



NATIONAL SOCIETY
FOR
HISTOTECHNOLOGY

**PREPARATION OF
TECHNICAL PROCEDURE MANUALS
IN A HISTOLOGY LABORATORY**

According to
NCCLS' *Clinical Laboratory Technical Procedure Manuals-Second Edition;*
Approved Guideline (GP2-A3, December 1996)

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This publication was designed by the Quality Control Committee as a guideline for preparing procedure manuals for the Histology Laboratory in accordance with the NCCLS Clinical Laboratory Technical Procedure Manuals, Third Edition (GPA2-A3) (1996).

Ethel Macrea, Chairman
Quality Control Committee

Acknowledgement from original version:

I am very grateful to the members of the 1990-1992 Quality Control Committee for their assistance in the productions of these guidelines.

I am particularly indebted to the following NSH members for their expert advise, encouragement and support: Joyce Eaton, Ada Feldman, Jerry Meade, Susan Meloan and Dr. Holde Puchtler.

It is my hope that these guidelines will offer to MSH members some realistic and workable recommendations to follow as they institute or revise the technical procedure manuals in their own laboratories.

Rhonda Rogers, Chairman (1988-1996)
Quality Control Committee

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INTRODUCTION

The intention of these Guidelines is to facilitate the development planning, maintenance, and utilization of histology laboratory technical procedure manuals written to conform with the standards published by the National Committee for Clinical Laboratory Standards (NCCLS) in *Clinical Laboratory Technical Procedure Manuals-Second Edition; Approved Guidelines (GP2-A3) (1996)*

The National Committee for Clinical Laboratory Standards defines itself as “a nonprofit, educational organization that provides a communication forum for recommending, evaluating, and implementing standards and guidelines designed to support the delivery of high quality patient care.” Representatives from the professional sector, government, and industry comprise the active membership of NCCLS. NSH is a member organization and participates through a delegated representative. The standards and guidelines result from the work of committee members who are knowledgeable in the subject and are published to provide dependable, sensible standards for laboratorians to follow in their daily work practices. NCCLS may be contacted at the following address:

National Committee for Clinical Laboratory Standards
Suite 1400
940 West Valley Road
Wayne, PA 19087

PHONE: (610) 688-0100
FAX: (610)-688-6400

Many federal and state agencies require written procedures and policies for licensure or accreditation. Laboratories accredited by the College of American Pathologists (CAP) are required to have procedure manuals “written in substantial compliance and meet the intent of NCCLS (GP2-A3) (1996) without having to precisely copy it.”

CAP specifies that the procedures manual should be available to, and used by personnel at the workbench and must include: Principle, clinical significance, specimen type, required reagents, calibration, quality control, procedural steps, calculations, reference ranges, and interpretation. Documentation of initial and annual reviews by the director (or technical supervisor, if designated by the director) is required.

The following is taken direct from the 1997.0 Edition of the CAP's Inspection Checklist section for Surgical Pathology Quality Control:

PROCEDURE MANUAL			
	PHASE	QUESTION NUMBER	CIRCLE ONE
Is a complete procedure manual written substantially in compliance with National Committee for Clinical Laboratory Standards (NCCLS) GP2-A2* available at the workbench or in the work area?	II	08.2000	N/A YES NO
<p>NOTE 1: Substantial compliance with NCCLS GP2-A2 (1992)* means that the components of that document are, where appropriate, included in the procedure manual. The format does not have to be identical to NCCLS GP2-A2 (1992)*.</p> <p>NOTE 2: The use of inserts provided by manufacturers is not acceptable in place of a procedure manual. However, such inserts may be used as part of a procedures description, if the insert is written in substantial compliance with NCCLS GP2-A2 (1992)*and describes the procedure as performed in the laboratory. Any variation from this printed procedure must be detailed in the procedure manual. In all cases, appropriate reviews must occur.</p> <p>NOTE 3: A manufacturer's procedure manual for an instrument/reagent system that complies with NCCLS GP2-A2 (1991)* may be acceptable as a component of the overall departmental procedures. Any modification to or deviation from the procedure manual, must be clearly documented.</p> <p>A. A complete manual is available for reference. B. The card file or similar system corresponds to the complete manual.</p>			
Is a copy of NCCLS GP2-A2 available to the person responsible for the preparation of the procedure manual?	II	08.2005	N/A YES NO
<p>NOTE: This does not imply that the histology laboratory must have its own copy of that document. One copy for each accredited laboratory is sufficient.</p>			

Both of the above questions are Phase II deficiencies. Accreditation is denied or revoked by CAP when a laboratory fails to correct Phased II deficiencies and does not meet the required Standards.

