Ruhof ATP Study: Phases I and II

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Overview:

Cleaning of flexible endoscopes is still predominately a manual process that if fraught with errors. As reviewed by Ofstead C. et al (ref), human factors play a significant role in the efficacy of the manual cleaning of flexible endoscopes and only 1.4% of all scopes reprocessed have all the steps properly performed. Recently, the development of rapid audit tools has allowed users to assess the efficacy of their manual cleaning such that improperly cleaned flexible endoscope channels can be re-cleaned prior to going to the High Level Disinfection (HLD) step. This process of rapidly auditing the cleaning and repeating it if needed ensures that an improperly cleaned endoscope would not be used on a subsequent patient. This reduces the risk of improperly reprocessed flexible endoscopes being responsible for transmission of infectious diseases. Testing for residual ATP is one method to rapidly audit cleaning compliance of flexible endoscopes. Ruhof has developed a novel sponge sampling method that can be used as part of ATP testing.

The Ruhof Test InstruSponge™ Channel Testing Sponge is designed to verify the cleanliness of interior channels of endoscopes. Sample collection involves passing the moistened sponge directly through the endoscope channel from the Biopsy Port to the distal end, with no additional flushing of the channel. The sponge tip is added directly to the Ruhof ATP test swab device and activated to determine the ATP value, as measured by a hand-held chemiluminometer (ATP Complete).

PHASE I: Identify optimal sample collection method:

Aim: In PHASE I, simulated use testing in a flexible endoscope was done to compare and validate the harvesting method employed by Ruhof (sponge only) versus the “flush only” method previously used by the St. Boniface Research Microbiology lab for harvesting of endoscope channels. Because the “sponge only” method involves no fluid, bioburden and protein values could not be determined from this sample without an elution process, which is not the process recommended by Ruhof.

Phase I was separated out into 3 different parts:
PART 1: Sponge only (no elution) for ATP only (5 replicates).
PART 2: Sponge only (elution in fluid) for ATP, bioburden and protein (5 replicates).
PART 3: Flush only for ATP, bioburden and protein (5 replicates).

For all the above harvesting validation experiments, the Suction/Biopsy (S/B) channel of the endoscope was soiled with Artificial Test Soil (ATS) containing 8 logs each of *Enterococcus faecalis* ATCC 29212 (EF) and *Pseudomonas aeruginosa* ATCC 27853 (PA). After 1 hour dry time, a partial clean (30 mL sterile tap water flush of the Suction/Biopsy BPD channel) was performed followed by harvesting. ATP testing was performed as per the manufacturer’s instructions. Bioburden was performed by serial dilutions and spread plate on Blood Agar (BA) media. Protein was evaluated using the QuantiPro™BCA Microassay method (Sigma).

Specifics of the Phase I Procedure are provided in Appendix I

RESULTS:

Table 1 Comparison of various channel sample collection methods: ATP

	ATP VALUES (RLU): L1 (Bx port to distal)	
	Sample Code:	**RLU DATA**
PART 1		
Sponge Only	RP1-SONE-BPD-1	9999
(No elution)	RP1-SONE-BPD-2	9999
	RP1-SONE-BPD-3	7709
	RP1-SONE-BPD-4	8815
	RP1-SONE-BPD-5	9999
	AVG:	**9304.20**
	STD:	**1028.62**
PART 2		
Sponge Only	RP1-SOE-BPD-1	2050
(Elution)	RP1-SOE-BPD-2	2122
	RP1-SOE-BPD-3	2041
	RP1-SOE-BPD-4	4543
	RP1-SOE-BPD-5	1801
	AVG:	**2511.40**
	STD:	**1142.13**
PART 3		
Flush Only	RP1-FO-BPD-1	4430
	RP1-FO-BPD-2	2259
	RP1-FO-BPD-3	1139
	RP1-FO-BPD-4	3619
	RP1-FO-BPD-5	2805
	AVG:	**2850.40**
	STD:	**1261.60**

Table 2 Comparison of various channel sample collection methods: Protein

PROTEIN VALUES:	SAMPLE:	TYPE OF TEST:	Protein (ug/mL)	Sample Volume (mL)	Protein (ug/cm^2)
PART 1 Sponge Only (No elution)	RP1-SONE-BPD-1	Sponge Only (No Elution)	NO PROTEIN TESTING WAS PERFORMED FOR THIS PART OF THE STUDY		
	RP1-SONE-BPD-2	Sponge Only (No Elution)			
	RP1-SONE-BPD-3	Sponge Only (No Elution)			
	RP1-SONE-BPD-4	Sponge Only (No Elution)			
	RP1-SONE-BPD-5	Sponge Only (No Elution)			
		AVG:			
		STD:			
PART 2 Sponge Only (Elution)	RP1-SOE-BPD-1	Sponge Only (Elution)	53.01	2.0	0.44
	RP1-SOE-BPD-2	Sponge Only (Elution)	47.95	2.0	0.40
	RP1-SOE-BPD-3	Sponge Only (Elution)	58.50	2.0	0.49
	RP1-SOE-BPD-4	Sponge Only (Elution)	83.60	2.0	0.69
	RP1-SOE-BPD-5	Sponge Only (Elution)	80.86	2.0	0.67
		AVG:	**64.78**		**0.54**
		STD:	**16.39**		**0.14**
PART 3 Flush Only	RP1-FO-BPD-1	Flush Only	359.20	20.0	29.83
	RP1-FO-BPD-2	Flush Only	224.12	24.5	22.80
	RP1-FO-BPD-3	Flush Only	238.47	19.5	19.31
	RP1-FO-BPD-4	Flush Only	333.88	25.0	34.66
	RP1-FO-BPD-5	Flush Only	267.79	24.0	26.69
		AVG:	**284.69**		**26.66**
		STD:	**59.292**		**5.979**

