IHC Optimization of SARS-CoV2 (COVID-19) Virus Spike Protein Antibody using Tissue-Tek Genie® Advanced Staining System

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Introduction & Objective

❖ COVID-19, a primarily respiratory and occasionally multisystemic disease, is caused by Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2).

❖ It remains unknown whether the SARS-CoV-2 Spike protein, the product of mRNA vaccines accumulates in the tissues after vaccination.

❖ Recent studies characterize histological changes related to COVID-19 but accurate immunohistochemical detection of SARS-CoV-2 remains to be challenging .

❖ The goal of this study is to develop an immunohistochemistry protocol for the detection of spike protein of SARS-CoV-2 Virus using the Tissue-Tek Genie® Advanced Staining System. This study does not promote any *in-vitro* diagnostic use.

Materials & Methods

Materials required

- Tissue-Tek Genie® Dewax Solution (8865-G001)
- Tissue-Tek Genie® Wash Buffer Solution (8874-G004)
- Tissue-Tek Genie® High pH Antigen Retrieval Solution (8744-G001)
- Tissue-Tek Genie® Citrate Antigen Retrieval Solution(8742-G001)
- Tissue-Tek Genie® *Pro* Detection Kit, DAB (8826-K250)
- Tissue-Tek Genie® Hematoxylin (8830-M250)
- Tissue-Tek Genie® Reagent Dispense Area [RDA] (8616-G090)
- Tissue-Tek Genie® Pro Antibody Diluent, (8866-G004)
- Genie Antibody Diluent 3.0 Bulk (GS-21027)

   Further information can be found on the Sakura Finetek USA website at www.sakuraus.com/Genie

Primary antibodies:
Two primary antibodies from available commercial vendors detecting the SARS-CoV2 Virus Spike Protein were evaluated for the detection of SARS-CoV2 virus on human FFPE tissues using Tissue-Tek Genie® Advanced Staining System.

1. **Mouse anti SARS-CoV-2 Spike Receptor binding domain (RBD) [1035423] Antibody**
   ❖ This antibody detects SARS-CoV-2 Spike RBD and SARS-CoV-2 B.1.1.529 S RBD (Omicron Variant) in direct ELISAs. (Source: available from the vendor datasheet)

2. **Mouse anti SARS-CoV-2 (COVID-19) spike protein [1A9] antibody**
   ❖ This antibody is raised against S2 fragment of spike protein of SARS-CoV which also cross reacts with SARS-CoV-2 and its multiple variants such as Omicron. (Source: available from the vendor datasheet)

FFPE Human Tissues:
   ❖ Two FFPE human placenta tissue specimens from clinically diagnosed COVID-19 positive patients and stained reference slide were received from our external collaborator, NordiQC (NQC). These slides were used as positive controls.
   ❖ Normal placenta, TMA- normal, TMA – neoplasia were used to evaluate the specificity of the antibody.
   ❖ Diluent control was used to evaluate the specificity of assay.
   ❖ Other tissues such as lung and placenta from COVID-19 patients from different resources were also tested.

