Document Number: **SASoM/METHOD/030.v4**

Title: **Using an in-House Quality Control Slide for Immunofluorescent Staining**

Version: **V4**

Author: **Peter Mullen**

Effective from:	01/02/2019
Valid to:	31/01/2021

SOP History		
Number	Date	Reason for Change
V1	01/02/2013	Original
V2	01/02/2015	Update
V3	01/02/2017	Update
V4	01/02/2019	Update

1.0 Purpose –

This SOP describes the current procedure for using an in-house quality control slide for immunofluorescent staining in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to all staff in the SASoM involved with an in-house quality control slide for immunofluorescent staining.

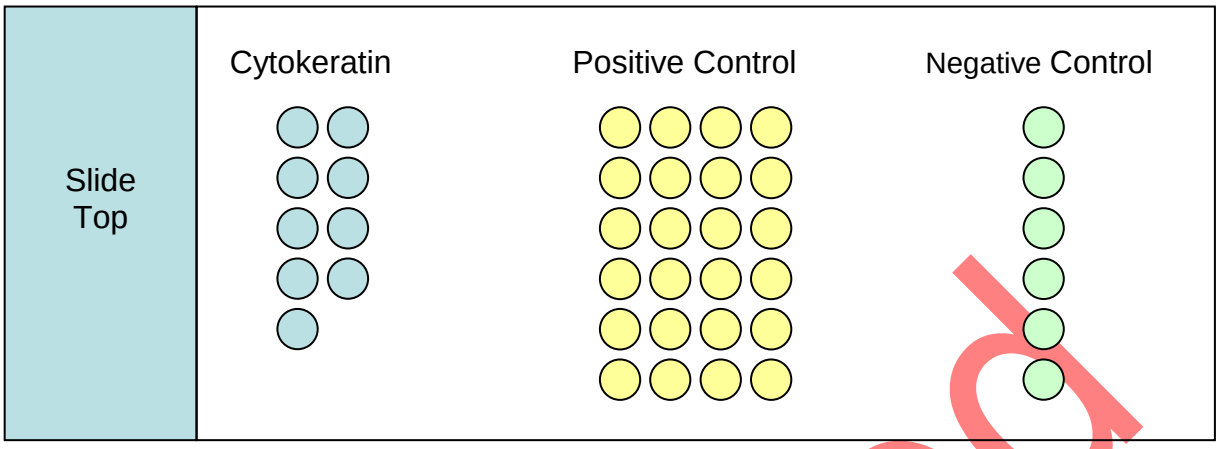
3.0 Responsibilities –

All staff involved in an in-house quality control slide for immunofluorescent staining are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.

4.0 Procedure –

An in-house quality control slide has three different TMAs as the figure below shows.



Dewax and Rehydration

Dewax in xylene for 2 x 5mins.

Rehydration for 2mins at 99%, 99%, 80%, 50% alcohol and running tap water.

Antigen Retrieval

Tris-EDTA pH9.0 or Sodium Citrate pH6.0 - pressure cooker for 5mins.

Cool down for 20mins.

Rinse in 0.05% PBS-Tween 20 for 5mins, in a coplin jar, on a rocker.

Blocking

Treat sections in 3% Hydrogen Peroxide for 10mins in a coplin jar.

Rinse in 0.05% PBS-T for 5mins.

Treat sections in Dako Serum free protein block for 10mins.

Primary Antibody and anti-cytokeratin (Tumour mask) incubation

a. Cytokeratin TMA - checking the quality of the tumour mask staining.
Incubate Mouse anti-cytokeratin (Dako, M3515) overnight at 4^{O}C.

b. Positive control TMA – checking the quality of one of each antibody you are staining.
Choose one of the already validated antibodies;

Cyclin D1	Dako, M3635	1:100	1hour incubation at room temperature
WT1	Dako, M3561	1:200	1hour incubation at room temperature
BCL2	Dako, M0887	1:100	1hour incubation at room temperature
ERK	CST, 9102	1:400	overnight incubation at 4^{O}C
PTEN	CST, 9559	1:200	overnight incubation at 4^{O}C
Cyclin B1	Epitomics, 1495-1	1:50	1hour incubation at room temperature

PgR	Epitomics, 1483-1	1:5	1hour incubation at room temperature
ER	Neomarkers RM-9101-s1	1:100	1hour incubation at room temperature

Incubate one of them appropriately according to their own conditions.

Rinse in 0.05% PBS-T 3 x 5mins.

Add the second primary antibody either Mouse Anti-cytokeratin (Dako, M3515) dilute 1:50 in Dako antibody diluent or Rabbit Anti-cytokeratin (Dako Z0622) dilute 1:150 combined with Rabbit anti-pan Cadherin (Cell signalling 4068) diluted 1:50 in Dako antibody diluent and incubate overnight at 4^{O}C.

c Negative control TMA - showing any non-specific binding as a result of unsuccessful blocking.
Do not add any primary antibodies or cytokeratin. Only add antibody diluent (Dako, S0809) during antibody incubation time.

Rinse in 0.05% PBS-T for 3 x 5mins.

<u>Epithelial mask visualisation</u>
a. CytokeratinTMA
Prepare a 1:25 dilution of the goat anti-mouse Alexa555 Ab (Invitrogen, A21422) in pre-diluted Envision goat-rabbit HRP antibody solution (Dako, K4003).

b. Positive control TMA
Prepare either 1 in 25 dilution of the goat anti-rabbit Alexa555 Ab (Invitrogen, A21428) in the pre-diluted Envision goat-mouse HRP antibody solution (Dako, K4001) or 1 in 25 dilution of the goat anti-mouse Alexa555 Ab (Invitrogen, A21422) in the pre-diluted Envision goat-rabbit HRP antibody solution (Dako, K4003) according to the antibody's species.

