

NDMRB-RA-006

Risk Assessment: Papanicolaou (Pap) Staining

Scope

Papanicolaou stain (also Papanicolaou's stain and Pap stain) is a multichromatic staining cytological technique developed by George Papanikolaou, the father of cytopathology.

Pap staining is used to differentiate cells in smear preparations of various bodily secretions; the specimens can be gynecological smears (Pap smears), sputum, brushings, washings, urine, cerebrospinal fluid, abdominal fluid, pleural fluid, synovial fluid, seminal fluid, fine needle aspiration material, tumor touch samples, or other materials containing cells.

A nuclear stain, haematoxylin, is used to stain cell nuclei. The unmordanted haematein may be responsible for the yellow color imparted to glycogen.

- First OG-6 counterstain (-6 denotes the used concentration of phosphotungstic acid). Orange G is used. It stains keratin. Its original role was to stain the small cells of keratinizing squamous cell carcinoma present in sputum.
- Second EA (Eosin Azure) counterstain, comprising three dyes; the number denotes the proportion of the dyes, e.g. EA-36, EA-50, EA-65.
 - Eosin Y stains the superficial epithelial squamous cells, nucleoli, cilia, and red blood cells.
 - Light Green SF yellowish stains the cytoplasm of other cells, including non-keratinized squamous cells. This dye is now quite expensive and difficult to obtain, therefore some manufacturers are switching to Fast Green FCF, however it produces visually different results and is not considered satisfactory by some.
 - Bismarck brown Y stains nothing and in contemporary formulations it is often omitted.

When performed properly, the stained specimen should display hues from the entire spectrum: red, orange, yellow, green, blue, and violet. The chromatin patterns are well visible, the cells from borderline lesions are easier to interpret and the photomicrographs are better. The staining results in very transparent cells, so even thicker specimens with overlapping cells can be interpreted.

On a well prepared specimen, the cell nuclei are crisp blue to black. Cells with high content of keratin are yellow, glycogen stains yellow as well. Superficial cells are orange to pink, and intermediate and parabasal cells are turquoise green to blue. Metaplastic cells often stain both green and pink at once.

Pap stain is not fully standardized; it comes in several versions, subtly differing in the exact dyes used, their ratios, and timing of the process.

The EA stain contains two mutually incompatible chemicals, Bismarck brown and phosphotungstic acid, which precipitate each other, impairing the useful life of the mixture and compromising the differential staining of eosin and light green. The descriptions of the compositions of the staining solutions vary by source and differ even in Papanicolaou's own publications. Mixtures of the same name from different vendors therefore can differ in composition, occasionally producing different or poor results

It is the users responsibility to ensure what controls are needed to ensure that the health of themselves and others around them. It is imperative that you **DO NOT** start any work until you are absolutely sure of the appropriate precautions that need to be employed. If you are unsure seek advice from your line/laboratory manager or your departmental safety officer (DSO).

Name of assessor:	Andrea Keepence-Keyte	Date of Assessment:	April 2016	Review Date:	Every three years
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Risk Matrix:

Risk Matrix		Likelihood			
		High	Medium	Low	Negligible
Consequence	Severe	High	High	Medium	Effectively Zero
	Moderate	High	Medium	Medium/low	Effectively Zero
	Insignificant	Medium/Low	Low	Low	Effectively Zero
	Negligible	Effectively Zero	Effectively Zero	Effectively Zero	Effectively Zero

Risk Assessment:

Hazard (Cause and consequence)	Affected Groups	Existing controls	Risk	Further Action
Exposure to hazardous chemicals	Staff/students/visitors	<p>Users must have signed their training records to confirm they have read, understood and will comply with local rules:</p> <p>NDMRB-SOP-004 Handling and Use of Chemicals</p> <p>TDI-GI-003 New hazard symbols vs. old hazard symbols</p> <p>NDMRB-SOP-008 Storage of Flammable Liquids in Containers</p> <p>NDMRB-RA-056 Xylene</p> <p>Staining through must be clearly labelled and, if left unattended, a clearly readable note with contact details must be left near the stain through.</p> <p>Flammable chemical over 0.5L will be kept in a lockable flammable cupboard, kept separate from acids and solvents.</p> <p><u>Xylene should be replaced by a safer alternative</u> <u>Histoclear must be considered. Use in Fume hood</u></p>	Medium	<p>Regular servicing of chemical fume hoods.</p> <p>COSHH is available on the NDMRB internal web pages</p>

Spillage of chemical	Staff, students and visitors	<p>Xylene: Accidental release measures: Use personal protective equipment. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Evacuate personnel to safe areas. Beware of vapours accumulating to form explosive concentrations. Vapours can accumulate in low areas.</p> <p>Methods and materials for containment and cleaning up Contain spillage, and then collect with an electrically protected vacuum cleaner or by wet-brushing and place in container for disposal according to local regulations.</p>	Medium	None
Potential cuts from glass slides	Staff, students and visitors	Glass slides will be used in this technique, users should be handle the slides carefully and dispose of any broken or cracked slides via clinical waste.	Low	None
Potential for items to be disposed of via incorrect waste stream	Staff, students and visitors	<p>Waste stream must follow NDMRB-POL-006 Handling, Storage and Disposal of Lab Waste</p> <p>Glass slide must be disposed of via the clinical waste bins (yellow sharps bin).</p> <p>Low volume of Flammable substances (<100ml) can be allowed to evaporate in the fume cupboard. Larger volume should be collected and disposed of via the Safety Office. Water-miscible solvents Can be disposed of down the drain, however, no more that 100ml/day/lab</p> <p>Eosin Azure EA50 Prevent further leakage or spillage if safe to do so. Do not let product enter drains. EA50 waste must be collected and disposed of via the Safety Office.</p>	Low	None

		<p>Xylene Environmental precautions Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided. Xylene waste must be collected and disposed of via the Safety Office.</p>		
Hazard resulting from lone working		<p>If using Xylene this technique must not be carried out outside of hours.</p> <p>Buddy system must be followed when lone working</p>	Medium	None

Signed By Author:

Approved by (sign and print):

Reviewed by:

Review date: