 10 minutes: the titre was now 1/16. This improvement reflects an increased sensitivity of the system as a whole; thus before tannic acid treatment, the total number of

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Au-Ag Dilutions	*Counter Immunoelectrophoresis Routine*				*Overnight Incubation*				*After Tannic Acid*			
	Anti-Au Dilutions											
	Neat		*1/4*		*Neat*		*1/4*		*Neat*		*1/4*	
Neat	···	−	+	+	−	·	−	+	+	·	+	+
	··	··	··	··	−	−	+	+	+	−	+	+
1/4	··	··	−	··	−	·	·	+	··	··	+	+
	−	−	−	··	−	−	··	+	··	··	+	+
1/16	−	−	−	−	−	−	−	−	±	±	±	+
	−	−	−	−	−	−	−	−	−	−	−	±
	Total positive		14				14				21	

Table I *Results on serial dilutions of Au-Ag and Anti-Au tested in a 'chessboard' fashion before and after tannic acid treatment*