

**SYSTEMS AND METHODS OF POINT-OF-CARE TESTING FOR URINARY
BIOMARKERS INCLUDING ULTRASENSITIVE IMMUNOMAG-CRISPR LATERAL
FLOW ASSAYS**

5 CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/544,862, filed October 19, 2023, which is incorporated by reference as if disclosed herein in its entirety.

10 STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH AND
DEVELOPMENT

[0002] This invention was made with U.S. Government support under Grant Numbers 1R01AI170424-01, 2U01AI063594-18, and 5U01AI063594-19 awarded by the National Institutes of Health. The United States Government has certain rights in the invention.

BACKGROUND

15 **[0003]** Rapid, accurate, and non-invasive detection of biomarkers in saliva, urine, or nasal fluid is highly beneficial for the identification, early diagnosis, and monitoring of conditions such as cancer, organ failure, transplant rejection, vascular diseases, autoimmune disorders, and infectious diseases. Advancements in sensing and detection platforms have advanced both point-of-care testing (POCT) and point-of-use (POU) technology. One such
20 advancement, clustered regularly interspaced short palindromic repeats/CRISPR-associated (CRISPR/Cas), has been harnessed for genome editing, gene regulation, imaging, and detection. In particular, indiscriminate single-stranded DNA cleavage activity of some CRISPR/Cas nucleases upon guide RNA-dependent DNA binding is attracting attention in biomarker sensing applications.

25 **[0004]** A major challenge in developing activatable CRISPR/Cas-based detection is broadening its use beyond nucleic acid targets, and to detecting other biomarkers including proteins, organic compounds, and metal ions. For example, in the case of protein detection, aptamers or antibodies can be used as capture or detection probes and coupled
30 with CRISPR/Cas-based detection system to achieve high sensitivity and specificity to identify target proteins. Another challenge is tailoring the amplification step for achieving high sensitivity and specificity. Traditional polymerase chain reaction (PCR)-based amplification methods are time-consuming and utilize temperature-controlled devices. Additionally, they can pose a risk of contamination by aerosols, which can compromise the accuracy of the sensor.

[0005] To overcome this problem, alternative isothermal amplification methods have been explored, such as hybridization chain reaction (HCR), T7 transcription amplification, and multiplication of CRISPR/Cas recognition sites. HCR uses DNA hairpins to generate long, branched DNA structures at room temperature, without the need for specialized equipment or enzymes. RNA amplification by T7 RNA polymerase transcription using DNA templates bound to antigen-antibody assemblies can enhance CRISPR/Cas collateral cleavage activity for protein detection. Such trans-cleavage activity is enabled by fluorophore-quencher (FQ) reporters as signaling molecules and a nucleic acid amplification step, such as PCR, LAMP (loop-mediated isothermal amplification) and RPA (recombinase polymerase amplification), to achieve atomolar or greater sensitivity. Overall, this technique offers a highly sensitive and specific way to amplify and detect nucleic acid signals, which has important applications in fields such as medical diagnostics and genetic analysis.

[0006] A Cas12a-coupled “Immuno-CRISPR assay” has also been developed to detect the urinary chemokine ligand 9 (CXCL9), which is a biomarker for non-invasive early detection of acute transplant rejection and cancer. The Immuno-CRISPR assay showed high sensitivity without an amplification step; however, it used specialized equipment for fluorescence detection.

[0007] Lateral flow assays (LFAs) offer the ability to detect biomarkers in an equipment-free, low-cost, and user-friendly manner. Unfortunately, LFAs are approximately three orders of magnitude less sensitive than, for example, enzyme-linked immunosorbent assay (ELISA) or PCR. Given that the limit of detection (LOD) of conventional horseradish peroxidase (HRP)-based ELISA for CXCL9 detection (100 pg/mL) is close to the threshold for borderline kidney transplant rejection (200 pg/mL), this low LOD poses a significant challenge for use of an LFA.

SUMMARY

[0008] Aspects of the present disclosure are directed to methods for detecting one or more target biomarkers in a sample using magnetic particle-antibody complexes and oligonucleotide-antibody complexes for naked-eye detection. The complexes enable magnetic enrichment of target biomarkers, e.g., urinary biomarkers such as chemokine ligand 9 (CXCL9) and chemokine ligand 10 (CXCL10), which can be detected in a lateral flow assay and the like. Embodiments of the present disclosure can achieve a limit of detection (LOD) of 0.6 pg/mL for CXCL9. This value surpasses the detection limits achieved by previously reported assays. The highly sensitive detection method in a lateral flow assay format produced a LOD of 18 pg/mL for CXCL9, thereby enabling non-invasive early detection of acute kidney transplant rejection.