Results

### 1. Mouse anti SARS-CoV-2 Spike Receptor binding domain RBD [1035423] Antibody
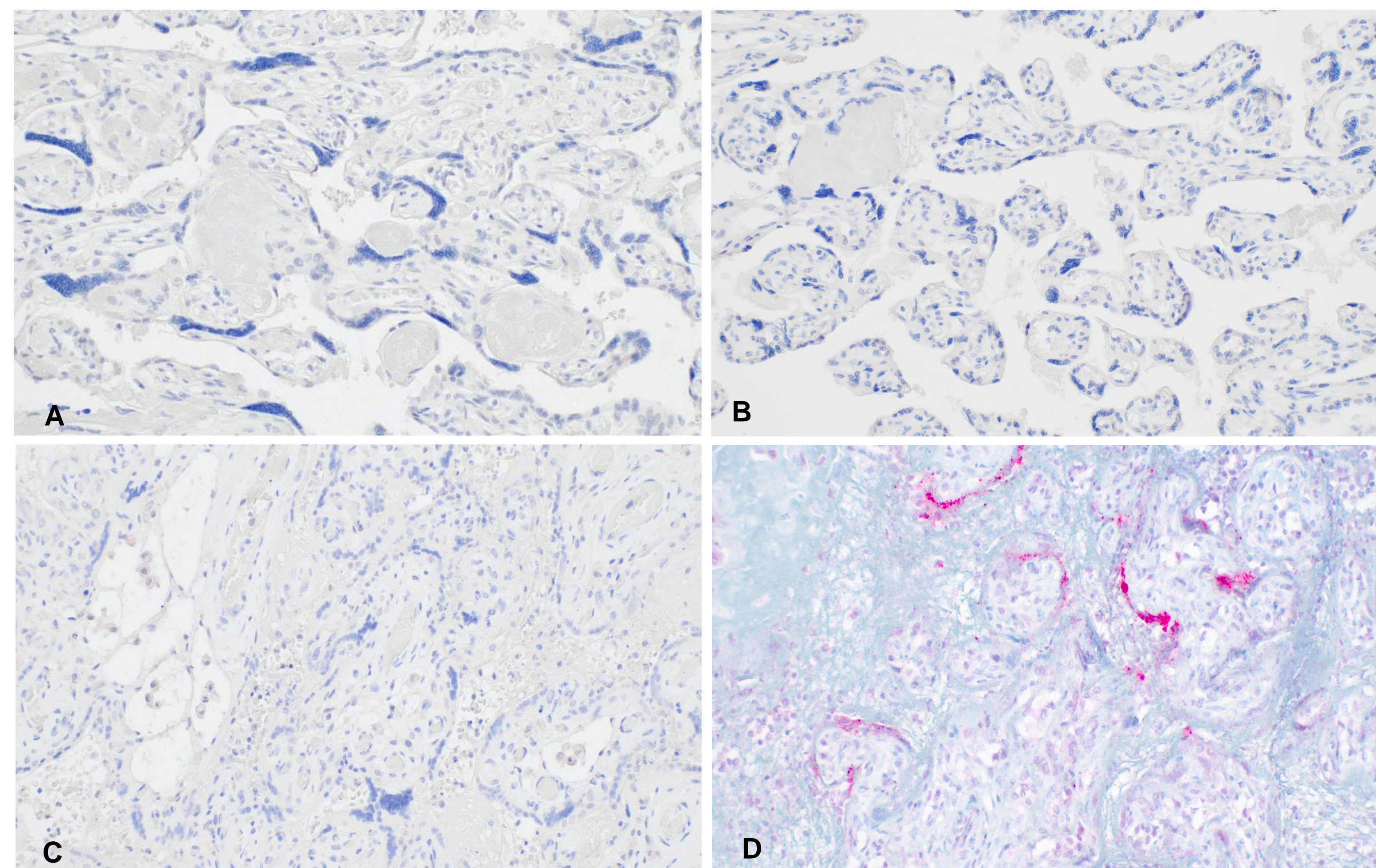
Figure-1. Specificity of the antibody [1035423] was evaluated by tittering the antibody using Tissue-Tek Genie® Pro Antibody Diluent, dilution factor (DF) 200 (2.5 µg/mL) on normal placenta using Tissue-Tek Genie® High pH Antigen Retrieval Solution for 45 min (**A, 200X**) and TMA–normal (data not shown). DF 200 (2.5 µg/mL) showed no to minimal background staining in normal placenta. Cytoplasmic staining in various cores of TMA normal was observed (data not shown). Specificity of the assay was evaluated using Tissue-Tek Genie® Pro Antibody Diluent (**B, 200X**). Sensitivity of the antibody [1035423] at DF 200 (2.5 µg/mL) was evaluated on NQC placenta tissue 1 (**C, 200X**). Reference-stained slide for NQC placenta tissue 1 from NQC is shown in (**D, 200X**). DF 200 (2.5 µg/mL) showed no to very weak mainly cytoplasmic staining pattern in NQC placenta tissue 1 when compared to NQC reference slide. Similar staining pattern was observed in NQC placenta tissue 2 (data not shown). Mouse anti- SARS-CoV-2 Spike Receptor binding domain RBD [1035423] Antibody showed reduced specificity and sensitivity on the tested tissues.