C. Negative control
Prepare a 1 in 25 dilution of the goat anti-mouse Alexa555 Ab (Invitrogen, A21422) in the pre-diluted Envision goat-rabbit HRP antibody solution (Dako, K4003).

Incubate slides in the dark for 1.5 hours at room temperature.

Rinse in 0.05% PBS-T 3 x 5mins.

<u>Target visualisation</u>
Dilute the Cy5 Tyramide 1:50 in target signal amplification diluent, use HistoRx tube F and E - AQUAntiplex #AQ-EMR1-0001. (Note: Tube E&F are very expensive, so use the minimum amount per slide.)

Vortex to mix thoroughly.

Incubate slides in the dark for 10mins at room temperature.

Rinse in 0.05% PBS-T for 3 x 5mins.

Dehydrate them in 80% IMS for 1 minute.

Air dry in the dark.

Counterstaining and cover slipping

Apply 45µl Prolong Gold anti-fade reagent with DAPI (Invitrogen, P36931), nuclear visualisation media, on the coverslip (22 x 40mm) and place the coverslip over the tissue. (For the smaller coverslips (22 x 26mm), apply 30µl).

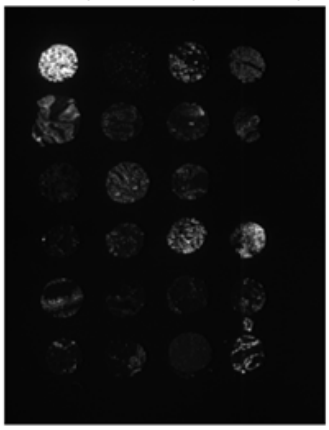
Let the mounted slide dry overnight in the dark.

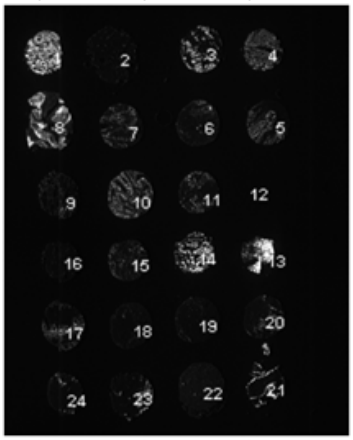
After slides are completely dried, seal the coverslips with nail polish.

Examples of Results

Positive control (CyclinD1)-AQUA normalised score and Cy5 low resolution image

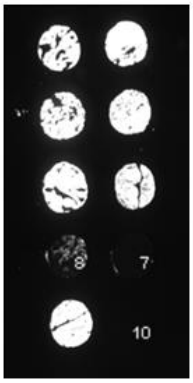
CyclinD1 in Nuclear AQUA_Norm	Control	PCC
8707.52	9039.497	0.964643
549.5453	584.0209	
2861.534	3839.598	
1778.112	2551.41	
861.0085	1487.702	
1519.821	1169.264	
474.2231	1065.87	
	3837.658	
295.3055	896.7928	
1198.135	2131.289	
997.3074	1602.071	
	6783.14	
	648.5911	
	471.1691	
	1466.43	
153.6703		
7940.615		
185.6933		
1114.3	3295.078	
	1316.034	





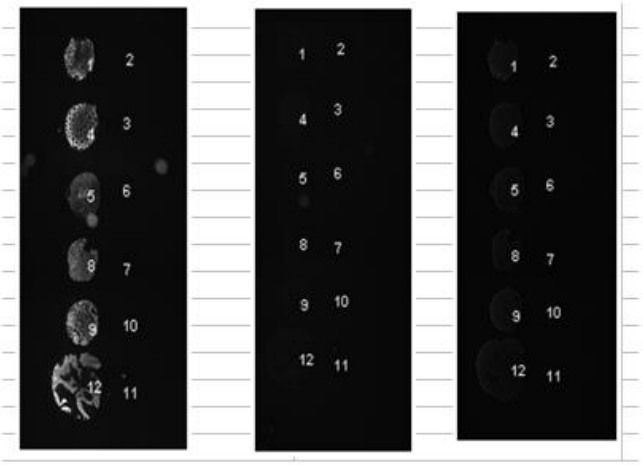
Cytokeratin control - AQUA normalised score and Cy5 low resolution image

CK in Cytoplasmic Compartment AQUA_		
72340.66	90186.14	PCC
59649.55	95578.4	0.944197
39957.97	64709.9	
132008	171505.2	
85138.57	105461.2	
53640.99	64917.87	
10738.55	34402.35	
20909.98	32089.24	
76857.48	68080.66	



Negative control- AQUA normalised score and Dapi, Cy3 and Cy5 low resolution image

Negative in Nuclear AQUA_Norm
25.85106
45.40625
34.68242
50.59087
43.5166



5.0 Personal protection –

A Howie coat must be worn at all times. Gloves as specified in the appropriate COSHH RA.

6.0 Spillages –

Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard.

Spillages should be mopped up with paper towel, disinfected with 70% ethanol and finally washed with detergent.

7.0 Training –

All staff should undergo training in this technique before performing the procedure.

8.0 Related documents –

8.1 Risk assessments COSHH/008

8.2 SOP SASoM/METHOD/024
Dewaxing and Rehydration of Paraffin Embedded Sections

8.3 SOP SASoM/EQUIP/015
Use of the Pressure Cooker for Antigen Retrieval

9.0 Approval and sign off –

Author:		
Name:	Peter Mullen	
Position:	Research Fellow	
Signature:		Date:
Management Approval:		
Name:	Peter Mullen	
Position:	SOP Administrator	
Signature:		Date:
QA release by:		
Name:	Alex MacLellan	
Position:	QA Manager	
Signature:		Date:

STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE