

- WATER DAMAGE RESTORATION -

PROTOCOL FOR BIO-REVEAL SAMPLING OF IICRC S520 CONDITION 1, 2 AND 3 MOLD 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 IICRC S520 defined condition of the indoor environment relative to the presence of mold and related biological contamination (Condition 1, 2 or 3 as defined by the IICRC S520 standard).

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 Bioreveal® 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.

<u>Methodology – Surface Sampling</u> <u>Investigation Phase</u>

Steps

- 1) 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
 - 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 NOT 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. The sample device should be inserted into the luminometer and read within 60 seconds after breaking the valve stem and activating the reagent as outlined in Step 5), for the most accurate results.
- 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 IICRC S520 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 Content of Sampled Surface (%)	Bio-reveal Testing Results (RLU)	IICRC S520 Indoor Environmental 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
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
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

^{*} 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

References utilized:

1) IICRC Standard for Professional Mold Remediation S520

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

Sampled Surface Condition	Moisture Content of Sampled Surface (%)	Bio-reveal Testing Results (RLU)	IICRC S520 Indoor Environmental 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
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%	100 - 500	2
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%	>500	3

^{*} 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

References utilized:

1) IICRC Standard for Professional Mold Remediation S520

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

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

Steps

- 11) 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:
 - d. Interior items not related to the building materials (ie: personal effects, etc.)
 - e. Remediation equipment and remediation devices
 - Other not mentioned above that may be site specific or specifically affected by water loss
- 12) 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 NOT directly touch the swab bud or swab shaft with your fingers or hand or it will become contaminated.
- 13) 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.
- 14) After swabbing place the plastic tube back over the swab bud and insert the open end back into the collar of the entire device.
- 15) 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.
- 16) Squeeze the bulb twice to expel the reagent in the bulk down into the collection tube covering the swab bud tip.
- 17) 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.
- 18) 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. The sample device should be inserted into the luminometer and read within 60 seconds after breaking the valve stem and activating the reagent as outlined in Step 5), for the most accurate results.
- 19) Close the lid of the Bio-reveal® luminometer.
- 20) 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 IICRC S520 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) Ideal Goal	Bio-reveal Surface Sampling Result (RLU)*	Interpretation Result
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.)	< 50** (Ideal surface hygiene condition)	PASS
	50 - 150 (Acceptable for most indoor environmental surfaces not used in healthcare patient care settings or food preparation situations)	CAUTION
	> 150	FAIL

^{*} RLU – Relative light unit or unit of measure for bioluminescent measurements

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

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) *FAIL* limits are determined by multiplying the *PASS* level by a factor of 2.
- Step 6) Caution is the region between the PASS and FAIL 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

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.

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