This application guide was developed using the systemSURE Plus luminometer. If using the EnSURE Luminometer, this unit has twice the sensitivity of the systemSURE Plus and the RLU pass / fail thresholds should be doubled if read on the EnSURE unit, e.g. a pass value 100 RLU's for the systemSURE equates to a pass value of 200 RLU's if read on the EnSURE unit.



- WATER DAMAGE RESTORATION -

PROTOCOL FOR BIO-REVEAL SAMPLING OF CONDITION 1, 2 AND 3 MOLD & FUNGI CONTAMINATION

Statement of Use

The Bio-reveal® Ultrasnap ATP swabs and the Bio-reveal® Systemsure Plus luminometer will be used to determine the level of surface contamination for viable fungi, molds, biofilms and related microbial organisms and environmental biological matter. The purpose of the sampling is to determine the level of biological surface contamination of building material and content surfaces that may be impacted by mold growth bioamplification. The Bio-reveal® system will allow water loss responders, restoration professionals, remediation contractors and the Indoor Environmental Professional (IEP) the real-time ability to quantify the mold and fungi contamination condition (Condition 1, 2 or 3) as defined by the IICRC S520 for the indoor environment relative to the presence of mold and related biological contamination.

The Bio-reveal® system also allows the user the ability to provide quality assurance for the remediation process and post remediation verification testing for cleaned and sanitized building materials and contents that are remaining or being restored. The Bio-reveal® bio-contamination detection system is designed to evaluate the level of surface cleanliness and sanitized hygiene in the indoor environment. The building materials suitable to be tested with the Bio-reveal® system include, but are not limited to, wall studs (metal and wood), concrete, vinyl and ceramic tile, laminate surfaces, stainless steel, masonry materials, plastic, gypsum board or sheetrock, wood sheathing, glass, etc.

Methodology – Surface Sampling Investigation Phase

Steps

- Identify the target surface (ie; wall studs (metal and wood), concrete, vinyl and ceramic tile, laminate, stainless steel, masonry materials, plastic, laminate, gypsum board or sheetrock, wood sheathing, glass, etc) to sample for determining the Condition present as well as the impacted surfaces that require cleaning and sanitizing:
 - a. Interior items not related to the building materials (ie: personal effects, etc.)
 - b. Remediation equipment and remediation devices
 - c. Other not mentioned above that may be site specific or specifically affected by water loss
- 2) Use aseptic techniques for all sample collection. Remove the plastic cover or tube from the Bio-reveal® Ultrasnap ATP swab. This will expose the collection end or swab bud, which is pre-moistened to assist in sample collection. Ensure to <u>NOT</u> directly touch the swab bud or swab shaft with your fingers or hand or it will become contaminated.
- 3) Thoroughly swab the desired sample surface over a 2" X 2" sampling area (4 inches square) using approximately 10 strokes vertical and 10 strokes horizontal over the sample area while rotating the swab over the surface. Allow the swab bud to "clean" the sampled surface in order to accurately reflect the sampled surface contamination potential.
- 4) After swabbing place the plastic tube back over the swab bud and insert the open end back into the collar of the entire device.
- 5) Grasp the bulb end of the sampling device and the small plastic stem inside the bulb. Then break the snap valve by bending this plastic stem forward and backward until the stem breaks off. Hold the device upright during this step.
- 6) Squeeze the bulb twice to expel the reagent in the bulk down into the collection tube covering the swab bud tip.
- 7) Gently shake the device to thoroughly mix the liquid contents in the base of the device for approximately 5 seconds. This ensures the swab bud is properly washed or bathed in the reagent solution.
- 8) Insert the entire sampling device into top of the Bio-reveal® Systemsure Plus luminometer. Be sure to insert the device completely into the open port hole before closing the lid of the luminometer. <u>The sample device should be inserted into the</u> <u>luminometer and read within 60 seconds after breaking the valve stem and</u> <u>activating the reagent as outlined in Step 5), for the most accurate results.</u>
- 9) Close the lid of the Bio-reveal® luminometer.
- 10) Press the "OK" button to read the sample results. This process will take 15 seconds from the time you press the "OK" button. Be sure to hold the instrument up and down (vertical position) to obtain the best results.

Environmental Surface Testing Interpretation of Bio-reveal Sampling Results

BIO-REVEAL FOR MOLD AND FUNGI TESTING INTERPRETATION GENERAL ENVIRONMENTAL SURFACE SAMPLING

Guideline for Surface Sampling of Building Materials or Contents Condition 1, 2 and 3 Mold Presence in Indoor Environments (Surface samples are collected using the Bio-reveal Ultrasnap swab from indoor environmental surfaces, building materials, furnishings, personal effects, etc.)

