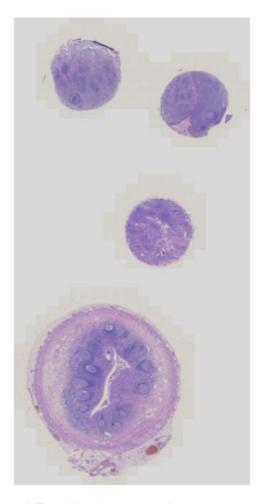
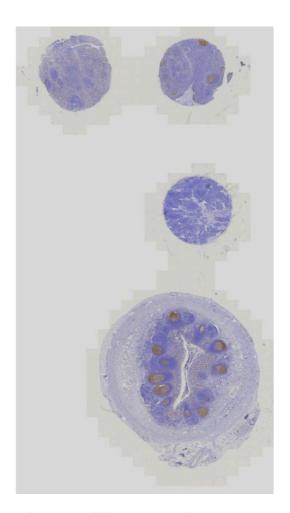


MIB-1 (Ki-67)



HE staining in scanner view.



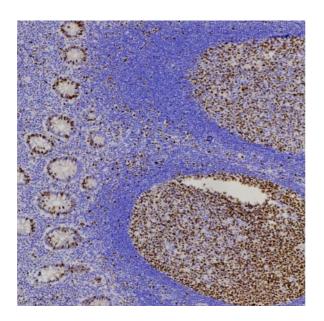
Immunostaining, scanner view

Provided sections in this run.

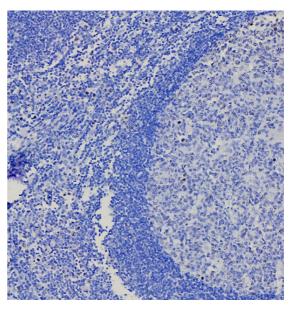
Follicular lymphoma, lymph node with reactive hyperplasia, Infiltrating duct carcinoma of breast and appendix were submitted. Out of these, follicular lymphoma and IDC were low proportion cases while the lymph node and appendix were high proportion cases.

General observations in Run 16

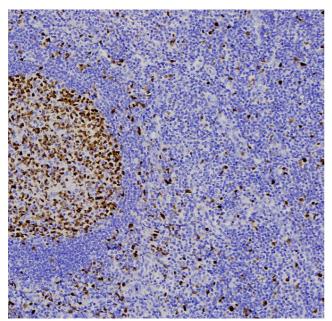




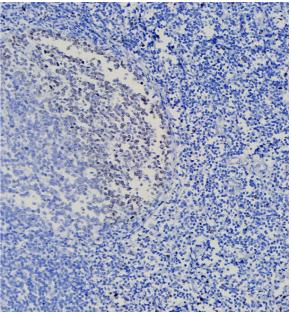
Optimum staining of appendix.



Suboptimal staining due to inferior antibody.

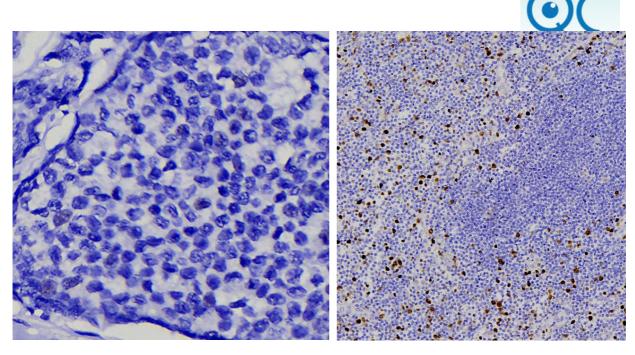


Optimum staining in appendix



Suboptimal staining due to inappropriate HIER. Note the irregular drying. The staining is less intense and less in proportion.

General observations in Run 16



Extremely weak intensity staining was noted in occasional tumour cells in case of IDC.

Optimal staining of follicular lymphoma

Protocol used by the top 3 in this run

| Variable | Protocol not submitted by this participant | 2 | 3 |
|----------------------|--|--|--|
| Technique | | Manual | Automated. Roche Ventana Benchmark XT |
| Clone | | MIB-1 | 30-9 |
| Vendor | | DAKO IS626 | VENTANA 790-4286 |
| Format | | RTU | RTU |
| Batch/Year | | 20079815 | H10583 |
| Expiry | | 31/12/2022 | 14/07/2023 |
| Dewaxing temperature | | 68 DEGREE CELSIUS | 75C in EZ Prep |
| Retrieval | | HIER | Heat Induced Epitope Retrieval (HIER) |
| Enzyme | | N/A | NA |
| HIER | | Microwave oven | CC1 STANDARD PROTOCOL |
| Peak T and Time | | 600watt/800; 8 minutes then dilute with distilled water to make volume then again 8 minutes | 120C 2 MIN |

General observations in Run 16



| Variable | Protocol not submitted by this participant | 2 | 3 |
|------------------------|--|---|---|
| Peak Pressure and Time | | N/A | NA |
| Retrieval Buffer | | Company provided, PathnSitu, Epitope Retrieval buffer, TRIS EDTA (50X) | Company Provided, Tris-EDTA Buffer |
| рН | | 9 | 7.8 |
| Blocking | | Polyexcel Hydrogen Peroxidase, 10 | Inhibitor Cm, 16 min |
| Wash sol | | Immunowash Buffer (25x) TRIS buffered saline with saponifying agent (TWEEN 20) | 10XSSC |
| Dilution of RTU | | NO | NO |
| Dilution of conc | | N/A | NA |
| Diluent | | N/A | NA |
| Inc time of Primary | | 1 HOUR | 32 MIN |
| Detection | | Polymer based system | LSAB |
| Cat No | | Polyexcel Target Binder , PathnSitu, IPS006,I08006CC, 4/2023 Polyexcel HRP labelled polymer, PathnSitu, IPS007,I08007CC, 4/2023 | Ultraview Universal DAB Detection kit F22794- F23052 14/07/2023 |
| Inc time of Sec | | Polyexcel Target Binder- 10 minutes Polyexcel HRP labelled polymer- 10 minutes | NA |
| Chro-substrate | | 5-10 MINUTES | 8 MIN |
| Post-treatment | | NO | COPPER Cm, 5min |
| Counterstain | | HAEMATOXYLIN | Haematoxylin |