Conclusions:

1. Elution of the sponge produced similar ATP levels compared to Flush sample collection. However, the protein levels from the sponge elution method were significantly lower than when Flush sample collection was used.
2. The sponge only channel sample collection method provided the best recovery of ATP.
3. To perform the Phase II and III testing it will be necessary to harvest one set of endoscope channels using the sponge to test for ATP and a second set of endoscope channels using the flush method to test for protein and bioburden levels.

Phase II: Use Simulated-use testing to determine the ATP cut-off for adequate cleaning

Aim:

In Phase II of the simulated use study, we will establish an ATP cutoff that indicates adequate cleaning for flexible GI endoscopes. Endoscope channel samples from fully cleaned endoscopes were compared to those that had been partially cleaned and those that had not been cleaned (positive control). Baseline samples were also be collected (negative control). Because the sponge method of harvesting (without elution) does not produce a fluid sample it was not possible to determine bioburden and protein levels from the sponge collected sample. In order to compare the ATP levels collected by sponge to level of bacteria (cfu/cm^2) and protein (ug/cm^2), one part of this study involved elution of a sponge sample collected from the same endoscope channel soiled in the same manner as used for the ATP testing. The eluted sample was tested for bioburden and protein.

For all parts of the simulated use study, the suction/biopsy (S/B) channel of a flexible GI endoscope was soiled with Artificial Test Soil (ATS) containing 8 logs each of *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853. After 1 hour dry time, a full manual clean (as per manufacturer's instructions) or a partial clean (30 mL sterile tap water flush of the S/B channel) was performed prior to harvesting. For positive controls, there was no cleaning of the channel prior to harvesting. Baseline samples were collected on 4 consecutive mornings from "patient-ready" (unsoiled) scopes. In the first part of this study, only ATP was evaluated. ATP testing was performed as per manufacturer's instructions. In the second part of this study, bioburden and protein was determined from a flush sample. Bioburden was determined by performing serial dilutions and spread plate on CHROMagar™ Orientation media. Protein was evaluated using the QuantiPro™ BCA Microassay method (Sigma).

Specifics of the Phase I Procedure are provided in Appendix II.

RESULTS:

To determine what the ATP cut-off for adequate cleaning should be set at, a flexible endoscope was soiled with ATS and then the RLUs, protein and bioburden levels were determined for fully cleaned, partially cleaned and uncleaned endoscope channels (5 replicates). The target was to correlate what RLU cutoff corresponded to a protein level of < 6.4ug/cm^2 and a bioburden level of < 4 Log/cm^2.

The results are presented in Table 3 and Table 4.

Table 3: Simulated-use Testing: ATP and Protein residuals in the Biopsy port to distal end of a flexible endoscope channel

		ATP* RLUs	**Protein*: µg/cm^2**
NO CLEAN Positive Control	AVG:	9747.00	335.42
	STD:	436.48	65.55
PARTIAL CLEAN	AVG:	6601.00	3.99
	STD:	1684.02	3.16
FULL CLEAN	AVG:	29.00	0.23
	STD:	36.39	0.07
		ATP:	
NEGATIVE CONTROL (Baseline)		14.00	0.04
		21.66	0.07

* Average of five replicates

Table 4 Simulated-use Testing: Bioburden* residuals in the Biopsy port to distal end of a flexible endoscope channel

		E. faecalis ATCC 29212	*P. aeruginosa* ATCC 27853	*Enterococcus faecalis* ATCC 29212		*Pseudomonas aeruginosa* ATCC 27853	
		Inoculum Count: Log_{10} cfu/mL	**Inoculum Count:** Log_{10} cfu/mL	**Recoverable Bioburden****: Log_{10} cfu/mL	**Log_{10} cfu/cm^2**	**Recoverable Bioburden*:** Log_{10} cfu/mL	**Log_{10} cfu/cm^2**
NO CLEAN Positive Control	AVG:	8.473	8.479	7.843	6.782	7.730	6.669
	STD:	0.145	0.157	0.000	0.212	0.200	0.192
PARTIAL CLEAN	AVG:	8.776	8.470	6.315	5.254	5.537	4.476
	STD:	0.199	0.280	0.133	0.142	0.245	0.253
FULL CLEAN	AVG:	8.657	8.802	1.816	0.787	2.638	1.608
	STD:	0.225	0.337	0.359	0.364	0.271	0.269
Any Organism: **NEGATIVE CONTROL (Baseline)**	AVG:			0.256	0.025		
	STD:			0.513	0.090		
OVERALL:	AVG:	8.635	8.584				
	STD:	0.190	0.258				

*Results represent the average of 5 replicates.

