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The science you expect.
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SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Final Report

FOR

**U.S. Food and Drug Administration
Office of Counterterrorism and
Emerging Threats (OCET)**

Robert W. Fisher, Ph.D.

Senior Advisor for CBRN and Pandemic Influenza
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MRIGlobal Project No. 110890.01.208.06.01.04

February 1, 2023

Preface

This final report was prepared at MRIGlobal for the work performed under MRIGlobal Project No. 110980.01.208.06.01.04, for the study entitled, “SARS-CoV-2 Infection Via the Oral Exposure In Hamsters.” Robert Fisher, Ph.D. was the sponsor representative for the study.

The study was initiated on December 21, 2021 and in life completed in January 12, 2022. Additional in-situ hybridization testing was conducted through September 2022. The study was managed by Kristina Eichhorst as study coordinator and by Rachel Olsen, Ph.D. at the University of Missouri Laboratory of Infectious Disease Research (LIDR). They were assisted by; Carl Gelhaus, Ph.D., Deborah Anderson, Ph.D., Paul Anderson, Ph.D., Bree Cox, Samantha Gem, Nathan Henderson, Molly Maloney, Kristy Solocinski, Ph.D., Steven Timotijevic, Jeff Whyte, Ph.D., and Jacob Wilkinson.

Although it was not required for this study to be performed in accordance with FDA GLP (21 CFR Part 58) regulations, MRIGlobal ensures that quality processes were in place for the performance of the study, and the work procedures, the data, and the final report were reviewed for quality. All raw data, including original study records, data sheets, work sheets, and copies of reports are stored at MRIGlobal.

MRIGLOBAL



Gene Olinger, Ph.D.
Study Director

Approved by:



Phillip H. Beske, Ph.D., D.A.B.T.
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February 1, 2023

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Section 1.

Abstract

COVID-19 is a global public health emergency caused by the SARS-CoV-2 virus. Food safety is a major consideration during a pandemic. Food products, such as produce, may become inadvertently contaminated when displayed in grocery stores here in the United States. Moreover, a fecal-oral transmission risk may exist due to the prolonged excretion of SARS-CoV-2 from the gastrointestinal tract: several reports have identified the presence of high levels of SARS-CoV-2 RNA in feces, although infectious virus has not yet been recovered. We do not currently know the risk posed by oral exposure to the SARS-CoV-2 virus. The SARS-CoV-2 virus infects human cells by binding to the ACE2 receptor. The hamster has a similar ACE2 gene to the human and therefore is a good candidate for an animal model of COVID-19. Our first study of oral inoculation of hamsters indicated shedding of viral RNA in the feces for at least 7 days with the UK variant. The objective of this study was to determine the duration of viral RNA shedding for up to 21 days as well as examine pathology following oral exposure to the virus at 7, 14, and 21 days after exposure. SARS-CoV-2 RNA was detected in the feces of hamsters up until the last day of study, 21 days after exposure. There were pathology findings in the lungs and nasal turbinates, but not in any of the gastro-intestinal (GI) tissues. Fluorescent in situ hybridization (FISH) was conducted on GI and other tissues. We found that virus was persistent in stomach and small intestine, as well as in kidney. These findings indicate that oral delivery of SARS-CoV-2 can establish infection in the GI as well as other (kidney) tissue. The infection results in shedding of virus for up to 21 days. The infection can also spread to the respiratory tract, but it is unclear how the infection travels from the GI to the respiratory tract.

Section 2.

Objective and Justification

The objective of this study was to determine the duration of viral RNA shedding for up to 21 days as well as examine pathology following oral exposure to the virus at 7, 14, and 21 days after exposure.

Section 3.

Sponsor, Testing Laboratory, and Personnel Responsibilities

3.1 Sponsor

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Senior Advisor for CBRN and Pandemic Influenza
10903 New Hampshire Avenue
Silver Springs, Maryland 20993

3.2 Sponsor's Representative

Robert Fisher, Ph.D.
Senior Advisor for CBRN and Pandemic Influenza
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U.S. Food and Drug Administration
Phone: (301) 796-8518

3.3 Testing Laboratory

MRIGlobal
425 Dr. Martin Luther King Jr. Blvd.
Kansas City, Missouri 64110
Phone: (816) 753-7600
Fax: (816) 753-8823

3.3.1 Testing Site

While MRIGlobal oversaw the testing, actual performance of the experiments was executed at:

Laboratory for Infectious Disease Research (LIDR)
1020 East Campus Loop
University of Missouri
Columbia, Missouri 65211

3.4 Personnel Responsibilities

H. Carl Gelhaus, Ph.D.
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Section 4.

Materials and Methods

4.1 Challenge agent

The challenge material was SARS-CoV-2 isolate USA/CA_CDC_5574/2020 from BEI Resources. The PANGO lineage for this isolate was the same as the UK variant (B.1.1.7). This virus has been passed through Vero cells once to obtain a master virus bank and the master virus bank was passed through Vero cells to obtain a working virus bank. The working virus bank was tested here. Details are in Table 1.

Table 1. Challenge Article

Name:	SARS-CoV-2
Virus Classification:	SARS-CoV-2
Strain/Isolate:	<i>Coronaviridae</i>
Lot No.	USA/CA_CDC_5574/2020
TCID ₅₀ /mL	To be included in the final report
Copy number/mL	1.1×10^7
Supplier	9.0×10^{10}
Storage Conditions:	MRIGlobal (derived from BEI Resources)

4.2 Test Animals

4.2.1 Species/Strain

Syrian Golden Hamsters

Species: *Mesocricetus auratus*

Strain: Outbred

4.2.2 Source

Charles River Laboratories
251 Ballardvale Street
Wilmington, Massachusetts 01887-1096

4.2.3 Sex and Number

15 male and 15 female

4.2.4 Age

The male hamsters used for this study were born on October 22, 2021. The female hamsters were born October 15, 2021. The animals were received on December 21, 2021.

4.2.1 Criteria

Hamsters were free from obvious clinical signs of ill health or malformation or any other condition that would interfere with the conduct of the study in the judgment of a veterinarian. Animals were replaced prior to challenge, if necessary.

4.2.2 Animal Identification

The hamsters were identified with ear tags with individual ID numbers.

The cages bore cards detailing the project number; the name of the study director, species, gender, and vendor where the animals were obtained; date of birth, date received, animal identification numbers, dose group (cohort) number, identification and concentration of test article received, route, and the date dosing occurred. The identification of each animal was verified at dosing, and upon death.

4.3 Animal Care

General procedures for animal care and housing were followed in accordance with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council, National Academic Press, 2011), the Animal Welfare Act (US Public Law 89-544 and all subsequent amendments), and the Public Health Service, “Policy on Humane Care and Use of Laboratory Animals” (revised 2015).

4.3.1 Diet

All animals were provided with Lab Diet 5008 Formulab Diet feed *ad libitum*.

Acidified reverse osmosis water was provided *ad libitum*.

4.3.2 Housing

Animals have been shown to transmit SARS-CoV-2 animal to animal. Therefore, all animals were individually housed in polycarbonate Tecniplast caging (Tecniplast, Phoenixville, PA) throughout the acclimation/quarantine period and post immunization and challenge. This microisolator caging ensured that animals cannot cross-contaminate each other or the room they are housed in. The hamsters were housed in environmentally controlled rooms. The rooms were maintained at a temperature of 68.0° to 79.0°F and a relative humidity of 50% ± 30% with a 12-h light/dark cycle per day.

4.4 Experimental Design

The study followed the protocol, which can be found in Appendix A. Hamsters were exposed to virus or PBS control on Study Day 0 via oral gavage. Hamsters were be individually housed in cages. There were six groups. Group 1-3 consisted of 3 male and 3 female hamsters each. Groups 4-6 consisted of 2 male and 2 female hamsters each (Table 2). Groups 1-3 were exposed to $\sim 1 \times 10^6$ TCID₅₀ of SARS-CoV-2. Groups 4-6 were exposed to PBS.

Every day post-infection, feces was be collected. Hamsters were removed from cages and weighed daily. At the time of body weight measurement, hamsters tended to release feces and two pellets of feces were collected, as possible. Fecal pellets were stored at $< -60^{\circ}\text{C}$ until analysis. All animals were observed twice daily for signs of clinical illness. Groups 1 and 4 were monitored for 7 days, Groups 2 and 5 were monitored for 14 days, and Groups 3 and 6 were monitored for 21 days. Nasal turbinates, trachea, lung, stomach, cecum, duodenum, colon, ileum, jejunum, and heart were collected and fixed in formalin. Tissues were sectioned and stained. Slides were analyzed by a board-certified pathologist. Additional selected

Table 2. Study Design

Group	N (50% male /50% female)	Challenge material	Challenge Route	Day of Sacrifice	Endpoints
1	6	~ 1 x 10 ⁶ TCID50 SARS-CoV-2 UK strain	Oral	7	Daily fecal collection
2	6			14	Daily body weight measurements
3	6			21	
4	4	PBS Control		7	Tissues collected on day of sacrifice and placed in formalin: nasal turbinates, trachea, lung, GI, and heart
5	4			14	
6	4			21	

4.4.1 Oral Gavage

On the day of challenge (Study Day 0), the UK variant virus was diluted in DPBS. The dilution and confirmation of dose was demonstrated by the TCID₅₀ assay of the challenge stock (i.e., back-titer). The backtiter was 1.0×10^5 TCID₅₀/mL. The challenge dose was inoculated by oral gavage, approximately 0.8 mL using a syringe with a gavage needle.

4.4.2 Clinical Observations

General health observations were performed at least twice daily after challenge from Study Day 0 through Study Day 21 on all surviving hamsters. Observations were made without opening cages, to prevent transmission of SARS-CoV-2 infection from one animal to another.

4.4.3 Feces Collection

Once per day, the feces will be collected into an individually labelled, pre-weighed tube for each hamster on each day. One aliquot will be made for TCID₅₀, a second aliquot for PCR, and all excess feces will be collected into a third tube (the excess feces tube will not be weighed). The PCR and TCID₅₀ tubes will be weighed after the feces is added to determine the mass of feces in each tube. Feces in tubes will be stored at $\leq -60^{\circ}\text{C}$ until analyzed.

4.4.4 Body Weights

Body weights were measured daily beginning on Study Day 0 through Study Day 21 on all surviving hamsters.

4.4.5 TCID₅₀

One aliquot of each feces sample was used to assess presence of infectious virus, in accordance with SOP MRI-8701, “50% Tissue Culture Infectious Dose (TCID₅₀) Titration of Biological Safety Level (BSL)-3 Coronaviruses.”

4.4.6 PCR

One aliquot of each feces sample was used to assess presence of infectious virus in a real time quantitative PCR (RT-qPCR) assay. RT-qPCR assays were performed in accordance with SOP MRI-9770, “Nucleic Acid Quantification using Real-Time Polymerase Chain Reaction (PCR).”

4.4.7 Euthanasia

Hamsters were euthanized on Study Day 7, 14, or 21 as shown in Table 2. Euthanasia was performed via CO₂ asphyxiation confirmed by vital organ harvest. There were no unscheduled deaths or euthanasia on study.

4.4.8 Histopathology

Tissues collected at necropsy included the nose/nasal cavity and turbinates, the trachea, lungs (all of which were infused with 10% neutral buffered formalin (NBF); plus stomach, intestinal tract, and heart. All these tissues were then preserved by immersion in additional NBF. Once fixed, the specimens were shipped to StageBio, Inc (Mount Jackson, VA) for routine paraffin processing. Slides were cut and stained with hematoxylin and eosin and sent to Toxpath Solutions for microscopic review and evaluation.

4.4.9 Fluorescent in situ hybridization

Sections of gastro-intestinal tissues were further analyzed by fluorescent in situ hybridization at the LIDR. Tissues fixed in paraffin blocks were further processed by the Invitrogen ViewRNA Tissue Assay user guide according to the manufacturer’s instructions:

ViewRNA Tissue Probe Sets were designed using the SARS-CoV-2 NCBI strain and sequence from Accession Number NC_045512, and cover multiple regions of the viral transcript. The positive strand was detected using the Type 1 (Fast Red; Red in BF/ Red FL) probe set and the negative strand using the Type 6 (Fast Blue; Aqua Blue in BF/ Far red in FL) probe set. Hematoxylin and DAPI were used as counter stains. All temperatures were monitored and confirmed using a calibrated digital thermometer; steps were performed at room temperature unless noted.

Day 1

Blocks were sliced in 5µm sections and affixed to slides. Slides were prepared by baking in a dry oven at 60°C for 60 minutes. A humidified hybridization system was prepared by placing pans of water in a static bacterial incubator set to 40°C. All solutions were prepared according to the manufacturer's instructions and recommendations. Tissue sections were deparaffinized by doing incubating with xylene for 5 minutes with agitation. Fresh xylene was used for a second incubation under the same conditions. Slides were then washed twice with 100% ethanol and allowed to air dry for 5 minutes. A hydrophobic barrier was then drawn on each slide using a wax pencil. Slides were submerged in 95°C pretreatment solution (provided in kit) for 20 minutes, washed twice for 1 minute in ddH₂O with frequent agitation, and transferred to 1X PBS for overnight storage, sealing film was used to prevent evaporation.

Day 2

One at a time, slides were removed from PBS (excess PBS was removed by tapping the edges and wiping the back with a laboratory wipe), placed flat on an incubator tray, and 400µL of 1X protease solution (provided in kit) was added with sufficient spread to cover the entire tissue section. Once all slides were prepared, the incubator tray was placed in the hybridization system and incubated at 40°C for 20 minutes. After incubation, protease solution was decanted and slides were washed twice in 1X PBS. Slides were then transferred to a 10% neutral-buffered formalin solution, fixed for 5 minutes, and washed twice in 1X PBS. One at a time, slides were removed from PBS (excess PBS was removed by tapping the edges and wiping the back with a laboratory wipe), placed flat on an incubator tray, and 400µL of pre-warmed probe set solution (provided in kit) was added with sufficient spread to cover the entire tissue section. Once all slides were prepared, the incubator tray was placed in the hybridization system and incubated at 40°C for 2 hours. After incubation, probe set solution was decanted from slides and they were placed in wash buffer. Slides were washed three times for two minutes each with constant and vigorous agitation. Slides were then placed in storage buffer (provided in kit) for overnight storage, sealing film was used to prevent evaporation.

Day 3

Slides were removed from storage buffer and washed three times for 2 minutes each in wash buffer with agitation. One at a time, slides were removed from wash buffer (excess buffer was removed by tapping the edges and wiping the back with a laboratory wipe), placed flat on an incubator tray, and 400µL of Pre-amplifier Mix QT (provided in kit) was added with sufficient spread to cover the entire tissue section. Once all slides were prepared, the incubator tray was placed in the hybridization system and incubated at 40°C for 25 minutes. After incubation, Pre-amplifier Mix QT was decanted from slides and they were placed in wash buffer. Slides were washed three times for two minutes each with constant and vigorous agitation. One at a time, slides were removed from wash buffer (excess buffer was removed by tapping the edges and wiping the back with a laboratory wipe), placed flat on an incubator tray, and 400µL of Amplifier Mix QT (provided in kit) was added with sufficient spread to cover the entire tissue section. Once all slides were prepared, the incubator tray was placed in the hybridization system and incubated at 40°C for 15 minutes. After incubation, Amplifier Mix QT was decanted from slides and they were placed in wash buffer. Slides were washed three times for two minutes each with constant and vigorous agitation. One at a time, slides were removed from wash buffer (excess buffer was removed by tapping the edges and wiping the back with a laboratory wipe), placed flat on an incubator tray, and 400µL of Working Label Probe 6-AP Solution (provided in

kit) was added with sufficient spread to cover the entire tissue section. Once all slides were prepared, the incubator tray was placed in the hybridization system and incubated at 40°C for 15 minutes. After incubation, Working Label Probe 6-AP Solution was decanted from slides and they were placed in wash buffer. Slides were washed three times for two minutes each with constant and vigorous agitation. One at a time, slides were removed from wash buffer (excess buffer was removed by tapping the edges and wiping the back with a laboratory wipe), placed flat on an incubator tray, and 400µL of Fast Blue Substrate (provided in kit) was added with sufficient spread to cover the entire tissue section. Once all slides were prepared, the incubator tray was placed in the dark at room temperature for 30 minutes. After incubation, Fast Blue Substrate was decanted from slides and they were placed in wash buffer. Slides were washed three times for two minutes each with constant and vigorous agitation. One at a time, slides were removed from wash buffer (excess buffer was removed by tapping the edges and wiping the back with a laboratory wipe), placed flat on an incubator tray, and 400µL of AP Stop QT (provided in kit) was added with sufficient spread to cover the entire tissue section. Once all slides were prepared, the incubator tray was placed in the dark at room temperature for 30 minutes. After incubation, AP Stop QT was decanted from slides and they were placed in 1X PBS. Slides were washed twice for two minutes each with constant and vigorous agitation. 1X PBS was replaced with wash buffer and slides were washed once for one minute with constant and vigorous agitation. One at a time, slides were removed from wash buffer (excess buffer was removed by tapping the edges and wiping the back with a laboratory wipe), placed flat on an incubator tray, and 400µL of Working Label Probe 1-AP Solution (provided in kit) was added with sufficient spread to cover the entire tissue section. Once all slides were prepared, the incubator tray was placed in the hybridization system and incubated at 40°C for 15 minutes. After incubation, Working Label Probe 1-AP Solution was decanted from slides and they were placed in wash buffer. Slides were washed three times for three minutes each with constant and vigorous agitation. One at a time, slides were removed from wash buffer (excess buffer was removed by tapping the edges and wiping the back with a laboratory wipe), placed flat on an incubator tray, and 400µL of AP Enhancer Solution (provided in kit) was added with sufficient spread to cover the entire tissue section. Once all slides were prepared, they were incubated room temperature for 5 minutes. After incubation, AP Enhancer Solution was decanted from slides and 400 µL of Fast Red Substrate (provided in kit) was immediately added onto each tissue section. Once all slides were prepared, the incubator tray was placed in the hybridization system and incubated at 40°C for 30 minutes. After incubation, Fast Red Substrate was decanted from slides and they were placed in 1X PBS. Slides were washed once for one minute with constant and vigorous agitation. Slides were transferred to a staining dish with Gill's hematoxylin and briefly stained. Slides were washed three times in ddH₂O for one minute each with constant and vigorous agitation. Slides were transferred to 0.01% ammonium hydroxide and incubated for 10 seconds. Slides were washed once in ddH₂O for one minute with constant and vigorous agitation. Slides were transferred to 3 µg/mL DAPI for one minute. Slides were washed once in ddH₂O for one minute with constant and vigorous agitation. One at a time, slides were removed from ddH₂O (excess ddH₂O was removed by tapping the edges and wiping the back with a laboratory wipe), placed flat on a paper towel on an incubator tray, and completely air dried for 20 min. Slides were mounted with Fluoromount-G mounting medium (Thermo Fisher) and edges sealed with clear nail polish.

Image Analysis of SARS-CoV-2 Probe (Negative-Strand) Fluorescence Intensity

Hamster tissue section images were acquired with a Lionheart FX Automated Microscope (Agilent/BioTek) and fluorescence was quantitatively analyzed with the instrument-associated Gen5 Image Prime software.

Briefly, slides were loaded into a slide adapter in a coverslip-down configuration. The Lionheart FX was used to image slides using 4x objective. A 15x15 montage of 225 image “tiles” (1973 μm x 1457 μm each) was acquired for four channels: (1) bright field, (2) DAPI nuclear staining with a DAPI filter cube (excitation 377/50 nm, emission 447/60 nm) equipped with a 365 nm LED cube, (3) Alexa Fluor 546 staining for SARS-CoV-2 positive strand with an RFP filter cube (excitation 531/40 nm, emission 593/40 nm) equipped with a 523 nm LED cube, and (4) Alexa Fluor 647 staining for SARS-CoV-2 negative strand with a Cy5 filter cube (excitation 628/40 nm, emission 685/40 nm) equipped with a 623 nm LED cube.

Optimal exposure settings were determined for each objective using a single slide and then applied to all images. Montage image tiles were stitched on the bright field channel using a linear blend method and 50% image downsizing to generate a single composite image montages with dimensions of 29,595 μm x 21,855 μm for each slide. Background fluorescence from each channel was corrected using image pre-processing settings with a rolling ball diameter of 24 μm and a background flattening priority set to fine results.

To quantify the SARS-CoV-2 negative strand fluorescence, a primary mask was defined on Alexa Fluor 647 stained objects (Cy5 channel) with a size from 3-100 μm and a signal threshold of 5000. Each slide contained up to four unique tissue type sections per montage area. Possible tissue sections on each slide included stomach, cecum, duodenum, jejunum, and ileum. For each tissue type in the montage, an outline was manually defined around the tissue section (i.e., a plug). Gen5 software metrics generated for each plug included object counts, mean Cy5 fluorescence intensity (MFI) per object, mean object size (μm) and area (μm^2), and total tissue plug area (μm^2). To calculate the total staining intensity of SARS-CoV-2 negative strand fluorescence in each tissue type, the sum of the individual MFI per object was divided by the total tissue plug area (MFI/ μm^2).

Section 5. Results

5.1 Clinical Observations

Animals were observed for signs of illness beginning on Study Day 0. Surviving animals were observed once a day until the day of sacrifice. In every instance, no unusual signs were observed.

5.2 Feces Collection

Initially, there was good collection of feces. Over time, it appeared the hamsters acclimated to handling and produced less feces during the weighing. The days each hamster had feces collection is shown in Figure 1. Individual animal collection data can be found in Appendix B. Initially, there were 60 pellets collected from the 30 hamsters on study, as planned. However, there was a drop in the number of pellets collected. Notice that there was a drop in pellets collected after Study Day 7, as Groups 1 and 4 were euthanized and after Study Day 14, as Groups 2 and 5 were euthanized. There were representative fecal samples from every group on every day of the study.

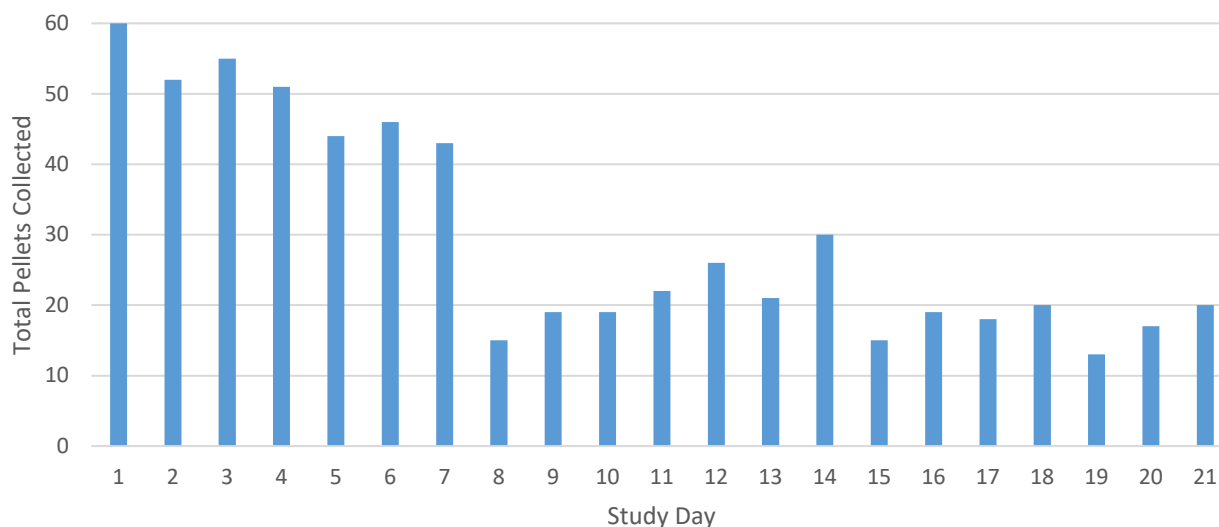


Figure 1. Total Fecal Pellets Collected

Every day on study, an attempt was made to collect two fecal pellets from each hamster at the time of body weight measurement. Initially, all 30 hamsters gave two fecal pellets each. However, as the study went on, less fecal pellets were produced. We believe this was due to hamsters acclimating to the daily handling. Note that after Study Day 7, the number of hamster reduced to 20 hamsters, with a maximum anticipated collection of 40 fecal pellets per day. After Study Day 14, the number of hamsters reduced to 10 hamsters with a maximum anticipated collection of 20 fecal pellets per day.

5.3 Body Weights

Body weights were collected from each animal on every day post-infection, for up to 21 days. Group average body weights are shown in Figure 2. Numerical body weight data for individual animals can be found in Appendix C. In general, animals gained weight over time, regardless of

dosing with SARS-CoV-2 or PBS. There appears to be no effect of SARS-CoV-2 oral gavage on body weight.

