SUMMARY

[0005a] In a first aspect, the present disclosure provides a method of detecting a plurality of target molecules comprising:

extracting target molecules in a dried biological sample from a collection device;

diluting the extracted target molecules into a first dilution and a second dilution;

contacting the first dilution with a first capture reagent to form a first capture reagent affinity complex with its target molecule if the target molecule is present in the first dilution;

contacting the second dilution with a second capture reagent to form a second capture reagent affinity complex with its target molecule if the target molecule is present in the second dilution;

incubating the first and second dilution samples separately to allow capture reagent affinity complex formation; wherein each of the first capture reagent affinity complex and the second capture reagent affinity complex are immobilized on separate first solid supports;

releasing and capturing the first capture reagent affinity complex on a second solid support;

after releasing the first capture reagent affinity complex, releasing and capturing the second capture reagent affinity complex on the second solid support; and

detecting for the presence of or determining the level of the first capture reagent, second capture reagent of the first or second capture reagent affinity complexes, or the presence or amount of the first or second capture reagent affinity complexes.

[0005b] In a second aspect, the present disclosure provides a method of preparing a biological sample for a multiplex assay comprising:

depositing the biological sample comprising a plurality of target molecules onto a collection device;

drying the biological sample on the collection device for a period of time to stabilize the dried sample prior to any temperature fluctuation;

wherein the biological sample is dried for at least 4 hours at about room temperature or below room temperature so that target molecules in the dried biological sample are detectable in the multiplex assay.

[0005c] In a third aspect, the present disclosure provides a method for preparing a liquid sample comprising:

drying the sample at a constant temperature of from -20^{o}C to room temperature for at least four (4) hours to create a dried sample; and

reconstituting the dried sample with a formulation comprising a buffering agent, one or more salts, a chelating agent, a protease inhibitor, a non-ionic surfactant, and an oligonucleotide.

[0005d] In a fourth aspect, the present disclosure provides a method for detecting an analyte in a sample comprising:

drying the sample at a constant temperature of from about -20°C to room temperature for at least four (4) hours to create a dried sample;

reconstituting the dried sample with a formulation comprising a buffering agent, one or more salts, a chelating agent, a protease inhibitor, a non-ionic surfactant, and an oligonucleotide; and

detecting the analyte from the reconstituted sample.

[0006] The foregoing and other objects, features, and advantages of the disclosure will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

[0007] Certain non-limiting exemplary embodiments are as follows:

[0008] In some embodiments, methods of preparing a biological sample for a multiplex assay are disclosed. The methods comprise depositing the biological sample comprising a plurality of target molecules onto a collection device, drying the biological sample on the collection device for a period of time to stabilize the dried sample prior to any temperature fluctuation, wherein the biological sample is dried for at least 4 hours at about room temperature or below room temperature so that target molecules in the dried biological sample are detectable in the multiplex assay.

[0009] In some embodiments, the biological sample is dried at about room temperature, 4°C-8°C, or -20°C. In some embodiments, the dried biological sample is stored at about room temperature or below room temperature prior to detection in the multiplex assay. In some embodiments, the dried biological sample is stored at about 4°C-8°C, or -20°C.

[0010] In some embodiments, the biological sample is dried for at least 6 hours, at least 8 hours, at least 10 hours, at least 12 hours, at least 14 hours, at least 16 hours, at least 18 hours, at least 20 hours, at least 22 hours, one day, at least two days, or at least three days.

[0011] In some embodiments, the biological sample is selected from plasma, serum, urine, whole blood, leukocytes, peripheral blood mononuclear cells, buffy coat, sputum, tears, mucus, nasal washes, nasal aspirate, semen, saliva, peritoneal washings, ascites, cystic fluid, meningeal fluid, amniotic fluid, glandular fluid, lymph fluid, nipple aspirate, bronchial aspirate, bronchial

brushing, synovial fluid, joint aspirate, organ secretions, cells, a cellular extract, and cerebrospinal fluid. In some embodiments, the biological sample is selected from plasma, serum, urine, and whole blood. In some embodiments, the plurality of target molecules are selected from a protein, a peptide, a carbohydrate, a polysaccharide, a glycoprotein, a hormone, a receptor, an antigen, an antibody, a virus, a bacteria, a metabolite, a cofactor, an inhibitor, a drug, a dye, a nutrient, a growth factor, a cell and a tissue.

[0012] In some embodiments, the dried biological sample is homogenized by the drying process. In some embodiments, the plurality of target molecules are extractable from the collection device after the biological sample is dried and detectable in the multiplex assay.

[0013] In some embodiments, methods of detecting a plurality of target molecules are disclosed. The methods comprise extracting target molecules in a dried biological sample from a collection

device, diluting the extracted target molecules into a first dilution and a second dilution, contacting the first dilution with a first capture reagent to form a first capture reagent affinity complex with its target molecule if the target molecule is present in the first dilution, contacting the second dilution with a second capture reagent to form a second capture reagent affinity complex with its target molecule if the target molecule is present in the second dilution, incubating the first and second dilution samples separately to allow capture reagent affinity complex formation; wherein each of the first capture reagent affinity complex and the second capture reagent affinity complex are immobilized on separate first solid supports, releasing and capturing the first capture reagent affinity complex on a second solid support, after releasing the first capture reagent affinity complex, releasing and capturing the second capture reagent affinity complex on the second solid support; and detecting for the presence of or determining the level of the first capture reagent, second capture reagent of the first or second capture reagent affinity complexes, or the presence or amount of the first or second capture reagent affinity complexes.

[0014] In some embodiments, the target molecules are extracted from the collection device in a formulation for at least 5 minutes. In some embodiments, the target molecules are extracted from the collection device in a formulation for at least 5 minutes, at least 10 minutes, at least 15 minutes, at least 20 minutes, at least 25 minutes, at least 30 minutes, at least 35 minutes, at least 40 minutes, at least 45 minutes, at least 50 minutes, at least 55 minutes, at least 60 minutes, at least 70 minutes, at least 80 minutes, at least 90 minutes, at least 100 minutes, at least 110 minutes, at least 120 minutes, at least 130 minutes, at least 140 minutes, at least 150 minutes, at least 160 minutes, at least 170 minutes, at least 180 minutes, at least 190 minutes, or at least 200 minutes.

[0015] In some embodiments, the formulation comprises a buffering agent, one or more salts, a chelating agent, a protease inhibitor, a non-ionic surfactant, and an oligonucleotide.

[0016] In some embodiments, the one or more salts are each independently selected from a sodium salt, a potassium salt and a magnesium salt.

[0017] In some embodiments, the one or more salts comprise a sodium salt, a potassium salt and a magnesium salt. In some embodiments, the sodium salt is NaCl, the potassium salt is KCl and the magnesium salt is MgCl2. In some embodiments, NaCl in the formulation is at a concentration of from about 10 mM to about 500 mM, or from about 50 mM to about 250 mM, or from about 100 mM to about 200 mM, or from about 75-125 mM, or about 100 mM. In some embodiments, the KCl in the formulation is at a concentration of from about 0.5 mM to about 30 mM, or from about 1 mM to about 20 mM, or from about 2 mM to about 15 mM, or from about 4 mM to about 10 mM, or about 5 mM. In some embodiments, the MgCl2 in the formulation is