

Supplementary Appendix

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This appendix has been provided by the authors to give readers additional information about the work.

Supplementary Appendix

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Detailed Case Reports

Patient 1

On the way to the emergency department (ED) of hospital A on July 10, 2020, Patient 1 was found to be in supraventricular tachycardia (>200bpm) requiring chemical and electrical cardioversion. On arrival, the patient was tachypneic (RR 40-60), hypoxic (SpO₂ 88%), and hypotensive (BP 99/57) and required emergent intubation for airway support and vasopressors. He was admitted to the intensive care unit (ICU) for the management of acute respiratory distress syndrome, sepsis, and multisystem organ failure, which required hemodialysis, continued vasopressor support, and mechanical ventilation.

Laboratory values indicated leukocytosis (10.2 x 10³/μl, hospital day 0; peak 37.2 x 10³/μl, hospital day 5), lactic acidosis (>12.2mmol/L), anemia (10.6g/dL), elevated Ferritin (>1098.5 ng/mL), elevated D-Dimer (>20 FEU ug/ML), transaminitis (AST 112 IU/L / ALT 58 IU/L), acute kidney injury (BUN

28mg/dL; Cr 2.54mg/dL), demand mediated non-ST-elevation myocardial infarction (troponin 8.82 ng/mL peak), and an elevated blood alcohol content of 226mg/dl. The initial chest radiograph (hospital day 0) and subsequent chest computed tomography (CT) (**Figure S1**) demonstrated bilateral multilobar pneumonia.

On hospital day 0, the patient tested positive for Influenza type A and blood was positive for gram-negative rods (GNR) which was sensitive only to ceftazidime, meropenem, Zosyn, Bactrim, and tetracycline. The patient was treated with IV meropenem 1g (Q 24 H, renal dosing), IV ceftazidime 1g (Q8H), and IV vancomycin 1-1.5g (as needed following dialysis). On hospital day 7, *B. pseudomallei* was presumptively identified on a MircoScan WalkAway Bacterial Identification panel and forwarded to the Mississippi Public Health Laboratory (MPHL) for confirmation. On hospital day 13, the patient was discharged home in stable condition with a midline on continued IV meropenem 1g (Q12 H, for 11 additional days) and oral doxycycline 100mg (Q12 H for 11 days). The following day, *B. pseudomallei* was confirmed by MPHL from the initial positive blood culture, and CDC was notified. Despite the patient not receiving adequate oral eradication therapy due in part to constraints with follow-up during the COVID-19 pandemic, the patient recovered from melioidosis. He however died approximately two years later from alcoholic cirrhosis and *Enterococcus gallinarum* bacteremia.

Patient 2

Patient 2 first presented to an outpatient clinic on April 18, 2022, with an 8-day history of cough and generalized weakness. He was diagnosed by chest radiograph with left upper lobe pneumonia and was sent home on oral azithromycin 250mg (2 tabs on day 1 followed by 1 tab for days 2-5) and oral amoxicillin 875mg (1-tab 2x/day). Two days later, following a fall at home, the patient presented to hospital A's emergency department (the same hospital as Patient 1). His physical exam was unremarkable except for tachycardia (110 bpm) and fever (100.4° F). Initial laboratory values were remarkable for leukocytosis (WBC 25.2 x 10³/μl), transaminitis (AST 195 U/L/ ALT 236 U/L°), and moderate hyponatremia (sodium 129mmol/L). Blood cultures obtained on hospital day 0 grew GNR and were

presumptively identified as *B. pseudomallei* on a MicroScan WalkAway Bacterial Identification panel and forwarded to MPHL for further identification. All subsequent blood, urine, and sputum cultures were negative. The initial chest CT without contrast (hospital day 0) showed a 6.5cm mass-like consolidation at the left lung apex with associated bulky hilar and mediastinal lymphadenopathy, which was concerning for necrotizing pneumonia or malignancy. He was treated initially with IV levofloxacin 750mg and broadened to piperacillin/tazobactam and vancomycin after the CT findings. On day 4, he was discharged home on five more days of oral levofloxacin 750mg (1 tab daily for a complete 10-day course).

Approximately one-week post-discharge, *B. pseudomallei* was confirmed at the MPHL and the patient was admitted to hospital B for the acute phase melioidosis treatment with IV meropenem 1g (Q8H) per MSDH and CDC guidance. Upon re-admission, he was noted to be febrile (102.3 °F), tachycardic (119 bpm), had profound weakness, myalgia, dry cough, and dyspnea, and laboratory values indicated leukocytosis (20.7 K/cm). Chest CT demonstrated persisting left lung apical consolidation with cavitating lesions (**Figure S2**). He was discharged home on hospital day 3 (afebrile with improving leukocytosis) with a peripherally inserted central catheter (PICC) to complete the 14-day course of IV meropenem 1g (Q8H) followed by three months of oral trimethoprim/sulfamethoxazole (Bactrim) 320mg/1600 mg (Q12H) as eradication therapy under the care of an infectious disease specialist.

Figure S1 - Patient 1: Chest CT without contrast on hospital Day 6 demonstrating bilateral lower lobe pneumonia

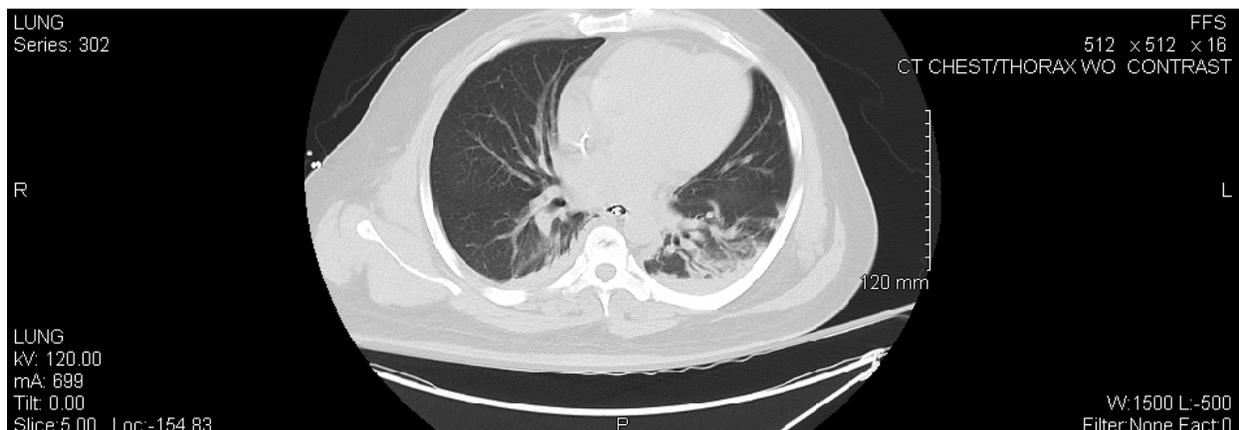
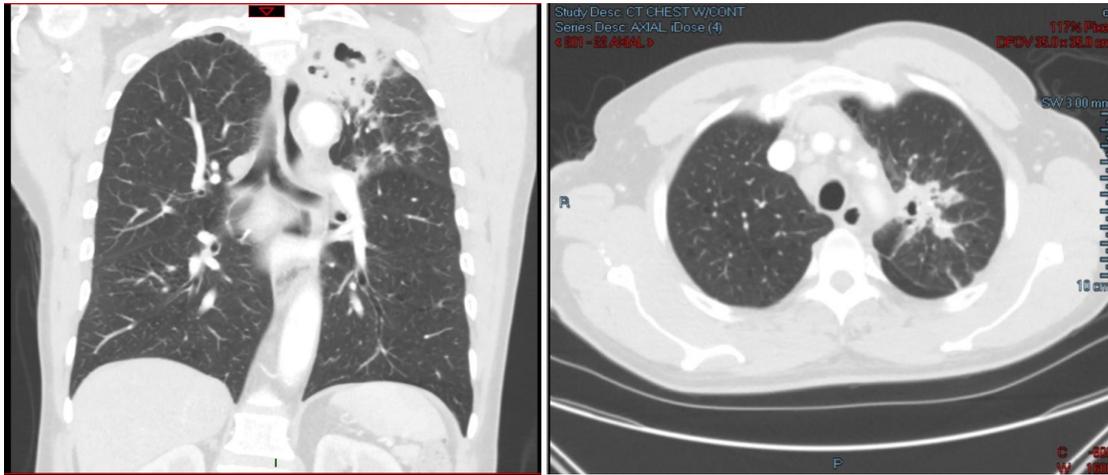