**The Fuji colonoscope Model EC-530HL from the Biopsy port to the distal end has a surface area of 240.80 cm^2.

Conclusions:

1. For the partially cleaned endoscope channel samples, the protein level was 3.99 ug/cm^2 which is lower than expected. The average protein level for an improperly reprocessed endoscope channel would be expected to be > 6.4 ug/cm^2. However, there was one of the replicates that had 7.59ug/cm^2 (i.e. above the 6.4 ug/cm^2 benchmark). As such it should be possible to correlate the ATP benchmark by ensuring that the RLUs for the partially cleaned endoscope are at least 2 standard deviations higher than the RLUs for the fully cleaned endoscope channel. For the partially cleaned endoscope channel there were an average of 6601 RLUs – indicating that there will be very high RLUs when a channel is only partially cleaned.
2. The fully cleaned endoscope channel had an average of 0.23 ug/cm^2 for protein and < 2 Log_{10}/cm^2 of either test organism. The fully cleaned endoscope channel had an average of 29 RLUs with a range of 7 to 71 RLUs.
3. The baseline data documents that after full reprocessing, the ATP, protein and bioburden levels returned to expected background values and there was no accumulation over the period of testing.
4. Based on the above findings the recommended cut-off for the Ruhof ATP test for a sponge collected sample from the biopsy port to distal end of the endoscope channel would be 100 RLUs. This represents two standard deviations above the average RLU for a totally cleaned endoscope channel and ensures the maximum RLU from the simulated-use testing is within this cutoff.

Appendix I

Phase I Procedure:

Supplies:

Endoscope used:	Flexible GI Endoscope provided by Ruhof:
	Fujinon Colonoscope - Model EC-530HL (2 scopes)
	- **#1**: S/N 1C517A129; **#2**: S/N 2C517A210
Channels evaluated:	Suction/Biopsy channel; Biopsy port to distal end only (BPD).
Bacteria used:	*Enterococcus faecalis* ATCC 29212
	Pseudomonas aeruginosa ATCC 27853
Media used:	Blood Agar media (BA)
Sponge used:	Ruhof Test Instrusponge™ Channel Testing Sponge
ATP device used:	Ruhof ATP Test Swab (ATP Complete)
Chemiluminometer:	Ruhof ATP Complete hand-held chemiluminometer
Protein Assay used:	QuantiPro™ BCA Microassay (Sigma)

Pre-experiment:

- Leak test the scope; pass thru the AER, alcohol flush, air dry and hang in closet until needed.
- Prepare and QC ATS (~ 50 mL required to soil the S/B channel).
- Subculture EF and PA from frozen stock onto Blood agar (BA) plates and subculture twice. (Note that both EF and PA should be 24 hours old on BA on the day of the experiment).
- Perform positive control ATP testing in the chemiluminometer.
- Label all media, collection tubes and eppendorf tubes for protein.
- Aliquot 450 µL sterile Phosphate Buffered Saline (sPBS; 0.01M; pH 7.5) for bioburden counts.
- Sterilize all connectors required for soiling/ harvesting of S/B channel; sterilize all eppendorfs, channel brushes (if required) etc..

Daily protocol: **(NOTE: Pre-warm the ATP devices at room temperature overnight)**

- Run a Diagnostic cycle in the AER (pre-warm water)
- Turn centrifuge ON to cool (4-8 ^{o}C) for spinning bacteria
- Charge the chemiluminometer so it is ready for ATP testing
- Subculture required number of BA (EF and PA) required for the next day
- Prepare soil/bug suspension as follows: (all flushed soil can be pooled with the original soil and re-used throughout the day).

  For ~ 100 mL (adjust volume accordingly):

  - Scrape 5 BA of EF and ~ 1 ½ BA of PA into 60 mL of sRO water.
  - Divide the suspension between 2 x 50mL conical tubes, balance and spin in a refrigerated centrifuge (3500 rpm, 17-18 minutes).
  - Carefully decant off the supernatant and discard.
  - Resuspend the pellets in a TOTAL of ~ 100-110 mL of ATS. Pool and perform an inoculum count.
- Inoculum count on soil-bug suspension: Serially 1:10 dilute (50 µL into 450 µL sRO water) from direct thru 10^{-6}. Plate 100 µL x 3 of the 10^{-3} thru 10^{-6} dilutions onto BA media. Spread inoculum and incubate 24 hours at 35-37 ^{o}C. Count all EF and PA colonies and record.
- Soil the endoscope: Lay the scope on a towel, raise control head and attach to stand. Plug the control head S/B port. Attach Biospy port connector (luer-lok). Draw up ~ 50 mL of well-mixed soil-bug suspension into a 60cc luer-lok syringe, and with distal end slightly lowered into a

sterile urine container, slowly push thru and out the endoscope (Biopsy port to distal end; BPD). Follow this with 50 mL of air. Pool the flushed soil with the original container and store in fridge.

- Lay scope flat and dry 1 hour.
  PERFORM EITHER PART 1 or PART 2 or PART 3 as per the following instructions:

PART 1: Sponge only (no elution) for ATP only:

- After 1 hour, attach a new sterile connector to the Biopsy port (luer-lok). Perform a partial clean on the S/B port (BPD) by pushing 30 mL of sterile tap water (with control head raised and distal end lowered into a sterile container) slowly thru the Biopsy port and out the distal end. Discard.
- Harvest by moistening a Ruhof Test Instrusponge™ Channel Testing Sponge in sterile RO water and passing thru the Biopsy port and out the distal end. Cut the end of the sponge off with sterile snippers, into an EMPTY sterile 12 x 75mm snap-cap tube.
- Pre-warm the Ruhof ATP Test Swab if required. Use sterile hemostats to transfer the sponge into the Test Swab. Activate as per manufacturer's instructions. Place in the Chemiluminometer and record the RLU (raw number). This is REPLICATE #1.
- RESOIL the endoscope with the pooled soil in the fridge (with no manual cleaning in between replicates) and dry 1 hour. Repeat testing of the S/B channel (BPD) exactly as for REPLICATE #1.