[0009] Aspects of the present disclosure are directed to a method for detecting one or more target biomarkers in a urinary sample. In some embodiments, the method includes obtaining a urinary sample from a patient. In some embodiments, the method includes preparing a plurality of magnetic particle-antibody complexes. In some embodiments, the magnetic particle-antibody complexes include a first biomarker antibody structured to bind to one or more target biomarkers. In some embodiments, the method includes preparing a plurality of oligonucleotide-antibody complexes. In some embodiments, the oligonucleotide-antibody complexes include one or more DNA barcodes and a second biomarker antibody structured to bind to the one or more target biomarkers. In some embodiments, the DNA barcodes include one or more CRISPR/Cas recognition sites. In some embodiments, the method includes preparing one or more reporter complexes. In some embodiments, the one or more reporter complexes include a control portion and a test portion. In some embodiments, the method includes preparing a plurality of Cas-RNA complexes. In some embodiments, the Cas-RNA complexes are configured to bind to the one or more DNA barcodes; be active when bound to the one or more DNA barcodes and inactive when not bound to the DNA barcodes; and cleave the control portion of the reporter complexes from the test portion of the reporter complexes when active. In some embodiments, the method includes contacting the sample with an amount of the magnetic particle-antibody complexes and an amount of the oligonucleotide-antibody complexes to form an amount of sandwich structure complexes with biomarker from the urinary sample bound between first biomarker antibody and second biomarker antibody. In some embodiments, the method includes applying a magnetic field to the sample to form an enriched portion of the sample and a depleted portion of the sample, the enriched portion having an elevated concentration of the sandwich structure complexes relative to the depleted portion. In some embodiments, the method includes contacting the enriched portion with the Cas-RNA complexes and the one or more reporter complexes to form a test urinary sample. In some embodiments, the method includes cleaving the test portion from the control portion of the one or more reporter complexes via active Cas-RNA complexes in the test urinary sample. In some embodiments, the method includes administering the test urinary sample to a lateral flow assay test strip including a control line configured to bind the control portion of the reporter and a test line configured to bind the test portion of the reporter. In some embodiments, the method includes detecting an amount of test portion bound to the test line.

[0010] In some embodiments, the one or more CRISPR/Cas recognition sites include a Cas9 recognition site, a Cas12a recognition site, a Cas13a recognition site, or combinations thereof. In some embodiments, the Cas-RNA complexes include a Cas9/crRNA complex,

a Cas12a/crRNA complex, a Cas13a/crRNA complex, or combinations thereof. In some embodiments, the one or more reporter complexes include a fluorophore-biotin (FB) reporter, fluorophore-quencher (FQ) reporter, or combinations thereof. In some embodiments, the one or more urinary biomarkers include CXCL9, CXCL10, or combinations thereof.

5 **[0011]** In some embodiments, the first biomarker antibody includes first heavy chain variable regions and first light chain variable regions, and the second biomarker antibody includes second heavy chain variable regions and second light chain variable regions. In some
10 embodiments, the first heavy chain variable regions and the second heavy chain variable regions have above about 85% sequence identity. In some embodiments, the first light chain variable
regions and the second light chain variable regions have above about 85% sequence identity.

[0012] Aspects of the present disclosure are directed to a biomarker assay kit. In some
embodiments, the kit includes a plurality of magnetic particle-antibody complexes, the magnetic
particle-antibody complexes including a first biomarker antibody structured to bind to one or
more target biomarkers; a plurality of oligonucleotide-antibody complexes, the oligonucleotide-
15 antibody complexes including one or more DNA barcodes and a second biomarker antibody
structured to bind to the target biomarkers, wherein the DNA barcodes include one or
more CRISPR/Cas recognition sites; one or more reporter complexes, wherein the reporter
complexes include a control portion and a test portion; and a plurality of Cas-RNA complexes.
As discussed above, in some embodiments, the Cas-RNA complexes are configured to: bind to
20 the DNA barcodes; be active when bound to the DNA barcodes and inactive when not bound to
the DNA barcodes; and cleave the control portion of the reporter complexes from the test portion
of the reporter complexes when active. In some embodiments, the kit includes a lateral flow
assay test strip, a microfluidic apparatus, or combinations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

25 **[0013]** The drawings show embodiments of the disclosed subject matter for the purpose
of illustrating the invention. However, it should be understood that the present application is not
limited to the precise arrangements and instrumentalities shown in the drawings, wherein:

[0014] FIG. 1 is a chart of a method for detecting one or more target biomarkers in a
sample according to embodiments of the present disclosure;

30 **[0015]** FIG. 2A portrays testing data demonstrating detection of urinary biomarkers such
as chemokine ligand 9 (CXCL9) and chemokine ligand 10 (CXCL10) in synthetic urine using
systems and methods according to embodiments of the present disclosure;

[0016] FIG. 2B portrays a schematic illustration of a magnetic-particle complex-based immunoassay consistent with embodiments of the present disclosure;

[0017] FIG. 2C is a graph showing the effect of incubation time in a magnetic-particle complex-based immunoassay consistent with embodiments of the present disclosure;

5 [0018] FIG. 3A portrays testing data demonstrating visual detection of urinary biomarkers (and corresponding calibration curves) using systems and methods according to embodiments of the present disclosure;

[0019] FIG. 3B portrays testing data demonstrating the benefits of enrichment of target biomarker and reduction of non-specific binding enabled by using systems and methods
10 according to embodiments of the present disclosure;

[0020] FIG. 4 portrays testing data demonstrating detection of urinary biomarkers in patient samples using systems and methods according to embodiments of the present disclosure;

[0021] FIG. 5 portrays testing data demonstrating the limit of detection (LOD) of systems and methods according to embodiments of the present disclosure;

15 [0022] FIG. 6 portrays testing data demonstrating detection of urinary biomarkers in patient samples using systems and methods according to embodiments of the present disclosure;

[0023] FIG. 7A is a chart of a method for detecting one or more target biomarkers in a sample according to embodiments of the present disclosure; and

[0024] FIG. 7B is a chart of a method for detecting one or more target biomarkers in a
20 sample according to embodiments of the present disclosure.

DETAILED DESCRIPTION

[0025] Referring now to FIG. 1, some embodiments of the present disclosure are directed to a method 100 for detecting one or more target biomarkers in a sample. In some embodiments, at 102, the sample is provided by and/or obtained from an individual, e.g., a patient. In some
25 embodiments, the individual is a human patient. In some embodiments, the sample is any substance obtained from an individual suspected of having a concentration of the target biomarker, e.g., human patients being monitored for acute kidney transplant rejection. In some embodiments, the sample is a urinary sample. In some embodiments, the target biomarker includes chemokine ligand 9 (CXCL9), chemokine ligand 10 (CXCL10), or combinations
30 thereof.

[0026] In some embodiments, at 104, a plurality of magnetic particle-antibody complexes are prepared. In some embodiments, the magnetic particle-antibody complexes include one or more first antibodies structured to bind to one or more target biomarkers. In some embodiments, the first biomarker antibody includes one or more first heavy chain variable regions and one or more first light chain variable regions, e.g., VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, VLCDR3, etc., configured to bind to the target biomarkers. In some embodiments, the first biomarker antibody is complexed to one or more magnetic particles. In some embodiments, the magnetic particles are composed of any suitable materials that can be complexed with the first biomarker antibodies and further enable the magnetic particle-antibody complexes to be spatially manipulated via application of a magnetic field. In some embodiments, the magnetic particles include magnetic beads. In some embodiments, the magnetic particles and first biomarker antibodies are complexed via any suitable means, e.g., via a linker, direct covalent binding, non-covalent protein-ligand interaction, simple adsorption, etc.

[0027] Still referring to FIG. 1, in some embodiments, at 106, a plurality of oligonucleotide-antibody complexes are prepared. In some embodiments, the oligonucleotide-antibody complexes include one or more oligonucleotides and a second biomarker antibody structured to bind to the one or more target biomarkers. In some embodiments, the second biomarker antibody is structured to bind the same target biomarker as the first biomarker antibody. In some embodiments, the second biomarker antibody includes one or more second heavy chain variable regions and one or more second light chain variable regions, e.g., VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, VLCDR3, etc., configured to bind to the target biomarkers. In some embodiments, the first biomarker antibody and the second biomarker antibody have sufficient sequence identity to display the same or substantially the same binding affinity for the target biomarkers. In some embodiments, the first heavy chain variable regions and the second heavy chain variable regions have above about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity. In some embodiments, the first light chain variable regions and the second light chain variable regions have above about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity. In some embodiments, the first biomarker antibody and the second biomarker antibody are configured to bind target biomarkers in a sample obtained from a patient, e.g., a human. In some embodiments, the first biomarker antibody and the second biomarker antibody are configured to bind urinary biomarkers. In some embodiments, the first biomarker antibody and the second biomarker antibody are configured to bind CXCL9, CXCL10, or combinations thereof.