Figure S2 - Patient 2: Chest CT on hospital day 3 of 2nd hospitalization demonstrating worsening upper left lobe consolidation with cavitating lesions.



Environmental Sampling Methods

Swabs used in sampling were Copan ESwab® regular flocked swab with liquid Amies medium. Water and biofilm samples were filtered by vacuum using PALL MicroFunnel™ filter funnels (<http://www.pall.com>, product ID 4818); filters were removed and placed into an enrichment broth, Galimand's selective broth (TBSS-C50). Swabs, sponge sticks, the bathmat sample, samples of individual plants and fish were directly added to selective broth. Twenty grams of soil was measured from each soil sample and were added to 30 ml broth each. Enriched samples were incubated in Celltreat Bio-Reaction tubes with 0.22- μ m hydrophobic vented membrane tops (<http://www.celltreat.com>), shaking, at 37°C for 6 days. After 6 days of enrichment, samples were extracted using a Qiagen EZ1 advanced XL instrument with the EZ1 advanced XL tissue card and EZ1 DNA tissue kit (QIAGEN, <https://qiagen.com>) or with a Promega Maxwell® CSC Instrument with the Maxwell® RSC Cultured Cells DNA kit (<http://www.promega.com>). Soil samples were processed on the QIAGEN QIAcube using a QIAamp Fast Stool Mini Kit (QIAGEN, <https://qiagen.com>). DNA was tested by the TTS1 PCR as previously described.^{1,2} All enriched samples were cultured on Ashdown's agar and suspect colonies were screened by the InBios Active Melioidosis *Detect*™ (AMD) Rapid Test Kit (<http://inbios.com>).

Results

Epidemiological Findings and Climate Observations

No pets, livestock, or other notable animal exposures of interest were identified for Patient 1 or 2. Patient 2 had a pet dog which was primarily kept outdoors in the yard. Patient 1 did not have any pets.

According to the National Centers for Environmental Information for this Mississippi county, the month of onset for Patient 1 had the highest recorded precipitation in the previous 6 years (11.41 inches), whereas the months of onset for Patient 2 and Patient 3 were relatively dry (4.12 inches, and 4.3 inches, respectively).

Figure S3 - Diagram of soil and water sample locations indicating *B. pseudomallei* positive sample locations on property of Patient 1 with associated photographs.

Images were taken on June 19, 2022, at time of sample collection. A) Maureen Ty collects surface water sample “W13” from a puddle in the front yard next to paved driveway B) Julia Petras collects soil sample “S22” with soil auger at a depth of 30 cm in the front yard of the property about 12 feet north from sample A C) Julia Petras collects soil sample “S27” with soil auger at a depth of 30cm in the back yard of the property next to a storm drain about 105 feet west from Samples “W13” and “S22.”