  NOTE: A total of **5 replicates** total will be performed. If all replicates cannot be performed the same day, discard soil from fridge at the end of the day and perform a full manual clean of the endoscope. Place in the AER and disinfect. Pass 70% Ethanol thru all channels and air dry. Hang in the closet overnight until the following morning. Prepare fresh soil-bug mix daily and perform an incoculum count.

PART 2: Sponge only (elution in fluid) for ATP, Bioburden and Protein

- After 1 hour, attach a new sterile connector to the Biopsy port (luer-lok). Perform a partial clean on the S/B port (BPD) by pushing 30 mL of sterile tap water (with control head raised and distal end lowered) slowly thru the Biopsy port and out the distal end. Discard.
- Harvest by moistening a Ruhof Test Instrusponge™ Channel Testing Sponge in sterile RO water and passing thru the Biopsy port and out the distal end. Cut the end of the sponge off with sterile snippers, into a PRE-DEFINED VOLUME OF STERILE RO WATER (eg. 2 mL).
- Vortex the sample well and remove 1 mL to a sterile 12 x 75mm snap-cap tube. Perform RECOVERABLE BIOBURDEN and PROTEIN on this aliquot as follows:
- ***Bioburden*****:** Under the BSC, vortex well and serially 1:10 dilute from direct thru 10^{-5} (50 µL into 450 µL sPBS) and plate 100 µL x 3 of the 10^{-1} thru 10^{-5} dilutions onto BA Media. Spread inoculum and incubate plates for 24 hours at 35-37 ^{o}C. Count all EF and PA colonies and record. This is REPLICATE #1.
- ***Protein*****:** Transfer remaining sample to a sterile eppendorf tube. Freeze aliquot at -20 ^{o}C. Within 2 weeks, perform a Protein assay using the Sigma QuantiPro™ BCA Microassay (Sigma) as per manufacturer's instructions. This is REPLICATE #1.
- ***ATP***: With the remaining 1.0 mL of sample (containing the sponge), use a sterile hemostat to remove the sponge from the water. Hold briefly over the tube so excess water drips off. Transfer this swab into the Ruhof ATP Test Swab (pre-warm the ATP Test Swab overnight at RT).

Activate as per manufacturer's instructions. Place in the Chemiluminometer and record the RLU (raw number). This is REPLICATE #1.

- RESOIL the endoscope with the pooled soil in the fridge (with no manual cleaning in between runs) and dry 1 hour. Repeat testing of the S/B channel (BPD) exactly as for REPLICATE #1.

NOTE: A total of **5 replicates** total will be performed. If all replicates cannot be performed the same day, discard soil from fridge at the end of the day and perform a full manual clean of the endoscope. Place in the AER and disinfect. Pass 70% Ethanol thru all channels and air dry. Hang in the closet overnight until the following morning. Prepare fresh soil-bug mix daily and perform an incoculum count.

Calculations for PART 2: In a separate experiment, we can determine what volume of water on average is absorbed by the Instrusponge sponge by determining the WEIGHT (1 mL of water weighs 1 gram). Based on the volume absorbed by the sponge (eg. 0.2 mL) we can calculate what the cfu/mL (bioburden) and µg/mL (protein) represent in terms of cfu/sponge and µg/sponge. These values can be related back to the raw ATP/sponge value.

PART 3: Flush only (no sponge)

- After 1 hour, attach a new sterile connector to the Biopsy port. Perform a partial clean on the S/B port (BPD) by pushing 30 mL of sterile tap water (with control head raised and distal end lowered into a sterile container) slowly thru the Biopsy port and out the distal end. Discard.
- Harvest by flushing 20 mL of sRO water thru the Biopsy port and out the distal end into a sterile 50 mL conical collection container. DO NOT SONICATE.
- Under the BSC, record the total sample volume. Aliquot 5 mL into a sterile 17 x 100 mm snap-cap tube for ATP testing (cover in foil). Vortex sample well and perform one of the following:
- ***Bioburden***: Vortex sample well. Serially 1:10 dilute from direct thru 10^{-5} (50 µL into 450 µL sPBS) and plate 100 µL x 3 of the 10^{-1} thru 10^{-5} dilutions onto BA Media.
  Spread inoculum and incubate plates for 24 hours at 35-37 ^{o}C. Count all EF and PA colonies and record. This is REPLICATE #1.
- ***Protein*:** Aliquot a portion of sample into a sterile eppendorf tube for chemistry. Freeze aliquot at -20 ^{o}C. Within 2 weeks, perform a Protein assay using the Sigma Qu antiPro™ BCA Microassay (Sigma) as per manufacturer's instructions. This is REPLICATE #1.
- ***ATP*:** Use the swab portion of the Ruhof ATP Test Swab (pre-warmed if required) to dip into the ATP aliquot. Activate the device as per manufacturer's instructions. Place in the Chemiluminometer and record the RLU (raw number). This is REPLICATE #1.
- RESOIL the endoscope with the pooled soil in the fridge (with no manual cleaning in between runs) and dry 1 hour. Repeat testing of the S/B channel (BPD) exactly as for REPLICATE #1.