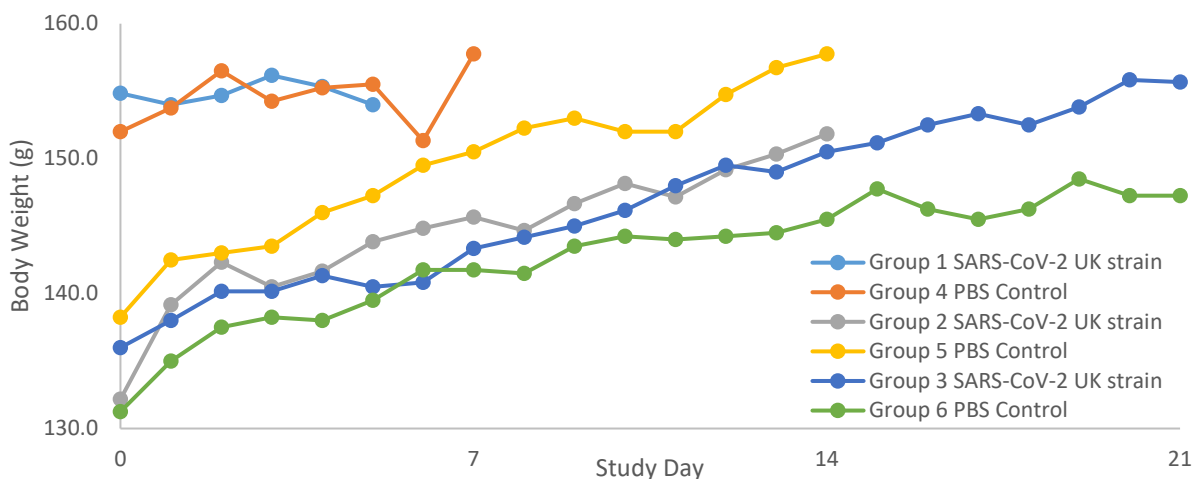


Figure 2. Group Average Body Weight

Every day on study, each animal was weighed. Group average body weights are displayed. Animals tended to increase in body weight regardless of group.

5.4 TCID₅₀

Fecal pellets were analyzed by TCID₅₀. No fecal pellet was ever observed to cause cytopathic effects on this study. As such, all samples were below the limit of detection (31.6 TCID₅₀/mL) and appear to lack the ability to infect cell culture.

5.5 PCR

Fecal pellets were analyzed by PCR. The geometric mean number of copies of SARS-CoV-2 RNA per gram of feces is shown in Figure 3. Numerical PCR results for individual fecal samples can be found in Appendix D. The data were variable, possibly due to the inconsistent harvesting of feces over time. However, the data suggest that continues to be shed for up to three weeks after oral infection.

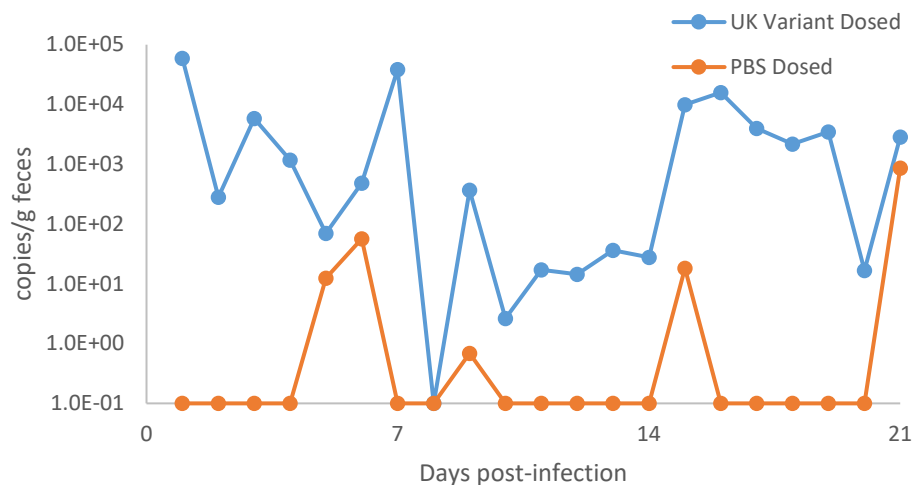


Figure 3. Group Geometric Mean PCR Titers in Feces

Every day on study, an attempt was made to collect two fecal pellets from each hamster at the time of body weight measurement. PCR was performed on the pellets collected to measure the copies of SARS-CoV-2 RNA per gram of feces. The geometric mean of all fecal samples obtained from animals dosed with SARS-CoV-2 UK variant was calculated as was the geometric mean of all fecal samples obtained from control animals dosed with PBS.

5.6 Necropsy and Histopathology

The complete pathologist's report can be found in Appendix E.

5.6.1 Necropsy

A variety of macroscopic findings in the lungs were recorded during the tissue collections, recorded in various animals as mottled and/or dark/red; a few lungs had notations of pale or purple or having red spots. Macroscopic notations in the lungs were recorded for all Group 1, 2, and 3 hamsters but none of the Group 4, 5, or 6 animals.

5.6.2 Histopathology

Most commonly, “mottled” or dark discoloration correlated microscopically with areas of interstitial lung inflammation; “pale discoloration” correlated with collections (aggregates) of alveolar macrophages; and red/purple correlated with multifocal areas of hemorrhage. Although not all gross lung findings had any confirmatory lesion found microscopically, many did, and all these were interpreted to be due to active Covid 19 pathology in the lungs.

To some degree, microscopic findings correlated with findings noted at necropsy, although not all animals had detectable microscopic changes even where gross lung mottling was recorded at necropsy. It is possible that some of the gross notations were only slight and actually due to vascular congestion, rather than Covid-related interstitial lung inflammation. In the 21-day cohorts, there were very few animals that displayed any microscopic evidence of Covid inflammation. This is consistent with prior studies in hamsters where lesions tended to begin

slowly over the first few days post-challenge, reach a maximum extent by about SD 7 or 8, and gradually recover to only minimal lesions by about SD 21.

Table 3. Summary Incidences of Selected Microscopic Findings (Males)

Group	1	2	3	4	5	6
Oral challenge agent	Virus	Virus	Virus	PBS	PBS	PBS
Termination day	7	14	21	7	14	21
Number examined	3	3	3	2	2	2
Lung						
Aggregate, alveolar macrophage	2	1	2	0	0	0
Inflammation, interstitial	2	0	1	0	0	0
Vasculitis	3	1	1	0	0	0
Nasal turbinate						
Exudate	2	0	0	0	0	0
Inflammation, olfactory epithelium	2	0	0	0	0	0
Inflammation, respiratory epithelium	2	0	0	0	0	0

Table 4. Summary Incidences of Selected Microscopic Findings (Females)

Group	1	2	3	4	5	6
Oral challenge agent	Virus	Virus	Virus	PBS	PBS	PBS
Termination day	7	14	21	7	14	21
Number examined	3	3	3	2	2	2
Lung						
Aggregate, alveolar macrophage	2	2	3	0	0	1
Inflammation, interstitial	1	1	1	0	0	0
Vasculitis	2	2	1	0	0	0
Nasal turbinate						
Exudate	1	0	0	0	0	0
Inflammation, olfactory epithelium	1	0	0	0	0	0
Inflammation, respiratory epithelium	2	0	0	0	0	0

One PBS (Group 6) female had a minimal, single focus of aggregated macrophages in the lungs as shown above. Such aggregates are often found in normal, untreated lungs and do not suggest accidental virus exposure.

In terms of the overall lung pathology observed, the UK variant of the SARS-CoV-2 virus caused the following pathologic changes in the lungs, similar to those seen in previous investigations of Covid infection in hamsters when challenged by the respiratory route:

1. Bronchiolar inflammation (the initial response to epithelial cell injury in the airways);
2. Bronchiolar epithelial cell hyperplasia (a hyperplastic response to epithelial cell injury or death as part of the repair process);
3. Aggregates of alveolar macrophages; (mostly alveolar macrophages early, but as time passes, these include more mixed cell lines such as monocytes and neutrophils, with a few multinucleated giant cells, which were coded separately because of their uniqueness);

4. Interstitial inflammation (interstitial pneumonia) with mild to marked thickening of alveolar walls by mixed but mostly mononuclear cell inflammation and minimal to mild “alveolar cell type II pneumocyte hyperplasia” (which was coded separately);
5. As noted in #3 above, the presence of small to large multinucleated giant cells;
6. Vasculitis, which ranged from minimal to severe *on an individual vessel basis*, consisting of perivascular infiltrates of mixed inflammatory cells; intraluminal monocyte margination of many pulmonary vessels; endothelial cell damage and significant transmigration of leukocytes (often neutrophils) through the vessel wall. While no obvious fibrin thrombosis of pulmonary vessels was found, vessel lumens were occasionally found with such severe leukocyte margination that no apparent patent passage was visible (possible “cell clots”);
7. Alveolar hemorrhage, generally found in hamsters with vasculitis; and
8. Mesothelial cell hypertrophy along the pleural surface, adjacent to where interstitial inflammatory foci were present.

5.6.3 Fluorescent In Situ Hybridization (FISH)

For animals that had PCR-positive fecal samples on the day of scheduled euthanasia, sections of the cecum, colon, duodenum, ileum, jejunum, and stomach were stained with a fluorescent marker specific for SARS-CoV-2. The images can be found in Appendix F. The fluorescence in each tissue is summarized in Table 5 below. All tissues demonstrated fluorescence, indicating infection of the GI tissues. There are no discernable trends in the fluorescence by day of infection or by tissue. These data do indicate that a SARS-CoV-2 infection of GI tissues does occur, even 21 days after oral infection.

Table 5. Summary of FISH Results

Slide Name	Tissue	Animal ID	Group	Days Post Infection	Sum of Fluorescence per Area of Tissue
Slide 3	Cecum	1207	1	7	78.6
Slide 5	Cecum	1201	1	7	90.0
Slide 6	Cecum	1204	1	7	93.4
Slide 7	Cecum	1207	2	14	108.9
Slide 11	Cecum	1216	3	21	109.7
Slide 12	Cecum	1204	1	7	87.9
Slide 13	Cecum	1201	1	7	97.1
Slide 14	Cecum	1216	3	21	156.3
Slide 15	Cecum	1202	1	7	91.6
Slide 17	Cecum	1207	2	14	115.7
Slide 2	Colon	1205	1	7	64.6
Slide 3	Colon	1207	1	7	102.0
Slide 8	Colon	1211	2	14	84.3
Slide 4	Duodenum	1211	2	14	141.1
Slide 5	Duodenum	1201	1	7	57.5
Slide 9	Duodenum	1211	2	14	39.2
Slide 14	Duodenum	1216	3	21	72.0
Slide 15	Duodenum	1202	1	7	63.5
Slide 16	Duodenum	1201	1	7	80.6
Slide 6	Duodenum of Jejunum	1204	1	7	53.7
Slide 17	Duodenum of Jejunum	1207	2	14	64.7
Slide 10	Duodenum†	1202	1	7	64.7
Slide 11	Duodenum†	1216	3	21	79.7
Slide 12	Duodenum†	1204	1	7	48.4
Slide 1	Ileum	1205	1	7	136.8
Slide 10	Ileum	1202	1	7	92.0
Slide 12	Ileum	1204	1	7	67.4
Slide 1	Jejunum	1205	1	7	78.8
Slide 2	Jejunum	1205	1	7	38.5
Slide 3	Jejunum	1207	1	7	74.1
Slide 8	Jejunum	1211	2	14	101.3
Slide 9	Jejunum	1211	2	14	30.1
Slide 13	Jejunum	1201	1	7	67.6
Slide 13	Jejunum	1201	1	7	56.8
Slide 14	Jejunum	1216	3	21	141.3
Slide 1	Stomach	1205	1	7	135.1
Slide 2	Stomach	1205	1	7	67.3
Slide 2	Stomach	1205	1	7	72.2
Slide 5	Stomach	1201	1	7	75.1
Slide 5	Stomach	1201	1	7	91.6
Slide 6	Stomach	1204	1	7	154.5
Slide 6	Stomach	1204	1	7	103.0
Slide 8	Stomach	1211	2	14	84.6
Slide 9	Stomach	1211	2	14	49.2
Slide 9	Stomach	1211	2	14	39.5
Slide 10	Stomach	1202	1	7	103.3
Slide 11	Stomach	1216	3	21	77.1
Slide 11	Stomach	1216	3	21	119.5
Slide 15	Stomach	1202	1	7	117.4
Slide 15	Stomach	1202	1	7	90.5
Slide 17	Stomach	1207	2	14	83.2
Slide 17	Stomach	1207	2	14	104.8

†These duodenum sections had pancreas attached.

Section 6. Conclusions

Oral infection of hamsters with SARS-CoV-2 UK variant resulted in no clinical disease. Necropsy found evidence of lung and nasal turbinate infection. There was no indication of infection in the GI tract. However, viral RNA of SARS-CoV-2 RNA was detected in the feces for up to 21 days. Previous experiments indicated a general level amount of RNA being shed in the feces over time. Here we found that the amount of RNA in the feces did decrease in after the first week, but one hamster was found to continue to shed RNA at 21 days after infection. FISH indicated that SARS-CoV-2 had successfully infected the cecum, colon, duodenum, ileum, jejunum, and stomach and that GI infections were present in one animal 21 days after oral infection. The GI infection did not result in observed microscopic pathology.

Section 7. Standards

This study did not require compliance with the FDA Good Laboratory Practice Regulations (21 *CFR* 58) and utilized University of Missouri Laboratory of Infectious Disease procedures.

Section 8. Archival

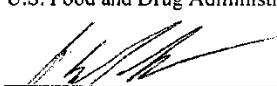
All data and observations were recorded. All data, records, documents, notes, or other materials generated by the study were collected and maintained by the study director for transfer to the MRIGlobal Records Center for archival upon completion of the study.

Appendix A. Study Protocol

MRIGlobal Study Protocol

Title	SARS-CoV-2 Infection Via the Oral Exposure In Hamsters
MRIGlobal Project No.	110890.01.208.06.01.03
Study Director	Carl Gelhaus, Ph.D.
Testing Facility	MRIGlobal 425 Dr. Martin Luther King Jr. Blvd. Kansas City, Missouri 64110
Sponsor	US Food and Drug Administration
Proposed In Life Start Date	August 9, 2021
Proposed In Life End Date	August 17, 2021

Approvals:

 Terry O'Neill for: _____ Claire Crutch, Ph.D. Medical Research Portfolio Director MRIGlobal	09 August 2021 _____ Date
Robert Fisher -S Digitally signed by Robert Fisher -S Date: 2021.08.09 12:11:26 -04'00' _____ Robert W. Fisher, Ph.D. Senior Advisor for CBRN and Pandemic Influenza Office of Counterterrorism and Emerging Threats (OCET) U.S. Food and Drug Administration	09 August 2021 _____ Date
 Carl Gelhaus, Ph.D. Study Director MRIGlobal	8/9/21 _____ Date

Study Protocol – MRIGlobal Project No. 110890.01.208.06.01.03

1. Objective and Justification

COVID-19 is a global public health emergency caused by the SARS-CoV-2 virus. Food safety is a major consideration during a pandemic. Food products, such as produce, may become inadvertently contaminated when displayed in grocery stores here in the United States. Moreover, a fecal-oral transmission risk may exist due to the prolonged excretion of SARS-CoV-2 from the gastrointestinal tract: several reports have identified the presence of high levels of SARS-CoV-2 RNA in feces, although infectious virus has not yet been recovered. We do not currently know the risk posed by oral exposure to the SARS-CoV-2 virus. The SARS-CoV-2 virus infects human cells by binding to the ACE2 receptor. The hamster has a similar ACE2 gene to the human and therefore is a good candidate for an animal model of COVID-19. Recent publications and our own experiments have demonstrated that hamsters develop a disease state that mimics that seen in humans infected with SARS-CoV-2 when instilled via the intranasal route. The objective of this study is examine the disease state that develops in the hamster following oral infection with SARS-CoV-2. Two variants of the SARS-CoV-2 will be tested.

2. Challenge Agents

Two isolates of the SARS-CoV-2 will be used. The first isolate challenge material will be SARS-CoV-2 isolate USA-WA1/2020 from BEI Resources. This virus has been passed through Vero cells once to obtain a master virus bank and the master virus bank was passed through Vero cells to obtain a working virus bank. The working virus bank is tested here.

Table 1. Challenge Article – WA1

Name:	SARS-CoV-2
Virus Classification:	<i>Coronaviridae</i>
Strain/Isolate:	USA-WA1/2020
Lot Number	20210214HCG
TCID ₅₀ /mL	1.67 x 10 ⁶
Copy number/mL	1.1 x 10 ¹⁰
Supplier	MRIGlobal (derived from BEI Resources)
Storage Conditions:	≤-70°C

The second isolate challenge material will be SARS-CoV-2 isolate USA/CA_CDC_5574/2020 from BEI Resources. The PANGO lineage for this isolate is the same as the UK variant (B.1.1.7). This virus has been passed through TMPRSS2 cells once to obtain a single virus bank, which is tested here.

Table 2. Challenge Article - UK

Name:	SARS-CoV-2
Virus Classification:	<i>Coronaviridae</i>
Strain/Isolate:	USA/CA CDC 5574/2020
Lot Number	To be included in the final report
TCID ₅₀ /mL	1.1 x 10 ⁷
Copy number/mL	9.0 x 10 ¹⁰
Supplier	MRIGlobal (derived from BEI Resources)
Storage Conditions:	<70°C

3. Test System

3.1 Specifications

Species:	<i>Mesocricetus auratus</i>
Strain:	Outbred
Source:	Envigo
Number of Animals Required for Study:	7 male and 7 female
Age Range at Challenge:	6-27 weeks

Fourteen (14) Syrian hamsters will be procured, 7 male and 7 female. All animals will be placed on study.

Weight is not a criteria for this study. Any extra hamsters will be transferred off study or euthanized.

3.2 Criteria

Hamsters must be free from obvious clinical signs of ill health or malformation or any other condition that would, in the judgment of a veterinarian or the Study Director, interfere with the conduct of the study. Animals can be replaced prior to challenge, if necessary.

3.3 Animal Identification

The identity of each animal will be confirmed at each procedure by ear tag and verified against cage cards illustrating at least the animal identification number, study number, and sex.

3.4 Animal Quarantine and Acclimation

Upon delivery, animals will be inspected for signs of ill-health and quarantined/acclimated in the Spencer conventional animal facility or ABSL-3 for no less than 44-48 h. The MRIGlobal Veterinarian will examine the health of the animals and release them for the study (see SOP MRI-1501, "Acclimation and / or Quarantine Procedures for Animals").

4. Animal Housing and Environmental Conditions

4.1 Feed

All hamsters will be provided with Envigo Teklad Food or equivalent. No contaminants which could affect the results of the study will be present in the feed, as verified by the certificate of

analysis (for each lot, provided by the vendor; see SOP MRI-1510, "Procedure for Feeding Animals"). Analytical results will be retained in the facility records.

Rodent food will be provided *ad libitum* and attending veterinarian approved supplement(s) will be provided as needed for enrichment and acclimation procedures.

4.2 Water

Municipal water will be available *ad libitum*.

4.3 Housing

Hamsters have been shown to transmit SARS-CoV-2 animal to animal. Therefore, all animals will be individually housed in polycarbonate Tecniplast caging (Tecniplast, Phoenixville, PA) throughout the acclimation/quarantine period and transferred to metabolic caging prior to infection. The metabolic caging will allow for collection of feces on a daily basis. The hamsters will be housed in environmentally controlled rooms. The rooms will be maintained at a temperature of 68.0°F to 79.0°F and a relative humidity of 50% ± 20% with a 12-h light/dark cycle per day. Room temperature, humidity, and light cycles will be monitored by the OceaView® monitoring system 24-h/day (see SOP MRI-1170, "Temperature and Humidity Monitoring in Animal Rooms"). Environmental enrichment will be provided as described in SOP MRI-1505.

4.4 Veterinary Care

Discomfort and distress should be limited to that which is unavoidable in the conduct of scientifically valuable research. Animals that develop non-study related illness or injury should be evaluated by a veterinarian for determination of treatment or disposition. In such cases, and if in the opinion of the Study Director and a veterinarian, an animal is in a moribund state due to the aforementioned circumstances, that animal should be euthanized.

5. Experimental Design

Hamsters will be weighed during the quarantine and acclimation period, prior to the initiation of this protocol.

Hamsters will be exposed to virus on Study Day 0 via oral gavage. Hamsters will be individually housed in metabolic cages. Six (3 male and 3 female) hamsters will be infected with SARS-CoV-2 WA1/2020. An additional six (3 male and 3 female) hamsters will be infected with SARS-CoV-2 UK B.1.1.7 variant. In addition, one male and one female will be orally dosed with sterile DPBS media as negative controls.

Every day post-infection, feces will be collected from metabolic cages. Hamsters will be anesthetized daily of the collection of rectal swabs and body weights. Rectal swabs will be collected using viral transport media kits according to manufacturer's instructions. Animals will be monitored for 7 days post infection. On Study Days 2 and 4, hamsters will also have the nasal passages washed with phosphate buffered saline (PBS). Feces will be weighed at collection and frozen at <-60°C until analysis. Nasal washes and rectal swabs in transport media will be stored at <-60°C. All nasal washes, feces homogenates, and rectal swab solutions will be enumerated by PCR and TCID₅₀ assays. Animals will be euthanized and carcasses disposed without examination.

Table 3. Study Design

Group	Size (N)	Route of Viral Administration	Isolate	Study Duration	Endpoints
1	6 (3/sex)	Oral up to 1 mL; likely 1×10^6 TCID ₅₀ /mL Challenge Concentration	Washington (Pass 7)	7 Day	Fecal Collection, Rectal Swabs, and BW Daily and Nasal Wash on SD 2 and 4
2	6 (3/sex)		B.117 UK (Pass 4)		
3	2 (1/sex)	Oral (control – DPBS)	Control –DPBS		

6. Methods

6.1 Anesthesia

Table 4. Inhaled Anesthetic

Drug	Dose (e.g., mg/kg or mL/kg)	Dose Volume (mL)	Route	Frequency
Isoflurane	1-5%	NA	Inhaled	Daily for 8 days (Study Day 0 through 7)

Use of sedation and/or anesthesia for performing various procedures in hamsters, includes but is not limited to oral gavage challenge, nasal washes, rectal swabs, body weight measurements and prior to euthanasia.

6.2 Randomization

Animals will be weighed and allocated to challenge groups according to the study design in Table 3. Animals will be allocated to challenge groups using a randomization schedule stratified by sex and body weight, such that average body weights within each treatment group and sex will be approximately equivalent.

6.3 Oral Gavage

On the day of challenge (Study Day 0), undiluted virus will be thawed and maintained on wet ice for challenge. The UK variant virus will be diluted in DPBS to $\sim 1 \times 10^6$ TCID₅₀/mL. The other challenge material will be diluted in DPBS to $\sim 1 \times 10^5$ TCID₅₀/mL. The dilution and confirmation of dose will be demonstrated by the TCID₅₀ assay of the challenge stock (i.e., back-titer) using a study specific laboratory procedure. The TCID₅₀ of the challenge materials will be determined in a manner to allow direct comparison of the titers.

Oral gavage challenge will be conducted according to SOP MRI-1835 (“Intravenous, Subcutaneous, Intramuscular, Intraperitoneal, Dermal, Intravenous, Intratracheal, and Oral Dose Administration to Rodents”), while the animals are anesthetized with isoflurane. The challenge dose will be inoculated by oral gavage, approximately 0.8 mL using a syringe with a gavage needle.

6.4 Clinical Observations

General health observations will be performed once daily during the quarantine and acclimation period and prior to the study start. Hamsters will be observed at least twice daily after challenge from Study Day 0 through Study Day 7.

All observations will be conducted in accordance with SOP MRI-1528 "Procedure for Observation of Animals". Observations will be made without opening cages, to prevent transmission of SARS-CoV-2 infection from one animal to another.

6.5 Feces Collection

Hamsters will be individually housed in metabolic cages with a feces collection tube. Once per day, the feces will be collected into an individually labelled, pre-weighed tube for each hamster on each day. One aliquot will be made for TCID₅₀, a second aliquot for PCR, and all excess feces will be collected into a third tube (the excess feces tube will not be weighed). The PCR and TCID₅₀ tubes will be weighed after the feces is added to determine the mass of feces in each tube. Feces in tubes will be stored at $\leq -60^{\circ}\text{C}$ until analyzed.

6.6 Body Weights

Prior to all studies, hamsters will be weighed during quarantine. Body weights will be measured daily beginning on Study Day 0 through Study Day 7. Body weights will be collected in accordance with SOP MRI-2020 "Weighing Rodents and Non-rodents".

6.7 Rectal Swabs

Beginning on Study Day 1 through Study Day 7, hamsters will be anesthetized and one swab from the viral transport kit will be inserted into the rectum of one hamster and rotated briefly. The swab will be placed in the viral transport media and stored at $2-8^{\circ}\text{C}$ until they are analyzed by TCID₅₀ and PCR.

6.8 Nasal Washes

Viral infection will be assessed by obtaining nasal washes on Study Days 2 and 4. Sterile DPBS will be administered intranasally to isoflurane anesthetized hamsters. Volumes for nasal wash administration will be approximately 600 μL , administered 300 μL /nostril via a 1 mL syringe attached to a 22 gauge intravenous catheter tip. The expelled fluid will be collected in a sterile petri dish and aliquoted into two cryogenic storage tubes at approximately 200 μL each. Both aliquots will be frozen at $\leq -60^{\circ}\text{C}$ until they are analyzed by TCID₅₀ and PCR (one aliquot for each assay). The nasal wash will follow the draft SOP entitled "Nasal Wash in Small Animal Models".

6.9 TCID₅₀

One aliquot of each feces sample, each nasal wash, and rectal swab will be used to assess presence of infectious virus, in accordance with SOP MRI-8701 "50% Tissue Culture Infectious Dose (TCID₅₀) Titration of Biological Safety Level (BSL)-3 Coronaviruses".

6.10 PCR

One aliquot of each feces sample, each nasal wash, and rectal swab will be used to assess presence of infectious virus in a real time quantitative PCR (RT-qPCR) assay. RT-qPCR assays

will be performed in accordance with SOP MRI-9770 “Nucleic Acid Quantification using Real-Time Polymerase Chain Reaction (PCR)”.