- [0028] In some embodiments, the oligonucleotides in the oligonucleotide-antibody complexes include one or more DNA barcodes. In some embodiments, the DNA barcode is self-assembled. In some embodiments, the DNA barcode includes one or more CRISPR/Cas recognition sites. In some embodiments, the one or more CRISPR/Cas recognition sites include a Cas9 recognition site, a Cas12a recognition site, a Cas13a recognition site, or combinations thereof. In some embodiments, the one or more CRISPR/Cas recognition sites include one or more Cas12a recognition sites. In some embodiments, the oligonucleotides and the second biomarker antibodies are complexed via any suitable means, e.g., via a linker, direct covalent binding, non-covalent protein-ligand interaction, simple adsorption, etc.
- 10 [0029] Referring again to FIG. 1, in some embodiments, at 108, one or more reporter complexes are prepared. In some embodiments, reporter complexes include a control portion and a test portion. In some embodiments, the control portion, the test portion, or combinations thereof are configured to be detectable by a predetermined mechanism or process. In some embodiments, the control portion is configured to be detected and confirm the presence of the reporter. In some embodiments, the test portion is configured to be detected and confirm presence of a concentration of a biomarker in a sample, as will be discussed in greater detail below. In some embodiments, the test portion includes one or more fluorophores. In some embodiments, one or more reporter complexes include a fluorophore-biotin (FB) reporter, fluorophore-quencher (FQ) reporter, or combinations thereof.
- 20 [0030] In some embodiments, at 110, a plurality of Cas-RNA complexes are prepared. In some embodiments, the Cas-RNA complexes are configured to bind to the oligonucleotides in the oligonucleotide-antibody complexes, e.g., one or more DNA barcodes. In some embodiments, the Cas-RNA complexes are configured to be active when bound to the DNA barcodes and inactive when not bound to the DNA barcodes. In some embodiments, the Cas-RNA complexes are configured to cleave the reporter complexes when active, e.g., upon DNA barcode binding. In some embodiments, the Cas-RNA complexes are configured to cleave the control portion of the reporter complexes from the test portion of the reporter complexes when active. In some embodiments, the Cas-RNA complexes include a Cas9/crRNA complex, a Cas12a/crRNA complex, a Cas13a/crRNA complex, or combinations thereof.
- 25 [0031] In some embodiments, at 112, the sample is contacted with an amount of the magnetic particle-antibody complexes and an amount of the oligonucleotide-antibody complexes. The magnetic particle-antibody and oligonucleotide-antibody complexes each bind to target biomarkers in the sample, forming sandwich structure complexes with biomarkers from
- 30

the sample bound between the first biomarker antibody and the second biomarker antibody. In some embodiments, at 114, a magnetic field is applied to the sample. The magnetic field acts upon the magnetic particle bound to the biomarker via the first biomarker antibody, causing spatial manipulation of the biomarker within the magnetic field to form a portion of sample “enriched” for the biomarkers, thus also forming a “depleted” portion of the sample having a reduced concentration of magnetic particles and, as a result, biomarkers. While embodiments of the present disclosure describe forming a sandwich structure complex with biomarkers bound between the first biomarker antibody and the second biomarker antibody prior to application of the magnetic field at 114, the present disclosure is not intended to be limited in this regard, as magnetic particle-antibody complexes can be bound to biomarkers in the sample and the magnetic field applied first to form the enriched portion, and then oligonucleotide-antibody complexes can be contacted with the enriched portion to form the sandwich structure complexes.

[0032] Still referring to FIG. 1, in some embodiments, at 116, the enriched portion of the sample is contacted with the Cas-RNA complexes and the one or more reporter complexes to form a test sample. As discussed above, the Cas-RNA complexes are configured to bind to the DNA barcodes in the oligonucleotide-antibody complexes. At contacting 116, the Cas-RNA complexes bind to DNA barcodes in the oligonucleotide-antibody complexes that are in turn bound to the biomarkers via the second biomarker antibody. Upon binding to the DNA barcodes, e.g., one or more CRISPR/Cas recognition sites, the Cas-RNA complexes in the test sample become active. In some embodiments, at 118, the test portion is cleaved from the control portion of reporter complexes in the test sample via the active Cas-RNA complexes.