In addition to satisfying the requirements of various regulatory agencies, compliance with NCCLS (GP20A3) (1996) will help produce uniformly consistent manuals that will augment existing Quality Control and Total Quality Management/Continuous Quality Improvement (TQM/CQI) programs; assist in orientation and instruction of new personnel and students; enable experienced laboratorians to perform an unfamiliar procedure with a minimum of supervision; and facilitate maintenance or revision of procedures.

The following is not intended to take the place of NCCLS (GP2-A3) (1996), but is designed to complement, expand, illustrate, and serve as a reference on how to employ the NCCLS Guidelines to design, prepare, and maintain a manual for practical, everyday use in a histology laboratory. Suggestions, ideas, and example should be modified so that each laboratory's individual requirements are satisfied.

PROCEDURE MANUAL DESIGN

Each laboratory should determine the form that its technical procedure manual or manuals shall take. This is usually best resolved by considering how the laboratory is organized and what needs are to be met.

Large laboratories may require several manuals and small laboratories may need only one manual. For example, an all encompassing manual which includes every procedure done within the entire laboratory may be sufficient for some laboratories. Others may require a number of manuals corresponding to the different divisions within the laboratory such as special stains, immunohistochemistry, or processing.

The style must be uniform for each procedure and once it is established, the style must remain constant for **each** procedure and **all** manuals throughout the laboratory. For instance, no matter how many different manuals there are in the laboratory, all procedures should appear similar on layout or design. Continuous use of the same margins, print font, and editorial style will help maintain consistency.

NCCLS requires uniform for **each procedure** includes the following:

1. Placement of the procedure adoption date (month and year) at the top of the first page.
2. Placement of the page number and total number of pages at the top or bottom of each page.
3. Notation of the author or preparer of the procedure and the initial approving authority.

4. Date and noted corrections in the review area.
5. Notation if the procedure replaces an earlier one.

NCCLS recommends organization of manuals into loose-leaf notebooks to facilitate changes and rearrangements. Tabs and tables of contents should be used to aid in location and organization procedures. A numbering system should be devised that will ease access to the procedures.

Electronic word processing systems are ideal for preparing and storing procedures. These systems help facilitate editing, revising, and amending.

Index card systems, flow-charts, and manufacturer inserts may be used **only** to supplement and complement written procedures. They **cannot replace** written procedures.

Procedures should be written clearly, precisely, and specifically for use at the bench level. Directions must be straightforward and easy to follow. All procedures should be complete, comprehensive, and detailed.

SOURCES

Sources may be consulted to prepare the procedures. These sources should contribute helpful information to the manual, but should not be used as a substitute. NCCLS approved sources include:

1. scientific journals
2. manufacturer product literature
3. textbooks
4. standard publications
5. research and validation data
6. written personal communications
7. workshops and seminars

COMTENT OF PROCEDURES

NCCLS (GP2-A3) (1996), Section 2.1, states that each technical procedure must include explicit information and unequivocal instructions in the following areas:

1. Principle of the test (summarized); clinical application and/or usefulness may be included
2. Specimen required and collection method; any special patient preparation or restriction
3. Reagents, standards, controls, and media used, as well as special supplies
4. Instrumentation, including calibration protocols and schedules
5. Ste-by-step directions
6. Calculations
7. Frequency and tolerance of controls; corrective action to be taken of tolerances are exceeded
8. Expected values; values requiring special notification; and interpretation of values
9. Procedure notes (e.g., linearly or detention limits)
10. Limitations of method (e.g., interfering substances and/or pitfalls and precautions)
11. Method validation
12. References
13. Effective date and schedule for review
14. Distribution
15. Author

