1. TITLE: Special Stains Overview.

2. DEFINITION :

Special staining is performed to visualize selected tissue elements, entities and microorganisms. Based on classical dye staining methods, special stains technique provide valuable information in the evaluation of numerous abnormal or disease conditions. The following special stains are of high quality, and they satisfactorily demonstrate (on each day of use), the tissue components or organisms for which they were designed.

a) Acid fast organisms
b) Iron
c) Bacteria
d) Elastic tissue
e) Fungi
f) Mucin
g) Connective tissue
h) Myelin
i) Nerve fibers
j) Glycogen
k) Reticulin fibers
l) Amyloids

3. POLICY :

Nil.

4. PROCEDURE :

4.1. SAFETY.

4.1.1. All specimens should be handled with care as if they are infectious.
4.1.2. All specimens should be handled with care as if they are infectious.
4.1.3. Always wear Personal Protection Equipment's (PPE).
4.1.4. For more safety information, refer to MSDS.

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4.2. QUALITY CONTROL.
4.2.1. Always run a known positive control with the test.
4.2.2. Positive tissue controls assess the performance of the stain. Special stains are performed on sections of control tissue known to contain components specific to each special stain.
4.2.3. Verification of tissue used as a positive control must be performed and documented before being used with clinical specimens.
4.2.4. Document the evaluation of positive control tissue block on Lab.Med.FRM-HIST-021.
4.2.5. Document daily QC on Lab.Med.FRM-HIST-032.
4.2.6. Check expiry date of the kit prior to run.
4.2.7. Perform maintenance of the machines used for special stains.

4.3. INSTRUMENT:
4.3.1. Dako Artisanlink Autostainer
(Refer to IPP 14.HIST 014 Dako Autostainer for Special stains).

5.

NO	SPECIAL STAIN	INTENDED USE	PRINCIPLE	CLINICAL SIGNIFICANCE	CONTROL	RESULTS
1.	**Acid Fast**	Demonstration of acid-fast Mycobacteria such as M.Tuberculosis, M. Leprose,M. Kansasii, M Ayium.	Bacteria of the genus mycobacterium have the unique property of being “acid fast” with certain stain. The term acid fact refer to the ability if the bacterium to retain a dye that washes	Acid Fast Bacillus stain is intended for use as a qualitative histologic stain to selectively demonstrate mycobacterium Tuberculosis and other acid-fast organisms or	A tissue containing acid- fast organism.	□ Acid fast bacilli: Red □ Nocardia Filaments: Red □ Leprae bacilli: Red □ Back ground: Blue

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			out of all other tissue element when the section is exposed to a dilute acid solution.	components in tissue section.		
2.	**Alcian Blue** Evaluation of Acid mucins and mucopolysaccharides		Alcian blue is a water – soluble amphoiteric copper phthalocyanin dye that stain by salt linkage with the acidic groups of acid mucopolysaccharides.	It stains basophiles, cartilae,, mucopolysaccharides and glycosaminoglycans	Umbilical Cord	Acid Mucopolysaccharides: Turquoise Blue
3.	**Alcian Blue / PAS**	Differentiation of neutral and acidic mucopolysaccharides	This is a combination of two standard techniques: Periodic Acid-Schiff and Alcian Blue pH 2.5 .	Non-small cell lung cancer (NSCLC) can be classified into several histological subtypes, most commonly adenocarcinoma (LADC) or squamous cell carcinoma (SqCC). With the introduction of targeted therapies that can result in dramatically different outcomes based on subtype, the importance of accurate classification has been amplified. Limited availability of tissue from needle core biopsies has challenged routine	Umbilical Cord or Appendix	· Acid mucopolysaccharides: Blue · Neutral polysaccharides: Magenta · Cartilage, ground substance, epithelial mucins: Shades of purple to very deep blue · Cell bodies of fungi: Red to purple · Mucoid capsule of Cryptococcus: Blue