Sampled Surface Condition	Moisture	Bio-reveal	Fungi and Mold
(as referenced by the IICRC S520)	Sampled Surface (%)	Results (RLU)	Contamination Condition
Normal Fungal Ecology (An indoor environment that may have settled spores, fungal fragments or traces of actual growth whose identity, location and quantity are reflective of a normal fungal ecology for a similar environment.)	<15%	1 – 50 (1 – 150)*	1 (TYPICAL FOR INDOOR ENVIRONMENTS)
Settled Spores (An indoor environment which is primarily contaminated with settled spores that were dispersed directly or indirectly from a Condition 3 area, and which may have traces of actual growth.)	<15%	50 - 150	2 (POTENTIALLY CROSS- CONTAMINATED INDOOR ENVIRONMENTS)
Actual Growth (An indoor environment contaminated with the presence of actual mold growth and associated spores. Actual growth includes growth that is active or dormant, visible or hidden.)	<15%	>150	3 (TYPICALLY NOT ACCEPTABLE)

DRY SUBSTRATE CONDITIONS

* Numerical definition of IICRC S520 Condition is dependent on indoor environmental hygiene or activities present at the site during the sampling event. Typical Condition 1 surfaces yielded ATP results that ranged from 1 RLU to 75 RLUs; however, there were samples that were as high as 150 RLUs

** RLU / 4 sq in = Relative light unit per recommended sampling area (4 square inches)

References utilized:

1) IICRC Standard for Professional Mold Remediation S520

Sampled Surface Condition	Moisture	Bio-reveal	Fungi and Mold
(as referenced by the IICRC S520)	Content of Sampled Surface	Testing Results	Contamination Condition
	(70)		
Normal Fungal Ecology	< 15%	1 - 50	1
(An indoor environment that may		(1 – 150)*	
have settled spores, fungal			(TYPICAL FOR
fragments or traces of actual			
growth whose identity, location and			ENVIRONMENTS)
quantity are reflective of a normal			
fungal ecology for a similar			
environment.)			
Settled Spores	< 15%	100 - 500	2
(An indoor environment which is			
primarily contaminated with settled			(POTENTIALLY
spores that were dispersed directly			CROSS-
or indirectly from a Condition 3			
area, and which may have traces of			
actual growth.)			
Actual Growth	≥ 15%	>500	3
(An indoor environment			
contaminated with the presence of			(TYPICALLY NOT
actual mold growth and associated			ACCEPTABLE)
spores. Actual growth includes			
growth that is active or dormant,			
visible or hidden.)			
* Numerical definition of IICRC S520 Condition is dependent on indoor environmental hygiene or			
activities present at the site during the sampling event. Typical Condition 1 surfaces yielded ATP			

WET SUBSTRATE CONDITIONS

RLUs
** RLU / 4 sq in = Relative light unit per recommended sampling area (4 square inches)

results that ranged from 1 RLU to 75 RLUs; however, there were samples that were as high as 150

References utilized:

1) IICRC Standard for Professional Mold Remediation S520

<u>Methodology – Surface Sampling</u> <u>Post Remediation Verification Phase</u>

Steps

- Identify the target surface (ie; wall studs (metal and wood), concrete, vinyl and ceramic tile, laminate, stainless steel, masonry materials, plastic, laminate, gypsum board or sheetrock, wood sheathing, glass, etc) to sample for determining the Condition present as well as the impacted surfaces that require cleaning and sanitizing:
 - a. Interior items not related to the building materials (ie: personal effects, etc.)
 - b. Remediation equipment and remediation devices
 - c. Other not mentioned above that may be site specific or specifically affected by water loss
- 2) Use aseptic techniques for all sample collection. Remove the plastic cover or tube from the Bio-reveal® Ultrasnap ATP swab. This will expose the collection end or swab bud, which is pre-moistened to assist in sample collection. Ensure to <u>NOT</u> directly touch the swab bud or swab shaft with your fingers or hand or it will become contaminated.
- 3) Thoroughly swab the desired sample surface over a 2" X 2" sampling area (4 inches square) using approximately 10 strokes vertical and 10 strokes horizontal over the sample area while rotating the swab over the surface. Allow the swab bud to "clean" the sampled surface in order to accurately reflect the sampled surface contamination potential.
- 4) After swabbing place the plastic tube back over the swab bud and insert the open end back into the collar of the entire device.
- 5) Grasp the bulb end of the sampling device and the small plastic stem inside the bulb. Then break the snap valve by bending this plastic stem forward and backward until the stem breaks off. Hold the device upright during this step.
- 6) Squeeze the bulb twice to expel the reagent in the bulk down into the collection tube covering the swab bud tip.
- 7) Gently shake the device to thoroughly mix the liquid contents in the base of the device for approximately 5 seconds. This ensures the swab bud is properly washed or bathed in the reagent solution.
- 8) Insert the entire sampling device into top of the Bio-reveal® Systemsure Plus luminometer. Be sure to insert the device completely into the open port hole before closing the lid of the luminometer. <u>The sample device should be inserted into the</u> <u>luminometer and read within 60 seconds after breaking the valve stem and</u> <u>activating the reagent as outlined in Step 5), for the most accurate results.</u>
- 9) Close the lid of the Bio-reveal® luminometer.
- 10) Press the "OK" button to read the sample results. This process will take 15 seconds from the time you press the "OK" button. Be sure to hold the instrument up and down (vertical position) to obtain the best results.