Section 5.1.11 further limits or defines histotechnology that Qualitative Testing Procedures shall include:

1. Title
2. Principle
3. Collection and Submission of Specimens
4. Quality Control
5. Reagents, Controls, Equipment
6. Stepwise Procedure
7. Results and Interpretation
8. Procedure Notes
9. Limitations/Interferences
10. References
11. Distribution
12. Author/Source

The guidelines are meant to be flexible and adaptable so that the finished procedures constitute a manual that is practical, realistic, and workable. However, when written technical procedures, an attempt should be made to stay within the guidelines and to include as many of the above areas as possible in each procedure.

TITLE:

The first word in the title should be the most significant or important word in the title. For example, it should be the name of the analyte or substance for which the testing (staining) is being done.

A logical, specific title will simplify retrieval and indexing the procedures. Specific methods may be used as a subtitle. The name of the instrument being used is also appropriate under certain circumstances.

Example: Fungus – Grocott's Methenamine Silver

Not: Grocott's Methenamine Silver for Fungus

Example: Automatic H&E Stainer – Daily Maintenance

Not: Daily Maintenance of Automatic H&E Stainer

Example: Specimen Numbering System

Not: How to Number Specimens

PRINCIPLE:

The principle should be written in paragraph form. It should be brief and to the point. The statement should incorporate the clinical reason for performing the procedure and should include chemical reactions involved in tests (stains).

Example: (from Acid Fast – Kinyoun's)

PRINCIPLE:

Mycobacterium, including *M. tuberculosis* and certain atypical mycobacteria, are pathogenic in humans. AIDS and other immunosuppressed patients are particularly vulnerable.

These rod-shaped organisms may be demonstrated due to the "acid fast" property of their cell walls. Carbol-fuchsin is applied to the tissue. At this point all tissue elements stain red. The tissue is then rinsed in acid alcohol which decolorizes all tissue elements except the "acid-fast" ones which remain red. The "acid-fastness" of *Mycobacterium* is probably due to the selective permeability of the cell wall or the lipid content of the cell wall.

Example: (from Fixation of Tissue Specimens)

PRINCIPLE:

Fixation is the first and probably most important step in the preparation of tissue sections for diagnosis. Fixation stabilizes the tissue proteins to inhibit autolysis, halts decay from bacteria, prevents distortion of tissue elements during subsequent processing, brings out the differences in the refractive index of tissues, and influences how tissues react with stains. A wide variety of fixatives are available for use in histology. The choice of which fixative to use depends on which tissue element is to be demonstrated.

SPECIMEN:

All instructions regarding the specimen should be noted in this section. This may include the following:

1. Preparation

- a. State if the specimen should be fixed in a particular fixative, kept fresh, frozen, or placed in a holding media.

Example: Specimen must be fixed in an alcohol based fixative, such as Carnoy's.

- b. State how thick the section should be sectioned.

Example: Cut paraffin sections at 5 microns.

2. Specimen Requirements

- a. Specify the particular type of specimen required.

Example: Peripheral blood or bone marrow smears are required. Blood may be collected in heparin.

- b. Define both the minimum and optimum amounts of specimen needed.

Example: Optimum amount of tumor required is 1.0 g. A minimum amount of 0.5 g will be accepted.

- c. Describe approved collection containers.

Example: The tissue must be collected in a sterile container.

- d. State the stability of the specimen and the storage requirements.

Example: Specimen may be stored indefinitely at 70° C or lower.

- e. Explain the standards for unacceptable specimens and what action will be taken by the laboratory.

Example: Only fresh, unfixed specimens will be accepted for Hormone Receptor Assay. Formalin fixed specimens will be processed in the usual manner and immunohistochemistry for Estrogen Receptors (ERICA) will be performed.

f. List any other characteristic of the specimen that may interfere with results.

Example: Calcified specimens that are overexposed to the acid in the decal solution may exhibit light nuclear staining.