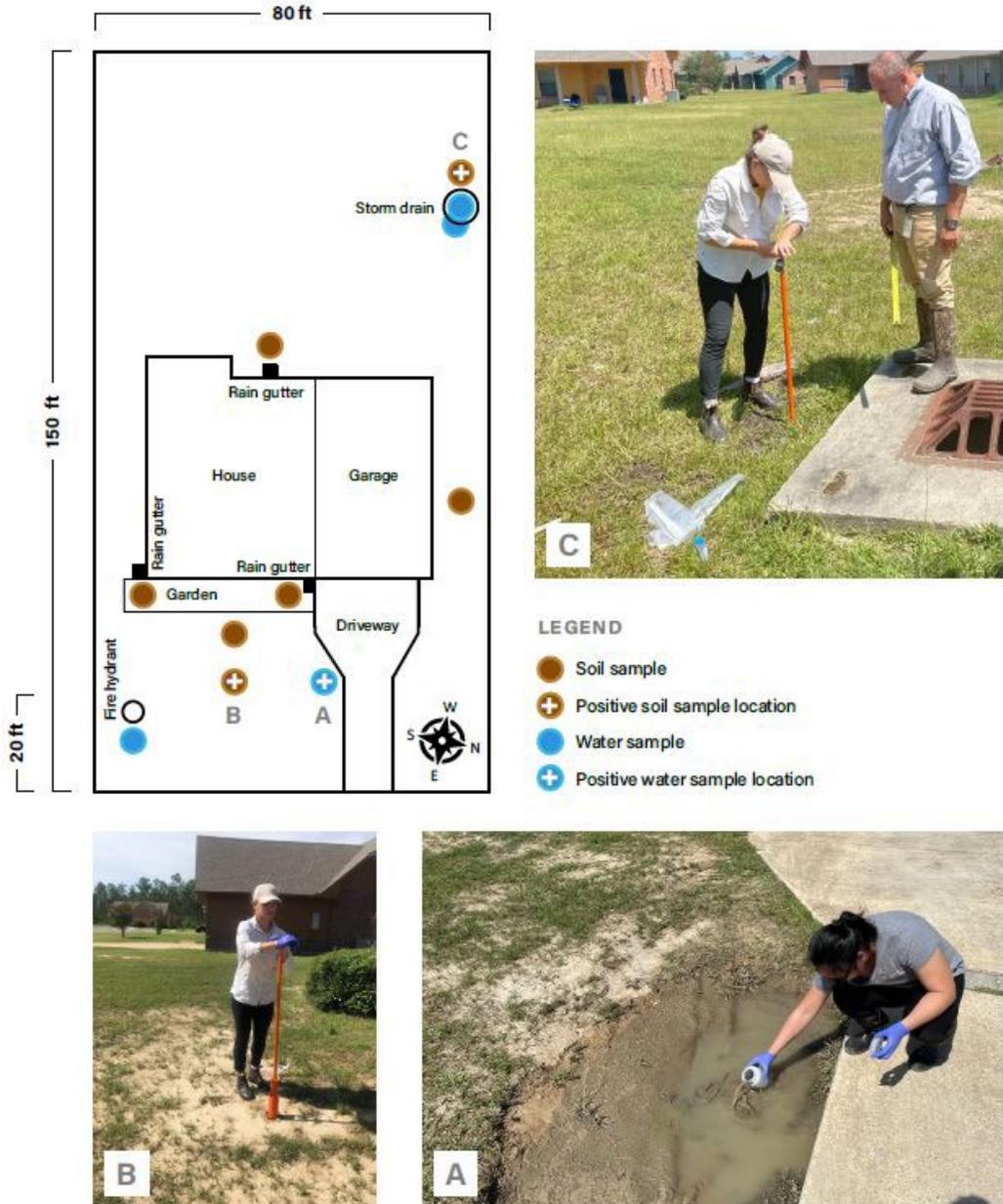


Table S1 - Samples collected with *B. pseudomallei* test results covering the 2020 and 2022 investigations.