2. Mouse anti SARS-CoV-2 (COVID-19) spike protein [1A9] antibody
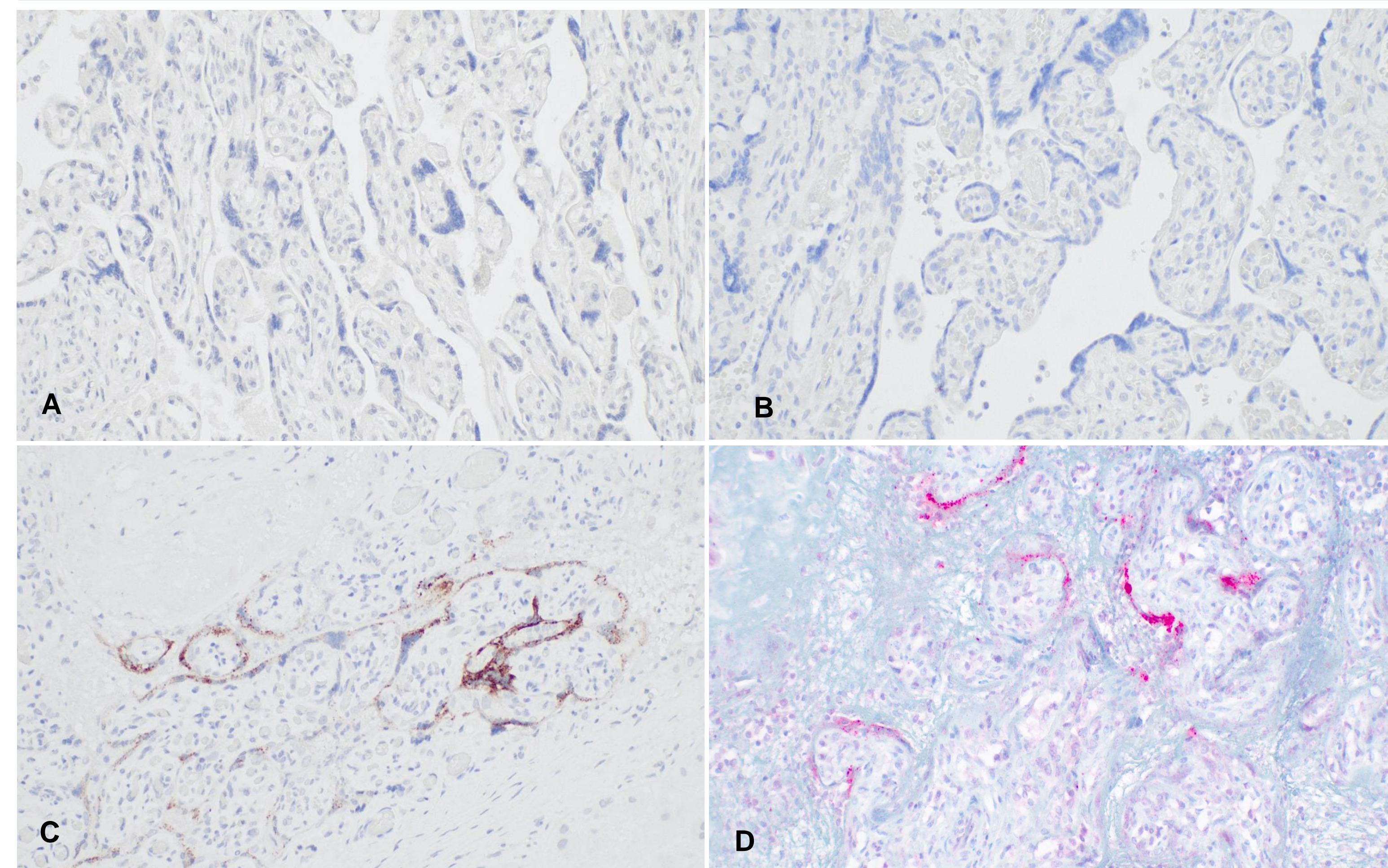
Figure-2. Specificity of the antibody [1A9] was evaluated by tittering the antibody using Tissue-Tek Genie® Pro Antibody Diluent (DF) 1000 (1µg/mL) on normal placenta (**A, 200X**) and TMA –normal (data not shown) with DF 2000 (0.5 µg/mL) (**B, 200X**). Sensitivity of the antibody [1A9] at DF 2000 (0.5 µg/mL) was evaluated on NQC placenta tissue 1 (**C, 200X**). Reference stained slide for NQC placenta tissue 1 from NQC is shown in (**D, 200X**). DF 2000 (0.5 µg/mL) was tested on TMA normal which showed no to minimal background with few cores particularly (tonsil) of TMA normal. DF 2000 (0.5 µg/mL) at high pH showed moderate to strong cytoplasmic staining in syncytiotrophoblasts in the NQC placenta tissue 1 and 2 when compared to NQC reference slides. Mouse anti SARS-CoV-2 (COVID-19) spike protein [1A9] antibody showed good specificity and sensitivity on the tested tissues at high pH with Tissue-Tek Genie® Pro Antibody Diluent using the Tissue-Tek Genie® Advanced Staining System.

