APPENDIX 1

SIALINIZED SLIDES PROCESSING

1. Soak slides in water with detergent (7x Decon 90) overnight.

2. Wash slides in running water until the detergent is completely removed for 10 minutes.

3. Rinse slides in 95% alcohol (2 changes for 5 minutes each).

4. Dry slides in oven at 60°C for 9 hours.

5. Coat slides in 2% APES (3-aminopropyl-triethoxy-silane A-3648, Lot 2540296) in acetone for 5 minutes (10ml silane in 500ml acetone).

6. Rinse in distilled water, 2-3 dips

7. Dry in oven at 60°C and pack slides ready for use.
APPENDIX 2

HAEMATOXYLIN AND EOSIN STAINING METHOD

1. Dewax sections in 2 changes of xylene for 5 and 3 minutes each

2. Hydrate sections with decreasing grades of ethanol; 2 minutes in 100%; 2 minutes in 95% and 2 minutes in 75%

3. Wash sections in running tap water for 3 minutes

4. Nuclear staining in Harris Haematoxylin for 3 minutes

5. Differentiating staining with acid alcohol for 2 dips

6. Wash sections in running tap water for 3 minutes

7. Blueing nuclear staining with potassium acetate for 2 minutes

8. Wash sections in running tap water for 3 minutes

9. Hydrate section in 80% ethanol for 3 dips

10. Cytoplasmic staining in eosin for 4 minutes

11. Dehydrate sections with increasing grades of ethanol; 2 changes of 95% 1 minute each and 2 changes of 100% 1 minute each

12. Clear sections in xylene. 3 changes of xylene 1 minute each.

MALIGNANT NEOPLASMS OF LIP, ORAL CAVITY AND PHARYNX

C00 Malignant neoplasm of lip (excluding skin of lip, C43.0, C44.0)

C00.0 External upper lip. Upper lip: NOS, lipstick area, vermilion border

C00.1 External lower lip. Lower lip: NOS, lipstick area, vermilion border

C00.2 External lip, unspecified: vermilion border NOS

C00.3 Upper lip, inner aspect. Upper lip: buccal aspect, frenulum, mucosa, oral aspect

C00.4 Lower lip, inner aspect. Lower lip: buccal aspect, frenulum, mucosa, oral aspect

C00.5 Lip, unspecific, inner aspect. Lip not specified whether upper or lower: buccal aspect, frenulum, mucosa and oral aspect.

C00.6 Commissure of lip

C00.8 Overlapping lesion of lip

C00.9 Lip, unspecified

C01 Malignant neoplasm of base of tongue

- dorsal surface of base of tongue

- fixed part of tongue NOS

- posterior third of tongue

C02 Malignant neoplasm of other and unspecified parts of tongue
C02.1 Border of tongue: tip of tongue

C02.2 Ventral surface of tongue: anterior two-thirds of tongue, ventral surface, frenulum linguae

C02.3 Anterior two-thirds of tongue, part unspecified: middle third of tongue NOS, mobile part of tongue NOS

C02.4 Lingual tonsil. Exclude tonsil NOS

C02.8 Overlapping lesion of tongue. Malignant neoplasm of tongue whose point of origin cannot be classified to any one of the categories C01-C02.4

C02.9 Tongue, unspecific

C03 Malignant neoplasm of gum. Includes alveolar (ridge) mucosa, gingival. Excludes malignant odontogenic neoplasms (C41.0-C41.1)

C03.0 Upper gum

C03.1 Lower gum

C03.9 Gum, unspecified

C04 Malignant neoplasm of floor of mouth

C04.0 Anterior floor of mouth: anterior to the premolar-canine junction

C04.2 Lateral floor of mouth

C04.8 Overlapping lesion of floor of mouth

C04.9 Floor of mouth, unspecified

C05 Malignant neoplasm of palate

C05.0 Hard palate

C05.1 Soft palate. Excludes nasopharyngeal surface of soft palate
**C05.2** Uvula

**C05.8** Overlapping lesion of palate

**C05.9** Palate, unspecified. Roof of mouth

**C06** Malignant neoplasm of other and unspecified parts of mouth

**C06.0** Cheek mucosa: buccal mucosa NOS, internal cheek

**C06.1** Vestibule of mouth: buccal sulcus (upper/lower), labial sulcus (upper/lower)

**C06.2** Retromolar area

**C06.8** Overlapping lesion of other and unspecified parts of mouth

**C06.9** Mouth, unspecified. Minor salivary gland, unspecific site, oral cavity NOS

(Adapted from WHO’s International Statistical Classification of Diseases and Related Health Problems, 10th Revision (ICD-10) http://www.who.int/classifications/apps/icd/icd10online)
## APPENDIX 4

### DATA COLLECTION SHEET FOR EXPRESSION OF Ki-67, p53, MDM-2 AND Bcl-2

1. Registration no:

2. Slide no and year:

3. Age:

4. Gender:  
   1. male  
   2. female

5. Ethnicity:  
   1. Malay  
   2. Chinese  
   3. Indian  
   4. Others

6. TNM:  
   T:  
   N:  
   M:  
   TNM Stage: I/II/III/IV

7. Broders’ Classification:  
   1. Well differentiated  
   2. Moderately differentiated  
   3. Poorly differentiated

8. Pattern of Invasion:  
   1. Pattern 1  
   2. Pattern 2  
   3. Pattern 3  
   4. Pattern 4

9. Ki-67 immunoreactivity:  
   1. Positive  
   Ki-67 LI (%):  
   2. Negative

10. p53 immunoreactivity  
    1. Positive

11. MDM-2 immunoreactivity:  
    1. Positive  
    2. Negative

12. Bcl-2 immunoreactivity:  
    1. Positive  
    2. Negative