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				diagnosis, particularly for poorly differentiated samples. Studies have shown that histochemical stains Alcian blue-PAS are useful in lung cancer diagnosis		
4.	**Amyloid (Congo Red)**	Demonstration of Amyloid in tissue sections	Congo red stain is a modification of the Highman's technique. The staining reaction is based on the application of congo red which stains the pattern of a typical proteins (Amyloid). The B-pleated sheets of amyloid are suitable in size and shape to accommodate the congo red molecules, which are held in the lattice work of B-pleated sheets. Birefringence is an intrinsic property of the amyloid fibril congo red complex. Without both the red staining congo red and the apple green birefringence under	Congo red stain is used as a qualitative histologic stain to selectively demonstrate AMYLOID in tissue sections	A tissue section containing amyloid Cut on 5 microns	*With normal light microscopy:* Amyloid Pink to red. Nuclei Blue · *With polarized light microscopy*: Under polarized light to pink areas change to green as the filter is rotated. The background is dark.

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			polarization, a definite identification cannot be used, a mayers Hematoxylin solution is applied to provide a contrasting blue nuclear stain.			
5.	**Giemsa**	Demonstration of Parasite and H.Pylori bacteria	"Romanowsky" stains are formed from a mixture of polychromed methylene blue and eosin, the resultant precipitate being dissolved in methyl alcohol. Such stains are acidic-basic stains (neutral dyes) and therefore stain both acidophilic and basophilic cellular components. Giemsa, a modification of the classic Romanowsky stain, is made from various azure compounds (thionine and its methyl derivatives) with eosin and methylene blue, In GIEMSA staining kit, a buffered thiazine-eosinate solution is used to stain cells differentially with a	GIEMSA staining kit differentiates leukocyte in bone marrow and other hematopoietic tissue (lymph nodes). The stain can also be used to demonstrate some microorganisms such as H.Pylori.	Bone Marrow, appendix, Gastric or stomach infected with H.Pylori	· Nuclei : Red-purple · Leukocytes : Purple · Erythrocyte : Pink · Mast cells : Blue · Pathogenic gram - § Negative spiral : Blue § Bacteria (H. Pylori).

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			characteristic blue or pink color.			
6.	**GMS** (Grocott methenamin silver)	Demonstrate of fungi	Tissue, fungal and microbial cell wall polysaccharides are oxidixed to aldehyde group by GMS oxidizer. Chronic acid suppresses weaker background staining of collagen fibers and basement membranes. Neutralizer removes excess chronic acid. GMS Silver A, provides the silver ions. GMS SILVER B provides the alkaline conditions which reduce the silver ions to metallic silver. Toner reagent contains gold chloride to form gold complex and removes yellow tones from the tissue. Fixer, with thiosulfate, staops the reaction and removes unreduced silver from the section. The staining reaction is based on aldehyde reduction of silver ions to	GMS stain is used as a qualitative histologic stain to selectively demonstrate polysaccharides in the cell walls of fungi and other opportunistic organisms, such as Pneumocystis carinie, Aspergillus and Blastomyces	tissue positive for pathogenic fungus or Pneumocystis carinii may be used as a positive control	· Fungus: Black against light green background · Mucin: Taupe to dark-grey · Aspergillus hypae: black filoments · Pneumocystis Carinii: clusters of helmet-shaped black spots

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			metallic silver under alkaline conditions.			
7.	**Gram**	Demonstration of Gram-positive and Gram-Negative bacteria	This is the most widely used bacteriological staining method, and most organisms are classified by their reaction to Gram stain. An organism that is gram-positive must have an intact cell wall. A similar organism with a wall damaged by any of various means will be gram-negative. This indicates the importance of the wall in the retention of dye lake. The Gram staining is based on the ability of bacteria cell wall to retaining the crystal violet dye during solvent treatment. The cell walls for Gram-positive microorganisms have a higher peptidoglycan and lower lipid content than gram-negative bacteria. Bacteria cell	Gram staining technique is used as a tool for the differentiation of gram-positive and gram-negative bacteria, a first step to determine the identity of a particular bacterial sample.	tissue section positive for Gram–positive or Gram–negative organisms.	· Gram–negative bacteria- -pink color to red. · Gram–positive bacteria: purple or intense blue – black. · Nuclei :Red · Erythrocytes: Yellow