NOTE: A total of **5 replicates** total will be performed. If all replicates cannot be performed the same day, discard soil from fridge at the end of the day and perform a full manual clean of the endoscope. Place in the AER and disinfect. Pass 70% Ethanol thru all channels and air dry. Hang in the closet overnight until the following morning. Prepare fresh soil-bug mix daily and perform an incoculum count.

Compare the average ATP values (5 replicates) from the 3 harvesting methods above. Determine which harvesting method recovers the most ATP from the endoscope sample.

Bioburden and Protein values can only be compared between the sponge (elution method) and the flush.

Summary Table: Phase I

- **1 endoscope**
- **1 channel (suction biopsy channel; biopsy port to distal end)**
- **5 replicates for each part of the study (3 parts)**

Experiment:	**ATP**	**Bioburden**	**Chemistry**
Part 1 (sponge only with no elution)	**√**	**-**	**-**
Part 2 (sponge only with elution)	**√**	**√**	**√**
Part 3 (flush only)	**√**	**√**	**√**
Total samples:	**15**	**10**	**10**

Sample Code List:

PART 1: RP1-SONE-BPD-x

PART 2: RP1-SOE-BPD-x

PART 3: RP1-FO-BPD-x

- RP1 = **R**uhof **P**hase **1**
- SONE = **S**ponge **O**nly (**N**o **E**lution)
- SOE = **S**ponge **O**nly (**E**lution)
- FO = **F**lush **O**nly
- BPD = **B**iopsy **P**ort to **D**istal End
- “x” = replicate # (5 replicates total will be performed)

Supply List:

PART 1: Ruhof Test Instrusponge™ Channel Testing Sponge = 5
Ruhof ATP Test Swab = 5
Chemiluminometer
Blood Agar (BA) for inoculum counts = 24 (assuming 2 days of testing)
Blood agar (BA) for subcultures of EF and PA = 20

PART 2: Ruhof Test Instrusponge™ Channel Testing Sponge = 5
Ruhof ATP Test Swab = 5
Chemiluminometer
Blood Agar (BA) = 15 x 5 = 75 (bioburden) + 24 (inoculum counts)
Blood agar (BA) for subculture of EF and PA = 20
QuantiPro™ BCA Microassay kit

PART 3: Ruhof ATP Test Swab = 5
Chemiluminometer
CHROMagar™ Orientation media = 15 x 5 = 75 (bioburden) + 24 (inoculum counts)
Blood agar (BA) for subculture of EF and PA = 20
QuantiPro™ BCA Microassay kit

TOTAL (3 parts):
Ruhof Test Instrusponge™ Channel Testing Sponge = 10
Ruhof ATP Test Swab = 15
Blood Agar (BA) = 282 minimum

Other supplies: Connectors, syringes, PPE, mechanical pipettors, pipette tips, disposable loops, AER including Biological and Chemical Indicators, 70% Ethanol, sonicator, vortex, incubator, tubes etc…

APPENDIX II

Phase II Procedure:

Supplies:

Endoscope used:	Flexible GI Endoscope provided by Ruhof (Fujinon Colonoscope, Model EC-530HL) – 2 scopes (**#1**: S/N 1C517A129; **#2**: S/N 2C517A210)
Channels evaluated:	Suction/Biopsy channel; Biopsy port to distal end (BPD)
Bacteria used:	*Enterococcus faecalis* ATCC 29212 (**EF**) *Pseudomonas aeruginosa* ATCC 27853 (**PA**)
Media used:	Blood agar (**BA**)
Sponge used:	Ruhof Test Instrusponge™ Channel Testing Sponge
ATP device used:	Ruhof ATP Test Swab (ATP Complete)
Chemiluminometer:	Ruhof ATP Complete hand-held chemiluminometer*
Protein Assay used:	QuantiPro™ BCA Microassay (Sigma)

* In Phase I, the chemiluminometers used (**#1**: S/N 035795; **#2**: S/N 035787) both had software loaded onto them that displayed a "max out" value of 9999. For Phase II, we will be using a new chemiluminometer with a higher readable range. This is especially important for the "partial clean" phase where ATP values may hover just around this 9999 RLU range.

Pre-experiment:

- Leak test the 2 scopes; pass thru the AER, alcohol flush, air dry and hang in closet until needed.
- Prepare and QC ATS (~ 110 mL required to soil the 2 scopes in parallel).
- Subculture the EF and PA from frozen stock onto Blood agar (BA) plates and subculture twice. (Note that both EF and PA should be 24 hours old on BA on the day of the experiment).
- Perform positive and negative control ATP testing in the chemiluminometer.
- Label all media, collection tubes and eppendorf tubes for protein.
- Aliquot 450 µL sterile Phosphate Buffered Saline (sPBS; 0.01M; pH 7.5) for bioburden counts.
- Sterilize all connectors used to soil/harvest/clean the endoscope.

Evening prior to study:

- Warm up the required number of Ruhof ATP Complete test swabs (overnight at room temperature)

Daily protocol:

- Run a Diagnostic cycle in the AER (pre-warm water)
- Turn centrifuge ON to cool (4-8 ^{o}C) for spinning bacteria
- Charge the chemiluminometer so it is ready for ATP testing
- Subculture the required number of BA (EF and PA) required for the next day
- Prepare soil/bug suspension as follows: (all flushed soil can be pooled with the original soil and re-used throughout the day).

  For ~ 110 mL (adjust volume accordingly): (this is sufficient for 2 scopes in parallel)

  - Scrape 5 BA of EF and ~ 1 ½ BA of PA into 60 mL of sRO water.