3. Special Handling

a. Specify any unusual timing considerations.

Example: Specimen must be received in the laboratory within 15 minutes of removal from the patient.

b. Define any special transport or storage conditions.

Example: Place in 70% alcohol following adequate fixation.

c. Indicate any unique special equipment required.

Example: Pick sections up on silane coated slides.

d. Explain precautions to be taken with biohazard materials.

Example: Gloves and a face shield must be worn while working with fresh tissue in the cryostat.

REAGENTS – SPECIAL SUPPLIES AND EQUIPMENT:

1. All reagents, supplies, and equipment used in the procedure should be recorded. They should be listed in order of use. The following details should be incorporated:

- a. Whenever possible, state both the name and formula of the chemicals required.

Example: Sodium Chloride (NaCl)
or
Sodium Sulfate, Anhydrous (Na₂SO₄)

- b. Designate the color index number for a dye.

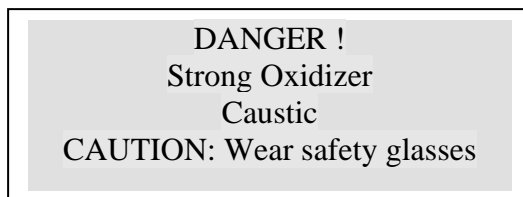
Example: Basic Fuchsin (C.I. 42510)

- c. Indicate the grade of the chemical, if it is relevant.

Example: U.S.P. or ACS or Technical

- d. Associated health and safety information, including the general category or class of hazard, should be indicated in a manner that is bold and will catch attention.

Example:



- e. Sources for reagent kits and commercially prepared solutions should be noted.

Example: X.Y.Z. Scientific Supply Co. (Cat. # 67B)

2. Give explicit instructions for reagents prepared in the laboratory.

- a. Note special instructions regarding preparation.

- State if heating is necessary.
- Specify types of glassware used.
- Indicate order of addition of chemicals.
- Stipulate whether to shake in a flask or stir on a magnetic stirrer.
- Note if solution should be filtered.
- State pH necessary.

- b. Designate special measuring devices needed, such as a volumetric flask or an analytical balance.³
- c. Specify the quality of the glassware required.
 - Indicate if acid clean glassware is required.
 - Note if plastic is unacceptable.
3. List physical or chemical parameter used to determine acceptable reagent performance.

Example: Discard if solution becomes cloudy.

or

Check pH daily before use.

4. Stipulate storage requirements by including the following information:
 - a. Specify type of container to be used.
 - b. State storage temperature.
 - c. Give stability or shelf-life of reagent.
5. Incorporate instructions on how the reagents are to be labeled.
 - a. State name and concentration of reagent.
 - b. Designate the lot number.
 - c. Record the date of preparation.
 - d. Indicate the expiration date.
 - e. Note the initials of technician who prepared the reagent.
 - f. List any special storage instructions.

Example: Store in Safety Cabinet for flammable material.

or

Store below eye level.

QUALITY CONTROL:

The Quality Control section of the procedure must include the following:

1. Identity of the control materials to be used.

Example: The quality control material consists of normal liver tissue.

2. Give instructions for preparing and handling the control materials,

Example: The control material must be fixed in an alcohol based fixative and cut a 5 microns.

3. State the frequency that controls are to be established.

Example: Controls will be run once daily.

4. Describe how tolerance limits for controls are to be established.

Example: The control is either positive or negative.

5. State corrective action taken when tolerance limits are exceeded.

Example: When the control is negative, the stain will be repeated.

6. Explain how to record and store quality control data.

Example: The control results are recorded on the requisition slip and stored with the patient's report.

7. An explanation must be given if controls are unavailable, or not used. Any alternatives should be listed.

Example: A control is not necessary because virtually every tissue has an internal control. If separate control is preferred, small intestine or uterus may be used.

PROCEDURE:

Detailed instructions for performing the procedure should be written in a logical, stepwise manner. The steps are numbered so there will be no doubt as to which step is next.