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			walls are stained by the crystal violet. Iodine is added as a mordant to form the crystal violet-iodine complex so that the dye cannot be removed easily. This step is referred to as fixing the dye. Subsequent treatment with a decolorizer, which is a mixed solvent of ethanol and acetone, dissolves the lipid layer from the gram-negative cells. The removal of the lipid layer enhances the leaching of the primary stain from the cells into the surrounding solvent. In contrast, the solvent dehydrates the thicker Gram-positive cell walls, closing the pores as the cell wall shrinks during dehydration. As a result, the diffusion of the violet-iodine complex is blocked, and the bacteria remains stained. The length of the decolonization				

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			is critical in differentiating the gram-positive bacteria from the gram-negative bacteria. Finally, a counterstain, of safranin is applied to the slide to give decolorized gram – negative bacteria a pink color.			
8.	**Iron (Perl's)**	Detection of ferric iron in tissue sections and blood or bone marrow films	Iron is absorbed through the small intestine and transported to the bone marrow. There, it is stored as hemosiderin, until erythropoiesis, when it is incorporated into hemoglobin molecules. Iron stain is based on the historic Prussian blue reaction. The iron is separated from protein by hydrochloric acid. The free ferric iron reacts with the potassium perrocyamide to foam on insoluble bright blue ferric ferrocyomide or	Iron stain is used as a qualitative histologic stain to detect iron in formalin-fixed paraffin embedded tissue and bone marrow. In the disease states of hemchromatosis and hemosiderosis, excessive amounts of ferric iron and present in the liver, spleen and lymph nodes. Iron may be found at any site where there has been local destruction of red cells, such as hemorrhage sites, infarction, long standing congestion	liver or spleen	· Iron pigment: Bright blue · Nuclei: Red · Cytoplasm: Light pink

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			Prussion blue.	and trauma.		
9.	**Reticulin**	Evaluation of reticulin fibers	Reticulum stain is a modification of Gordon and Sweet Stain. The staining reaction is based on the affinity of silver for glycoproteins. Oxidizer, with potassium permanganate, oxidizes the tissue to enhance staining of reticular fibers. Decolorizer, with oxalic acid, removes excess potassium permanganate. Sensitizer, with ferric ammonium sulfate, is added to form a metal – organic compound. The metal – organic compound is replaced by the silver in Reticulum II Silver A. Reducer is applied to develops the deposited silver into visible silver. Toner reagent contains gold chloride for better contrast and clarity. Fixer, with thiosulfate, stops the reaction and removes	Reticulum stain is used as a qualitative histologic stain to demonstrate reticular fibers in formalin – fixed paraffin embedded tissue. It aids in the differential diagnosis of certain tumor types. Also, it can be used to show disease states in organs such as the liver, spleen and kidney by demonstrating reticular patterns not normal to the organ. In normal liver, the fibers are well-defined strands, but in necrotic or cirrhosis liver, the fibers have discontinuous patterns.	liver	· Reticular fibers: Black · Background: Pink to red

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			any unreacted silver from the section. Nuclear Fast Red counterstain is applied to provide contrasting background.			
10.	**Trichrome (masson's)**	Evaluation of collagen and muscle fibers	Trichrome III GREEN staining kit is a modification of Masson's Trichrome stain. The staining reaction is based on the differential effect of acid dye on muscle and collagen. Bouin's solution is applied to tissue sections to intensify the final coloration. Cytoplasm and muscle are stained with Trichrome Red, containing Biebrich scarlet and acid fuchsin. Nuclei are stained with iron hematoxylin. After application of Trichrome III mordant, the collagen is stained with Trichrome III Green, which contains fast green	Trichrome stain is used as a qualitative histologic stain to study connective tissue, muscle and collagen fibers in formalin-fixed, paraffin – embedded tissue. It is also used to differentiate collagen from muscle tissue. The stains are useful for indicating fibrotic change, that is, an increase in collagen like that which occurs in liver cirrhosis and pyelopnephretis. Thrichrome stains can be useful for distinguishing histologic changes that occur in neuromuscular disease. They are also useful for differentiating	Colon Kidney Liver Esophagus.	· Basement membranes: Black · Reticulum: Black · Nuclei: Blue-Purple ·Cytoplasm, Collagen ,Connective tissue: Pink to orange

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			FCF	tumors that originated in muscle cells from tumors that originated in fibroblasts.		
12.	**Mucicarmine**	Evaluation of mucin, mucopolysaccharides and capsule of cryptococcus	Mucin is a term given to a secretion produced by a variety of epithelial and connective tissue cells. The rationale underlying the remarkable specificity of mucicarmine for mucin is not fully understood. Aluminum salts is believed to form a chelate complex with mucin, to which carmine attaches by dye-lake formation. In this method, iron – hematoxylin is used for the nuclear stain and Tartrazine for the counterstain.	Mucicarmine stain is used as a qualitative histologic stain in the identification of primary tumor sites and distinguishing mucin-negative undifferentiated squamous cell lesions from mucin – positive adenocarcinomas. The stain displays a specificity towards mucins of epithelial origin whereas mucins of fibroblastic origin stain poorly.	Appednix or Colon	· Mucin: Deep rose to red · Nuclei: Deep rose to red · Capsule of Cryptococcus: Deep rose to red · Other tissue element: Yellow
13.	**PAS**	Undigested; evaluation of mucopolysaccharides and glycoprotien	PAS stain uses Periodic Acid to oxidize glycols to aldehydes. Schiff's reagents forms a colorless dialdehyde	PAS stain in tissue and digestion with Diastase is used as a qualitative histologic stain to demonstrate the presence of	Liver	· Glycogen: Magenta · Mucin: Red purple · Base Membrane: Red to purple

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			compound that is transformed to the colored final staining of glycol – containing cellular components.	glycogen storage disease in tissue. PAS–positive reticular fibers, basement membrane, fungus, and neutral muco-polysaccharides are also detected. It may also be used to aid in distinguishing a PAS-positive, secreting adenocarcinoma from undifferentiated PAS-negative, squamous carcinoma.		· Fungi: Red to purple (Bright Magenta)
14.	**PAS/ Diastase**	Diastase digested; evaluation of glycogen and glycogen storage disease	The staining reaction is based on the ovidation of glycol to adehyde followed by selective staining of the aldehyde groups by schiff's Reagent. Diastase digests the glycogen present in the tissue so it is washed out of the tissue before PAS staining occurs. Diastase digestion is used to differentially determine if the PAS – positive component is	PAS staining in tissue sections and digestion with diastose is useful as an aid in the determination and diagnosis of glycogen storage disease in tissue.	Liver	· Glycogen: Bright Magenta · Nuclei: Light purple

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			glycogen.			
15.	**Elastic Fiber**	Evaluation of elastic fiber	Elastic staining kit is a modification of HART's method for elastic fibers. The staining reaction is based on the offinity of resorcin fuchsin towards elastic fibers. Van GIESON's solution is applied to provide contrasting yellow background tissue.	Elastic stain is used as a qualitative histologic stain to demonstrate elastic fibers in tissue section. This stain is useful in demonstrating atrophy of elastic tissue in cases of emphysema, and the thinning and loss of elastic fibers in arteriosclerosis and other vascular diseases	section from aorta skin or kidney	· Elastic Fiber: dark blue-purple against a yellow background (muscles & RBC) · Collagen: Pink to red · Nuclei: Blue black.
16.	**Warthin Starry**	Demonstration of H.Pylori	The Warthin-Starry stain is a modification of the original warthin-starry procedure which overlays silver nitrate around organism. This stain is optimized for use on the Dako Artisanlink staining system. Wash steps follow all of the	The warthin-starry stain is used to identify Helicobacter Pylori, spirochetes and other organisms in the tissue.	tissue section with H.Pylori and spirochetes	· Helicobactor Pylori: Black · Spirochetes: Black · Background: Golden yellow

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			staining steps. Following staining, the air dried slides are removed from the instrument cleared with xylene and mounted with appropriate mounting media. Results are interpreted using a standard light microscope.			

6. RESPONSIBILITY :

6.1 All Technologists.

7. ATTACHMENTS :

7.1 Lab.Med.FRM-HIST-032 Special stains Daily QC review.

8. DISTRIBUTION :

8.1 LMD Administration Office
8.2 Histopathology Laboratory Section
8.3 DQSA.

9. REFERENCES :

9.1 Theory and Practice of Histopathology techniques Bancroft and Stevens 2004 edition.

9.2 College of American Pathologist checklist 2012.

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9.3 College of American Pathologist checklist 04.21.2014

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