Patient	Site	Sample ID	Sample Type	Sample Location	PCR and Culture Result
2020 Investigation – Vessel, Louisiana					
1	1	E001	Floor mat	Crew Bathroom Showerhead external surface	Negative
1	1	E002	Swab	Crew Bathroom Shower soap dish	Negative
1	1	E003	Swab	Crew Bathroom Showerhead neck	Negative
1	1	E004	Swab	Crew Bathroom Shower drain	Negative
1	1	E005	Swab	Crew Bathroom Shower floor	Negative
1	1	E006	Tap Water	Crew Bathroom Shower floor mat	Negative
1	1	E007	Tap Water	Crew Bathroom Exterior showerhead	Negative
1	1	E008	Tap Water	Crew Bathroom Exterior showerhead surface	Negative
1	1	E009	Tap Water	Crew Bathroom Showerhead neck	Negative
1	1	E010	Tap Water	Crew Bathroom Shower stem	Negative
1	1	E011	Tap Water	Crew Bathroom Shower drain external surface	Negative
1	1	E012	Tap Water	Crew Bathroom Shower drain underside surface	Negative
1	1	E013	Sponge Stick	Crew Bathroom Shower drain interior	Negative
1	1	E014	Sponge Stick	Crew Bathroom Shower drain interior	Negative
1	1	E015	Sponge Stick	Crew Bathroom Shower floor in front of drain	Negative
1	1	E016	Sponge Stick	Crew Bathroom Shower floor around drain	Negative
1	1	E017	Sponge Stick	Crew Bathroom Soap dish and shower stem	Negative
1	1	E018	Swab	Crew Bathroom Shower knobs and intake pipe	Negative
1	1	E019	Swab	Crew Bathroom Shower water	Negative
1	1	E020	Swab	Crew Bathroom Sink knobs and area around it	Negative
1	1	E021	Swab	Crew Bathroom Sink basin	Negative
1	1	E022	Swab	Crew Bathroom Sink drain	Negative
1	1	E023	Swab	Crew Bathroom Sink trap	Negative
1	1	E024	Swab	Crew Bathroom Sink faucet aerator exterior	Negative

1	1	E025	Swab	Crew Bathroom Sink faucet aerator exterior	Negative
1	1	E026	Sponge Stick	Crew Bathroom Sink drain exterior surface	Negative
1	1	E027	Sponge Stick	Crew Bathroom Sink drain interior surface	Negative
1	1	E028	Swab	Crew Bathroom Sink basin	Negative
1	1	E029	Swab	Crew Bathroom Sink water	Negative
1	1	E030	Swab	Crew Bathroom Toilet tank water line	Negative
1	1	E031	Swab	Crew Bathroom Toilet tank rubber flapper	Negative
1	1	E032	Swab	Crew Bathroom Toilet tank water line	Negative
1	1	E033	Swab	Crew Bathroom Toilet tank water line	Negative
1	1	E034	Swab	Crew Bathroom Toilet tank rubber flapper	Negative
1	1	E035	Swab	Crew Bathroom Toilet tank rubber flapper	Negative
1	1	E036	Swab	Galley Left sink basin	Negative
1	1	E037	Sponge Stick	Galley Sink faucet aerator exterior	Negative
1	1	E038	Swab	Galley Sink faucet aerator interior	Negative
1	1	E039	Sponge Stick	Galley Left sink drain exterior surface	Negative
1	1	E040	Sponge Stick	Galley Left sink drain interior surface	Negative
1	1	E041	Sponge Stick	Galley Left sink basin	Negative
1	1	E042	Swab	Galley Sink faucet water	Negative
1	1	E043	Swab	Galley Right sink basin	Negative
1	1	E044	Swab	Galley Right sink drain	Negative
1	1	E045	Swab	Galley Sink trap	Negative
1	1	E046	Swab	Galley Sink sprayer nozzle exterior	Negative
1	1	E047	Sponge Stick	Galley Sink sprayer nozzle exterior	Negative
1	1	E048	Sponge Stick	Galley Right sink drain exterior	Negative
1	1	E049	Swab	Galley Right sink drain interior	Negative
1	1	E050	Swab	Galley Right sink basin	Negative
1	1	E051	Swab	Galley Sink sprayer water	Negative
1	1	E052	Swab	Galley Lid door interior	Negative
1	1	E053	Swab	Galley Lid rim interior perimeter	Negative

1	1	E054	Swab	Tank Room Drainage pipe interior	Negative
1	1	E055	Swab	Tank Room Circular surface with red interior	Negative
1	1	E056	Swab	Tank Room Railing under drainage pipe	Negative
1	1	E057	Swab	Tank Room Water from tank drainage pipe	Negative
1	1	E058	Swab	Tank Room Water from tank drainage pipe	Negative
1	1	E059	Swab	Tank Room Water from tank filter pump	Negative