Each step is written using the present imperative form. Action verbs are employed to begin each step. Action verbs, for example, may include the following: stain, rinse, place, wash, differentiate, dehydrate, mordant, impregnate, decolorize, and remove.

Example: Wash the slides in running tap water for 10 minutes.

Not: The slides are washed in running tap water for 10 minutes.

The directions must be precise and accurate, not too verbose. Any lengthy explanations and justifications that are necessary should be included in the Procedure Note section. Divide complicated steps into simple, easy to understand ones.

Incorporate step by step directions for preparing working solutions from stock solutions into the procedure. Stipulate which measuring, filtering, or heating devices are needed. Note any special glassware required.

4. Prepare working Mucicarmine Solution
 - a. Measure, with a volumetric pipette, 5.0 ml stock Mucicarmine Solution into a 50.0 ml glass beaker.
 - b. Dilute with 15.0 ml of reagent grade water, Type I.
5. Place slides in staining rack.
6. Apply working Mucicarmine Solution to the slides using a plastic transfer pipette. Make sure that the tissue is amply covered.

State length of staining time and temperature required. When time ranges are given, also explain the criteria for determining the stopping point.

Example: Wash in running tap water for 10 - 15 minutes or until all yellow color is removed from the sections.

If an instrument is required, describe how it is employed within the procedure. Keep this part simple and not nearly as detailed as the manual for the instrument. Indicate settings, state temperatures, or give simple programming directions.

Example: Turn on water bath and preheat to 45° C ($\pm 1^\circ$ C).

Note any hazards that exist and relate the actions to be taken regarding handling and disposal of these hazards. Designate what safety equipment is to be used and what protective clothing should be worn. State disposal, spill containment, and clean-up procedures. Safety notes should be positioned in a very noticeable manner, perhaps placed in a box or underlined.

Example: **WARNING:** Wear safety glasses when handling.

RESULTS:

The anticipated results of a staining procedure may be given as a list or, when necessary, stated in a simple sentence. The results for the most important tissue element should be given first.

Example:

mucin – deep rose to red
capsule of Cryptococcus – deep rose to red
nuclei – blue-black
background – pale yellow

or

Glycogen will stain magenta in the “undigested” slide and the staining will be abolished on the “digested” slide.

The results of procedures, other than stains, should include expected accomplishments, achievements, benefits, and goals.

Example:

Surgical specimens stored according to the Surgical Pathology Specimen Storage Procedure can be quickly and easily retrieved for additional sections or for disposal.

PROCEDURE NOTES:

The Procedure Notes section includes:

1. any information that is too extensive or complex to include in the Principle or Procedure Sections
2. explanations of reasons for special precautions
3. Lists of possible sources of error
4. suggestions of where procedural problems may occur
5. helpful technical hints
6. descriptions of situations that may influence the results, such as fixatives, temperatures, etc.
7. names of acceptable alternate procedures
8. additional clinical applications
9. supplementary information that does not fit in other sections

LIMITATIONS OF PROCEDURE:

In this section list known substances that might interfere with the procedure. These include any chemical or *in vivo* substances that could potentially be a source of error.

Example: fixation, heat, drying, drugs, or cauterization

REFERENCES:

The following items should be included in the Reference section when used as sources of information. The format for references should be modeled on those acceptable for scientific publication. Refer to *Council of Biology Editors Style Manual*, fifth edition, 1983, or American Chemical Society's *Handbook for Authors*, 1978.

Sources of information may include:

1. Scientific literature

Example:

Swisher BL: Modified Steiner procedure for microwave staining of spirochetes and nonfilamentous bacteria. *J Histotechnol* **10**:241-243, 1987.

2. Textbooks

Example:

Sheehan DC, Hrapchak BB: *Theory and Practice of Histotechnology*, 2nd ed, CV Mosby, St. Louis, 1980, pp 514-518

3. Manufacturer Product Literature

Example:

Sigma Diagnostics: Silver Stain, Procedure No. HT100. St. Louis, revised April, 1990.