[0033] The control portions and the test portions of the reporter complexes, e.g., cleaved and uncleaved reporter complexes, can then be detected to confirm the presence and/or absence of a concentration of target biomarkers in the sample. The control portions and/or test portions of the reporter complexes can be detected via any suitable means. In some embodiments, the control portions and/or test portions of the reporter complexes are detected using a lateral flow assay, microfluidic apparatus, or combinations thereof. In some embodiments, at 120, the test sample is administered to a lateral flow assay test strip. In some embodiments, the lateral flow assay test strip includes a control line configured to bind the control portion of the reporter complex and a test line configured to bind the test portion of the reporter complex. When administered to and migrated along the lateral flow assay test strip, the control line binds control portions from the reporter complexes, whether bound to the test portion or not. Test portions from reporter complexes cleaved by the Cas-RNA complexes, however, do not bind to the

control line and instead proceed to and are finally bound by the test line. In some embodiments, at 122, an amount of test portion bound to the test line is detected. In some embodiments, the lateral flow assay test strip is a commercially available paper-based strip including a streptavidin-coated control line and an anti-rabbit antibody-coated test line.

5 [0034] Firstly, binding of the control portions to the control line enables confirmation that the testing portions should also be detectable on the strip. Secondly, if the testing portion is detected on the control line, then the reporter complex was not cleaved, indicating that the Cas-RNA complexes in the test sample were not active, which further indicates that there was little or no target biomarker in the sample to which the first and second biomarker antibodies could bind and form sandwich structure complexes for magnetic enrichment. If the testing portion is
10 detected on the test line, then the reporter complexes were cleaved, indicating the presence of a concentration of target biomarker in the sample. Further, the amount of testing portion bound to the test line relative to testing portion bound to the control line (via the control portion) is an indicator of the concentration of target biomarker in the sample, since increased binding of
15 testing portion at the testing line indicates greater activation of Cas-RNA complexes, i.e., more reporter complexes are cleaved, which corresponds to formation of a larger/enriched amount of sandwich structure complexes including bound target biomarkers.

[0035] Referring now to FIG. 2A-2C, in an exemplary embodiment, target biomarkers in urine, such as CXCL9 and CXCL10, were captured and concentrated by magnetic particle-
20 antibody complexes consistent with embodiments of the present disclosure, followed by the formation of the sandwich structure complexes with oligonucleotide-antibody complexes. As used herein, systems and methods for detecting biomarkers utilizing the magnetic particle-antibody complexes and the oligonucleotide-antibody complexes consistent with some embodiments of the present disclosure are also referred to as the “ImmunoMag-CRISPR assay.”
25 Similarly, the use of the ImmunoMag-CRISPR assay in combination with a lateral flow assay, e.g., with a lateral flow assay test strip as described above, consistent with some embodiments of the present disclosure, is also referred to as “ImmunoMag-CRISPR LFA.”

[0036] In such embodiments, e.g., for the detection of urinary CXCL9 and CXCL10, the first biomarker antibody was immobilized on three types of magnetic beads: carboxylate-
30 functionalized, streptavidin-coated, and polystyrene-coated magnetic beads, via covalent attachment, biotin-streptavidin binding, and simple adsorption, respectively. To evaluate the detection sensitivity of each magnetic bead-based immunoassay, a detection antibody-HRP complex (Ab-HRP) was used. The signal-to-noise ratio (SNR) was calculated by dividing the

activity in the presence of the target biomarker by the activity in the absence of the target biomarker. In these exemplary embodiments, through the evaluation of HRP activity, the magnetic bead-based immunoassay using carboxylate-functionalized magnetic beads showed the best performance (see FIG. 2A) for detecting CXCL9 in synthetic urine when compared to
5 assays using SA-coated and polystyrene-coated magnetic beads (see FIG. 2B). The capture antibody loading of carboxylate-functionalized magnetic beads was measured to be 5.3 $\mu\text{g}/\text{mg}$ bead, which is higher than that of both streptavidin-coated and polystyrene-coated magnetic beads. Without wishing to be bound by theory, the improved SNR of magnetic bead-based immunoassay using carboxylate-functionalized magnetic beads was due to a combination of
10 increased signal from the binding of CXCL9 and a lower background signal. In some embodiments, the magnetic enrichment time, e.g., of CXCL9, and the binding time, e.g., of the Ab-HRP, is about 20 min each (see FIG. 2C).