2022 Investigation – Mississippi

2	2	1A	Swab	Body Lotion	Negative
2	2	1B	Grab-sample	Body Lotion	Not tested
2	2	2A	Swab	Conditioner	Negative
2	2	2B	Grab-sample	Conditioner	Not tested
2	2	3A	Swab	Pain Relief	Negative
2	2	3B	Grab-sample	Pain Relief	Not tested
2	2	4	Grab-sample	Wax - Wall Plug-in scented	Not tested
2	2	5A	Swab	Scent Splash-On	Negative
2	2	5B	Grab-sample	Scent Splash-On	Not tested
2	2	6A	Swab	Scent Plug-In	Negative
2	2	6B	Grab-sample	Scent Plug-In	Not tested
2	2	7A	Swab	Cologne 1 (Spray Bottle)	Negative
2	2	7B	Grab-sample	Cologne 1 (Spray Bottle)	Not tested
2	2	8A	Swab	Cologne 2 (Spray Bottle)	Negative
2	2	8B	Grab-sample	Cologne 2 (Spray Bottle)	Not tested
2	2	9A	Swab	Cologne 3 (Spray Bottle)	Negative
2	2	9B	Grab-sample	Cologne 3 (Spray Bottle)	Not tested
2	2	10A	Swab	After Shave	Negative
2	2	10B	Grab-sample	After Shave	Not tested
2	2	11	Grab-sample	Free Testosterone Booster Pill	Negative
2	2	12A	Swab	Cough Syrup	Negative
2	2	12B	Grab-sample	Cough Syrup	Not tested

2	2	13A	Swab	Cleaning Spray	Negative
2	2	13B	Grab-sample	Cleaning Spray	Not tested
2	2	14A	Swab	Dental Adhesive	Negative
2	2	14B	Grab-sample	Dental Adhesive	Not tested
2	2	15A	Swab	Cold Medicine	Negative
2	2	15B	Grab-sample	Cold Medicine	Not tested
2	2	16A	Swab	Ant & Roach Killer	Negative
2	2	16B	Grab-sample	Ant & Roach Killer	Not tested
2	2	W1A	Swab	Kitchen Faucet aerator screen	Negative
2	2	W1B	Water	Kitchen Faucet aerator screen	Negative
2	2	S1	Soil	Next to PVC pipe at side of house	Negative
2	2	S2	Swab	From inside of PVC pipe	Negative
2	2	S3	Swab	From exterior of PVC pipe	Negative
2	2	S4	Soil	Rooted area under tree, front yard	Negative
2	2	S5	Soil	Soft area under tree, front yard	Negative
2	2	S6	Soil	Front garden	Negative
2	2	S7	Soil	Front garden	Negative
2	2	S8	Soil	Tree well next to dog kennel	Negative
2	2	S9	Soil	From backyard under awning next to house	Negative
2	3	S10	Soil	Fishing Area A River water's edge	Negative
2	3	W2A	Swab	Fishing Area A River water's edge	Negative
2	3	W2B	Water	Fishing Area A River water's edge	Negative
2	3	W3	Water	Fishing Area A River right side	Negative
2	3	W4	Water	Fishing Area A River under tree by boat dock	Negative
2	3	W5	Water	Fishing Area A River shore edge at left side	Negative
2	3	W6	Water	Fishing Area A River under covered boat dock	Negative
2	3	W18	Water	Fishing Area A Deep water sample	Negative
2	3	W19	Water	Fishing Area A Under the dock	Negative
2	3	P2	Plant	Fishing Area A Plant with roots from boat dock	Negative