4. Standard Publications

Example:

National Committee for Clinical Laboratory Standards (NCCLS):
Labeling of Clinical Laboratory Materials, 2nd ed.: Approved
Standard. NCCLS publication GP1-A2. Villanova, PA, 1980

5. Written Personal Communications

Example:

Johnson T: The State University of New York at Buffalo, personal
communication, 1988.

6. Research Findings (Unpublished papers)

Example:

Brown AB: *Automated Immunoperoxidase*. Paper presented at
3rd Annual Meeting of the American Cancer Society. New York, 1990

7. Workshops

Example:

Grizzle WE & Staples TC: *Silver Staining Techniques*. Workshop
presented at the National Society for Histotechnology Annual Symposium,
Orlando, FL, September 28 – October 4, 1991

8. Personal Modifications

Example:

Smythe ZQ: Kinyoun's Acid Fast – personal modification
(unpublished)> City hospital, Histology Laboratory,
Somewhere, CA, 1991.

SUPPLEMENTAL MATERIALS

Certain information extracted from the technical procedure manual may be used at the bench. This includes flow diagrams, index cards, and manufacturer product literature. These supplemental materials may **not** be submitted for the procedure manual.

Supplemental materials must be current and referenced to the technical procedure manual by date, procedure number, reviewer's initials, etc.

1. Manufacturer Product Literature

Products or package literature must be current with and applicable to kits and reagents actually in use. A change in the product could result in changes in the literature. Therefore, a system must be developed to assure that literature and kits and reagents match. For example, lot numbers of kits or reagents could be cross-referenced to the literature.

- a. Set up title page using the laboratory's standard format.
- b. Make sure that all required information is included to meet the technical procedure manual standards of content (for example, principle, specimen required, reagents, etc.)
- c. Clearly mark those parts of the products literature that indicate the method followed by the laboratory.
- d. Cross out or black out irrelevant information or alternative procedures, unless the alternates serve as back-up procedures. Alternative procedures must be clearly marked as such.
- e. Document any revisions by the manufacturer or modifications by the laboratory. When a manufacturer's protocol is modified, the modification must be clearly justified in writing.
- f. A copy of the above material must be a part of the master technical procedure manual.

2. Flow diagrams

- a. Flow diagrams usually contain only the step-by-step working procedure and not all essential elements of the technical procedure manual. As such, flow diagrams must be included as part of the overall procedure manual.
- b. Waterproof materials should be considered when producing a flow diagram. Consider the use of plastic or other "reagent-proof" coverings.

3. Index Card Systems

- a. At minimum, each card must contain the following: principle, specimen required, reagents, quality control, and step-by-step directions.
- b. Each procedure must be on a separate card.
- c. Cards may be color coded or have alphabetical tabs for easy retrieval.
- d. A system should be devised to assure that cards are returned to the proper place in the files.
- e. A duplicate of the card must be part of the master technical procedure manual.

PROCEDURE REVIEW AND UPDATE

Each laboratory must establish who has the authority to review procedures. This will vary from laboratory to laboratory depending on the administrative structure. In some laboratories, the laboratory director conducts the reviews, while in others, the reviewer might be the section manager, supervisor, or a delegated committee.

The procedure must be reviewed whenever a change occurs. For instance, any changes in methodology, instrumentation, or reagents would require that a review is conducted.

Each procedure **must** be reviewed at least annually. During review of the procedure, decide if it is to be reapproved as written, if revisions are necessary, or if it is out of date. It is imperative to ask the following questions while conducting a procedure review:

1. Does the procedure comply with the prescribed guidelines?
2. Does the procedure conform with the current methodology used in the laboratory?

Minor changes may be added by hand to the original. Changes are signed and dated by the proper authority and, in addition, noted in the review section. Major changes require retyping the entire procedure.

There should be a mechanism in place to document that the bench technicians and technologists acknowledge and understand any changes in procedures.

Documentation is required as confirmation of the review process. The documentation consists of signature and date. For convenience, these might be arranged into a date box on the first page of the procedure.