6.11 Euthanasia

6.11.1 Unscheduled Euthanasia

The determination to euthanize an animal prior to scheduled termination will be made independently for each animal contingent on progression of disease. Animals meeting any one of the following criteria will be euthanized:

- Presence of any convulsions (denoting meningitis or encephalitis)
- Limb paralysis/paresis which prevents the animal from obtaining food and water
- Respiratory distress
- Unresponsive to touch or external stimuli
- Moribundity
- Weight loss > 20% from the baseline

All hamsters judged to meet the euthanasia criteria will be euthanized per SOP MRI-2330 “Euthanasia of Rodents”. No necropsy will be performed.

6.12 Scheduled/Terminal Euthanasia and Tissue Collection

Hamsters will be euthanized on Study Day 7. Euthanasia will be performed via Isoflurane anesthesia followed by bilateral pneumothorax in accordance with SOP MRI-2330 “Euthanasia of Rodents”. No necropsy will be performed.

7. Statistical Analysis of Data

7.1 Body Weight

Changes in body weight will be visualized by graphing subject-specific trajectories over time, with study groups identified using different color lines. Descriptive statistics (mean $\pm$ SD, median, minimum, and maximum) for body weight will be generated. Body weight on Study Day 0 will be considered the baseline weight. The study is not designed for statistical model analysis of body weights.

7.2 Fecal, Nasal Wash, and Rectal Swab Viral Burden

Descriptive statistics (mean $\pm$ SD, median, minimum, and maximum) for infectious viral load in feces, nasal washes, and rectal swabs will be generated for both TCID₅₀ and PCR results for each study group. Graphs will be generated that show the mean $\pm$ standard error of these variables as a function of sacrifice day, treatment (treated vs. placebo). Results will be visually inspected by the Study Director to assess the likelihood of statistically significant differences. Statistical analysis will be at the Study Director’s discretion in consultation with the sponsor.

7.3 Clinical Observations

The number and proportion of animals showing clinical signs and symptoms of infection (e.g., increased respiration, lethargy) in each study group on each study day will be tabulated.

8. Other Materials and Equipment

Materials and equipment to be used in performing the tests include but are not limited to:

- Hamsters
- Metabolic cages
- Balance
- Pipettes and tips
- Plastic vials
- Ice
- Absorbent padding
- Syringes
- Catheters for nasal washes
- Petri dishes or weigh boats to catch nasal washes
- Oral gavage needles
- Isoflurane
- PBS
- Surgical instruments
- Biosafety cabinets
- CO₂ incubators

9. SOPs

Methods and laboratory procedures to be used in performing the tests include but are not limited to the SOPs contained within animal user job roles. These include but are not limited to:

- MRI-1117, Light Cycle Maintenance in Animal rooms
- MRI-1170, Temperature and Humidity Monitoring in Animal Rooms
- MRI-1504, Procedure for Identification of Animals and Cages
- MRI-1505, Performance and Documentation of Animal Husbandry and Animal Room Maintenance
- MRI-1510, Procedure for Feeding and Watering Animals
- MRI-1528, Procedures for Observations of Animals
- MRI-1568, Procedures for Handling Rodents
- MRI-1835, Intravenous, Subcutaneous, Intramuscular, Intraperitoneal, Dermal, Intranasal, and Oral Dose Administration to Rodents
- MRI-2020, Weighing of Rodents and Non-Rodents
- MRI-2330, Euthanasia of Rodents
- MRI-8701 "50% Tissue Culture Infectious Dose (TCID₅₀) Titration of Biological Safety Level (BSL)-3 Coronaviruses"
- MRI-9502, Data Recording Procedures for In Vitro Work Performed in Biological Safety Cabinets and In Vivo Work Performed in the Animal Facilities
- MRI-9501, Data Review Requirements for In Vitro and In Vivo Work in the Medical Countermeasures Division
- MRI-9770, Nucleic Acid Quantification using Real-Time Polymerase Chain Reaction (PCR)

10. Records to Be Maintained

Records to be maintained by the testing facility will include:

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Study Protocol

- Signed protocol and any amendments
- Description of equipment and instruments used
- All raw data
- All correspondence, memos, notes, and telephone notes relating to the study
- Full descriptions of the experimental designs and procedures, including a description of the test equipment used, and of deviations, if any, from the protocol
- All instrument logs and calibration information
- Storage conditions of test article and samples
- Test article and reagent receipt and use documentation
- Professional information about study participants
- Mathematical equations used in generating and analyzing the data as well as representative calculations using the equations
- Manufacturers information and any characterization data for the test article
- All SOPs applicable to this study

NOTE: All recording of data, except for automated data collection, will be made directly, promptly, and legibly in ink. All data entries will be dated on the date of entry and signed or initialed by the person responsible for the entry. Automated data collection systems must identify the person responsible for the direct data input. Any changes to written or automated data entries will be made so as to not obscure original entry, will indicate the reason for the change, and will be signed or initialed, and dated by the person responsible.

11. Protocol Amendments/Deviations

All changes or revisions to this protocol, and the reasons for the change, shall be documented, signed by the Study Director, dated, and maintained with the protocol, in accordance with SOP MRI-0080. If changes in the protocol are required which would change the objectives and/or scope of work, prior approval will be obtained from Sponsor.

12. Standards

This study will not require compliance with the FDA Good Laboratory Practice Regulations (21 CFR 58); however, the studies will be performed in compliance with MRIGlobal QA procedures. All operations pertaining to this study, unless specifically defined in this protocol, will be performed according to the Standard Operating Procedures of MRIGlobal, and any deviations will be documented.

13. Archival

All data and observations will be recorded and stored at the testing facility (MRIGlobal). All data, records, documents, notes, or other materials generated by the study will be collected and maintained by the study director for transfer to the MRIGlobal Records Center for archival upon completion of the study. The sponsor will receive a copy of the final report.

14. Reports

At the conclusion of each study, a brief letter report will be prepared representing the raw data and analysis.

Appendix B. Fecal Collection

110890.01.208.06-02

Fecal Pellet Collection

Animal ID	Group	12/21/21 Pellet Count	12/22/21 Pellet Count	12/23/21 Pellet Count	12/24/21 Pellet Count	12/25/21 Pellet Count	12/26/21 Pellet Count	12/27/21 Pellet Count	12/28/21 Pellet Count	12/29/21 Pellet Count	12/30/21 Pellet Count	12/31/21 Pellet Count	1/01/22 Pellet Count	1/02/22 Pellet Count	1/03/22 Pellet Count	1/04/22 Pellet Count
1201	1															
1202	1															
1203	1															
1204	1															
1205	1															
1206	1															
1207	2	NA	2	2	2	2	2	2	2	0	2	2	2	2	2	2
1208	2	NA	2	2	2	2	2	2	1	0	2	2	0	2	2	2
1209	2	NA	2	2	2	2	2	2	2	0	0	0	0	0	0	2
1210	2	NA	2	2	2	2	2	2	1	2	1	1	2	2	2	2
1211	2	NA	2	2	0	2	0	2	2	0	1	1	1	2	2	2
1212	2	NA	2	2	2	2	2	2	2	1	1	1	0	1	1	2
1213	3	NA	2	2	1	2	0	0	1	0	0	0	0	0	0	2
1214	3	NA	2	0	0	2	2	0	2	0	2	0	2	0	2	0
1215	3	NA	2	2	2	2	2	2	0	2	1	0	2	2	2	0
1216	3	NA	2	2	2	2	2	2	2	0	1	1	1	2	1	1
1217	3	NA	2	0	2	0	0	0	2	1	1	1	1	2	1	2
1218	3	NA	2	2	2	2	0	2	2	1	2	2	2	2	2	2
1219	4															
1220	4															
1221	4															
1222	4															
1223	5	NA	2	2	2	1	0	2	0	0	0	0	2	0	1	2
1224	5	NA	2	2	2	2	2	2	0	0	0	2	0	0	0	2
1225	5	NA	2	2	2	2	2	2	2	2	2	1	2	2	1	2
1226	5	NA	2	2	2	2	2	2	0	1	0	1	2	1	0	2
1227	6	NA	2	2	2	2	2	2	2	2	0	0	0	2	0	0
1228	6	NA	2	2	2	2	2	2	0	0	0	0	0	0	0	0
1229	6	NA	2	2	2	2	2	2	0	2	2	2	2	2	1	2
1230	6	NA	2	2	2	2	2	2	0	1	1	2	1	2	1	1

Entered by: KE 1.21.2022 KE 1.21.2022 KE 1.21.2022 KE 1.21.2022 KE 1.21.2022 KE 1.21.2022 KE 1.21.2022 KE 1.21.2022 KE 1.21.2022 KE 1.21.2022 KE 1.21.2022 KE 1.21.2022 KE 1.21.2022 KE 1.21.2022 KE 1.21.2022 KE 1.21.2022 KE 1.21.2022
QC'd by: ZG 1/26/2022 ZG 1/26/2022 ZG 1/26/2022 ZG 1/26/2022 ZG 1/26/2022 ZG 1/26/2022 ZG 1/26/2022 ZG 1/26/2022 ZG 1/26/2022 ZG 1/26/2022 ZG 1/26/2022 ZG 1/26/2022 ZG 1/26/2022 ZG 1/26/2022 ZG 1/26/2022 ZG 1/26/2022

110890.01.208.06-02

Fecal Pellet Collection

Animal ID	Group	1/05/22 Pellet Count	1/06/22 Pellet Count	1/07/22 Pellet Count	1/08/22 Pellet Count	1/09/22 Pellet Count	1/10/22 Pellet Count	1/11/22 Pellet Count	1/12/22 Pellet Count
1201	1	NA	2	1	2	0	0	0	2
1202	1	NA	2	0	2	0	0	0	2
1203	1	NA	2	2	2	2	2	2	2
1204	1	NA	2	2	2	2	1	1	2
1205	1	NA	2	2	2	0	1	2	2
1206	1	NA	2	2	2	2	2	2	2
1207	2								
1208	2								
1209	2								
1210	2								
1211	2								
1212	2								
1213	3	1	2	2	2	1	0	2	
1214	3	2	2	2	2	1	2	2	
1215	3	2	2	2	2	2	2	2	
1216	3	2	2	2	2	1	2	2	
1217	3	2	2	1	2	1	2	2	
1218	3	1	2	2	2	1	2	2	
1219	4	NA	2	2	2	2	2	0	2
1220	4	NA	2	2	2	2	2	1	2
1221	4	NA	2	2	2	2	2	2	2
1222	4	NA	2	1	2	2	2	2	2
1223	5								
1224	5								
1225	5								
1226	5								
1227	6	1	2	2	2	2	2	2	
1228	6	0	2	2	2	1	1	2	
1229	6	2	2	2	2	1	2	2	
1230	6	2	1	1	2	2	2	2	

Entered by: KE 1.21.2022KE 1.21.2022KE 1.24.2022KE 1.24.2022KE 1.24.2022KE 1.24.2022KE 1.24.2022KE 1.24.2022

QC'd by: ZG 1/26/202ZG 1/26/202ZG 1/26/202ZG 1/26/202ZG 1/26/202ZG 1/26/202ZG 1/26/202ZG 1/26/202

Appendix C. Body Weights

110890.01.208.04-02
Body Weights

Body Weight in g																
Animal ID	Group	12/21/2021	12/22/2021	12/23/2021	12/24/2021	12/25/2021	12/26/2021	12/27/2021	12/28/2021	12/29/2021	12/30/2021	12/31/2021	1/1/2022	1/2/2022	1/3/2022	1/4/2022
1201	1															
1202	1															
1203	1															
1204	1															
1205	1															
1206	1															
1207	2	148	150	151	150	150	153	153	152	153	154	154	150	154	157	157
1208	2	140	142	142	142	140	145	148	150	147	148	151	153	155	156	158
1209	2	119	152	158	156	161	164	165	164	165	174	174	173	178	180	183
1210	2	126	127	133	132	132	133	134	139	136	137	138	136	136	138	139
1211	2	130	134	136	132	133	132	132	133	132	133	136	137	138	136	139
1212	2	130	130	134	131	134	136	137	136	135	134	136	134	134	135	135
1213	3	147	148	148	150	153	150	150	156	159	158	156	161	165	163	164
1214	3	133	133	136	136	133	133	133	129	127	133	136	137	140	137	135
1215	3	148	153	158	156	159	156	156	169	171	166	172	174	174	175	180
1216	3	132	136	137	136	137	137	136	135	136	137	137	138	140	141	143
1217	3	127	129	130	130	131	131	132	132	133	134	134	135	134	134	135
1218	3	129	129	132	133	135	136	138	139	139	142	142	143	144	144	146
1219	4															
1220	4															
1221	4															
1222	4															
1223	5	155	163	163	161	168	171	173	173	173	179	175	174	180	184	184
1224	5	142	147	148	150	151	150	154	154	158	156	158	158	160	160	163
1225	5	132	133	133	136	139	140	143	146	150	149	149	150	152	154	154
1226	5	124	127	128	127	126	128	128	129	128	128	126	126	127	129	130
1227	6	129	134	139	141	140	143	147	146	148	150	151	152	153	152	153
1228	6	137	142	146	148	143	147	149	150	148	148	151	152	150	150	153
1229	6	124	125	125	124	126	124	124	124	123	123	124	121	121	122	122
1230	6	135	139	140	140	143	144	147	147	147	153	151	151	153	154	154
Entered by:		KE 1.21.2022	KE 1.21.2022	KE 1.21.2022	KE 1.21.2022	KE 1.21.2022	KE 1.21.2022	KE 1.21.2022	KE 1.21.2022	KE 1.21.2022	KE 1.21.2022	KE 1.21.2022	KE 1.21.2022	KE 1.21.2022	KE 1.21.2022	KE 1.21.2022
QC'd by:		ZG 1/26/2022	ZG 1/26/2022	ZG 1/26/2022	ZG 1/26/2022	ZG 1/26/2022	ZG 1/26/2022	ZG 1/26/2022	ZG 1/26/2022	ZG 1/26/2022	ZG 1/26/2022	ZG 1/26/2022	ZG 1/26/2022	ZG 1/26/2022	ZG 1/26/2022	ZG 1/26/2022

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Body Weights

Body Weight in g									
Animal ID	Group	1/5/2022	1/6/2022	1/7/2022	1/8/2022	1/9/2022	1/10/2022	1/11/2022	1/12/2022
1201	1	166	168	170	171	170	173	172	169
1202	1	171	176	176	177	177	184	186	181
1203	1	163	164	165	164	165	172	170	171
1204	1	134	138	137	135	136	134	130	129
1205	1	143	145	141	138	140	135	134	134
1206	1	140	140	140	139	140	139	140	140
1207	2								
1208	2								
1209	2								
1210	2								
1211	2								
1212	2								
1213	3	166	170	171	170	170	179	179	
1214	3	138	139	140	140	140	145	142	
1215	3	182	184	185	185	190	190	190	
1216	3	142	141	145	140	142	141	142	
1217	3	133	135	135	131	131	130	129	
1218	3	146	146	144	149	150	150	152	
1219	4	171	177	180	175	175	177	100	179
1220	4	162	164	169	167	168	172	179	175
1221	4	145	145	146	144	146	142	144	144
1222	4	130	129	131	131	132	131	131	133
1223	5								
1224	5								
1225	5								
1226	5								
1227	6	156	155	154	154	155	155	153	
1228	6	154	153	152	154	158	154	156	
1229	6	125	120	120	121	123	122	124	
1230	6	156	157	156	156	158	158	156	

Entered by: KE 1.21.2022 KE 1.21.2022 KE 1.24.2022 KE 1.24.2022 KE 1.24.2022 KE 1.24.2022 KE 1.24.2022 KE 1.24.2022
QC'd by: ZG 1/26/2022 ZG 1/26/2022 ZG 1/26/2022 ZG 1/26/2022 ZG 1/26/2022 ZG 1/26/2022 ZG 1/26/2022 ZG 1/26/2022

Appendix D. PCR Results

110890.01.208.04-02
Quantitative PCR Results from Fecal Samples - Copies per gram of feces

Animal ID	Group	Study Day																					
		SD1	SD2	SD3	SD4	SD5	SD6	SD7	SD8	SD9	SD10	SD11	SD12	SD13	SD14	SD15	SD16	SD17	SD18	SD19	SD20	SD21	
1201	1	8.1E+04	-	6.0E+05	-	-	-	3.6E+04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1202	1	-	-	-	-	-	-	1.6E+03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1203	1	-	-	1.2E+03	-	-	2.0E+04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1204	1	1.5E+05	3.7E+05	2.0E+06	3.4E+06	7.5E+05	1.6E+05	6.1E+04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1205	1	8.2E+05	1.0E+06	7.4E+05	-	2.0E+05	4.2E+05	1.9E+04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1206	1	-	1.3E+03	1.7E+04	-	-	4.1E+04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1207	2	-	9.3E+02	6.3E+03	8.7E+05	1.5E+06	8.9E+05	1.5E+05	-	2.3E+04	8.6E+03	7.9E+02	2.4E+03	1.1E+03	-	-	-	-	-	-	-	-	
1208	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1209	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1210	2	-	-	-	-	-	-	5.3E+04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1211	2	6.6E+04	1.6E+05	-	1.8E+06	-	5.9E+04	5.9E+04	-	6.8E+03	-	3.1E+03	2.2E+03	9.9E+02	1.6E+04	-	-	-	-	-	-	-	
1212	2	-	-	-	1.1E+03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1213	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1214	3	-	-	-	9.0E+06	1.3E+06	-	1.2E+05	-	6.2E+03	-	1.1E+03	-	-	-	1.2E+03	-	-	-	-	-	-	
1215	3	-	-	-	1.9E+03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1216	3	8.1E+04	4.0E+05	6.8E+06	8.4E+05	4.5E+05	6.0E+05	4.8E+04	-	2.5E+04	1.0E+04	2.9E+04	1.7E+03	5.8E+03	3.7E+03	8.1E+04	1.6E+04	7.7E+03	2.2E+03	3.5E+03	2.8E+03	2.8E+03	
1217	3	1.1E+03	-	-	-	-	-	-	-	3.7E+03	-	-	-	-	-	-	-	5.2E+03	-	-	-	-	
1218	3	-	-	-	7.8E+02	-	4.9E+03	-	-	-	-	-	-	-	-	-	-	1.6E+03	-	-	-	-	
1219	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1220	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1221	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1222	4	-	-	-	-	1.6E+03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1223	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1224	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1225	5	-	-	-	-	-	1.1E+03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1226	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1227	6	-	-	-	-	-	1.6E+03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1228	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8.7E+02	-	
1229	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1230	6	-	-	-	-	-	-	-	-	1.1E+04	-	-	-	-	-	3.4E+03	-	-	-	-	-	-	-

*Orange = MS2 Drop Out
*Yellow = Inconclusive
**INCLUDES REPLATED SAMPLE DATA

Appendix E. Pathology Report

**MRIGlobal (MRI) Study Number 110890.01.208.06.01.04:
SARS-CoV-2 Infection Via the Oral Exposure in Hamsters**

PATHOLOGY REPORT

Singer & Associates Toxpath Consulting Solutions
388 Eagle Pass Dr
Ponte Vedra, FL 32081

Testing Facility

MRIGlobal
425 Dr Martin Luther King Jr Blvd
Kansas City, Missouri 64110

Sponsor

US Food and Drug Administration

Final

January 29, 2023

Singer & Associates Toxpath Consulting Solutions
388 Eagle Pass Dr, Ponte Vedra FL 32081
singer@toxpathsolutions.com

**Study Number 110890.01.208.06.04:
SARS-CoV-2 Infection Via the Oral Exposure in Hamsters**

Pathology Report

Introduction

COVID 19 is a global pandemic caused by the SARS-CoV-2 virus. In any pandemic, food supplies may become a major consideration. Food supplies may be contaminated with virus anywhere along the distribution and sales systems. Further, the degree of risk relative to food supplies and oral transmission of the virus is unknown, although the acute fecal shedding of virus has been documented. As the SARS-CoV-2 virus infects human cells by initially binding to surface ACE2 receptors, and since hamsters have a similar ACE2 gene to humans, they have been tested in initial studies which have demonstrated that they do indeed develop a disease progression similar to that seen in humans. The objective of this study, therefore, was to purposely expose groups of hamsters to the UK variant of SARS-CoV-2 via the oral route of administration and determine the duration of viral RNA shedding for up to 21 days, as well as examine the pathology, if any, at 7-, 14-, and 21-days post administration.

The study was conducted at MRIGlobal, 425 Dr Martin Luther King Jr Blvd, Kansas City, MO 64110; the sponsor was the US Food and Drug Administration. Pathology evaluations were conducted by Allen W. Singer, DVM, DACVP of Singer & Associates Toxpath Consulting Solutions (*dba* Toxpath Solutions).

In-life activities, necropsy, and tissue collection activities were conducted by trained technical staff. Protocol-specified tissues were collected, fixed in 10% neutral buffered formalin (NBF), and shipped to Toxpath Solutions for evaluation and reporting. This report presents the anatomic pathology results only.

Standards

Per protocol, this study was not intended to require compliance with 21 CFR Parts 11 or 58 of the FDA (Good Laboratory Practice Guidelines). However, study procedures were performed in accordance with appropriate standard operating procedures (SOPs) and any deviations were documented.

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Experimental Design

Thirty hamsters (15 males and 15 females) were divided into 6 groups and challenged PO with either 1×10^6 TCID₅₀ SARS-CoV-2 UK variant (3/sex/group; Groups 1, 2, and 3) or PBS (2/sex/group; Groups 4, 5, and 6), as shown below in Table 1. Animals were humanely terminated at SD 7 (Groups 1 and 4), SD 14 (Groups 2 and 5) and SD 21 (Groups 3 and 6) from each group were terminated and necropsied at either 2-, 4-, 7- or 14-days post-exposure, as presented below.

Table 1. Experimental Design

Group	Number of Hamsters	Challenge Article Dose (TCID ₅₀) ^a	Termination Day
1	3M/3/F	1×10^6 TCID ₅₀ SARS-CoV-2 UK variant	7
2	3M/3/F	1×10^6 TCID ₅₀ SARS-CoV-2 UK variant	14
3	3M/3/F	1×10^6 TCID ₅₀ SARS-CoV-2 UK variant	21
4	2M/2/F	PBS	7
5	2M/2/F	PBS	14
6	2M/2/F	PBS	21

^a Challenge agents were administered by oral gavage at 0.8 mL using a syringe and gavage needle.

Other endpoints, collected at the various timepoints but not further discussed here, included daily fecal collections for virus or PCR quantification and body weight determinations. Necropsies at each termination interval included gross examinations of the various tissues and organs. Tissues collected at necropsy included the nose/nasal cavity and turbinates, the trachea, lungs (all of which were infused with 10% neutral buffered formalin (NBF); plus stomach, intestinal tract, and heart. All these tissues were then preserved by immersion in additional NBF. Once fixed, the specimens were shipped to StageBio, Inc (Mount Jackson, VA) for routine paraffin processing. Slides were cut and stained with hematoxylin and eosin and sent to Toxpath Solutions for microscopic review and evaluation.

Results

Mortality

There were no early, unscheduled deaths reported among the 30 hamsters scheduled and necropsied for the pathologic examinations.

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Necropsy Observations

A variety of macroscopic findings in the lungs were recorded during the tissue collections, recorded in various animals as mottled and/or dark/red; a few lungs had notations of pale or purple or having red spots. Macroscopic notations in the lungs were recorded for all Group 1, 2, and 3 hamsters but none of the Group 4, 5, or 6 animals. A few animals had notations made relative to reddish-brown discoloration in the stomach, and two had a “white soft palate.” These macroscopic findings are not listed individually here but are presented by animal number and organ in the attached Appendix A1 as the *Individual Data Listing of Gross to Microscopic Correlation*. The gross to microscopic data listing also includes the correlate, if any, that was noted during the microscopic examination. Most commonly, “mottled” or dark discoloration correlated microscopically with areas of interstitial lung inflammation; “pale discoloration” correlated with collections (aggregates) of alveolar macrophages; and red/purple correlated with multifocal areas of hemorrhage. Although not all gross lung findings had any confirmatory lesion found microscopically, many did, and all these were interpreted to be due to active Covid 19 pathology in the lungs. Stomach lesions correlated with incidental mineralization, a common finding in rodents (McInnes, 2012); the palates were not processed per Study Director instructions.

Histopathology

Microscopic diagnoses were recorded directly into the Xybion® (Lawrenceville, NJ) Pristima® version 7.2 computer capture system as they were observed microscopically. Individual animal gross and microscopic pathology data are presented below in Appendix A3 (*Individual Gross and Microscopic Observations*). Lesions were graded on a 1-5 scale, where 1 = minimal, with up to 5% of the tissue mass affected; 2 = mild, 6 - 10% of the tissue affected; 3 = moderate, 11 - 25% of the tissue affected; 4 = marked, 26 - 50% of the tissue or organ affected, and 5 = severe, with greater than 50% of the tissue area affected. Summaries of the microscopic findings with expanded severities are presented by group and sex in Appendix A2 as the *Expanded Summary Report of Histopathology*. For simplicity, a truncated summary incidence table of selected findings is presented below by sex in Table 2. Because of the number and complexity of the microscopic findings, the reader is referred to Appendix A2 (*Expanded Summary Report of Histopathology*) for the complete summary incidences of microscopic findings.