- Divide the suspension between 2 x 50 mL conical tubes, balance and spin in a refrigerated centrifuge (3500 rpm, 17-18 minutes).
- Carefully decant off the supernatant and discard.
- Resuspend the pellets in a TOTAL of ~100-110 mL of ATS. Pool and perform inoculum count.

- Incoculum count on soil-bug suspension: Serially 1:10 dilute (50 µL into 450 µL sPBS) from direct thru 10^{-6}. Plate 100 µL x 3 of the 10^{-3} thru 10^{-6} dilutions onto Blood agar media. Spread inoculum and incubate 24 hours at 35-37 ^{o}C. Count all EF and PA colonies and record.
- Soil 2 endoscopes in parallel: Lay the scopes side by side on a towel, and soil 1 scope at a time. Raise control head and attach to stand. Plug the control head S/B port with a small yellow plug. Attach Biopsy port luer-loc connector. Draw up ~ 40-50 mL of well-mixed soil-bug suspension into a 60cc luer-lok syringe (do not draw air), and with distal end slightly lowered into a sterile urine container, slowly push thru and out the endoscope (Biopsy port to distal end; BPD). Follow this with ~50 mL of air. Repeat exactly for the second scope. Pool all the flushed soil with the original container and store in fridge. This same suspension can be used throughout the day.

- Lay the 2 scopes flat and dry 1 hour.

PERFORM ONE OF THE FOLLOWING:

Scope #1*: Sponge only (no elution) for ATP

*** alternate daily which scope is used as Scope #1 and Scope #2**

- After 1 hour dry time, perform one of the following (in triplicate):
POS CONTROL: Harvest the unwashed scope by moistening a Ruhof Test Instrusponge™ Channel Testing Sponge in sRO water and passing thru the Biopsy port and out the distal end. Cut the end of the sponge off into an EMPTY sterile 12 x 75mm snap-cap tube.
Pre-warm the Ruhof ATP Test Swab if required. Use sterile hemostats to transfer the sponge into the Test Swab. Activate as per manufacturer's instructions. Place in the Chemiluminometer and record the RLU (raw number). This is REPLICATE #1.
If time permits, RESOIL the endoscope with the pooled soil in the fridge and dry 1 hour (do not manually clean the endoscopes between replicates but do use suction to remove all fluid from the scope prior to re-soiling). Repeat the testing of the S/B channel (BPD) exactly as for REPLICATE #1 (3 replicates total).
PARTIAL CLEAN: Attach a Biopsy port connector and perform a **50 mL sterile tap water flush** from the Biopsy port to the distal end (control head plugged and raised; distal end lowered into a sterile container). Discard. Harvest the partially washed scope as per the POS CONTROL scope above.
Perform ATP testing as per POS CONTROL. This is REPLICATE #1.
If time permits, RESOIL the endoscope with the pooled soil in the fridge and dry 1 hour (do not manually clean the endoscope between replicates but do use suction to remove all fluid from the scope prior to re-soiling). Repeat the testing of the S/B channel (BPD) as per REPLICATE #1 (3 replicates total).
POST CLEAN: Perform a full manual clean on the endoscope as per APPENDIX 1 (use syringes). Harvest the fully washed scope as per the POS CONTROL scope above. Perform ATP testing as per POS CONTROL. This is REPLICATE #1.

If time permits, RESOIL the endoscope with the pooled soil in the fridge and dry 1 hour (do not manually clean the endoscope between replicates but do use suction to remove all fluids from the scope prior to re-soiling). Repeat the testing of the S/B channel (BPD) as per REPLICATE #1 (3 replicates total).
BASELINE: On 3 separate mornings, harvest the BPD channel using the **sponge only (no elution)** method from a "patient-ready" endoscope directly from the scope cabinet. These are the BASELINE samples (3 replicates total).
Perform ATP testing as per POS CONTROL above.

- **END OF DAY PROCESSING OF ENDOSCOPE**: After all samples are collected for the day, the endoscope should be fully manually cleaned (as per Appendix 1) if not already done so, placed in an AER for a full disinfection, then have an alcohol flush and air dry. Hang in the scope cabinet until required.

Scope #2*: Flush Only for Bioburden and Protein (no ATP)

