



- WATER DAMAGE RESTORATION -

PROTOCOL FOR BIO-REVEAL SAMPLING OF CATEGORY 1, 2 AND 3 WATER LOSS LIQUID/WATER & SURFACE TESTING

Statement of Use

The Bio-reveal® Aquasnap ATP liquid sampling devices, Ultrasnap ATP swabs and the Bio-reveal® Systemsure Plus luminometer will be used to determine the level of surface contamination for viable biological matter, primarily bacteria and biofilms that may be present in contaminated water or on surfaces impacted by Category 1, 2 or 3 water. The use of the Bio-reveal® testing system will allow water loss responders, restoration professionals, remediation contractors and the Indoor Environmental Professional (IEP) the real-time ability to quantify the level of Category 1, 2 or 3 water loss upon affected building materials and contents as well as provide quality assurance to the remediation process and post remediation verification testing.

The Bio-reveal® bio-contamination detection system is designed to evaluate the level of surface cleanliness and sanitized hygiene in the indoor environment. This system will not detect specific strains of bacterial, viral or other micro-organisms, rather will measure and document the total surface or liquid conditions where these types of pathogenic organisms may be detected or harbored as a result of dirty, unhygienic or where direct impaction of Category 1, 2 or 3 water contamination may have occurred. Additionally, the Bio-reveal® bio-contamination detection system can be used to generally quantify the total bacterial concentrations of Category 1, Category 2 and Category 3 water as defined and referenced by the IICRC S500 Standard and Reference Guide for Professional Water Damage Restoration.

Methodology – Liquid Sampling

Steps

- 1) Identify the target liquid to sample for determining the water loss category present:
 - a. Extracted water from building material
 - b. Ponded water from affected surface
- 2) Use aseptic techniques for all sample collection. Remove the plastic cover or tube from the Bio-reveal® Aquasnap ATP liquid sampling device. This will expose the collection end, which is shaped like a spiral cone to assist in sample collection. Ensure to NOT directly touch the collection end or device shaft with your fingers or hand or it will become contaminated.
- 3) For sampling potentially contaminated liquids, dip the Aquasnap ATP liquid sampling device collection end completely into the target liquid for approximately 5 seconds to collect approximately 0.10 ml of sample liquid. Once completed, follow step 4) below.
- 4) After collecting the liquid sample, place the plastic tube back over the collection end of the sample device and insert the open end back into the collar of the entire Aquasnap ATP liquid sampling 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 collection 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 collected liquid sample 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.

Interpretation of Bio-reveal Sampling Results

LIQUIDS / WATER

BIO-REVEAL FOR CATEGORY 1, 2 AND 3 WATER LOSS TESTING LIQUID SAMPLING

Bio-reveal Guideline for Evaluating Category 1, 2 and 3 Water Loss

(Liquid samples collected through extraction or from settled or ponded water, etc. The water samples are then analyzed by dipping the Bio-reveal Aquasnap into the collected liquid)

Water Loss Category	Definition of Water Loss * (Field Conditions)	Bio-reveal Result (RLU / 0.10 ml)**
Category 1	Clean Water (broken water supply lines, tub or sink overflows with no contaminants, appliance malfunctions involving water supply lines, melting ice or snow, falling rainwater, broken toilet tanks, toilet bowls that do not contain contaminants or additives, etc.)	< 5
Category 2	Gray Water (discharged water from dishwashers, washing machines, overflows from toilet bowls with some urine-no feces, sump pump failures, seepage due to hydrostatic pressure, broken aquariums, punctured water beds, etc.)	≥ 5 and < 500
Category 3	Black Water (sewage or other contaminated water sources entering or affecting the indoor environment, toilet backflows that originate beyond the trap, flooding from seawater, ground surface water and rising water from rivers or streams, etc.)	≥ 500

* Definition of water loss is dependent on time and temperature characteristics present at the site. Category 1 and Category 2 water loss situations can become Category 3 water losses after sufficient time as defined and referenced by the IICRC S500 standard.

** RLU / 0.10 ml = Relative light unit per volume collected on sampling swab equal to 0.10 ml.

References utilized:

- 1) IE Connections article titled: *H. Quantifying Bacteria Levels in Water Categories 1–3*, Table 10: Summary of suggested Category 1, 2 and 3 bacterial ranges in water, *Brandys, Dr. Robert*, February 2007
- 2) IICRC Standard for Professional Water Damage Restoration S500

Methodology – Surface Sampling

Steps

- 1) Identify the target surface to sample for determining the water loss category present as well as the impacted surfaces that require cleaning and sanitization:
 - a. Flooring materials
 - b. Wall materials
 - c. Ceiling materials
 - d. Concrete materials
 - e. Tile materials
 - f. Wood building materials
 - g. Furnishings
 - h. Interior items not related to the building materials (ie: personal effects, etc.)
 - i. Remediation equipment and remediation devices
 - j. 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.

Interpretation of Bio-reveal Sampling Results

SURFACES

BIO-REVEAL FOR CATEGORY 1, 2 AND 3 WATER LOSS TESTING SURFACE SAMPLING

**Guideline for Surface Sampling of Building Materials or Contents
Includes Initial Assessments & Post Remediation Verification Testing
of Surfaces for Water Damaged Building Materials**

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

Sampled Surface Condition	Bio-reveal Surface Sampling Result (RLU)*	Interpretation Result
Not affected by water loss Or (Final hygiene goal for water loss restoration or remediation of building materials or contents to be salvaged)	< 50 (Category 1 losses)	PASS
	< 15 (Category 2 or 3 losses – threshold lowered to reduce potential for pathogen presence)	PASS
Moderately affected by water loss (Suggests restoration or remediation of remaining building materials or contents is not completely satisfactory)	≥ 50 and < 150 (Category 1 losses)	CAUTION
	≥ 15 (Category 2 or 3 losses – threshold lowered to reduce potential for pathogen presence)	FAIL
Severely affected by water loss (Suggests the building materials or contents are heavily contaminated and should be restored, remediated or disposed of)	≥ 150 (Category 1 losses)	FAIL
	≥ 15 (Category 2 or 3 losses – threshold lowered to reduce potential for pathogen presence)	FAIL

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

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:

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Or

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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 PASS 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