

Alkaline Congo Red Method (Puchtler et al)

Purpose

The demonstration of amyloid in tissues.

Principle

Green birefringence following Congo red staining is considered the most specific technique for the demonstration of amyloid. However, false-positive results may be obtained, and the method used is of the utmost importance. Congo red is a benzidine derivative that can react with cellulose; Virchow regarded amyloid as an isomer of cellulose, and amyloid resembles cellulose in its chemical reactions (Vacca). In this method, pretreatment with alkali aids in the release of native internal hydrogen bonds between adjacent protein chains; as a result more potential sites are available for dye binding. Congo red is a linear molecule, and this configuration allows azo and amine groups of the dye to form hydrogen bonds with similarly spaced hydroxyl radicals of the amyloid. Whether the binding of the dye occurs with the polysaccharide or the protein component of amyloid is indefinite at this time.

Fixative

Alcohol or Carnoy solution is preferred: 10% neutral-buffered formalin, Bouin solution, or Zenker solution may be used. Prolonged storage in 10% formalin will cause a gradual decrease in staining intensity.

Equipment

Mechanical stirrer, Coplin jars, filter paper, Erlenmeyer flasks, graduated cylinders.

Technique

Cut paraffin sections at 8 to 10 μ m. Sections not in this range may not show the green birefringence.

Quality Control

Sections containing amyloid must be used. According to Bancroft and Stevens, it is better not to keep too many control sections cut, as the staining intensity has been reported to decrease with the age of the sections. Also, massive, presumably longstanding deposits give less intense histochemical reactions than small, newly formed deposits.

Reagents

Stock 80% Alcohol Saturated with Sodium Chloride

Sodium Chloride	20 g
Distilled water	.5 mL

Stir until the salt is dissolved; then, with continued stirring on a magnetic stirrer, add:

Ethyl alcohol, 100%	800 mL
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*Some salt should precipitate out, indicating that the solution is saturated. This method ensures a saturated solution (Lillie and Fullmer).

Alkaline Salt Solution

Stock 80%

Stock Congo Red Staining Solution

Working Congo Red Staining Solution

Procedure

Results

Notes