To some degree, microscopic findings correlated with findings noted at necropsy, although not all animals had detectable microscopic changes even where gross lung mottling was recorded at necropsy. It is possible that some of the gross notations were only slight and actually due to vascular congestion, rather than Covid-related interstitial lung inflammation. In the 21-day cohorts, there were very few animals that displayed any microscopic evidence of Covid inflammation. This is consistent with prior studies in hamsters where lesions tended to begin slowly over the first few days post-challenge, reach a maximum extent by about SD 7 or 8, and gradually recover to only minimal lesions by about SD 21.

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Table 2. Summary Incidences of Selected Microscopic Findings (Males)

Group	1	2	3	4	5	6
Oral challenge agent	Virus	Virus	Virus	PBS	PBS	PBS
Termination day	7	14	21	7	14	21
Number examined	3	3	3	2	2	2
Lung						
Aggregate, alveolar macrophage	2	1	2	0	0	0
Inflammation, interstitial	2	0	1	0	0	0
Vasculitis	3	1	1	0	0	0
Nasal turbinate						
Exudate	2	0	0	0	0	0
Inflammation, olfactory epithelium	2	0	0	0	0	0
Inflammation, respiratory epithelium	2	0	0	0	0	0

Table 2. Summary Incidences of Selected Microscopic Findings (Females)

Group	1	2	3	4	5	6
Oral challenge agent	Virus	Virus	Virus	PBS	PBS	PBS
Termination day	7	14	21	7	14	21
Number examined	3	3	3	2	2	2
Lung						
Aggregate, alveolar macrophage	2	2	3	0	0	1
Inflammation, interstitial	1	1	1	0	0	0
Vasculitis	2	2	1	0	0	0
Nasal turbinate						
Exudate	1	0	0	0	0	0
Inflammation, olfactory epithelium	1	0	0	0	0	0
Inflammation, respiratory epithelium	2	0	0	0	0	0

One PBS (Group 6) female had a minimal, single focus of aggregated macrophages in the lungs as shown above. Such aggregates are often found in normal, untreated lungs and do not suggest accidental virus exposure.

In the turbinates, Covid-related changes consisting of mixed cell inflammation (with some epithelial cell degeneration, not coded separately) was found throughout the respiratory epithelium lining much of the nasal cavity, as well as mixed cell inflammation of the olfactory epithelium (again, with degeneration of the olfactory cells, not coded separately) in many of the Group 1 (SD 7 termination) hamsters. In a few animals, the inflammation was sufficiently widespread and acute that a mild exudate of neutrophils and serum filled the rear portions of the nasal cavity. The inflammation with degeneration of the olfactory epithelium was sufficient that it likely would have affected the sense of smell in the affected animals. The underlying olfactory nerve fibers were not affected.

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In the trachea, acute inflammation was seen in one animal; this has been seen in previous natural history progression studies of SARS-CoV-2 when hamsters were challenged by the nasal route, typically beginning very early (SD 3) and recovering to normal by around day 7, so the observed incidence in this study was consistent with previous work.

In terms of the overall lung pathology observed, the UK variant of the SARS-CoV-2 virus caused the following pathologic changes in the lungs, similar to those seen by the undersigned in previous investigations of Covid infection in hamsters when challenged by the respiratory route:

1. Bronchiolar inflammation (the initial response to epithelial cell injury in the airways);
2. Bronchiolar epithelial cell hyperplasia (a hyperplastic response to epithelial cell injury or death as part of the repair process);
3. Aggregates of alveolar macrophages; (mostly alveolar macrophages early, but as time passes, these include more mixed cell lines such as monocytes and neutrophils, with a few multinucleated giant cells, which were coded separately because of their uniqueness);
4. Interstitial inflammation (interstitial pneumonia) with mild to marked thickening of alveolar walls by mixed but mostly mononuclear cell inflammation and minimal to mild "alveolar cell type II pneumocyte hyperplasia" (which was coded separately);
5. As noted in #3 above, the presence of small to large multinucleated giant cells;
6. Vasculitis, which ranged from minimal to severe *on an individual vessel basis*, consisting of perivascular infiltrates of mixed inflammatory cells; intraluminal monocyte margination of many pulmonary vessels; endothelial cell damage and significant transmigration of leukocytes (often neutrophils) through the vessel wall. While no obvious fibrin thrombosis of pulmonary vessels was found, vessel lumens were occasionally found with such severe leukocyte margination that no apparent patent passage was visible (possible "cell clots");
7. Alveolar hemorrhage, generally found in hamsters with vasculitis; and
8. Mesothelial cell hypertrophy along the pleural surface, adjacent to where interstitial inflammatory foci were present.

Unrelated to the Covid virus challenge, a number of animals from all groups had mineralized areas of gastric epithelium or muscle coat. This change is a common finding in rodents (McInnes, 2012).

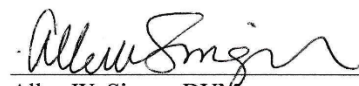
Conclusions

Gross and microscopic changes observed during this investigation demonstrated a pattern of infection in the hamsters consistent with Covid virus exposure. Lesions consisted of nasal mucosa inflammation, tracheal inflammation, bronchiolitis and bronchiolar epithelial hyperplasia in the lung, consistent with a mucosal response to cellular injury. Also present were increased aggregates of alveolar macrophages with some monocytes, neutrophils, and even multinucleated giant cells. Interstitial inflammation began near the terminal airways, occasionally spreading into the deeper

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parenchyma, and approached the pleural surface in a few animals. Many pulmonary blood vessels displayed abnormalities, such as perivascular mixed cell infiltrates, margination of mononuclear leukocytes along the endothelial surfaces, (which was often accompanied by hypertrophic endothelial lining cells), and progressing to packed collections of such mononuclear cells to the point of occasionally occluding the vascular lumen. Often, there was transmigration of leukocytes through the vessel wall by neutrophils. The impression here was that the mononuclear cells were attaching or attacking the endothelial cells lining some blood vessels; perhaps there was SARS-CoV-2 antigen at this site, but the reason for this presentation was unclear. All these vascular changes were coded simply "vasculitis." As the study progressed through SD 21, the lung lesions resolved with most lesions disappearing and lung alveoli often becoming quite normal.

Tissues examined from the gastrointestinal tract were essentially free from any Covid-related pathology. It was unclear whether the lungs were infected via the blood stream following oral/gastrointestinal absorption or by inhalation of virus from the feces present in the cage or possible gavage-related regurgitation and inhalation, however the clear Covid changes noted in the nasal cavity suggests some respiratory mechanism was likely.



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1-29-23

Date

Citation

McInnes EF. *Background Lesions in Laboratory Animals, A Color Atlas*. Edinburgh: Saunders Elsevier, 2012.

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Appendix A1. Individual Data Listing of Gross to Microscopic Correlation

Key to Group Challenge Assignments:

- PBS = Groups 4, 5, 6
- 1×10^6 TCID₅₀ SARS-CoV-2 UK = Groups 1, 2, 3

Key to Group Endpoint Assignment:

- Termination Day 7 = Groups 1, 4
- Termination Day 14 = Groups 2, 5
- Termination Day 21 = Groups 3, 6

Individual Data Listing of Gross to Microscopic Correlation

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Study: 110890.01.208.06.01.04

StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Test Article: SARS-CoV-2

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Individual Data Listing of Gross to Microscopic Correlation

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Study: 110890.01.208.06.01.04

StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Test Article: SARS-CoV-2

Hamster/Golden Syrian			Single Dose/Oral Exposure	
Gross Observations			Status	Microscopic Observations
Animal #	Sex	Group		
1212	M	2		
		Lungs, Mottled, Darker color	Not Correlated	
1216	M	3		
		Lungs, Mottled, Noted	Correlated	Lungs, Inflammation, interstitial, minimal
1217	M	3		
		Lungs, Mottled, slightly purple, dark	Not Correlated	
1218	M	3		
		Lungs, Dark spot, red, upper left lobe	Not Correlated	
		Lungs, Mottled, throughout	Not Correlated	
1201	F	1		
		Lungs, Mottled, Darker, right lobe	Correlated	Lungs, Inflammation, interstitial, mild
		Lungs, Mottled, slightly, left lobe	Correlated	Lungs, Inflammation, interstitial, mild
1202	F	1		
		Lungs, Dark spot, Right top lobe, approximately 1cm in diameter	Not Correlated	
		Lungs, Mottled, slightly, Left lobe and right bottom lobe	Not Correlated	
		Nasal Turbinate, Broken, Noted	Not Correlated	

Individual Data Listing of Gross to Microscopic Correlation

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Study: 110890.01.208.06.01.04

StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Test Article: SARS-CoV-2

Hamster/Golden Syrian			Single Dose/Oral Exposure	
Gross Observations			Status	Microscopic Observations
Animal #	Sex	Group		
1203	F	1		
		Lungs, Mottling, slight, left lobe	Correlated	Lungs, Infiltrate, mixed cell, minimal
		Lungs, Pale, Noted	Correlated	Lungs, Aggregate, alveolar macrophage, minimal
1207	F	2		
		Lungs, Mottled, Noted	Correlated	Lungs, Inflammation, interstitial, moderate
		Stomach, Dark area, reddish-brown	Correlated	Stomach, Mineralization, minimal
1208	F	2		
		Lungs, Mottled, very dark red	Correlated	Lungs, Hemorrhage, minimal
		Soft Palate, White, Noted/Microscopic evaluation of macroscopic observation not required	Not Applicable	
		Stomach, Dark area, reddish-brown	Correlated	Stomach, Mineralization, mild
1209	F	2		
		Lungs, Mottled, Darker color	Not Correlated	
		Soft Palate, White, Noted/Microscopic evaluation of macroscopic observation not required	Not Applicable	
		Stomach, Dark area, reddish-brown	Correlated	Stomach, Mineralization, minimal

Individual Data Listing of Gross to Microscopic Correlation

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StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Test Article: SARS-CoV-2

Hamster/Golden Syrian			Single Dose/Oral Exposure	
Gross Observations			Status	Microscopic Observations
Animal #	Sex	Group		
1213	F	3		
		Lungs, Mottled, slightly darker than normal	Not Correlated	
1214	F	3		
		Lungs, Mottled, very dark red	Correlated	Lungs, Inflammation, interstitial, minimal
1215	F	3		
		Lungs, Mottled, pale	Not Correlated	

Appendix A2. Expanded Summary Report of Histopathology

Key to Group Challenge Assignments:

- PBS = Groups 4, 5, 6
- 1×10^6 TCID₅₀ SARS-CoV-2 UK = Groups 1, 2, 3

Key to Group Endpoint Assignment:

- Termination Day 7 = Groups 1, 4
- Termination Day 14 = Groups 2, 5
- Termination Day 21 = Groups 3, 6

Expanded Summary Report of Histopathology

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Study: 110890.01.208.06.01.04

StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Test Article: SARS-CoV-2

Hamster/Golden Syrian

Single Dose/Oral Exposure

Males			
	Dosage Group:	1	4
	Number of Animals:	3	2
	Number Examined:	3	2
	Number Unremarkable:	0	1
Large Intestine, Cecum	Number examined:	3	2
	Number unremarkable:	3	2
Large Intestine, Colon	Number examined:	3	2
	Number unremarkable:	3	2
Lungs	Number examined:	3	2
	Number unremarkable:	0	2
Aggregate, alveolar macrophage	3>	2	0
	Total Finding Incidence	2	0
Bronchiolitis	1>	2	0
	Total Finding Incidence	2	0
Hemorrhage	1>	1	0
	2>	1	0
	Total Finding Incidence	2	0
Hyperplasia, alveolar type II cell	2>	1	0
	Total Finding Incidence	1	0
Hyperplasia, respiratory epithelium, bronchioles	2>	1	0
	3>	1	0
	Total Finding Incidence	2	0
Hypertrophy, mesothelial cell	1>	2	0
	Total Finding Incidence	2	0
Infiltrate, mixed cell	3>	2	0

Expanded Summary Report of Histopathology

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Study: 110890.01.208.06.01.04

StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Test Article: SARS-CoV-2

Hamster/Golden Syrian

Single Dose/Oral Exposure

Males			
	Dosage Group:	1	4
	Number of Animals:	3	2
	Number Examined:	3	2
	Number Unremarkable:	0	1
Lungs	Number examined:	3	2
	Number unremarkable:	0	2
Infiltrate, mixed cell	Total Finding Incidence	2	0
Inflammation, interstitial	3>	2	0
	Total Finding Incidence	2	0
Multinucleated Giant Cell	1>	2	0
	Total Finding Incidence	2	0
Vasculitis	1>	1	0
	3>	2	0
	Total Finding Incidence	3	0
Nasal Turbinate	Number examined:	3	2
	Number unremarkable:	1	2
Exudate, nasal cavity	2>	2	0
	Total Finding Incidence	2	0
Inflammation, olfactory epithelium	3>	2	0
	Total Finding Incidence	2	0
Inflammation, respiratory epithelium	2>	2	0
	Total Finding Incidence	2	0
Small Intestine, Duodenum	Number examined:	3	2
	Number unremarkable:	3	2

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Study: 110890.01.208.06.01.04

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Test Article: SARS-CoV-2

Hamster/Golden Syrian

Single Dose/Oral Exposure

Males			
	Dosage Group:	1	4
	Number of Animals:	3	2
	Number Examined:	3	2
	Number Unremarkable:	0	1
Small Intestine, Ileum	Number examined:	3	2
	Number unremarkable:	3	2
Small Intestine, Jejunum	Number examined:	3	2
	Number unremarkable:	3	2
Soft Palate	Number examined:	0	0
	Number unremarkable:	0	0
Stomach	Number examined:	3	2
	Number unremarkable:	3	1
Mineralization	1>	0	1
	Total Finding Incidence	0	1
Trachea	Number examined:	3	2
	Number unremarkable:	2	2
Inflammation	1>	1	0
	Total Finding Incidence	1	0

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Study: 110890.01.208.06.01.04

StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Test Article: SARS-CoV-2

Hamster/Golden Syrian

Single Dose/Oral Exposure

Females			
	Dosage Group:	1	4
	Number of Animals:	3	2
	Number Examined:	3	2
	Number Unremarkable:	1	0
Large Intestine, Cecum	Number examined:	3	2
	Number unremarkable:	3	2
Large Intestine, Colon	Number examined:	3	2
	Number unremarkable:	3	2
Lungs	Number examined:	3	2
	Number unremarkable:	1	1
Aggregate, alveolar macrophage	1>	1	0
	2>	1	0
	Total Finding Incidence	2	0
Bronchiolitis	2>	1	0
	Total Finding Incidence	1	0
Hyperplasia, respiratory epithelium, bronchioles	1>	1	0
	Total Finding Incidence	1	0
Hypertrophy, mesothelial cell	1>	1	0
	Total Finding Incidence	1	0
Infiltrate, mixed cell	1>	1	1
	2>	1	0
	Total Finding Incidence	2	1
Inflammation, interstitial	2>	1	0
	Total Finding Incidence	1	0
Multinucleated Giant Cell	1>	1	0

Expanded Summary Report of Histopathology

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Study: 110890.01.208.06.01.04

StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Test Article: SARS-CoV-2

Hamster/Golden Syrian

Single Dose/Oral Exposure

Females			
	Dosage Group:	1	4
	Number of Animals:	3	2
	Number Examined:	3	2
	Number Unremarkable:	1	0
Lungs	Number examined:	3	2
	Number unremarkable:	1	1
Multinucleated Giant Cell	Total Finding Incidence	1	0
Vasculitis	1>	1	0
	3>	1	0
	Total Finding Incidence	2	0
Nasal Turbinate	Number examined:	3	2
	Number unremarkable:	1	2
Exudate, nasal cavity	2>	1	0
	Total Finding Incidence	1	0
Inflammation, olfactory epithelium	3>	1	0
	Total Finding Incidence	1	0
Inflammation, respiratory epithelium	2>	1	0
	3>	1	0
	Total Finding Incidence	2	0
Small Intestine, Duodenum	Number examined:	3	2
	Number unremarkable:	3	2
Small Intestine, Ileum	Number examined:	3	2
	Number unremarkable:	3	2

Expanded Summary Report of Histopathology

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Study: 110890.01.208.06.01.04

StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Test Article: SARS-CoV-2

Hamster/Golden Syrian

Single Dose/Oral Exposure

Females			
	Dosage Group:	1	4
	Number of Animals:	3	2
	Number Examined:	3	2
	Number Unremarkable:	1	0
Small Intestine, Jejunum	Number examined:	3	2
	Number unremarkable:	3	2
Soft Palate	Number examined:	0	0
	Number unremarkable:	0	0
Stomach	Number examined:	3	2
	Number unremarkable:	3	1
Mineralization	2>	0	1
	Total Finding Incidence	0	1
Trachea	Number examined:	3	2
	Number unremarkable:	3	2

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Study: 110890.01.208.06.01.04

StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Test Article: SARS-CoV-2

Hamster/Golden Syrian

Single Dose/Oral Exposure

Males			
	Dosage Group:	2	5
	Number of Animals:	3	2
	Number Examined:	3	2
	Number Unremarkable:	1	0
Large Intestine, Cecum	Number examined:	3	2
	Number unremarkable:	3	2
Large Intestine, Colon	Number examined:	3	2
	Number unremarkable:	3	2
Lungs	Number examined:	3	2
	Number unremarkable:	2	2
Aggregate, alveolar macrophage	1>	1	0
	Total Finding Incidence	1	0
Hemorrhage	1>	1	0
	Total Finding Incidence	1	0
Vasculitis	1>	1	0
	Total Finding Incidence	1	0
Nasal Turbinate	Number examined:	3	2
	Number unremarkable:	3	2
Small Intestine, Duodenum	Number examined:	3	2
	Number unremarkable:	3	2

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Study: 110890.01.208.06.01.04

StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Test Article: SARS-CoV-2

Hamster/Golden Syrian

Single Dose/Oral Exposure

Males			
	Dosage Group:	2	5
	Number of Animals:	3	2
	Number Examined:	3	2
	Number Unremarkable:	1	0
Small Intestine, Ileum	Number examined:	3	2
	Number unremarkable:	3	2
Small Intestine, Jejunum	Number examined:	3	2
	Number unremarkable:	3	2
Soft Palate	Number examined:	0	0
	Number unremarkable:	0	0
Stomach	Number examined:	3	2
	Number unremarkable:	1	0
Mineralization	1>	1	2
	2>	1	0
	Total Finding Incidence	2	2
Trachea	Number examined:	3	2
	Number unremarkable:	3	2

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Study: 110890.01.208.06.01.04

StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Test Article: SARS-CoV-2

Hamster/Golden Syrian

Single Dose/Oral Exposure

Females			
	Dosage Group:	2	5
	Number of Animals:	3	2
	Number Examined:	3	2
	Number Unremarkable:	0	0
Large Intestine, Cecum	Number examined:	3	2
	Number unremarkable:	3	2
Large Intestine, Colon	Number examined:	3	2
	Number unremarkable:	3	2
Lungs	Number examined:	3	2
	Number unremarkable:	1	2
Aggregate, alveolar macrophage	1>	1	0
	3>	1	0
	Total Finding Incidence	2	0
Hemorrhage	1>	2	0
	Total Finding Incidence	2	0
Hyperplasia, alveolar type II cell	2>	1	0
	Total Finding Incidence	1	0
Hypertrophy, mesothelial cell	1>	1	0
	Total Finding Incidence	1	0
Infiltrate, mixed cell	1>	1	0
	Total Finding Incidence	1	0
Inflammation, interstitial	3>	1	0
	Total Finding Incidence	1	0
Multinucleated Giant Cell	1>	1	0
	Total Finding Incidence	1	0

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Study: 110890.01.208.06.01.04

StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Test Article: SARS-CoV-2

Hamster/Golden Syrian

Single Dose/Oral Exposure

Females			
	Dosage Group:	2	5
	Number of Animals:	3	2
	Number Examined:	3	2
	Number Unremarkable:	0	0
Lungs	Number examined:	3	2
	Number unremarkable:	1	2
Vasculitis	1>	1	0
	2>	1	0
	Total Finding Incidence	2	0
Nasal Turbinate	Number examined:	3	2
	Number unremarkable:	3	2
Small Intestine, Duodenum	Number examined:	3	2
	Number unremarkable:	3	2
Small Intestine, Ileum	Number examined:	0	2
	Number unremarkable:	0	2
Small Intestine, Jejunum	Number examined:	3	2
	Number unremarkable:	3	2
Soft Palate	Number examined:	0	0
	Number unremarkable:	0	0

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StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Test Article: SARS-CoV-2

Hamster/Golden Syrian

Single Dose/Oral Exposure

Females			
	Dosage Group:	2	5
	Number of Animals:	3	2
	Number Examined:	3	2
	Number Unremarkable:	0	0
Stomach	Number examined:	3	2
	Number unremarkable:	0	0
Mineralization	1>	2	1
	2>	1	1
	Total Finding Incidence	3	2
Trachea	Number examined:	3	2
	Number unremarkable:	3	2

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Study: 110890.01.208.06.01.04

StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Test Article: SARS-CoV-2

Hamster/Golden Syrian

Single Dose/Oral Exposure

Males			
	Dosage Group:	3	6
	Number of Animals:	3	2
	Number Examined:	3	2
	Number Unremarkable:	1	0
Large Intestine, Cecum	Number examined:	3	2
	Number unremarkable:	3	2
Large Intestine, Colon	Number examined:	3	2
	Number unremarkable:	3	2
Lungs	Number examined:	3	2
	Number unremarkable:	1	2
Aggregate, alveolar macrophage	1>	2	0
	Total Finding Incidence	2	0
Infiltrate, mixed cell	1>	1	0
	Total Finding Incidence	1	0
Inflammation, interstitial	1>	1	0
	Total Finding Incidence	1	0
Vasculitis	1>	1	0
	Total Finding Incidence	1	0
Nasal Turbinate	Number examined:	3	2
	Number unremarkable:	3	2

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Pristima® Version 7.2.0 Build 31

Study: 110890.01.208.06.01.04

StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Test Article: SARS-CoV-2

Hamster/Golden Syrian

Single Dose/Oral Exposure

Males			
	Dosage Group:	3	6
	Number of Animals:	3	2
	Number Examined:	3	2
	Number Unremarkable:	1	0
Small Intestine, Duodenum	Number examined:	3	2
	Number unremarkable:	3	2
Small Intestine, Ileum	Number examined:	3	2
	Number unremarkable:	3	2
Small Intestine, Jejunum	Number examined:	3	2
	Number unremarkable:	3	2
Soft Palate	Number examined:	0	0
	Number unremarkable:	0	0
Stomach	Number examined:	3	2
	Number unremarkable:	3	0
Mineralization	2>	0	2
Total Finding Incidence		0	2
Trachea	Number examined:	3	2
	Number unremarkable:	3	2

Expanded Summary Report of Histopathology

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StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Test Article: SARS-CoV-2

Hamster/Golden Syrian

Single Dose/Oral Exposure

Females			
	Dosage Group:	3	6
	Number of Animals:	3	2
	Number Examined:	3	2
	Number Unremarkable:	0	0
Large Intestine, Cecum	Number examined:	3	2
	Number unremarkable:	3	2
Large Intestine, Colon	Number examined:	3	2
	Number unremarkable:	3	2
Lungs	Number examined:	3	2
	Number unremarkable:	0	1
Aggregate, alveolar macrophage	1>	3	1
	Total Finding Incidence	3	1
Hyperplasia, alveolar type II cell	1>	1	0
	Total Finding Incidence	1	0
Infiltrate, mixed cell	1>	2	0
	Total Finding Incidence	2	0
Inflammation, interstitial	1>	1	0
	Total Finding Incidence	1	0
Vasculitis	1>	1	0
	Total Finding Incidence	1	0
Nasal Turbinate	Number examined:	2	2
	Number unremarkable:	2	2

Expanded Summary Report of Histopathology

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Study: 110890.01.208.06.01.04

StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Test Article: SARS-CoV-2

Hamster/Golden Syrian

Single Dose/Oral Exposure

Females			
	Dosage Group:	3	6
	Number of Animals:	3	2
	Number Examined:	3	2
	Number Unremarkable:	0	0
Small Intestine, Duodenum	Number examined:	3	2
	Number unremarkable:	3	2
Small Intestine, Ileum	Number examined:	3	1
	Number unremarkable:	3	1
Small Intestine, Jejunum	Number examined:	3	1
	Number unremarkable:	3	1
Soft Palate	Number examined:	0	0
	Number unremarkable:	0	0
Stomach	Number examined:	3	2
	Number unremarkable:	0	1
Mineralization	1>	3	1
	Total Finding Incidence	3	1
Trachea	Number examined:	3	2
	Number unremarkable:	3	2

Appendix A3. Individual Gross and Microscopic Observations

All animals, all groups

Key to Group Challenge Assignments:

- PBS = Groups 4, 5, 6
- 1×10^6 TCID₅₀ SARS-CoV-2 UK = Groups 1, 2, 3

Key to Group Endpoint Assignment:

- Termination Day 7 = Groups 1, 4
- Termination Day 14 = Groups 2, 5
- Termination Day 21 = Groups 3, 6