Example:

Prepared by:

ACTION	DATE	INITIAL

Supersedes:

As an alternative to documentation of review on each individual procedure, a single, signed cover page may be used for documentation of review of **all** procedures contained in the technical procedure manual. When the single page option is chosen there must be very clear indication as to the version of each procedure referenced. A list of changes made over the last year must also be included.

MANUAL REVIEW

Each manual must also be reviewed at least annually. The reviewer may be the same person appointed to review each procedure or may be a different person.

The following questions must be asked during the manual review process:

1. Is all required material included in the manual?
2. Are all the procedures comprising the manual currently in use?
3. Has each procedure been reviewed or revised on an annual basis?
4. Is there any out-of-date or obsolete material in the manual?

5. If bench manuals, flow diagrams, or card files are in use, do they accurately reflect current master manual procedures?

If multiple copies of a manual exist, each copy must be reviewed in the above described manner. A log of all existing copies should be kept by the responsible person.

Documentation of manual review should be on the approval and review page. This page should be the first page of each manual. The authorized reviewer should date and sign as evidence of examination.

Example:

IMMUNOPEROXIDASE PROCEDURE MANUAL		
PREPARED:	October, 1995	
BY:	Jane Doe, HT (ASCP) HTL	_____
TITLE:	Supervisor, Histology	
ACCEPTED:	December, 1995	
BY:	Mary Jones, M.D.	_____
TITLE:	Laboratory Director	
REVIEWED:	DATE:	SIGNATURE:
	_____	_____
	_____	_____
	_____	_____
	_____	_____
	_____	_____

A file must be maintained for obsolete or superseded procedures removed from manuals. Initial date of use and retirement date should be recorded. These records should be maintained for historical review and for liability reasons. Retention of these files for a minimum of two years in the recommended standard.

BIBLIOGRAPHY

College of American Pathologists (CAP): *Commission on Laboratory Accreditation Inspection Checklist; Anatomic Pathology, Section 8*. CAP, Laboratory Accreditation Program, Northfield, IL., 1997, p 10-14.

Council of Biology Editors (CBE): *Council of Biological Editors Style Manual: A guide for Authors, Editors, and Publishers in the Biological Sciences*, 5th ed., CBE, Washington, distributed by the American Institute of Biological Sciences, 1983.

Eaton JD: *Writing Procedure Manuals for the Histopathology Laboratory*. Workshop presented at the National Society for Histotechnology Annual Symposium: San Antonio, TX, September 8-14, 1990.

National Committee for Clinical Laboratory Standards (NCCLS): *Clinical Laboratory Technical Procedure Manuals-Second Edition; Approved Guideline*. NCCLS document GP2-A3. Wayne, PA, 1996

Appendix B – Sample Procedure

Prepared by: R. Rogers 02/90

AMYLOID – Puchtler’s Alkaline Congo Red

PRINCIPLE:

Assorted pathological conditions may cause amyloid to be deposited in tissues. These deposits are intracellular and may become large enough to cause injury to the surrounding

Supersedes 10/87

tissue.

Amyloid is selectively stained by congo red due to the linear configuration of the dye molecule. This permits hydrogen bonding of the azo and amine groups of the dye to the similarly spaced hydroxyl radical of the amyloid. Pretreatment in alkaline alcohol releases native internal hydrogen bonding and creates, therefore, more sites for potential binding with dye.

SPECIMEN:

Any well fixed tissue may be used; however, alcoholic based fixatives yield better results. Cut paraffin sections at 5 microns.

QUALITY CONTROL:

Control material consists of a slide made from tissue known to contain amyloid. One known positive slide is run with each set of patient unknowns.

Small newly formed deposits of amyloid stain more intensely than older, usually longer deposits.

Record the results (either negative or positive) of the control slide on the Special Stain Worksheet. If the control slide results are negative, the stain will be repeated in accordance with established policy (reference Administrative Manual: Quality Control Procedure).