2	3	W20	Water	Fishing Area A Right side of boat ramp	Negative
2	3	S17	Soil	Fishing Area A Right side of boat ramp	Negative
2	3	W21	Water	Fishing Area A Under tree at slope on left side	Negative
2	3	W22	Water	Fishing Area A Off boat ramp, left of center	Negative
2	3	W23	Water	Fishing Area A Right of boat ramp	Negative
2	3	W24	Water	Fishing Area A Scum patch on surface of water	Negative
2	3	W25	Water	Fishing Area A Base of cypress stump	Negative
2	3	W26	Water	Fishing Area A Inside drainage pipe	Negative
2	3	W27	Water	Fishing Area A Wooden post holding	Negative
2	4	W11	Water	Fishing Area C Pond near bank	Negative
2	4	W12	Water	Fishing Area C Pond next to bridge	Negative
2	4	P1	Plant	Fishing Area C Pond plant near bank	Negative
2	4	W17	Water	Fishing Area C Pond under bridge	Negative
1	5	W28	Water	Front Yard puddle next to open fire hydrant	Negative
1	5	P3	Plant	Front Yard Puddle besides open fire hydrant	Negative
1	5	S18	Soil	Front Yard, in-line with front door	Negative
1	5	W13	Water	Front Yard Standing water left of driveway	Positive
1	5	S19	Soil	Front Yard Garden on edge of front porch	Negative
1	5	S20	Swab	Front Yard Gutter right side of front porch	Negative
1	5	S21	Soil	Front Yard Garden left side of front porch	Negative
1	5	S22	Soil	Front Yard Middle of yard	Positive
1	5	S23	Swab	Front Yard Gutter left side of house	Negative
1	5	W14	Water	Back Yard Flood drain	Negative
1	5	W15	Water	Back Yard Flood drain	Negative
1	5	S24	Soil	Back Yard Drainage line from flood drain	Negative
1	5	S25	Swab	Back Yard Flood drain	Negative
1	5	S26	Soil	Back Yard Right side of back patio by gutter	Negative
1	5	W16	Swab	Back Yard Inside open flood drain	Negative
1	5	S27	Soil	Back Yard Behind flood drain (mixed sand/clay)	Positive

1	6	W29	Water	Fishing Area D River at base of rocks	Negative
1	6	W30	Water	Fishing Area D River off the bank slope	Negative
1	6	W31	Water	Fishing Area D River	Negative
1	6	S28	Soil	Fishing Area D River between rocks at water edge	Negative
1	6	P4	Plant	Fishing Area D River plants from crevice	Negative
1	6	S29	Soil	Fishing Area D River right side of wooden pier	Negative
1	6	S30	Soil	Fishing Area D River right side of wooden pier	Negative
1	6	W32	Water	Fishing Area D River under wooden pier	Negative
1	6	S31	Soil	Fishing Area D River from left side of wooden pier	Negative
1	6	W33	Water	Fishing Area D River from left side of wooden pier	Negative
1	6	D1	Swab	Fishing Area D River dock left side	Negative
1	6	P5	Plant	Fishing Area D River plant w/ roots	Negative
1	6	F2	Fish	Fishing Area D River dead fish left of wooden pier	Negative
1	7	W34	Water	Fishing Area E Lake under tree	Negative
1	7	S32	Soil	Fishing Area E Lake between tree roots	Negative
1	7	W35	Water	Fishing Area E Lake front of tree	Negative
1	7	P6	Plant	Fishing Area E Lake from bank	Negative
1	7	S33	Soil	Fishing Area E Lake sandy area	Negative

References

1. Novak RT, Glass MB, Gee JE, et al. Development and evaluation of a real-time PCR assay targeting the type III secretion system of *Burkholderia pseudomallei*. *J Clin Microbiol.* 2006;44(1):85-90. doi:10.1128/JCM.44.1.85-90.2006
2. Dawson P, Duwell MM, Elrod MG, Thompson RJ, Crum DA, Jacobs RM, et al. Human Melioidosis Caused by Novel Transmission of *Burkholderia pseudomallei* from Freshwater Home Aquarium, United States. *Emerg Infect Dis.* 2021;27(12):3030-3035. doi: [10.3201/eid2712.211756](https://doi.org/10.3201/eid2712.211756)