Individual Gross and Microscopic Observations				Printed: 01/27/2023 02:51:41 PM	
Study: 110890.01.208.06.01.04				Pristima® Version 7.2.0 Build 31	
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2				Single Dose/Oral Exposure	
Hamster/Golden Syrian		Group: 1	Day Of Death:	Dosing Phase, Day 7	
Animal #: 1204		Sex: M	Death Status:	Day 7	
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Lungs				Aggregate, alveolar macrophage , moderate Bronchiolitis, minimal Hyperplasia, respiratory epithelium, bronchioles, mild Hypertrophy, mesothelial cell, minimal Infiltrate, mixed cell, moderate Multinucleated Giant Cell, minimal Vasculitis, moderate Hemorrhage, minimal Inflammation, interstitial, moderate	
Nasal Turbinate	Within Normal Limits			Exudate, nasal cavity, mild Inflammation, olfactory epithelium, moderate Inflammation, respiratory epithelium, mild	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum	
Small Intestine, Jejunum	Stomach	Trachea			
Required tissues not microscopically examined:					
None					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum	
Stomach	Trachea				

Individual Gross and Microscopic Observations				Printed: 01/27/2023 02:51:41 PM	
Study: 110890.01.208.06.01.04				Pristima® Version 7.2.0 Build 31	
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2				Single Dose/Oral Exposure	
Hamster/Golden Syrian		Group: 1		Day Of Death: Dosing Phase, Day 7	
Animal #: 1205		Sex: M		Death Status: Day 7	
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Lungs				Bronchiolitis, minimal Hyperplasia, alveolar type II cell, mild Hyperplasia, respiratory epithelium, bronchioles, moderate Hypertrophy, mesothelial cell, minimal Infiltrate, mixed cell, moderate Multinucleated Giant Cell, minimal Vasculitis, moderate	
	Dark spot, red, right lower lobe, approximately 1/2cm in diameter		Correlated	Hemorrhage, mild	
	Lesions, right and left lobe, clear, pale		Correlated	Aggregate, alveolar macrophage , moderate	
	Mottled, dark		Correlated	Inflammation, interstitial, moderate	
Nasal Turbinate	Within Normal Limits			Exudate, nasal cavity, mild Inflammation, olfactory epithelium, moderate Inflammation, respiratory epithelium, mild	
Trachea	Within Normal Limits			Inflammation, minimal	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum	
Small Intestine, Jejunum	Stomach	Trachea			
Required tissues not microscopically examined:					
None					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum	

Individual Gross and Microscopic Observations				Printed: 01/27/2023 02:51:41 PM
Study: 110890.01.208.06.01.04				Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters				
Test Article: SARS-CoV-2				Single Dose/Oral Exposure
Hamster/Golden Syrian	Group: 1		Day Of Death:	Dosing Phase, Day 7
Animal #: 1205	Sex: M		Death Status:	Day 7
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments
Continued from previous page ...				
Stomach				

Individual Gross and Microscopic Observations					Printed: 01/27/2023 02:51:41 PM
Study: 110890.01.208.06.01.04					Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2					Single Dose/Oral Exposure
Hamster/Golden Syrian	Group: 1		Day Of Death:	Dosing Phase, Day 7	
Animal#: 1206	Sex: M		Death Status:	Day 7	
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Lungs	Mottled, right lobes Mottled, slightly, left lobe		Not Correlated Not Correlated	Vasculitis, minimal	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum	
Small Intestine, Jejunum	Stomach	Trachea			
Required tissues not microscopically examined:					
None					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum	
Small Intestine, Jejunum	Stomach	Trachea			

Individual Gross and Microscopic Observations				Printed: 01/27/2023 02:51:41 PM	
Study: 110890.01.208.06.01.04				Pristima® Version 7.2.0 Build 31	
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2					
Hamster/Golden Syrian				Single Dose/Oral Exposure	
Animal #: 1201	Group: 1	Day Of Death:	Dosing Phase, Day 7		
	Sex: F	Death Status:	Day 7		
Tissue	Gross Observations/Comments	Status	Microscopic Observations/Comments		
Lungs			Tissue Comment: Vaculitis consists of transmural infiltration of mixed cells (monocytes, neutrophils), perivascular infiltration of mixed cells, endothelial hyperplasia/hypertrophy, and the apparent occlusion of the lumen of some small vessels with mononuclear "cell clots." Aggregate, alveolar macrophage, mild Bronchiolitis, mild Hyperplasia, respiratory epithelium, bronchioles, minimal Hypertrophy, mesothelial cell, minimal Infiltrate, mixed cell, mild Multinucleated Giant Cell, minimal Vasculitis, moderate		
	Mottled, Darker, right lobe	Correlated	Inflammation, interstitial, mild		
	Mottled, slightly, left lobe	Correlated	Inflammation, interstitial, mild		
Nasal Turbinate	Within Normal Limits		Exudate, nasal cavity, mild Inflammation, olfactory epithelium, moderate Inflammation, respiratory epithelium, moderate		
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum	
Small Intestine, Jejunum	Stomach	Trachea			
Required tissues not microscopically examined:					
None					

Individual Gross and Microscopic Observations					Printed: 01/27/2023 02:51:41 PM
Study: 110890.01.208.06.01.04					Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					Single Dose/Oral Exposure
Test Article: SARS-CoV-2					
Hamster/Golden Syrian	Group: 1		Day Of Death:	Dosing Phase, Day 7	
Animal #: 1201	Sex: F		Death Status:	Day 7	
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Continued from previous page ...					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum	
Stomach	Trachea				

Individual Gross and Microscopic Observations					Printed: 01/27/2023 02:51:41 PM
Study: 110890.01.208.06.01.04					Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2					Single Dose/Oral Exposure
Hamster/Golden Syrian	Group: 1		Day Of Death: Dosing Phase, Day 7		
Animal #: 1202	Sex: F		Death Status: Day 7		
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Lungs	Dark spot, Right top lobe, approximately 1cm in diameter		Not Correlated	No Abnormalities Detected	
	Mottled, slightly, Left lobe and right bottom lobe		Not Correlated		
Nasal Turbinate				Tissue Comment:Nasal turbinate is fractured on the slide presented, however there is no hemorrhage or inflammation, suggesting this was a post-mortem incident.	
	Broken, Noted		Not Correlated	No Abnormalities Detected	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum	
Stomach	Trachea				
Required tissues not microscopically examined:					
None					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum	
Small Intestine, Ileum	Small Intestine, Jejunum	Stomach	Trachea		

Individual Gross and Microscopic Observations					Printed: 01/27/2023 02:51:41 PM
Study: 110890.01.208.06.01.04					Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2					Single Dose/Oral Exposure
Hamster/Golden Syrian	Group: 1		Day Of Death: Dosing Phase, Day 7		
Animal #: 1203	Sex: F		Death Status: Day 7		
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Lungs	Mottling, slight, left lobe Pale, Noted		Correlated Correlated	Vasculitis, minimal Infiltrate, mixed cell, minimal Aggregate, alveolar macrophage , minimal	
Nasal Turbinate	Within Normal Limits			Inflammation, respiratory epithelium, mild	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon		Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Stomach		Trachea		
Required tissues not microscopically examined:					
None					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon		Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum
Stomach	Trachea				

Individual Gross and Microscopic Observations					Printed: 01/27/2023 02:52:27 PM
Study: 110890.01.208.06.01.04					Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2					Single Dose/Oral Exposure
Hamster/Golden Syrian		Group: 2		Day Of Death: Dosing Phase, Day 14	
Animal #: 1210		Sex: M		Death Status: Day 14	
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Lungs	Mottled, dark red		Correlated	Aggregate, alveolar macrophage , minimal Vasculitis, minimal Hemorrhage, minimal	
Nasal Turbinate	Within Normal Limits			Tissue Comment:Only one nasal section provided. No Abnormalities Detected	
Stomach	Within Normal Limits			Mineralization, mild	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum	
Small Intestine, Jejunum	Stomach	Trachea			
Required tissues not microscopically examined:					
None					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum	
Small Intestine, Jejunum	Trachea				

Individual Gross and Microscopic Observations					Printed: 01/27/2023 02:52:27 PM
Study: 110890.01.208.06.01.04					Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2					Single Dose/Oral Exposure
Hamster/Golden Syrian	Group: 2		Day Of Death:	Dosing Phase, Day 14	
Animal #: 1211	Sex: M		Death Status:	Day 14	
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Lungs	Darker color, Noted		Not Correlated	No Abnormalities Detected	
Nasal Turbinate	Within Normal Limits			Tissue Comment: Only one nasal section provided. No Abnormalities Detected	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon		Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Stomach		Trachea		
Required tissues not microscopically examined:					
None					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon		Lungs	Nasal Turbinate	Small Intestine, Duodenum
Small Intestine, Ileum	Small Intestine, Jejunum		Stomach	Trachea	

Individual Gross and Microscopic Observations					Printed: 01/27/2023 02:52:27 PM
Study: 110890.01.208.06.01.04					Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2					Single Dose/Oral Exposure
Hamster/Golden Syrian	Group: 2		Day Of Death: Dosing Phase, Day 14		
Animal #: 1212	Sex: M		Death Status: Day 14		
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Lungs	Mottled, Darker color		Not Correlated	No Abnormalities Detected	
Nasal Turbinate	Within Normal Limits			Tissue Comment: Only one nasal section provided. No Abnormalities Detected	
Stomach	Within Normal Limits			Mineralization, minimal	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum	
Small Intestine, Jejunum	Stomach	Trachea			
Required tissues not microscopically examined:					
None					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum	
Small Intestine, Ileum	Small Intestine, Jejunum	Trachea			

Individual Gross and Microscopic Observations				Printed: 01/27/2023 02:52:27 PM	
Study: 110890.01.208.06.01.04				Pristima® Version 7.2.0 Build 31	
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2					
Hamster/Golden Syrian		Group: 2		Day Of Death: Dosing Phase, Day 14	
Animal #: 1207		Sex: F		Death Status: Day 14	
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Lungs				Aggregate, alveolar macrophage , moderate Hemorrhage, minimal Hyperplasia, alveolar type II cell, mild Hypertrophy, mesothelial cell, minimal Infiltrate, mixed cell, minimal Multinucleated Giant Cell, minimal Vasculitis, mild Inflammation, interstitial, moderate	
	Mottled, Noted		Correlated		
Nasal Turbinate	Within Normal Limits			Tissue Comment:Only one nasal section presented. No Abnormalities Detected	
Small Intestine, Ileum	Within Normal Limits			Tissue Comment:Recut for missing ileum produced normal colon. Missing	
Stomach				Tissue Comment:Area of chronic inflammation with mineralization present.	
	Dark area, reddish-brown		Correlated	Mineralization, minimal	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon		Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Trachea				
Required tissues not microscopically examined:					
Small Intestine, Ileum					

Individual Gross and Microscopic Observations					Printed: 01/27/2023 02:52:27 PM
Study: 110890.01.208.06.01.04					Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2					Single Dose/Oral Exposure
Hamster/Golden Syrian	Group: 2		Day Of Death:	Dosing Phase, Day 14	
Animal #: 1207	Sex: F		Death Status:	Day 14	
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Continued from previous page ...					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Jejunum	
Trachea					

Individual Gross and Microscopic Observations				Printed: 01/27/2023 02:52:27 PM	
Study: 110890.01.208.06.01.04				Pristima® Version 7.2.0 Build 31	
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2				Single Dose/Oral Exposure	
Hamster/Golden Syrian		Group: 2		Day Of Death: Dosing Phase, Day 14	
Animal #: 1208		Sex: F		Death Status: Day 14	
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Lungs	Mottled, very dark red		Correlated	Aggregate, alveolar macrophage , minimal Vasculitis, minimal Hemorrhage, minimal	
Nasal Turbinate	Within Normal Limits			Tissue Comment:Only one nasal section presented. No Abnormalities Detected	
Small Intestine, Ileum	Within Normal Limits			Tissue Comment:Recut for missing ileum produced normal colon. Missing	
Soft Palate	White, Noted /Comments: Microscopic evaluation of macroscopic observation not required		Not Applicable	Missing	
Stomach	Dark area, reddish-brown		Correlated	Mineralization, mild	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum	
Small Intestine, Jejunum	Trachea				
Required tissues not microscopically examined:					
Small Intestine, Ileum	Soft Palate				
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Jejunum	

Individual Gross and Microscopic Observations				Printed: 01/27/2023 02:52:27 PM
Study: 110890.01.208.06.01.04				Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters				
Test Article: SARS-CoV-2				Single Dose/Oral Exposure
Hamster/Golden Syrian	Group: 2	Day Of Death:	Dosing Phase, Day 14	
Animal #: 1208	Sex: F	Death Status:	Day 14	
Tissue	Gross Observations/Comments	Status	Microscopic Observations/Comments	

Continued from previous page ...

Trachea

Individual Gross and Microscopic Observations				Printed: 01/27/2023 02:52:27 PM	
Study: 110890.01.208.06.01.04				Pristima® Version 7.2.0 Build 31	
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2				Single Dose/Oral Exposure	
Hamster/Golden Syrian Animal #: 1209		Group: 2	Day Of Death:	Dosing Phase, Day 14	
		Sex: F	Death Status:	Day 14	
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Lungs	Mottled, Darker color		Not Correlated	No Abnormalities Detected	
Nasal Turbinate	Within Normal Limits			Tissue Comment: Only one nasal section provided. No Abnormalities Detected	
Small Intestine, Ileum	Within Normal Limits			Tissue Comment: Recut for missing ileum produced normal colon. Missing	
Soft Palate	White, Noted /Comments: Microscopic evaluation of macroscopic observation not required		Not Applicable	Missing	
Stomach	Dark area, reddish-brown		Correlated	Mineralization, minimal	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum	
Small Intestine, Jejunum	Trachea				
Required tissues not microscopically examined:					
Small Intestine, Ileum	Soft Palate				
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum	
Small Intestine, Jejunum	Trachea				

Individual Gross and Microscopic Observations					Printed: 01/27/2023 02:53:17 PM
Study: 110890.01.208.06.01.04					Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2					Single Dose/Oral Exposure
Hamster/Golden Syrian	Group: 3			Day Of Death:	Dosing Phase, Day 21
Animal#: 1216	Sex: M			Death Status:	Day 21
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Lungs	Mottled, Noted		Correlated	Aggregate, alveolar macrophage, minimal	
Infiltrate, mixed cell, minimal					
				Vasculitis, minimal	
				Inflammation, interstitial, minimal	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum	
Small Intestine, Jejunum	Stomach	Trachea			
Required tissues not microscopically examined:					
None					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum	
Small Intestine, Jejunum	Stomach	Trachea			

Individual Gross and Microscopic Observations					Printed: 01/27/2023 02:53:17 PM
Study: 110890.01.208.06.01.04					Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2					Single Dose/Oral Exposure
Hamster/Golden Syrian	Group: 3		Day Of Death:	Dosing Phase, Day 21	
Animal #: 1217	Sex: M		Death Status:	Day 21	
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Lungs	Mottled, slightly purple, dark		Not Correlated	No Abnormalities Detected	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum	
Small Intestine, Jejunum	Stomach	Trachea			
Required tissues not microscopically examined:					
None					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum	
Small Intestine, Ileum	Small Intestine, Jejunum	Stomach	Trachea		

Individual Gross and Microscopic Observations					Printed: 01/27/2023 02:53:17 PM
Study: 110890.01.208.06.01.04					Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2					Single Dose/Oral Exposure
Hamster/Golden Syrian	Group: 3			Day Of Death:	Dosing Phase, Day 21
Animal #: 1218	Sex: M			Death Status:	Day 21
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Lungs	Dark spot, red, upper left lobe		Not Correlated	Aggregate, alveolar macrophage , minimal	
	Mottled, throughout		Not Correlated		
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon		Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Stomach		Trachea		
Required tissues not microscopically examined:					
None					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon		Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Stomach		Trachea		

Individual Gross and Microscopic Observations					Printed: 01/27/2023 02:53:17 PM
Study: 110890.01.208.06.01.04					Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2					Single Dose/Oral Exposure
Hamster/Golden Syrian	Group: 3			Day Of Death:	Dosing Phase, Day 21
Animal#: 1213	Sex: F			Death Status:	Day 21
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Lungs	Mottled, slightly darker than normal		Not Correlated	Aggregate, alveolar macrophage, minimal Infiltrate, mixed cell, minimal	
Stomach	Within Normal Limits			Mineralization, minimal	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon		Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Stomach		Trachea		
Required tissues not microscopically examined:					
None					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon		Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Trachea				

Individual Gross and Microscopic Observations				Printed: 01/27/2023 02:53:17 PM	
Study: 110890.01.208.06.01.04				Pristima® Version 7.2.0 Build 31	
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2				Single Dose/Oral Exposure	
Hamster/Golden Syrian		Group: 3		Day Of Death: Dosing Phase, Day 21	
Animal #: 1214		Sex: F		Death Status: Day 21	
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Lungs	Mottled, very dark red		Correlated	Aggregate, alveolar macrophage , minimal Hyperplasia, alveolar type II cell, minimal Infiltrate, mixed cell, minimal Vasculitis, minimal Inflammation, interstitial, minimal	
Stomach	Within Normal Limits			Mineralization, minimal	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon		Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Stomach		Trachea		
Required tissues not microscopically examined:					
None					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon		Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Trachea				

Individual Gross and Microscopic Observations					Printed: 01/27/2023 02:53:17 PM
Study: 110890.01.208.06.01.04					Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2					Single Dose/Oral Exposure
Hamster/Golden Syrian	Group: 3		Day Of Death: Dosing Phase, Day 21		
Animal #: 1215	Sex: F		Death Status: Day 21		
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Lungs	Mottled, pale		Not Correlated	Aggregate, alveolar macrophage , minimal	
Nasal Turbinate	Within Normal Limits			Missing	
Stomach	Within Normal Limits			Mineralization, minimal	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum	
Small Intestine, Jejunum	Stomach	Trachea			
Required tissues not microscopically examined:					
Nasal Turbinate					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum	
Trachea					

Individual Gross and Microscopic Observations					Printed: 01/27/2023 02:54:20 PM
Study: 110890.01.208.06.01.04					Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2					Single Dose/Oral Exposure
Hamster/Golden Syrian	Group: 4		Day Of Death:	Dosing Phase, Day 7	
Animal #: 1221	Sex: M		Death Status:	Day 7	
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Stomach	Within Normal Limits			Mineralization, minimal	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum	
Small Intestine, Ileum	Small Intestine, Jejunum	Stomach	Trachea		
Required tissues not microscopically examined:					
None					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum	
Small Intestine, Ileum	Small Intestine, Jejunum	Trachea			

Individual Gross and Microscopic Observations					Printed: 01/27/2023 02:54:20 PM
Study: 110890.01.208.06.01.04					Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2					Single Dose/Oral Exposure
Hamster/Golden Syrian	Group: 4		Day Of Death:	Dosing Phase, Day 7	
Animal #: 1222	Sex: M		Death Status:	Day 7	
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum	
Small Intestine, Ileum	Small Intestine, Jejunum	Stomach	Trachea		
Required tissues not microscopically examined:					
None					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum	
Small Intestine, Ileum	Small Intestine, Jejunum	Stomach	Trachea		

Individual Gross and Microscopic Observations					Printed: 01/27/2023 02:54:20 PM
Study: 110890.01.208.06.01.04					Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2					Single Dose/Oral Exposure
Hamster/Golden Syrian					
Animal #: 1219	Group: 4		Day Of Death:	Dosing Phase, Day 7	
	Sex: F		Death Status:	Day 7	
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Lungs	Within Normal Limits			Infiltrate, mixed cell, minimal	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum	
Small Intestine, Ileum	Small Intestine, Jejunum	Stomach	Trachea		
Required tissues not microscopically examined:					
None					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Nasal Turbinate	Small Intestine, Duodenum	Small Intestine, Ileum	
Small Intestine, Jejunum	Stomach	Trachea			

Individual Gross and Microscopic Observations					Printed: 01/27/2023 02:54:20 PM
Study: 110890.01.208.06.01.04					Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2					Single Dose/Oral Exposure
Hamster/Golden Syrian	Group: 4			Day Of Death:	Dosing Phase, Day 7
Animal #: 1220	Sex: F			Death Status:	Day 7
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Stomach	Within Normal Limits			Mineralization, mild	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum	
Small Intestine, Ileum	Small Intestine, Jejunum	Stomach	Trachea		
Required tissues not microscopically examined:					
None					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum	
Small Intestine, Ileum	Small Intestine, Jejunum	Trachea			

Individual Gross and Microscopic Observations						Printed: 01/27/2023 02:55:15 PM	
Study: 110890.01.208.06.01.04						Pristima® Version 7.2.0 Build 31	
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters							
Test Article: SARS-CoV-2						Single Dose/Oral Exposure	
Hamster/Golden Syrian		Group: 5		Day Of Death: Dosing Phase, Day 14			
Animal #: 1225		Sex: M		Death Status: Day 14			
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments			
Nasal Turbinate	Within Normal Limits			Tissue Comment: Only one nasal section provided. No Abnormalities Detected			
Stomach	Within Normal Limits			Mineralization, minimal			
Tissues grossly within normal limits:							
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum			
Small Intestine, Ileum	Small Intestine, Jejunum	Stomach	Trachea				
Required tissues not microscopically examined:							
None							
Tissues microscopically examined and within normal limits:							
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum			
Small Intestine, Ileum	Small Intestine, Jejunum	Trachea					

Individual Gross and Microscopic Observations						Printed: 01/27/2023 02:55:15 PM
Study: 110890.01.208.06.01.04						Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters						
Test Article: SARS-CoV-2						
Single Dose/Oral Exposure						
Hamster/Golden Syrian		Group: 5		Day Of Death: Dosing Phase, Day 14		
Animal #: 1226		Sex: M		Death Status: Day 14		
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments		
Nasal Turbinate	Within Normal Limits			Tissue Comment: Only one nasal section provided. No Abnormalities Detected		
Stomach	Within Normal Limits			Mineralization, minimal		
Tissues grossly within normal limits:						
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum		
Small Intestine, Ileum	Small Intestine, Jejunum	Stomach	Trachea			
Required tissues not microscopically examined:						
None						
Tissues microscopically examined and within normal limits:						
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum		
Small Intestine, Ileum	Small Intestine, Jejunum	Trachea				

Individual Gross and Microscopic Observations						Printed: 01/27/2023 02:55:15 PM	
Study: 110890.01.208.06.01.04						Pristima® Version 7.2.0 Build 31	
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters							
Test Article: SARS-CoV-2						Single Dose/Oral Exposure	
Hamster/Golden Syrian		Group: 5		Day Of Death: Dosing Phase, Day 14			
Animal #: 1223		Sex: F		Death Status: Day 14			
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments			
Nasal Turbinate	Within Normal Limits			Tissue Comment:Only one nasal section submitted. No Abnormalities Detected			
Stomach	Within Normal Limits			Mineralization, mild			
Tissues grossly within normal limits:							
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum			
Small Intestine, Ileum	Small Intestine, Jejunum	Stomach	Trachea				
Required tissues not microscopically examined:							
None							
Tissues microscopically examined and within normal limits:							
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum			
Small Intestine, Ileum	Small Intestine, Jejunum	Trachea					

Individual Gross and Microscopic Observations						Printed: 01/27/2023 02:55:15 PM
Study: 110890.01.208.06.01.04						Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters						
Test Article: SARS-CoV-2						
Single Dose/Oral Exposure						
Hamster/Golden Syrian						
Animal #: 1224						
Group: 5						
Sex: F						
Day Of Death: Dosing Phase, Day 14						
Death Status: Day 14						
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments		
Nasal Turbinate	Within Normal Limits			Tissue Comment:Only one nasal section presented. No Abnormalities Detected		
Stomach	Within Normal Limits			Mineralization, minimal		
Tissues grossly within normal limits:						
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum		
Small Intestine, Ileum	Small Intestine, Jejunum	Stomach	Trachea			
Required tissues not microscopically examined:						
None						
Tissues microscopically examined and within normal limits:						
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum		
Small Intestine, Ileum	Small Intestine, Jejunum	Trachea				

Individual Gross and Microscopic Observations					Printed: 01/27/2023 02:55:59 PM
Study: 110890.01.208.06.01.04					Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2					
Hamster/Golden Syrian				Single Dose/Oral Exposure	
Animal #: 1229	Group: 6		Day Of Death:	Dosing Phase, Day 21	
	Sex: M		Death Status:	Day 21	
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Stomach	Within Normal Limits			Mineralization, mild	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum	
Small Intestine, Ileum	Small Intestine, Jejunum	Stomach	Trachea		
Required tissues not microscopically examined:					
None					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum	
Small Intestine, Ileum	Small Intestine, Jejunum	Trachea			

Individual Gross and Microscopic Observations					Printed: 01/27/2023 02:55:59 PM
Study: 110890.01.208.06.01.04					Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2					Single Dose/Oral Exposure
Hamster/Golden Syrian	Group: 6		Day Of Death:	Dosing Phase, Day 21	
Animal #: 1230	Sex: M		Death Status:	Day 21	
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Stomach	Within Normal Limits			Mineralization, mild	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum	
Small Intestine, Ileum	Small Intestine, Jejunum	Stomach	Trachea		
Required tissues not microscopically examined:					
None					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum	
Small Intestine, Ileum	Small Intestine, Jejunum	Trachea			

Individual Gross and Microscopic Observations				Printed: 01/27/2023 02:55:59 PM	
Study: 110890.01.208.06.01.04				Pristima® Version 7.2.0 Build 31	
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2				Single Dose/Oral Exposure	
Hamster/Golden Syrian		Group: 6		Day Of Death: Dosing Phase, Day 21	
Animal #: 1227		Sex: F		Death Status: Day 21	
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Lungs	Within Normal Limits			Aggregate, alveolar macrophage , minimal	
Small Intestine, Ileum	Within Normal Limits			Missing	
Small Intestine, Jejunum	Within Normal Limits			Tissue Comment:Recuts of missing jejunum and ileum produced additional (normal) duodenum and colon. Missing	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum	
Small Intestine, Ileum	Small Intestine, Jejunum	Stomach	Trachea		
Required tissues not microscopically examined:					
Small Intestine, Ileum	Small Intestine, Jejunum				
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Nasal Turbinate	Small Intestine, Duodenum	Stomach	
Trachea					