EQUIPMENT AND SUPPLIES:

graduated cylinders, flasks, pipettes, balance, Coplin jars, filter paper, funnels

ACTION	DATE	INITIAL
<i>Received</i>	<i>6/90</i>	<i>R.R.</i>
<i>Received</i>	<i>6/91</i>	<i>R.R.</i>
<i>Received</i>	<i>6/92</i>	<i>R.R.</i>
<i>Received</i>	<i>6/93</i>	<i>R.R.</i>

REAGENTS:

Label all laboratory prepared reagents with the name of the solution as underlined, date of preparation, expiration date, storage information, and preparer's initials. Store all reagents appropriately sized bottle glass bottle, unless otherwise noted.

Anatech Hematoxylin – Extra Strength

Purchased from:

Anatech, LTD
Battle Creek, MI
Cat. No. 822

FILTER PRIOR TO USE.

Shelf-life: see expiration date indicated by manufacturer

Storage: room temperature

Alkaline Alcohol Stock

Ethanol, 100% (C ₂ H ₅ OH)	400.0 ml
Reagent Water, Type I	100.0 ml
Sodium Chloride (NaCl)	to saturate

Shelf-life: 1 year

Storage: room temperature

Congo Red Stock

Congo Red (C.1.22120)	7.5 g
Reagent Water, Type I	100.0 ml

Shake and let stand for 5 minutes.

Then add:

Ethanol, 100% (C ₂ H ₅ OH)	400.0 ml
Sodium Chloride (NaCl)	20.0 g

LET STAND 24 HOURS BEFORE USE

Shelf-life: 1 year

Storage: room temperature

1% Aqueous Sodium Hydroxide

Sodium Hydroxide (NaOH)	1.0 g
Reagent Water, Type I	100.0 ml

Shelf-life: 1 year

Storage: room temperature

HEALTH AND SAFETY PRECAUTIONS:

Ethanol – flammable
Sodium Chloride – irritant
Sodium Hydroxide – oxidizer

For additional information, see MSDS Manual.

PROCEDURE:

1. Deparaffinize and hydrate to water.
2. Stain in Anatech Hematoxylin – Extra Strength in a glass Coplin jar for 30 seconds
3. Rinse in three changes of Reagent Water, Type I.
4. Prepare Alkaline Alcohol Working solution.
 - a. Vigorously shake the Alkaline Alcohol Stock solution prior to measuring 50.0 mL into a glass flask.
 - b. Pipet 0.5 mL of 1% Aqueous Sodium Hydroxide into the above solution and stir.
 - c. Filter and use within 15 minutes.
5. Stain the sections in the freshly filtered Alkaline Alcohol Working solution in a glass Coplin jar for 20 minutes.
6. Prepare Congo Red Working solution.
 - a. Vigorously shake the Congo Red Stock solution prior to measuring 50.0 mL into a glass flask.
 - b. Pipet 0.5 mL of 1% Aqueous Sodium Hydroxide into the above solution and stir.
 - c. Filter and use within 15 minutes.
7. Place slides directly (no rinse) into the freshly filtered Congo Red Working solution in a glass Coplin jar for 20 minutes.
8. Dehydrate rapidly in three changes of 100% Ethanol, clear in two changes of Xylene, and mount in a synthetic mounting media.

PROCEDURE NOTES:

1. Saturation of both the Alkaline Alcohol Stock and the Congo Red Stock is very important. Make sure an excess of dye and Sodium Chloride is precipitated in the bottom of the solution.
2. The intensity of the staining is increased by shaking the solutions where indicated in the instructions.
3. Following Congo Red staining, amyloid exhibits an apple-green birefringence under polarized light.

LIMITATIONS OF PROCEDURE:

1. Prolonged fixation in 10% buffered formalin may decrease the staining
2. Sections cut too thin will give pale red staining, while sections cut too thick will give yellow birefringence.

RESULTS:

amyloid – deep pink to red

elastic tissue – pale pink

nuclei – blue

REFERENCE:

Puchtler H, Sweat F, Levine M: On the binding of Congo Red by Amyloid. J Histochem Cytochem 10:355-364, 1962

Rogers R: Puchtler's Alkaline Congo Red – personal modification (unpublished). University Hospital, Histology Laboratory, Augusta, GA, 1990.