Evaluation of [1A9] antibody with various FFPE human tissues
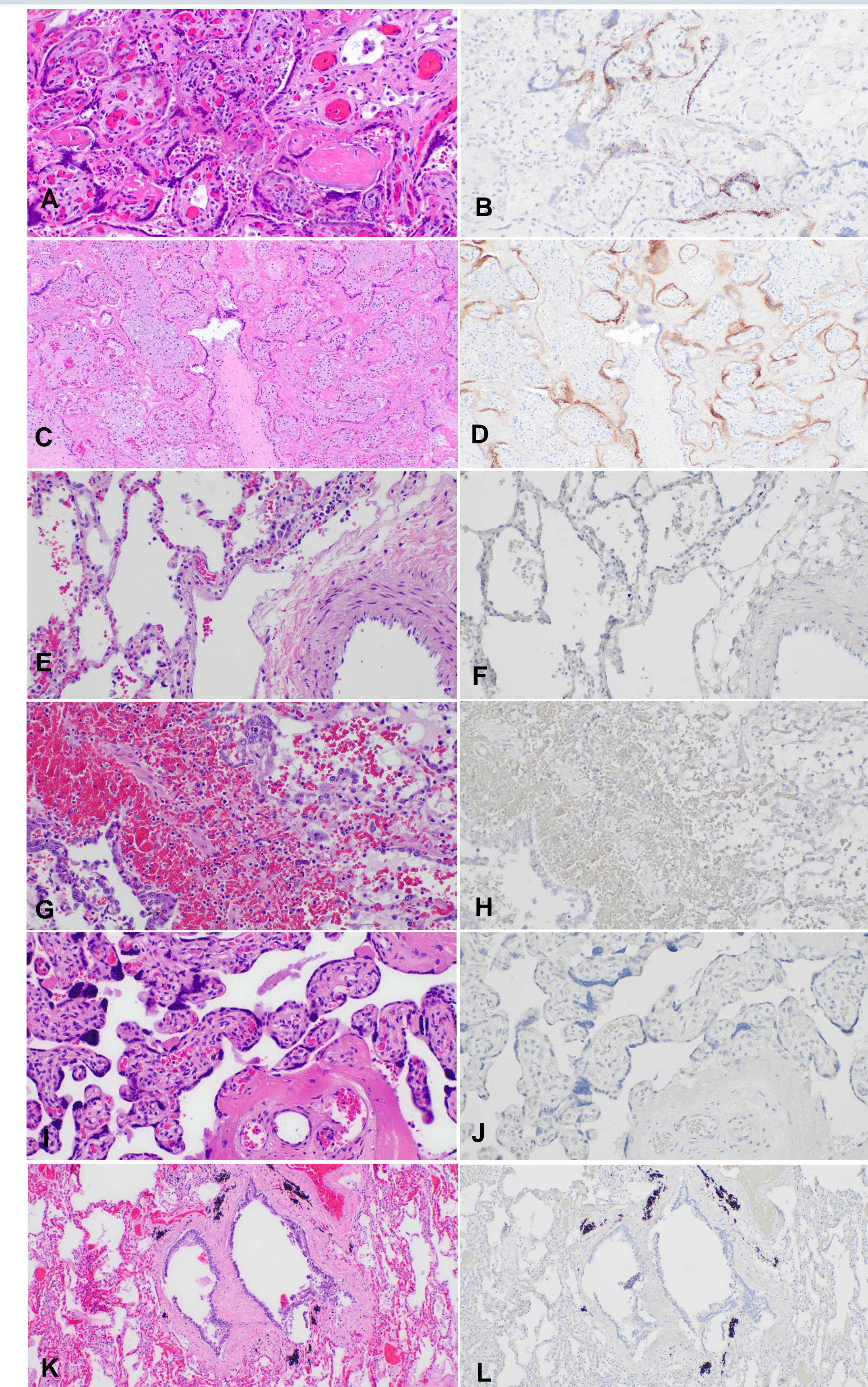
Figure-3. Acute and chronic intervillositis and perivillous fibrin deposition with necrosis and viral changes exhibiting multinucleated cells with glassy cytoplasm (H&E, 200x) (**A**) Moderate to strong cytoplasmic positivity for SARS-CoV-2 in placental syncytiotrophoblasts (IHC, **200X**) (**B**). Acute and chronic intervillositis and perivillous fibrin deposition and early intervillous thrombus (H&E, **200X**) (**C**). Moderate to strong cytoplasmic positivity for SARS-CoV-2 in placental syncytiotrophoblasts (IHC, **200X**) (**D**). Mild, focal emphysematous changes and vascular congestion with rare peribronchial lymphocytic inflammation (H&E, **200X**) (**E**). Negative SARS-CoV-2 staining was observed (IHC, **200X**) (**F**). Lung with diffuse alveolar damage (cellular and proteinaceous exudates, extensive intraalveolar, subpleural and perivascular hemorrhage, reactive pneumocytes, and vasculitis) (H&E, **200X**) (**G**), Negative SARS-CoV-2 staining was observed (IHC, **200X**) (**H**). Mild placental syncytial knot hyperplasia (H&E, **200X**) (**I**), Negative SARS-CoV-2 staining was observed (IHC, **200X**) (**J**). Lung exhibiting emphysema with reactive macrophages, vascular congestion and focal perivasculitis and bronchial mucous plug (H&E, **200X**) (**K**), Negative SARS-CoV-2 staining was observed (IHC, **200X**) (**L**). Citrate and high pH antigen retrieval solutions and antibody diluents were tested with anti-SARS-CoV-2 antibody [1A9]. Heat-induced epitope retrieval (HIER) with Tissue-Tek Genie® High pH Antigen Retrieval Solution and with Tissue-Tek Genie® Pro Antibody Diluent showed best staining pattern.

Conclusion

The preliminary data suggest that the optimized protocol using anti-SARS-CoV-2 antibody [1A9] with the Tissue-Tek Genie Advanced Staining System may be useful for the detection of multiple variants of the SARS-CoV-2. However, testing of more samples are required to determine sensitivity and specificity.

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