Individual Gross and Microscopic Observations					Printed: 01/27/2023 02:55:59 PM
Study: 110890.01.208.06.01.04					Pristima® Version 7.2.0 Build 31
StudyTitle: SARS-CoV-2 Infection Via the Oral Exposure In Hamsters					
Test Article: SARS-CoV-2					Single Dose/Oral Exposure
Hamster/Golden Syrian	Group: 6		Day Of Death:	Dosing Phase, Day 21	
Animal #: 1228	Sex: F		Death Status:	Day 21	
Tissue	Gross Observations/Comments		Status	Microscopic Observations/Comments	
Stomach	Within Normal Limits			Mineralization, minimal	
Tissues grossly within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum	
Small Intestine, Ileum	Small Intestine, Jejunum	Stomach	Trachea		
Required tissues not microscopically examined:					
None					
Tissues microscopically examined and within normal limits:					
Large Intestine, Cecum	Large Intestine, Colon	Lungs	Nasal Turbinate	Small Intestine, Duodenum	
Small Intestine, Ileum	Small Intestine, Jejunum	Trachea			

Appendix F. FISH Images

Appendix F Introduction

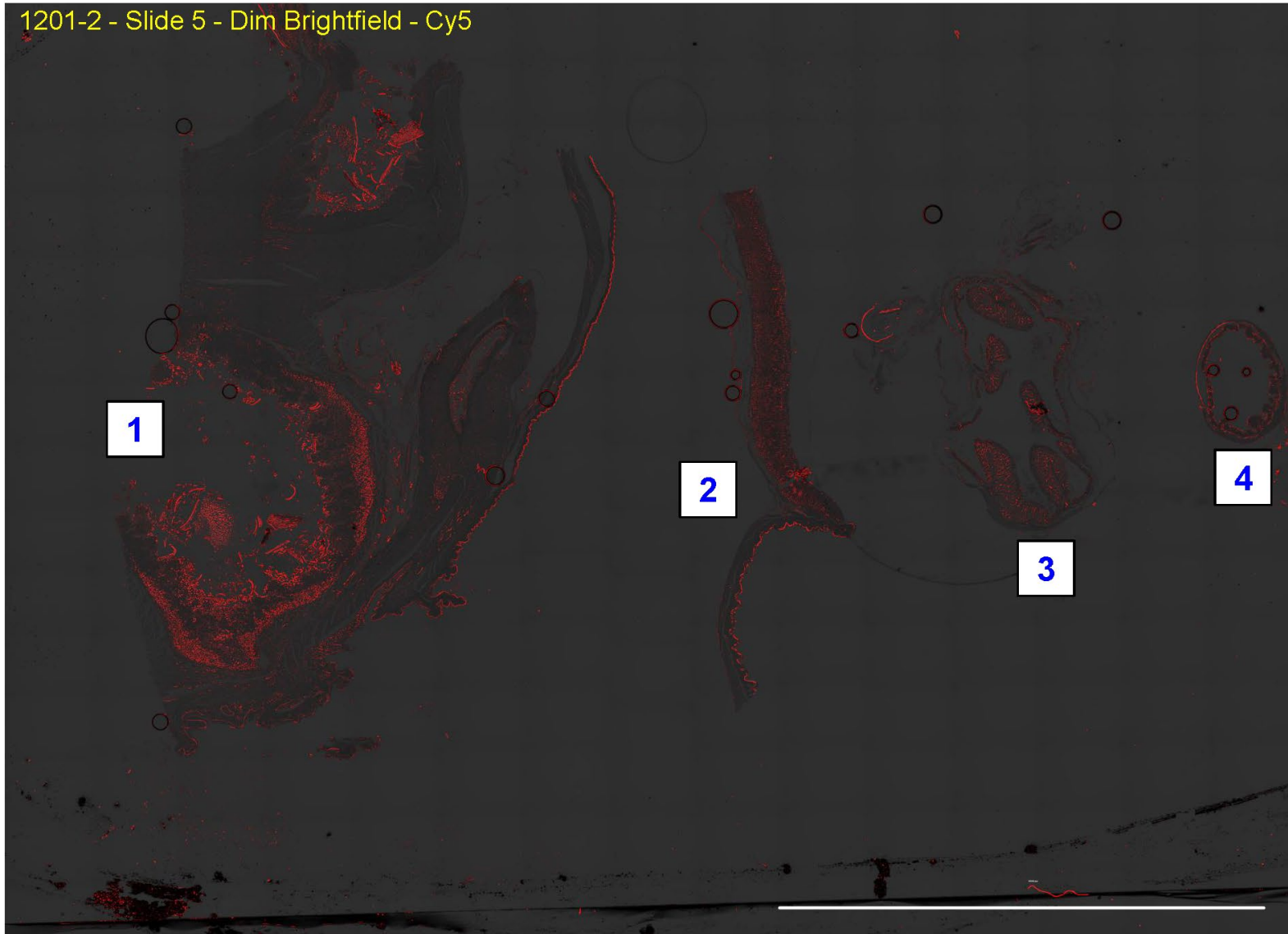
Images taken on the Lionheart are as follows. The animal ID is at the top right corner of the page. The slide number and type of light captured are next. The tissues are individually identified by number. That number and animal ID can be found on this table.

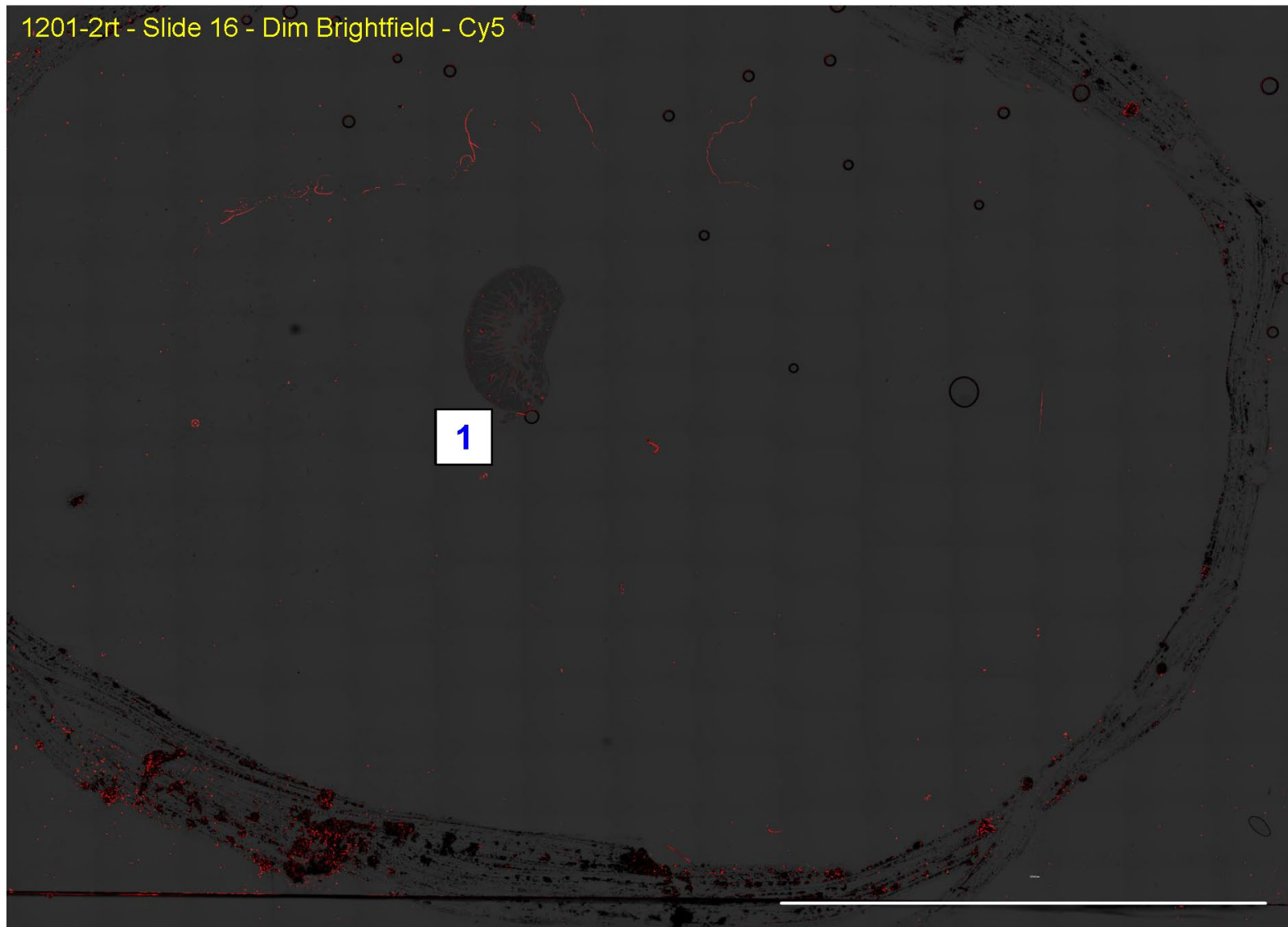
Slide Image Name	Tissue Number on Slide Image				Animal ID	Group Number	Euthanasia Day Post-Infection
	1	2	3	4			
1201-2 slide 5	Stomach	Stomach	Cecum	Duodenum	1201	1	7
1201-2rt slide 16	Duodenum				1201	1	7
1201-3 slide 13	Jejunum	Cecum	Jejunum		1201	1	7
1202-2 slide 15	Stomach	Stomach	Cecum	Duodenum	1202	1	7
1202-3 slide 10	Duodenum [†]	Stomach	Ileum		1202	1	7
1204-2 slide 6	Stomach	Stomach	Cecum	Duodenum or Jejunum	1204	1	7
1204-3 slide 12	Duodenum [†]	Cecum	Ileum		1204	1	7
1205-2 slide 2	Stomach	Stomach	Jejunum	Colon	1205	1	7
1205-3 slide 1	Jejunum	Stomach	Ileum		1205	1	7
1207-2 slide 17	Stomach	Stomach	Cecum	Duodenum or Jejunum	1207	2	14
1207-3 slide 3	Colon	Cecum	Jejunum		1207	2	14
1207-3rt slide 7	Cecum				1207	2	14
1211-2 slide 9	Stomach	Stomach	Duodenum	Jejunum	1211	2	14
1211-3 slide 8	Colon	Stomach	Jejunum		1211	2	14
1211-3rt slide 4	Duodenum				1211	2	14
1216-2 slide 11	Stomach	Stomach	Cecum	Duodenum [†]	1216	3	21
1216-3 slide 14	Duodenum	Cecum	Jejunum		1216	3	21

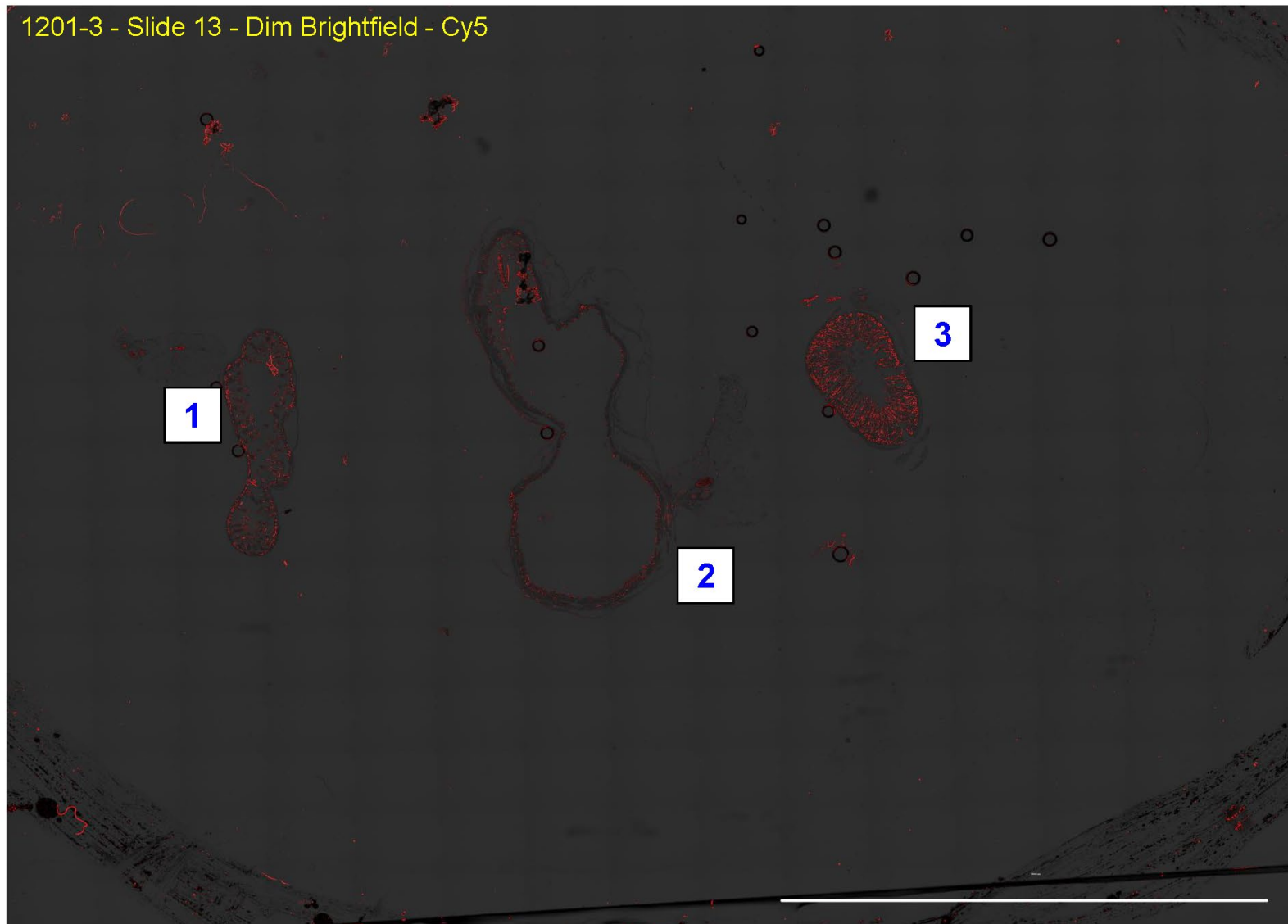
[†]These duodenum sections had pancreas attached.

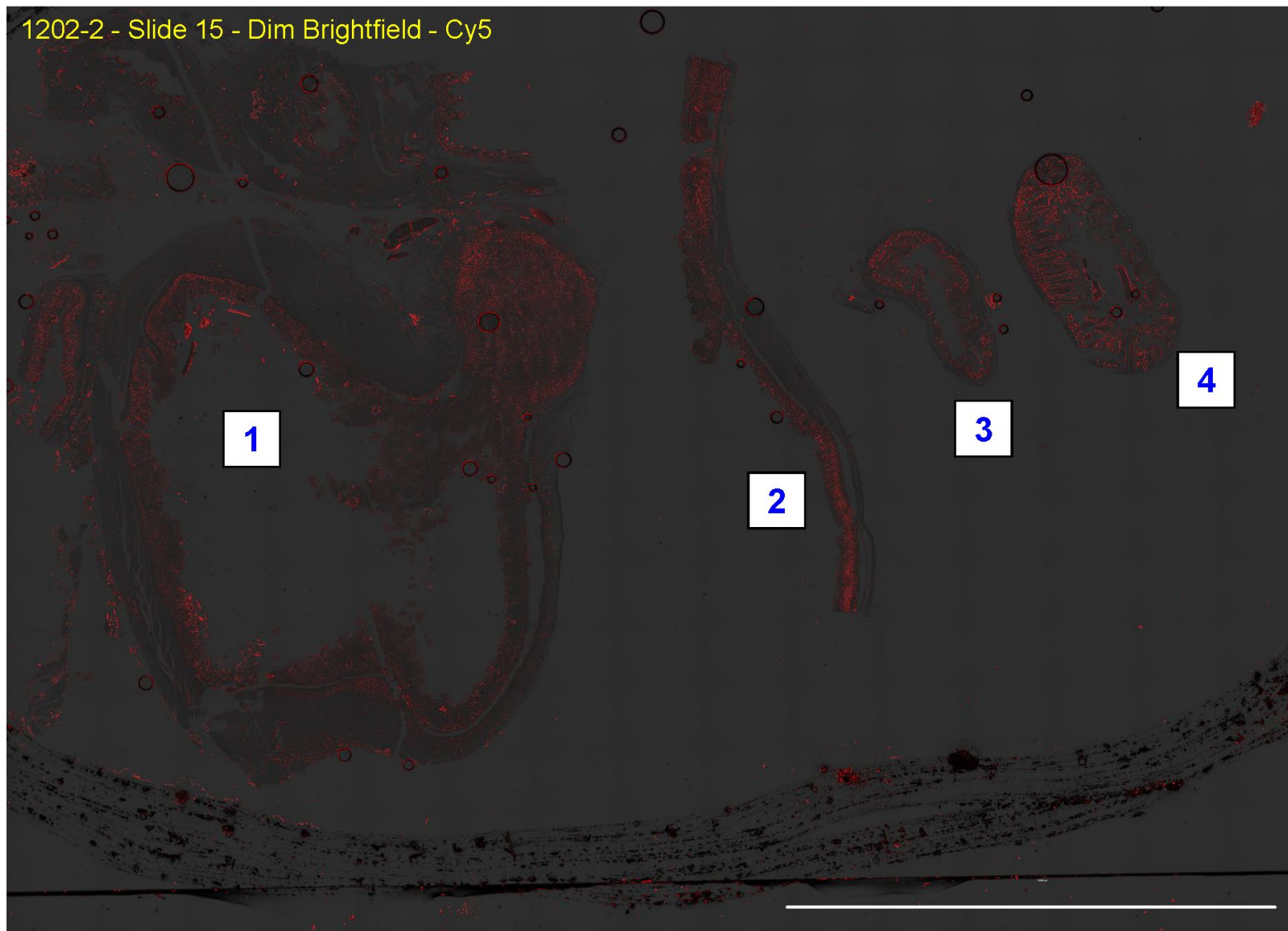
The images in this appendix have a dim bright field channel to show the tissue structure as well as fluorescence channel (Cy5) to show where the SARS-CoV-2 negative strand specific fluorescent probes have bound (Alexa Fluor 647). Following the images is a table of fluorescent parameters. The Sum Cy5/Area column represents the total staining intensity of SARS-CoV-2 negative strand fluorescence in each tissue type, calculated as the sum of the individual Cy5 MFI per object divided by the total tissue plug area ($\text{MFI}/\mu\text{m}^2$). . There are no discernable trends, except for the possible increase in Cy5 over time in the cecum. However, given the few number of animals (only 1 cecum at day 21 and only 2 ceca at Day 14), this observation is not definitive. With as few as one cecum at a time point, statistical analysis was not possible.