*** alternate daily which scope is used as Scope #1 and Scope #2**

- After 1 hour dry time, perform one of the following (in triplicate):
POS CONTROL: Harvest the unwashed scope by flushing 20 mL of sRO water + 20 mL of air thru the Biopsy port and out the distal end into a sterile 50 mL conical collection container. Sonicate the sample 3 x 5 seconds; vortex 1 min; sonicate 3 x 5 seconds.
***Bioburden*:** Under the BSC, vortex sample well and aliquot 1 mL x 2 into sterile eppendorf tubes for chemistry testing. Serially dilute 1:10 (50 µL into 450 µL sPBS) from direct thru 10^{-6}. Plate 100 µL x 3 of the 10^{-2} thru 10^{-6} dilutions onto BA media. **FREEZE EPPENDORF SAMPLE FOR PROTEIN TESTING (-20 ºC)**. Spread inoculum and incubate 24 hours at 35-37 ºC. Count all EF and PA colonies and record. This is REPLICATE #1.
***Protein Assay*:** Within 2 weeks of freezing aliquot, perform the QuantiPro™ BCA Microassay (Sigma) as per manufacturer's instructions. Perform dilutions if required.
This is REPLICATE #1. If time permits, RESOIL the endoscope with the pooled soil from the fridge and dry 1 hour (do not manually clean the endoscope between replicates but do use suction to remove all fluids from the scope prior to re-soiling). Repeat the testing of the S/B channel (BPD) as per REPLICATE #1 (3 replicates total).
PARTIAL CLEAN: Attach a Biopsy port luer connector and perform a **50 mL tap water flush** from the Biopsy port to the distal end (control head plugged and raised; distal end lowered into a sterile container). Discard. Harvest the partially washed scope as per the POS CONTROL scope above **(20 mL flush only).**
Perform bioburden* and protein testing exactly as for POS CONTROL.
This is REPLICATE #1. RESOIL the endoscope with the pooled soil in the fridge and dry 1 hour (do not manually clean the endoscope between replicates). Repeat the testing of the S/B channel (BPD) as per REPLICATE #1 (3 replicates total).
* Serially 1:10 dilute from direct thru 10^{-4} and plate 100 µL x 3 of the direct thru 10^{-4} onto BA media.
POST CLEAN: Perform a full manual clean on the endoscope as per APPENDIX 1 (use syringes). Harvest the fully washed scope as per the POS CONTROL scope above **(flush only).**
Perform bioburden** and protein testing exactly as for POS CONTROL.
This is REPLICATE #1. If time permits, RESOIL the endoscope with the pooled soil in the fridge and dry 1 hour (do not manually clean the endoscope between replicates but do use suction to

remove fluid from the scope prior to re-soiling). Repeat the testing of the S/B channel (BPD) as per REPLICATE #1 (3 replicates total).
** Serially 1:10 dilute from direct thru 10^{-2} and plate 100 µL x 3 of the direct thru 10^{-2} onto BA media.
BASELINE: On 3 separate mornings, harvest the BPD channel using the **flush only method** from a "patient-ready" endoscope directly from the scope cabinet. These are the BASELINE samples (3 replicates total).
Perform bioburden*** and protein testing exactly as for POS CONTROL.
*** Plate direct x 3 onto Blood agar (BA) plates only. Incubate 48 hours and count TOTAL VIABLE COUNT.

END OF DAY PROCESSING OF ENDOSCOPE: After all samples are collected for the day, the endoscope should be fully manually cleaned (as per Appendix 1) if not already done so, placed in an AER for a full disinfection, then have an alcohol flush and air dry. Hang in the scope cabinet until required.

How to relate the bioburden and protein values back to ATP

Based on preliminary testing, we know the average volume of fluid a Ruhof Test Instrusponge™ Channel Testing Sponge absorbs (based on weight). We can convert the cfu/mL (bioburden) and µg/mL (protein) values back to cfu/sponge and µg/sponge, and compare this to the raw ATP/sponge value.

Ex: If the sponge absorbs 0.2 mL on average, and the POS CONTROL sample shows a bioburden count of 524,000 cfu/mL, this is equivalent to 524,000 x 0.2 = 104,800 cfu/sponge (5.020 logs).

Summary Table: Phase II

- **2 endoscopes in parallel**
- **1 channel (suction biopsy channel; biopsy port to distal end)**
- **1 parts to the study, 4 tests per part (full clean, partial clean, no clean, baseline), 3 replicates per test.**

Experiment:	**Harvesting method:**	**ATP**	**Bioburden**	**Chemistry**
Scope #1: Test 1: Positive control (No clean) **(n = 3)**	Sponge only (no elution)	**√**	**-**	**-**
Scope #2: Test 1: Positive control (No clean) **(n = 3)**	Flush only	**-**	**√**	**√**
Scope #1: Test 2: Partial clean **(n = 3)**	Sponge only (no elution)	**√**	**-**	**-**
Scope #2: Test 2: Partial clean **(n = 3)**	Flush only	**-**	**√**	**√**
Scope #1: Test 3: Full Clean **(n = 3)**	Sponge only (no elution)	**√**	**-**	**-**
Scope #2: Test 3: Full Clean **(n = 3)**	Flush only	**-**	**√**	**√**
Scope #1: Test 4: Negative Control (Baseline) **(n = 3)**	Sponge only (no elution)	**√**	**-**	**-**
Scope #2: Test 4: Negative Control (Baseline) **(n = 3)**	Flush only	**-**	**√**	**√**
Total Samples:		**12**	**12**	**12**

Sample Codes:

SPONGE ONLY (no elution) – for ATP:

Positive Control: RP2-SONE-BPD-P-x
Partial Clean: RP2-SONE-BPD-PC-x
Full Clean: RP2-SONE-BPD-FC-x
Baseline: RP2-SONE-BPD-Base-x

FLUSH ONLY – for Bioburden & Protein

Positive Control:	RP2-FO-BPD-P-x
Partial Clean:	RP2-FO-BPD-PC-x
Full Clean:	RP2-FO-BPD-FC-x
Baseline:	RP2-FO-BPD-Base-x

- RP2 = **R**uhof **P**hase **2**
- SONE = **S**ponge **O**nly (**N**o **E**lution)
- FO = **F**lush **O**nly
- BPD = **B**iopsy **P**ort to **D**istal End
- P = **P**ositive Control
- PC = **P**artial **C**lean
- FC = **F**ull **C**lean
- Base = **Base**line
- "x" = replicate # (3replicates total will be performed)

Supply List:

- ATP Test Swabs = 12 total
- Chemiluminometer
  - Blood agar plates = 126 (bioburden counts) + 72 minimum (for inoculum counts).
  - Blood agar plates = 13/day for EF & PA subcultures and inoculum preps x 6 days minimum = 78 minimum.
    TOTAL BA = 276
- Artificial Test Soil (~ 160 mL for 2 replicate days; ~ 110 mL for 1 replicate days)
  TOTAL ATS = ~810 mL

Other supplies: Syringes for soiling and partial clean, endoscope cleaning connectors, sterile urine containers, sterile 50 mL conical tubes, leak tester, AER including Biological and Chemical Indicators, Steris System I sterilant, PPE, sterile 12 x 75mm glass tubes, 12 x 75mm snap-cap tubes, sterile eppendorf tubes, QuantiPro™ BCA Microassay kit (Sigma), 70% Ethanol, Vacuum source, sonicator, vortexer, mechanical pipettors, pipette tips, disposable loops, incubator etc…

APPENDIX A: Manual cleaning protocol for the Fujinon EC-530HL Colonoscope

FUJINON EC-530HL COLONOSCOPE: CLEANING INSTRUCTIONS

Supplies Required:

- Cleaning basins (3 total).
- Enzymatic detergent (Ruhof Endozime® Bio-Clean) – used at a 6 mL/Liter concentration.
- Fujinon Hand-held Leak Tester (LT-7).
- Gauze for wipe down of the external surfaces of the endoscope.
- Short brushes for cleaning of control head ports and biopsy port.
- Two-ended disposable long brushes (provided by Ruhof) (not autoclaved).
- CA-510 Cleaning Adaptor for G5 series colonoscopes, which includes the following parts, inter-linked together:

  - CA-503S/A (connects to suction/biopsy and air/water ports at control head)
  - CA-503B/C (connects to the biopsy port)
  - CA-500J/A (jet cleaning adaptor)
  - Cleaning Tubing (for Manual Syringe cleaning only)
- 30 cc syringe (slip tip or luer-lok acceptable) (for Manual Syringe cleaning).

Part 1: LEAK TEST (dry and wet)

1) Lay scope out flat on the table.
2) Attach the Hand-held Leak Tester (LT-7) to the ventilation connector of the endoscope.
3) Feed air until the gauge on the Leak Tester reads ~ 150 mm Hg (middle of black line).
4) Let stand 30 seconds [reading should not drop by more than 2 mm Hg – if it does, do NOT use the scope and consider it a leaking scope. At this point, do NOT submerse the scope in water].
5) Submerse the scope in a basin of tap water (with gauge still reading ~ 150 mm Hg).
6) Manipulate the up-down and right-left controls on the scope, so that the distal end rotates in all directions while submerged. Watch for air bubbles streaming from the distal end. Also press down on the RC, MM and FR switches at the control head. Again watch for bubbles emerging from these switches.
7) Remove the scope from the water and lay on towels. Release the air using the red trigger on the Leak Tester.

Part 2: MANUAL CLEAN (using SYRINGES)

1) Fill one basin with enzymatic detergent (recommended dilution), one basin with tap water and leave one basin empty.
2) Submerse the endoscope into the enzymatic detergent (be sure the cap is tightly secured on the EVE connector).
3) Wipe down the external surface of the entire endoscope with lint-free cloth.
4) Use the short brush to brush the suction/biopsy port and air/water port on the control head, as well as the biopsy port while the scope is submerged (place brush into port, rotate 360° and remove; wipe brush end with finger tips and repeat 2 more times = 3 times total).
5) Use the two-ended disposable long brush (non-sterile) as follows:
   - Push thru the biopsy port out the distal end, pull out from the distal end and repeat x 2 = 3 total (clean brush with finger tips after it emerges from the scope each time).
   - Push thru the suction/biopsy port STRAIGHT thru out the distal end, pull out from the distal end and repeat x 2 = 3 total (clean brush with finger tips after if emerges from the scope each time).
   - Push thru the suction/biopsy port at a RIGHT ANGLE out the umbilical end, pull out from the distal end and repeat x 2 = 3 total (clean brush with finger tips after it emerges from the scope each time).
6) Attach the CA-503S/A connector to the suction/biopsy and air/water ports on the control head (clicks into place).
7) Attach the CA-503B/C connector to the biopsy port (clicks and slides into place).
8) Attach the CA-500J/A connector to the feed water connector on the umbilical end of the scope (clicks and twists into place).
9) Now attach the tubing ends (red and blue) to the corresponding luer-lok ends of the CA-503S/A connector.

10) Attach a 30cc slip tip or luer-lok syringe to the **blue port** of the cleaning tube; with scope submerged, draw up and dispense 30cc of enzymatic detergent x 3 (90 cc total) – be sure the distal ends of the tubing is under fluid. Repeat 30cc x 3 for the **red port**. Also draw up 30cc detergent and push thru the CA-500J/A luer-lok end.
11) Transfer the scope into the tap water. Wipe down the external surface of the entire endoscope using a lint-free cloth.
12) Repeat step 10 above in the tap water (90cc of water thru the blue and red ports; 30cc of water thru the water connector).
13) Transfer the scope into an empty basin and repeat the above syringe pushes for air.
14) Harvest the samples or high level disinfect the endoscope at this point (Steris System I; use Steris Quick Connect # QFC1744).
15) **Alcohol flush (70% Ethanol) and air dry**. Hang in closet.