Post Remediation Verification Interpretation of Bio-reveal Sampling Results

BIO-REVEAL FOR MOLD TESTING INTERPRETATION PRV SURFACE SAMPLING

Guideline for Surface Sampling of Building Materials or Contents Post Remediation Verification Testing of Building Materials and Contents Surfaces

(Surface samples are collected using the Bio-reveal Ultrasnap swab from indoor environmental surfaces, building materials, furnishings, personal effects, etc.)

Sampled Surface Condition (Post Remediation Verification)	Bio-reveal Surface Sampling Result	Interpretation Result
Ideal Goal	(RLU)*	
Normal Fungal Ecology	< 50**	PASS
(An indoor environment that may have settled spores, fungal fragments or traces of actual growth whose identity, location and quantity are reflective of a normal fungal ecology for a similar environment.)	(Ideal surface hygiene condition)	
	50 - 150	CAUTION
	(Acceptable for most indoor environmental surfaces not used in healthcare patient care settings or food preparation situations)	
	> 150	FAIL

RLU – Relative light unit or unit of measure for bioluminescent measurements

* Surface hygiene goal after restoration or remediation cleaning and sanitizing activities.

Considerations when using the Bio-reveal sampling system

- a. Avoid collecting large amounts of sample debris on the swab bud. Too much sampled material may reduce signal strength of test and provide inaccurate readings or false negatives.
- b. Damaged or accidental activations of the sampling swab device should not be used and should be disposed of.
- c. Disposal of the sampling swab device can be in general waste. No special precautions are required for disposal.
- d. Hold the Bio-reveal® Systemsure II upright during Step 10).
- e. Hold the Bio-reveal® Ultrasnap ATP swab device upright when activating in Step 5).
- f. The Bio-reveal® Ultrasnap ATP swabs will tolerate room temperature storage for up to two months but all unused sampling devices should be stored in the refrigerator, where they will remain viable for up to 12 months.

For Technical Questions or Customer Service, please contact Slade Smith at: Phone number: 866-989-5567, ext 2. Cell number: 320-309-5448

Or

email at info@bio-reveal.com

ALTERNATIVE INTERPRETATION GUIDELINE SETTINGS

Recommended Threshold Setting Procedure

- Step 1) Identify the sample points or critical control points.
- Step 2) Clean the sample point surfaces thoroughly. This procedure may be repeated 2 or more times to achieve the best possible cleanliness.
- Step 3) Conduct ATP sampling at each location identified and cleaned, using 10 test replicates.
- Step 4) Calculate the average RLU. This will be considered the <u>PASS</u> level.
- Step 5) <u>FAIL</u> limits are determined by multiplying the <u>PASS</u> level by a factor of 2.
- Step 6) Caution is the region between the <u>PASS</u> and <u>FAIL</u> calculated limits.
- Step 7) Monitor results and assess the trends. Recalculation of the PASS and FAIL limits may be warranted to optimize the results and improve the quality standards.

Alternative Threshold Setting Procedure

- Step 1) Identify the sample points or critical control points.
- Step 2) Clean the sample point surfaces thoroughly. This procedure may be repeated 2 or more times to achieve the best possible cleanliness.
- Step 3) Conduct ATP sampling at each location identified and cleaned several times and over several days, using a minimum of 50 test replicates.
- Step 4) Calculate the average and standard deviation for the documented RLUs.
- Step 5) Set limits as follows:

Pass	<= Mean RLU
Caution	>= Mean RLU < Mean + 3 standard deviations
Fail	>= Mean RLU + 3 standard deviations

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