1201-2 - Slide 5 - Dim Brightfield - Cy5

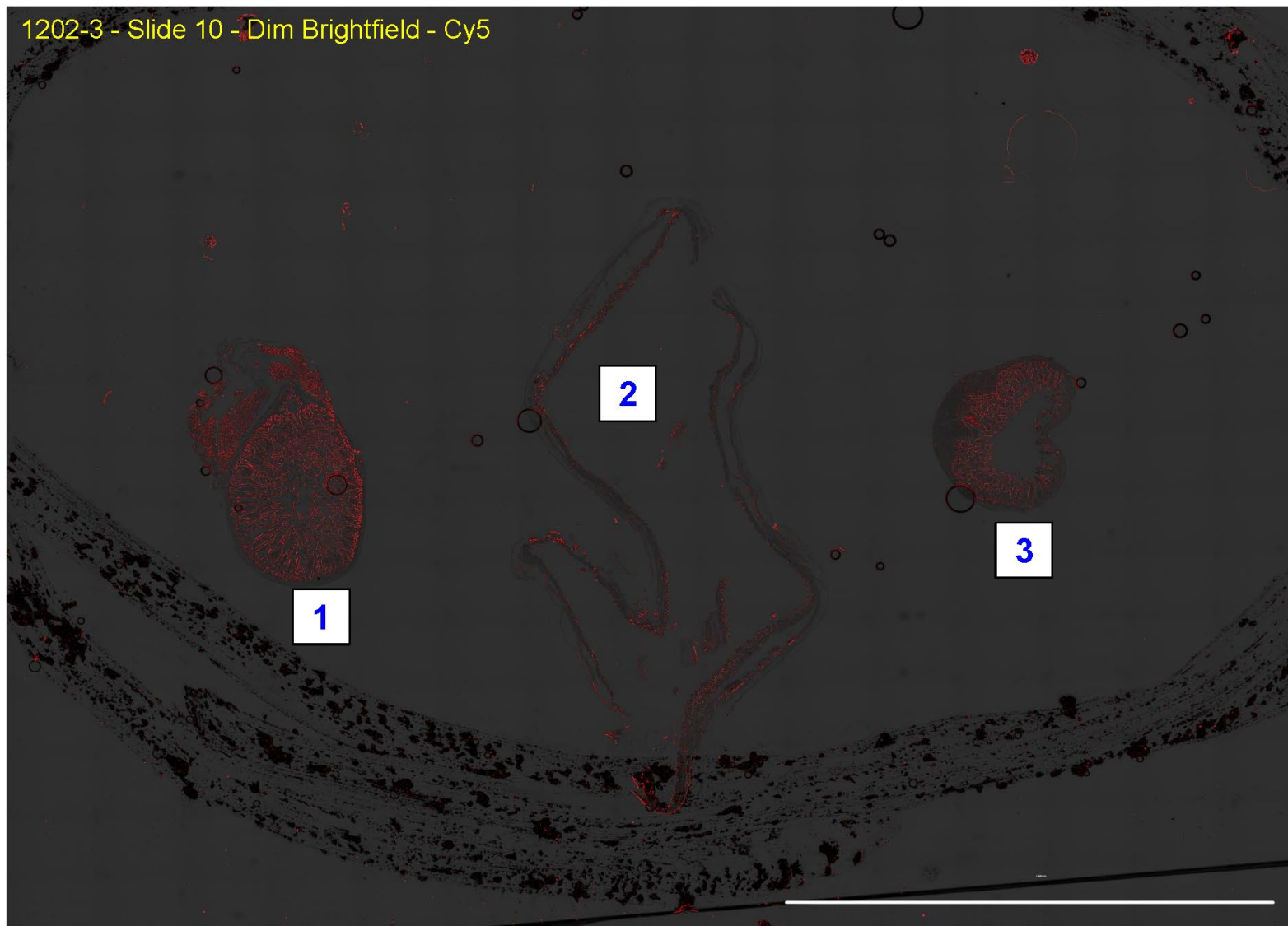




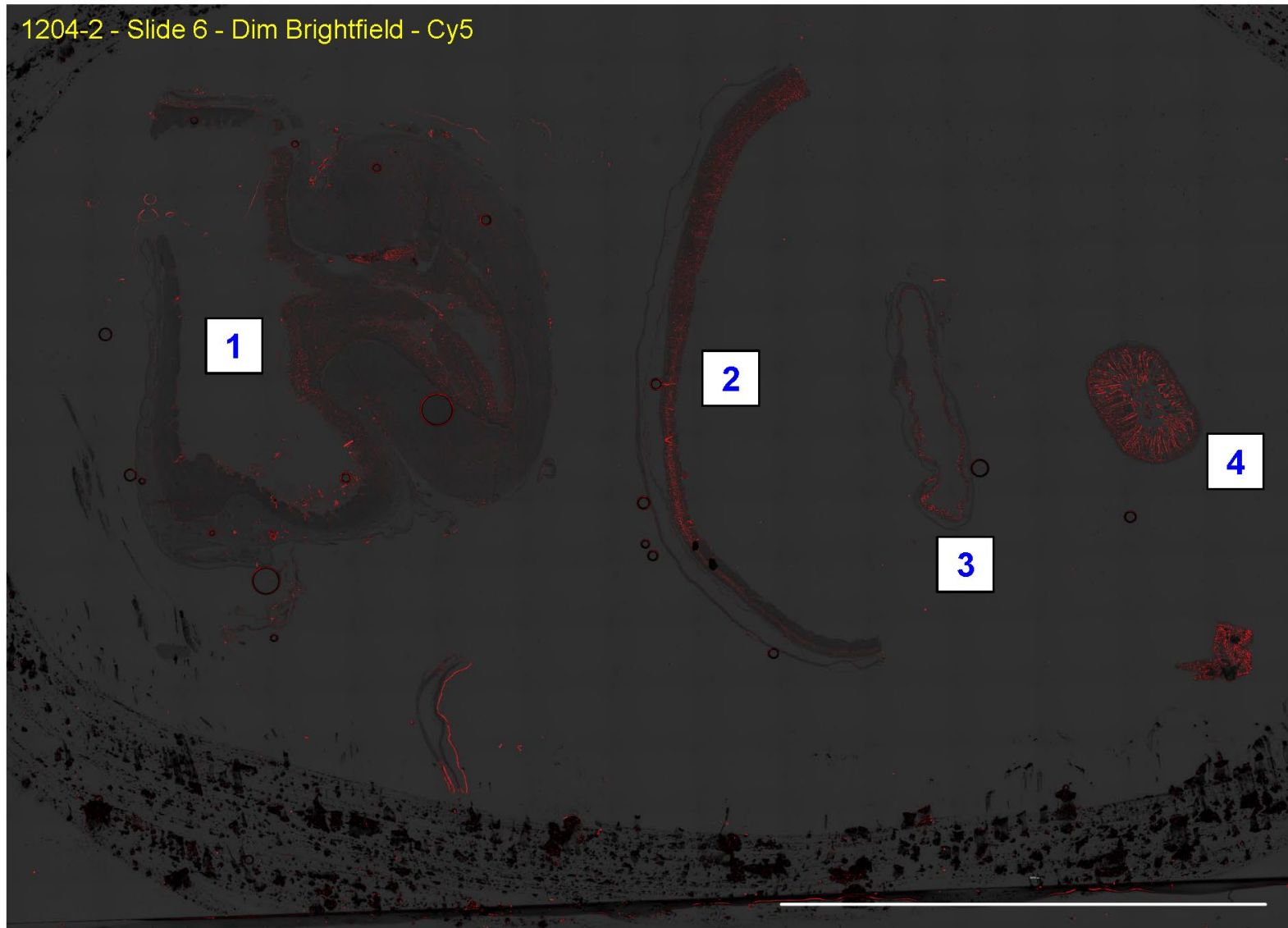




1202-3 - Slide 10 - Dim Brightfield - Cy5

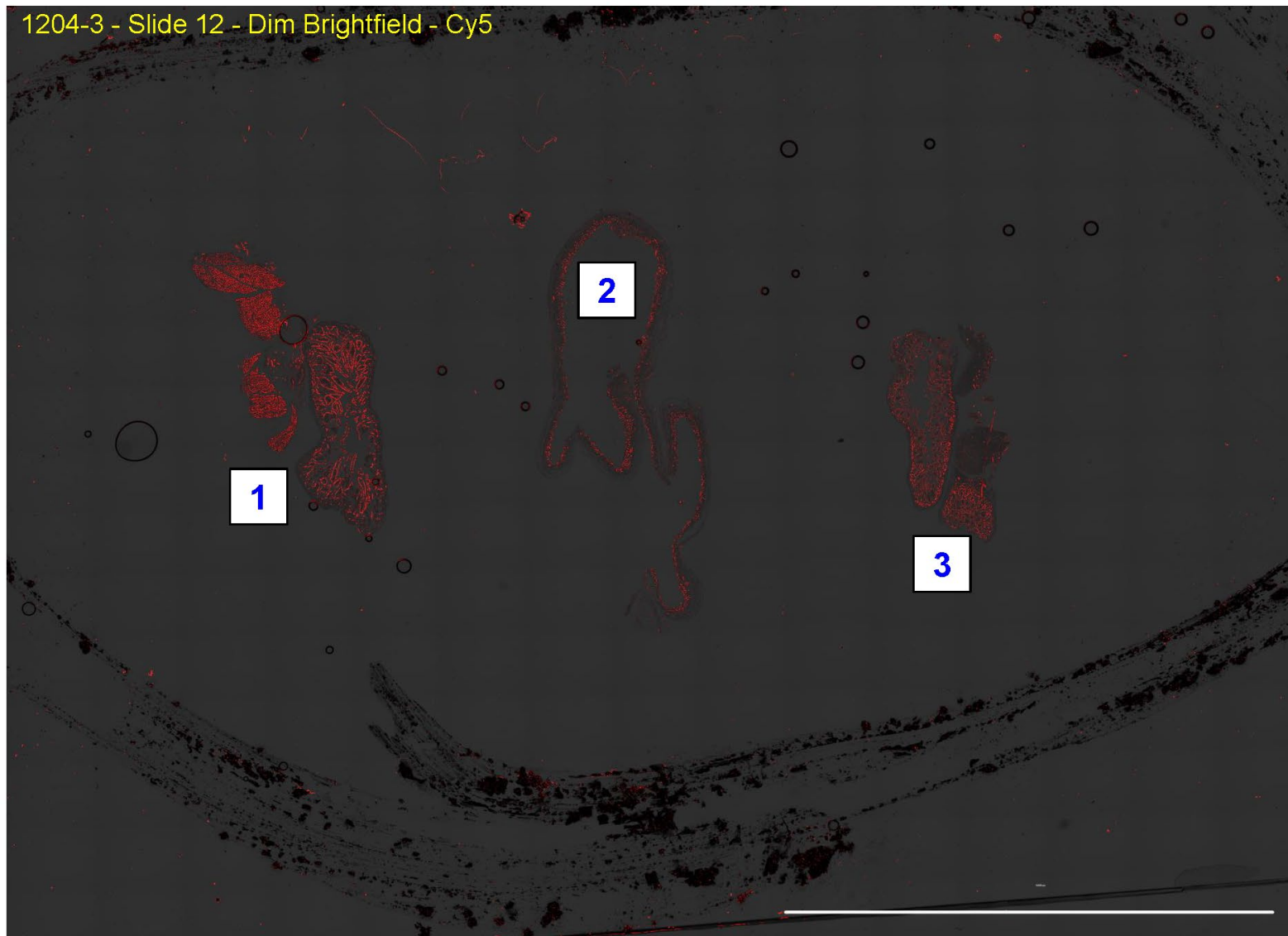


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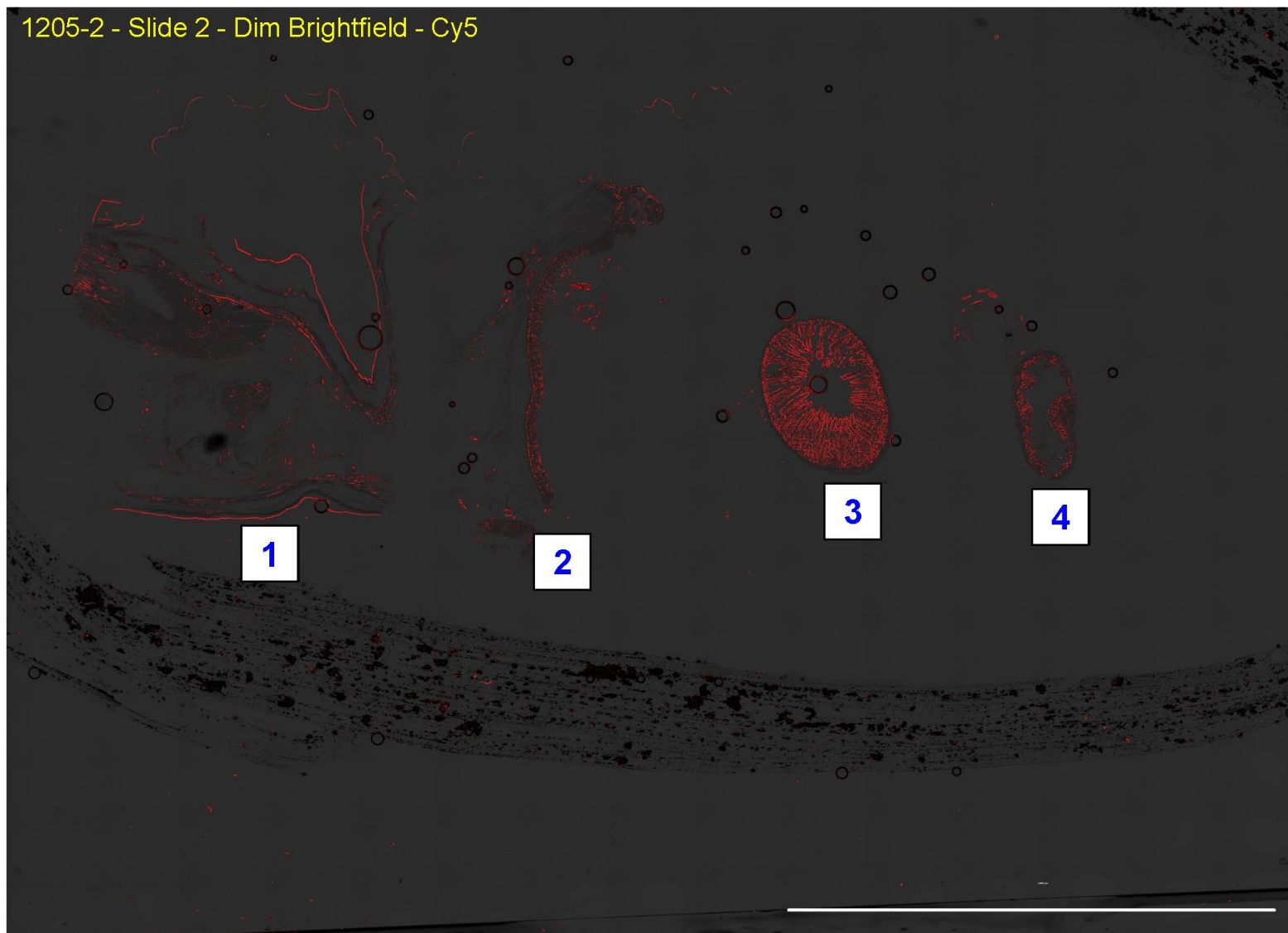


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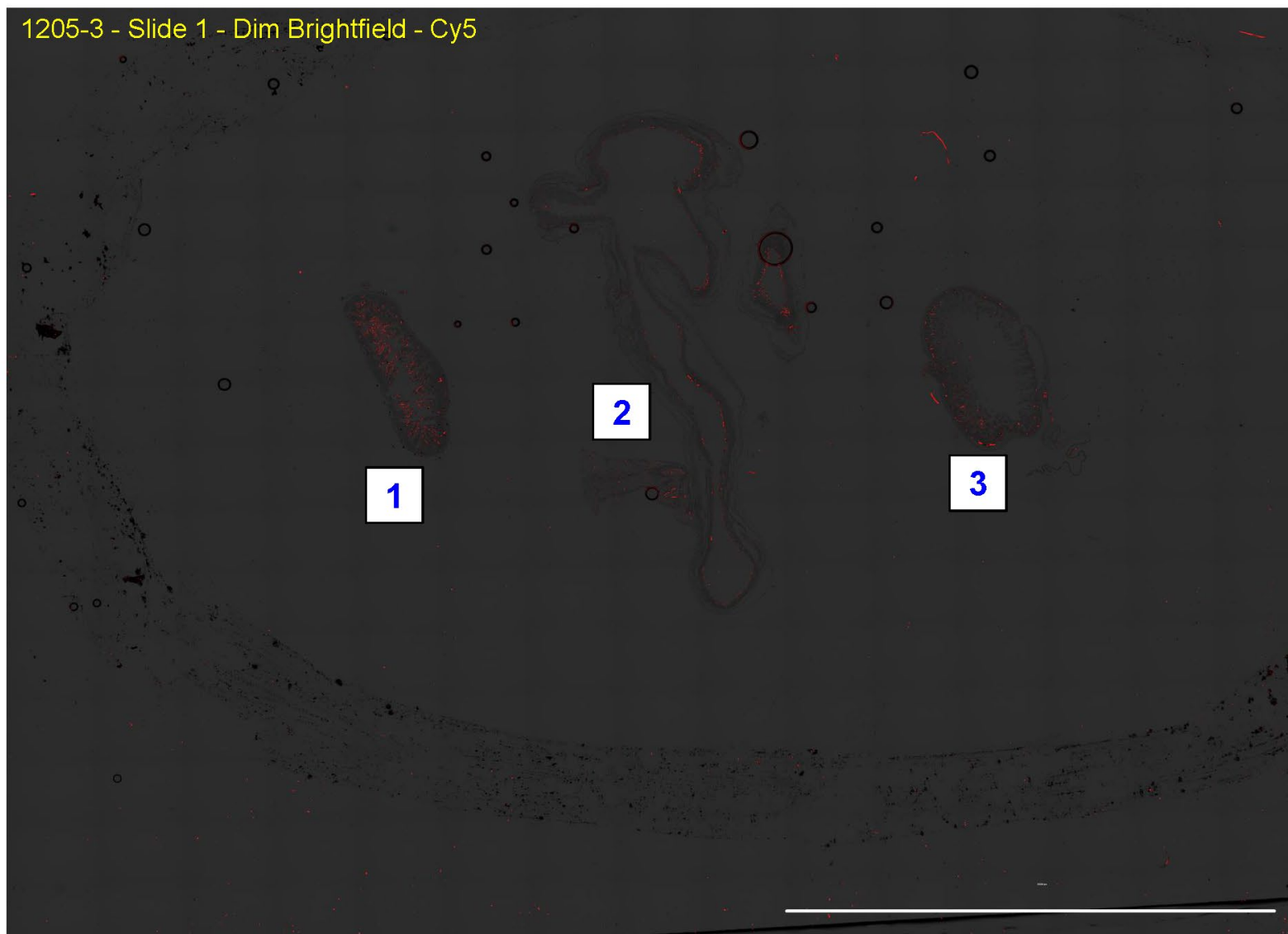
1204-3 - Slide 12 - Dim Brightfield - Cy5



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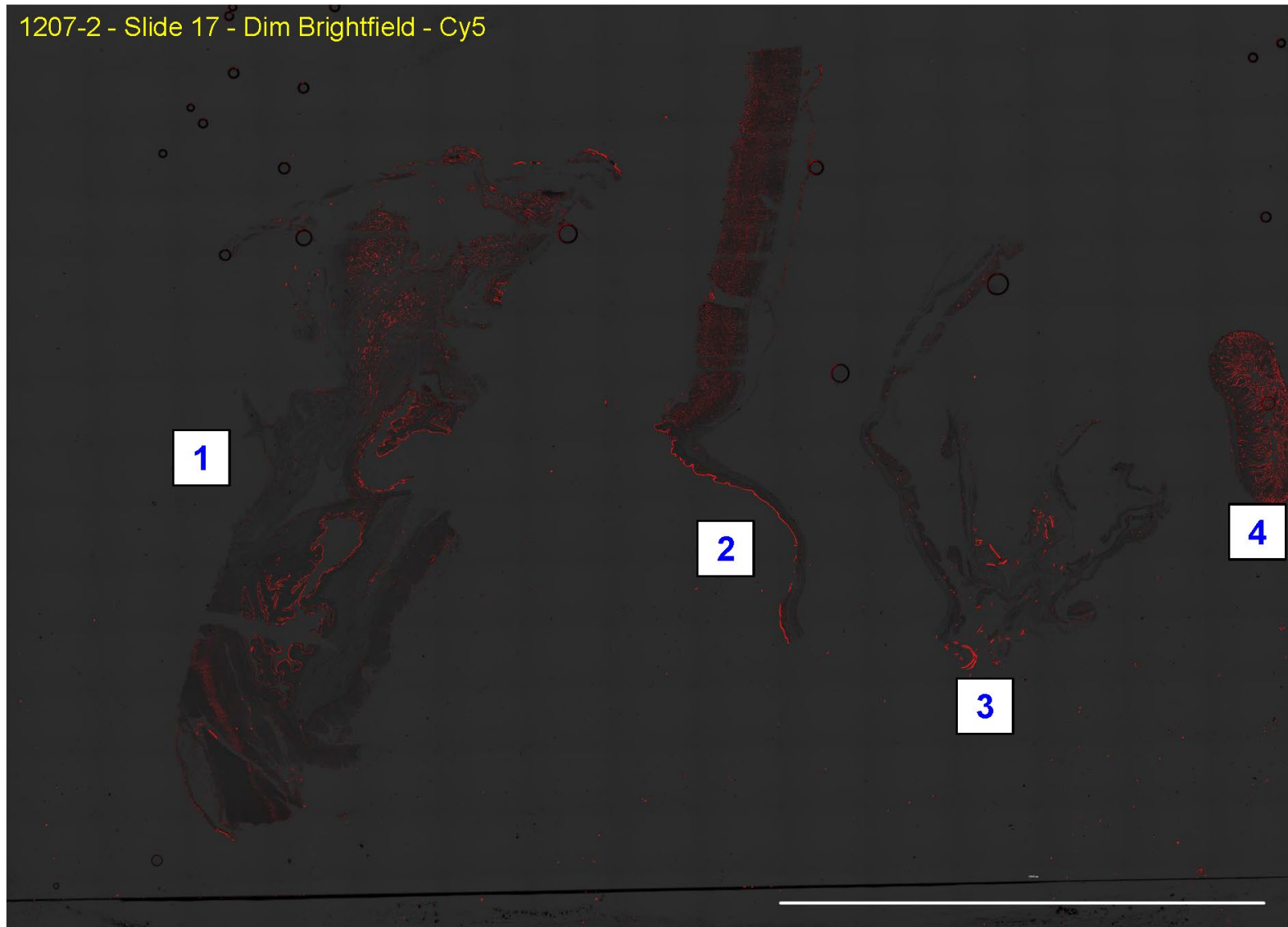


1205-3 - Slide 1 - Dim Brightfield - Cy5

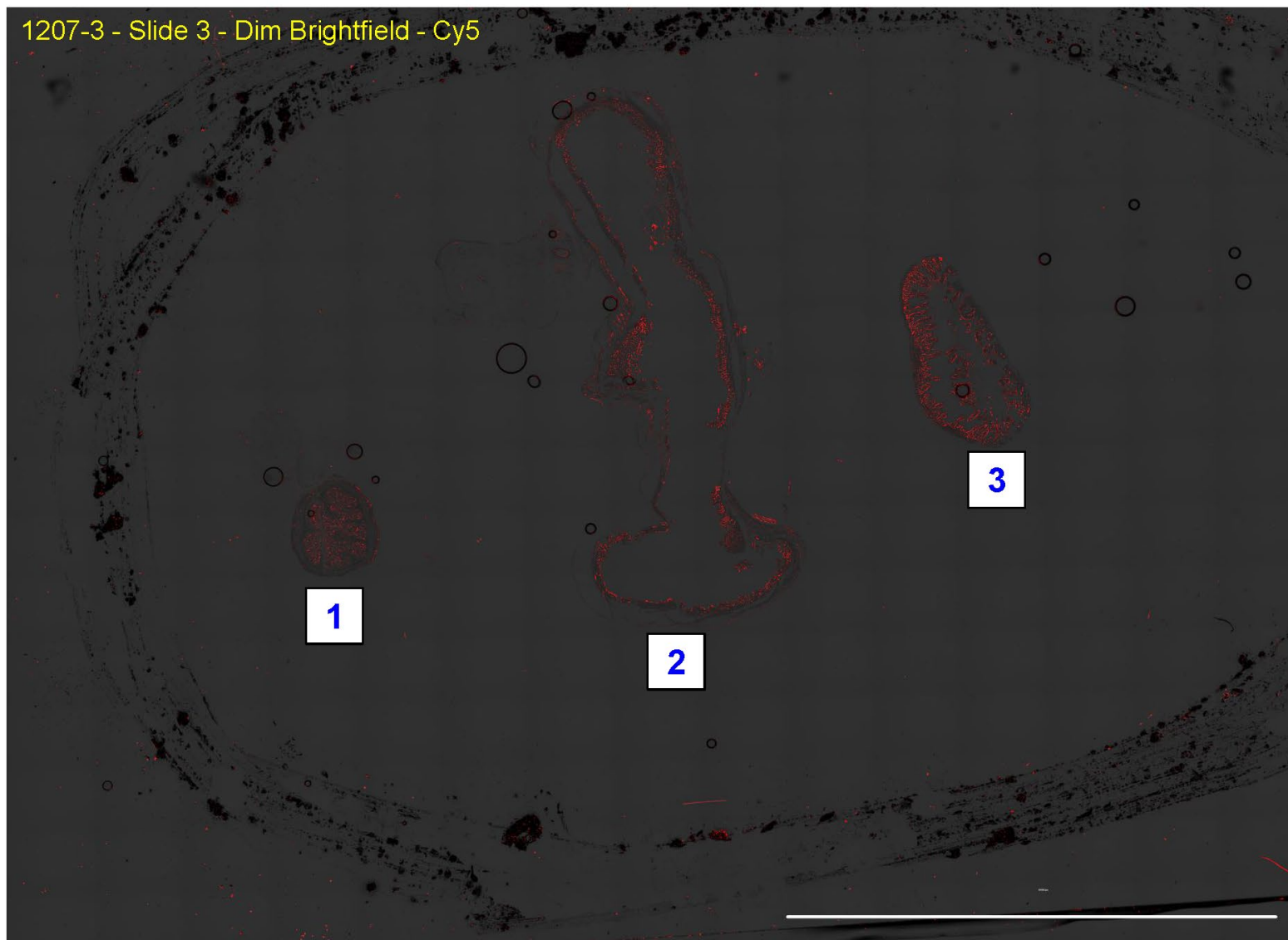


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1207-2 - Slide 17 - Dim Brightfield - Cy5

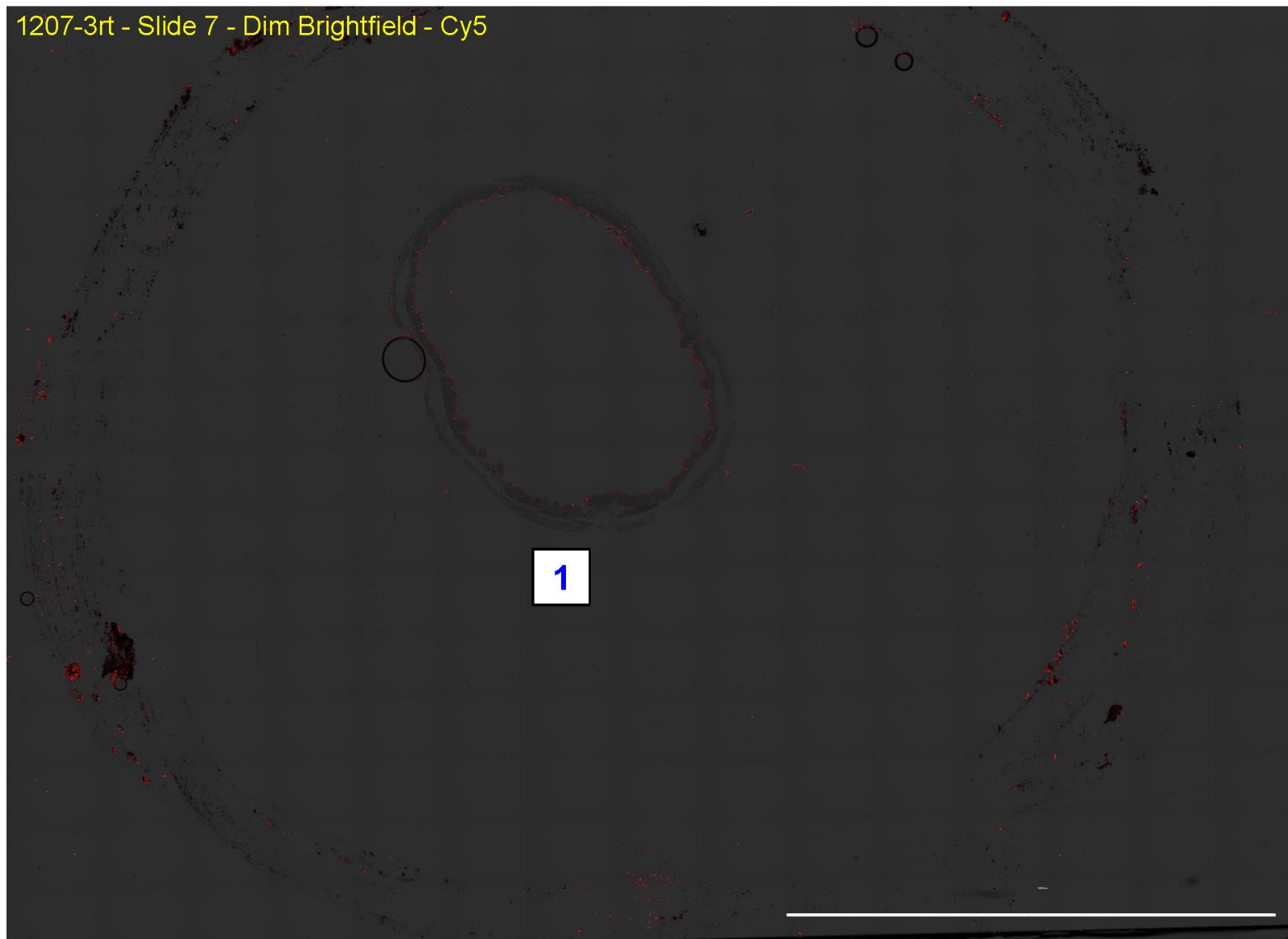


1207-3 - Slide 3 - Dim Brightfield - Cy5

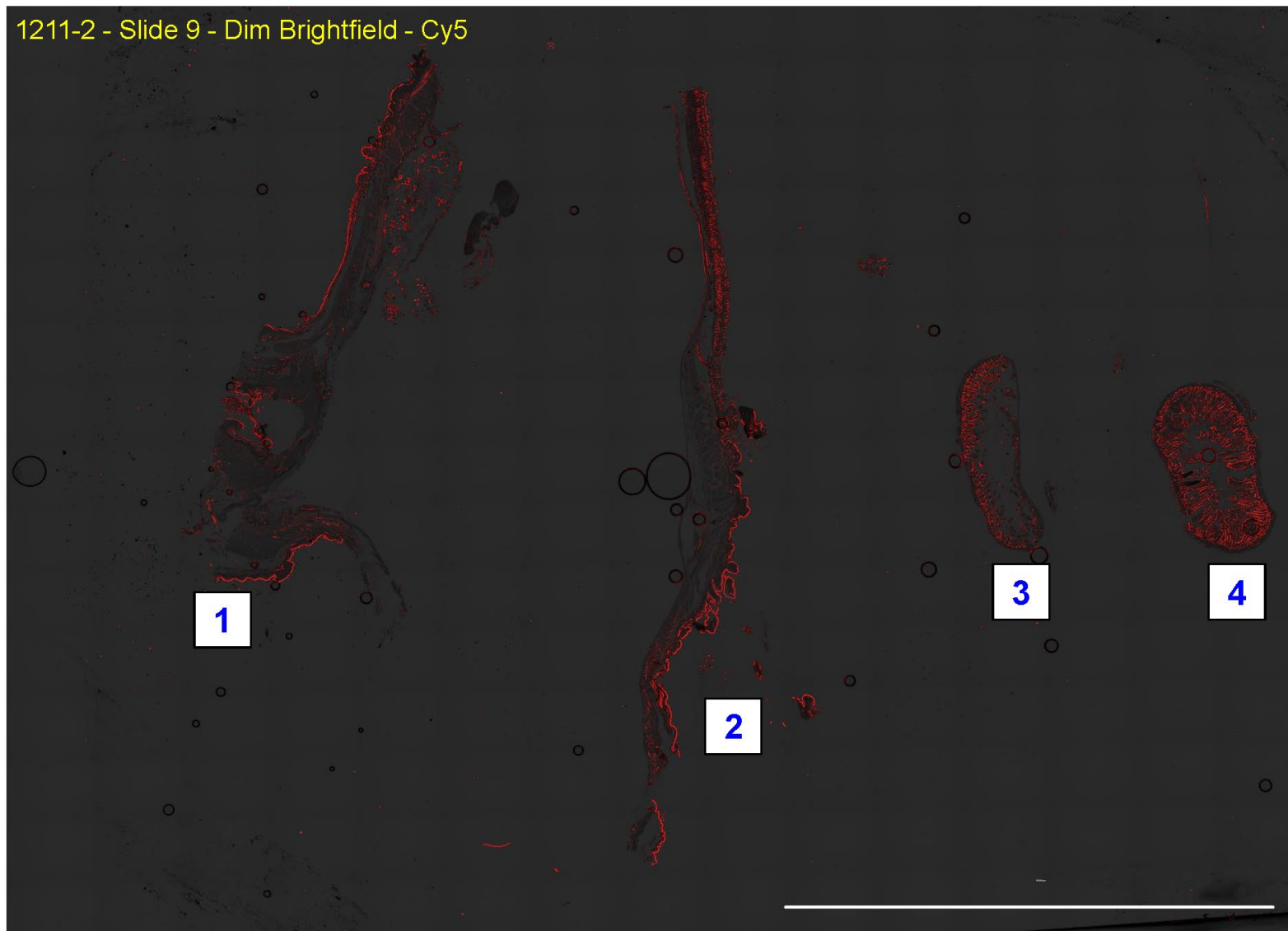


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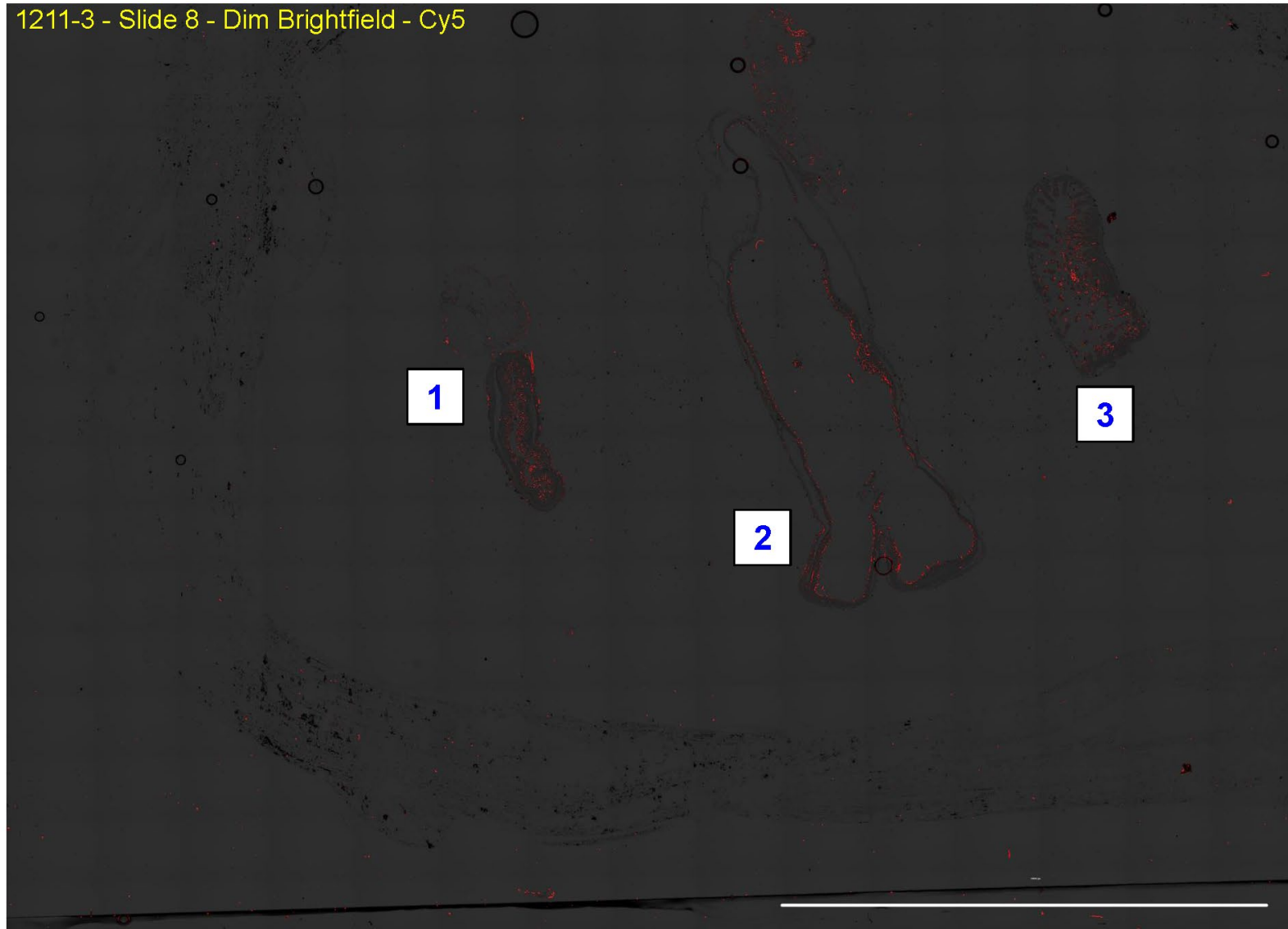
1207-3rt - Slide 7 - Dim Brightfield - Cy5



1211-2 - Slide 9 - Dim Brightfield - Cy5

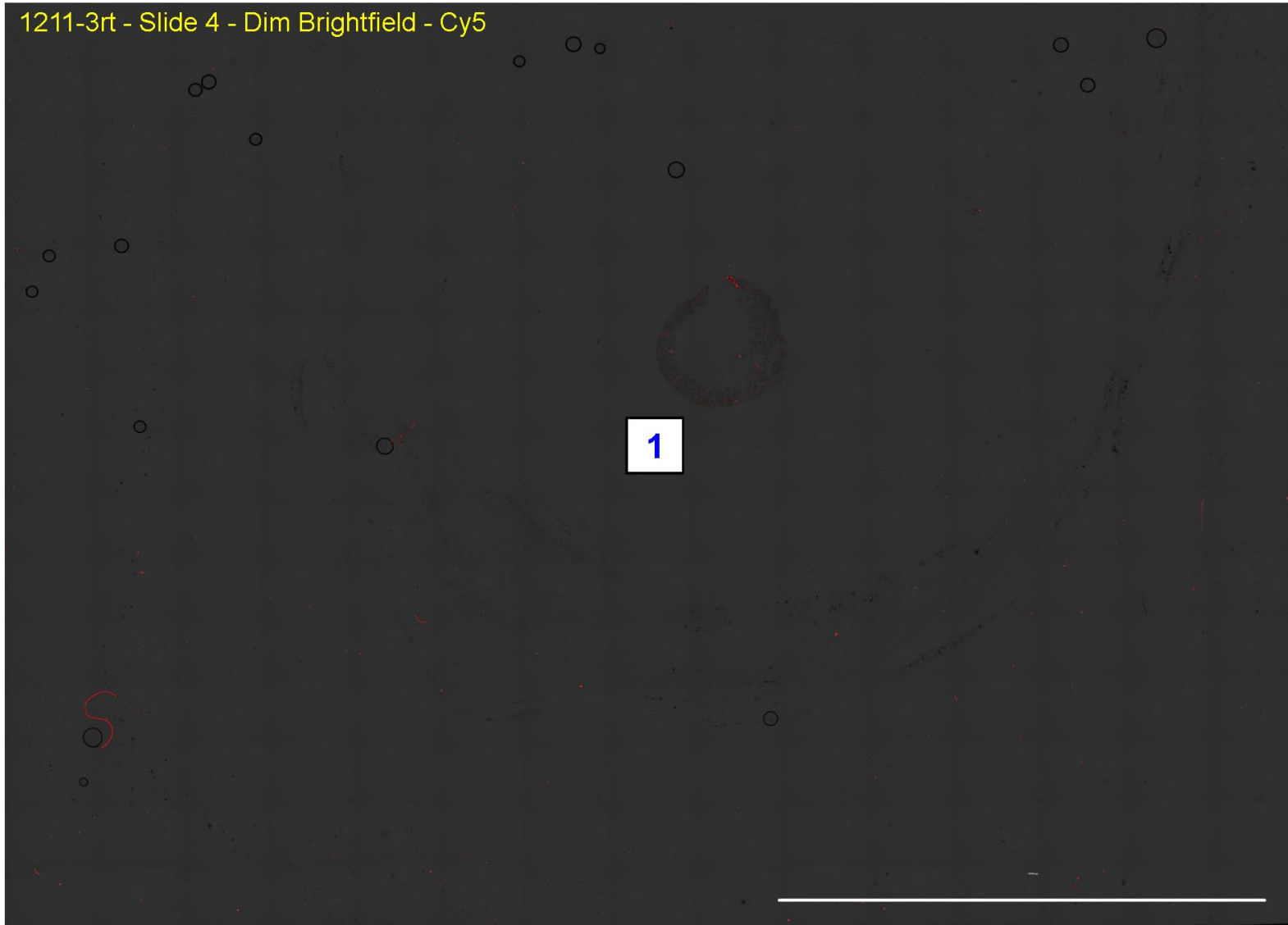


1211-3 - Slide 8 - Dim Brightfield - Cy5



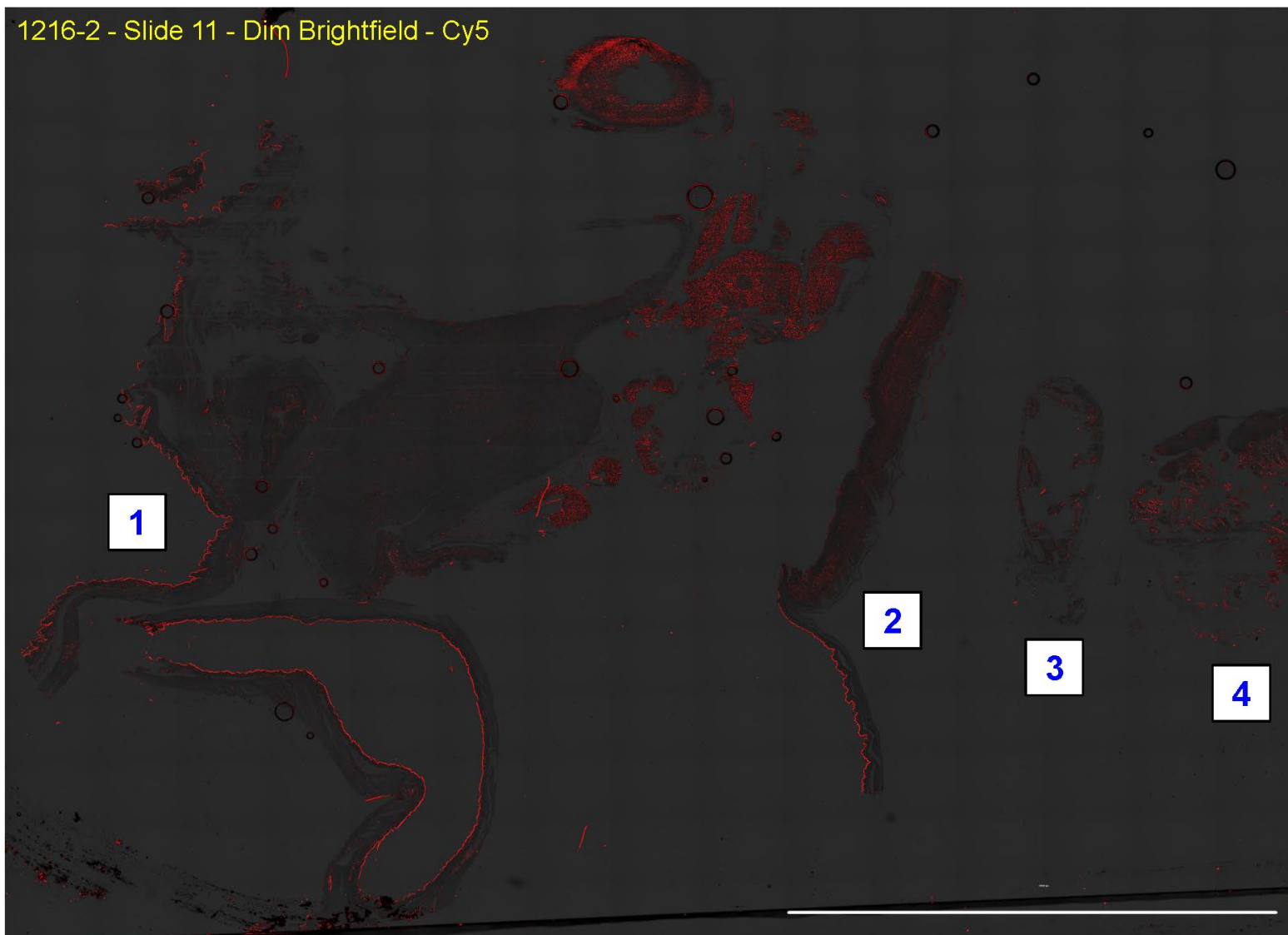
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1211-3rt - Slide 4 - Dim Brightfield - Cy5

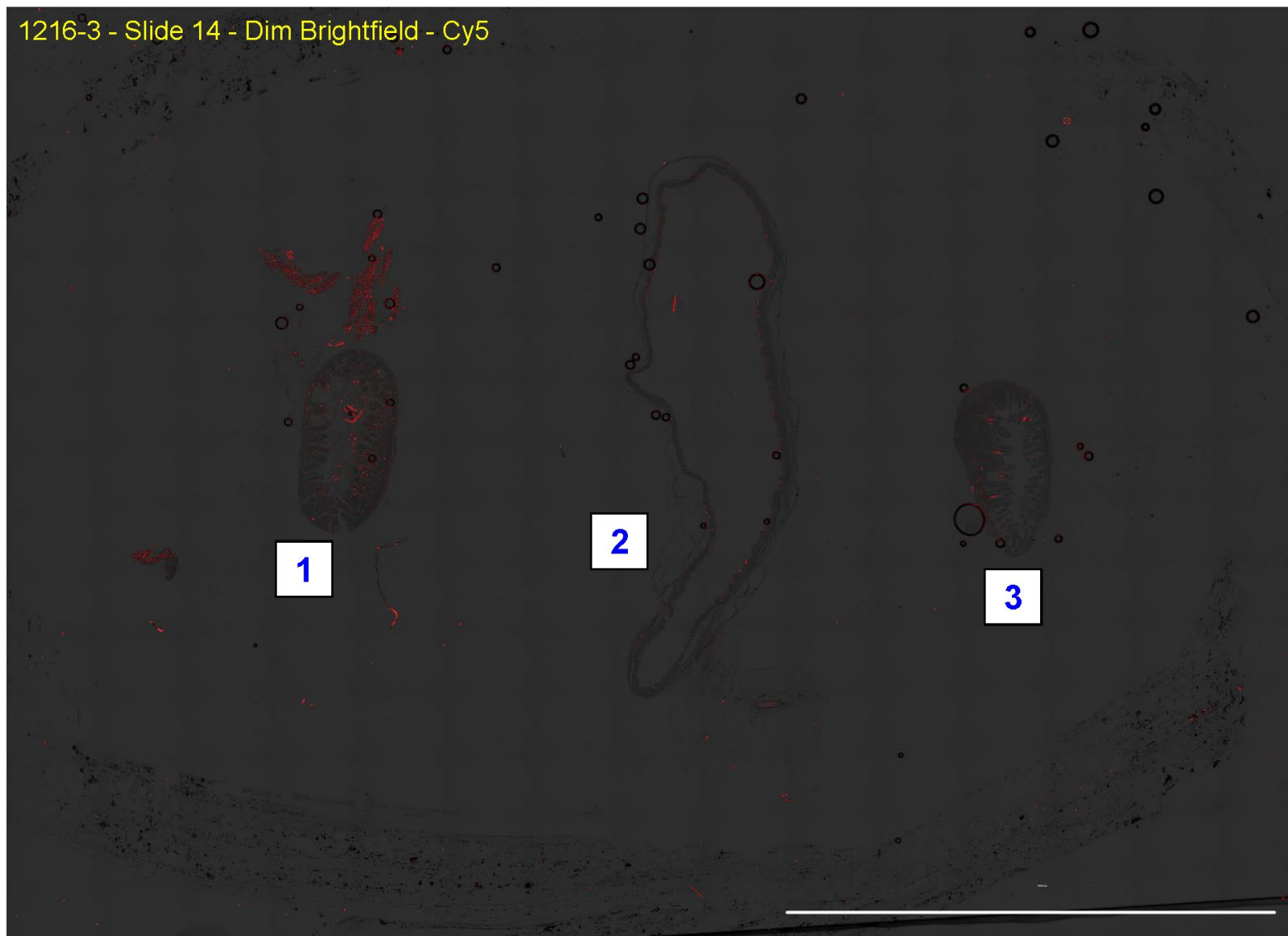


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1216-2 - Slide 11 - Dim Brightfield - Cy5



1216-3 - Slide 14 - Dim Brightfield - Cy5



110890.01.208.06
FISH Results

Slide Name	Sample ID	Tissue Number	Tissue	Animal ID	Group Number	Euthanasia Day Post-Infection	Cy5 Object Count	Mean Object Size (µm)	Mean Object Area (µm ²)	Sum Tissue Area (µm ²)	Mean Cy5 (MFI*)	Sum Cy5 (MFI*)	Sum Cy5 / Area
Slide 5	1201-2	3	Cecum	1201	1	7	10594	8.3	81	858000	7291	77239011	90.0
Slide 13	1201-3	2	Cecum	1201	1	7	8217	8	76.2	626000	7397	60781629	97.1
Slide 15	1202-2	3	Cecum	1202	1	7	5280	9	88.7	468000	8121	42881384	91.6
Slide 6	1204-2	3	Cecum	1204	1	7	1703	8.1	77.5	132000	7240	12329607	93.4
Slide 12	1204-3	2	Cecum	1204	1	7	8310	8.8	89.7	745000	7883	65504852	87.9
Slide 3	1207-3	2	Cecum	1207	2	14	12926	9.2	102	1320000	8025	103733298	78.6
Slide 7	1207-3rt	1	Cecum	1207	2	14	4044	7.3	70.8	286000	7702	31146229	108.9
Slide 17	1207-2	3	Cecum	1207	2	14	5552	6.8	62.6	347000	7232	40153545	115.7
Slide 11	1216-2	3	Cecum	1216	3	21	1747	7.5	65.9	115000	7219	12612146	109.7
Slide 14	1216-3	2	Cecum	1216	3	21	3955	6.1	45.6	180000	7113	28132263	156.3
Slide 2	1205-2	4	Colon	1205	1	7	2440	10.6	132	322000	8531	20816147	64.6
Slide 3	1207-3	1	Colon	1207	2	14	3895	8.3	76.5	298000	7807	30409048	102.0
Slide 8	1211-3	1	Colon	1211	2	14	3383	8.8	89.8	304000	7573	25621213	84.3
Slide 5	1201-2	4	Duodenum	1201	1	7	2329	9.9	125	291000	7185	16735090	57.5
Slide 16	1201-2rt	1	Duodenum	1201	1	7	1360	7.9	76.4	104000	6167	8386529	80.6
Slide 10	1202-3	1	Duodenum	1202	1	7	15169	10.9	132	2010000	8568	129966647	64.7
Slide 15	1202-2	4	Duodenum	1202	1	7	8930	10.2	123	1090000	7752	69228785	63.5
Slide 12	1204-3	1	Duodenum	1204	1	7	10684	13.3	201	2140000	9693	103555524	48.4
Slide 4	1211-3rt	1	Duodenum	1211	2	14	1531	6.4	49.5	75700	6977	10682219	141.1
Slide 9	1211-2	3	Duodenum	1211	2	14	2451	13.3	204	499000	7981	19560642	39.2
Slide 11	1216-2	4	Duodenum	1216	3	21	5477	8.6	90.9	498000	7245	39683544	79.7
Slide 14	1216-3	1	Duodenum	1216	3	21	6544	9.6	107	697000	7670	50195317	72.0
Slide 6	1204-2	4	Duodenum or Jejunum	1204	1	7	4697	11.1	146	684000	7820	36732995	53.7
Slide 17	1207-2	4	Duodenum or Jejunum	1207	2	14	5335	10.7	131	700000	8493	45310600	64.7
Slide 10	1202-3	3	Ileum	1202	1	7	6714	9.2	91.8	616000	8440	56669238	92.0
Slide 12	1204-3	3	Ileum	1204	1	7	7942	10.5	125	989000	8389	66628502	67.4
Slide 1	1205-3	3	Ileum	1205	1	7	2493	6.6	52.5	131000	7188	17920735	136.8

* MFI = Mean Fluorescence Intensity

110890.01.208.06
FISH Results

Slide Name	Sample ID	Tissue Number	Tissue	Animal ID	Group Number	Euthanasia Day Post-Infection	Cy5 Object Count	Mean Object Size (µm)	Mean Object Area (µm ²)	Sum Tissue Area (µm ²)	Mean Cy5 (MFI*)	Sum Cy5 (MFI*)	Sum Cy5 / Area
Slide 13	1201-3	1	Jejunum	1201	1	7	4284	9.9	113	486000	7668	32851235	67.6
Slide 13	1201-3	3	Jejunum	1201	1	7	5248	11.4	149	782000	8463	44413999	56.8
Slide 1	1205-3	1	Jejunum	1205	1	7	2812	8.8	95.2	268000	7513	21126040	78.8
Slide 2	1205-2	3	Jejunum	1205	1	7	7724	15	250	1930000	9616	74270361	38.5
Slide 3	1207-3	3	Jejunum	1207	2	14	6539	10.1	116	760000	8614	56327889	74.1
Slide 8	1211-3	3	Jejunum	1211	2	14	4469	7.7	72.9	326000	7390	33025200	101.3
Slide 9	1211-2	4	Jejunum	1211	2	14	4800	15.8	272	1310000	8219	39449319	30.1
Slide 14	1216-3	3	Jejunum	1216	3	21	1803	6.2	49.3	89000	6975	12576341	141.3
Slide 5	1201-2	1	Stomach	1201	1	7	60983	9.1	104	6320000	7788	474915458	75.1
Slide 5	1201-2	2	Stomach	1201	1	7	13535	8.3	80.1	1080000	7309	98929021	91.6
Slide 10	1202-3	2	Stomach	1202	1	7	11876	8.1	77.6	921000	8008	95103449	103.3
Slide 15	1202-2	1	Stomach	1202	1	7	102130	7.4	62.9	6430000	7390	754741969	117.4
Slide 15	1202-2	2	Stomach	1202	1	7	14057	8.6	82.8	1160000	7468	104971645	90.5
Slide 6	1204-2	1	Stomach	1204	1	7	35206	6.5	47.8	1680000	7375	259636094	154.5
Slide 6	1204-2	2	Stomach	1204	1	7	11449	7.9	69.3	793000	7133	81664429	103.0
Slide 1	1205-3	2	Stomach	1205	1	7	5485	6.5	51.3	281000	6920	37955048	135.1
Slide 2	1205-2	1	Stomach	1205	1	7	14471	9.1	109	1580000	7351	106371827	67.3
Slide 2	1205-2	2	Stomach	1205	1	7	7444	9.2	102	761000	7385	54977871	72.2
Slide 17	1207-2	1	Stomach	1207	2	14	24451	8.6	90.2	2210000	7518	183815232	83.2
Slide 17	1207-2	2	Stomach	1207	2	14	16578	7.9	70.7	1170000	7393	122565756	104.8
Slide 8	1211-3	2	Stomach	1211	2	14	6989	8.3	89.6	626000	7575	52939823	84.6
Slide 9	1211-2	1	Stomach	1211	2	14	9665	11	151	1460000	7438	71890947	49.2
Slide 9	1211-2	2	Stomach	1211	2	14	7749	12.7	195	1510000	7706	59716605	39.5
Slide 11	1216-2	1	Stomach	1216	3	21	45779	8.8	98	4490000	7562	346168661	77.1
Slide 11	1216-2	2	Stomach	1216	3	21	10958	6.8	55.9	612000	6676	73158999	119.5

* MFI = Mean Fluorescence Intensity