[0037] Referring again to FIG. 2A, as discussed above, in some embodiments, the “detection” or second biomarker antibody-conjugated DNA barcode complex includes
15 multiple Cas12a recognition sites. Such Cas12a-coupled ImmunoMag-CRISPR assay embodiments combined “capture” antibody-immobilized magnetic beads and “detection” antibody-streptavidin-DNA barcode (Ab-DNA barcode) complexes. FQ reporter was used as a substrate to measure *trans*-cleavage activity of Cas12a activated by the DNA barcode complexes. The SNR of Cas12a-coupled ImmunoMag-CRISPR assay (170) was higher than that
20 of the HRP-coupled magnetic bead-based immunoassay (67), indicating that magnetic bead-based immunoassay incorporated the Ab-DNA barcode complex. In another exemplary embodiment, this Cas12a-coupled assay was used to detect CXCL10 and observed an SNR for CXCL10 (85), which was also functional.

[0038] In another exemplary embodiment, the ImmunoMag-CRISPR assay was
25 evaluated under conditions of varying CXCL9 concentrations spiked into synthetic urine, and compared to a magnetic bead-based immunoassay using an Ab-HRP complex. The LODs of CXCL9 using the ImmunoMag-CRISPR assays with Ab-DNA barcode complex and the magnetic bead-based immunoassay using Ab-HRP complex were estimated to be 0.6 and 8.0 pg/mL , respectively, demonstrating the improvement in sensitivity achieved as a result
30 of the embodiments of the present disclosure. The LOD of previous well plate-based Immuno-CRISPR assays was 14 pg/mL . Hence, the LOD of embodiments of the ImmunoMag-CRISPR assay from the present disclosure is more than 20-times lower than that of the well plate-based Immuno-CRISPR assay, without wishing to be bound by theory, as a result of the enhanced capture and concentration of target CXCL9 proteins by magnetic enrichment. The sensitivity of

ImmunoMag-CRISPR assay for detection of CXCL9 is significantly improved over chemiluminescence-based single-plex micropatterned aqueous two-phase (LOD: 28 pg/mL) and AlphaLISA (LOD: 20 pg/mL) assays. In another exemplary embodiment, the ImmunoMag-CRISPR assay was then applied to detect CXCL10, which resulted in an LOD of 2.4 pg/mL, which is nearly 10-fold lower than that of the HRP-based ELISA.

[0039] Referring now to FIGs. 3A-3B, ImmunoMag-CRISPR LFA consistent with embodiments of the present disclosure was tested as a function of CXCL9 or CXCL10 concentration spiked into synthetic urine. ImmunoMag-CRISPR LFA was also compared with a lateral flow assay combined with the well plate-based Immuno-CRISPR assay ("Immuno-CRISPR LFA") as a control. The LOD of each assay was estimated by evaluating the ratio of the test to control line signal intensity (T/C ratio) for various concentrations of CXCL9 or CXCL10 using ImageJ density analysis. The LOD of ImmunoMag-CRISPR LFA for CXCL9 was calculated to be 18 pg/mL. In contrast, the LOD of 96 well plate-based Immuno-CRISPR LFA for CXCL9 detection was 570 pg/mL. This significant enhancement of detection sensitivity, without wishing to be bound by theory, resulted from enrichment of the target biomarker (CXCL9) and reduction of non-specific binding in ImmunoMag-CRISPR LFA (see FIG. 3B). A urinary CXCL9 level of 200 pg/mL is a threshold value for early detection of kidney transplant rejection using the traditional HRP-based ELISA assay, and corresponds to a T/C ratio of 0.9 ± 0.1 in the ImmunoMag-CRISPR LFA. Exemplary embodiments of the ImmunoMag-CRISPR LFA were then used to detect CXCL10 spiked into urine. A similar trend as with CXCL9 detection was observed. No cross-reactivity between urinary CXCL9 and CXCL10 (1000 pg/mL each) in the ImmunoMag-CRISPR LFA, as well as the ImmunoMag-CRISPR assay (see again, FIG. 3B), was observed. Specifically, CXCL9 with the CXCL10 capture antibody and CXCL10 with CXCL9 capture antibody did not result in a signal above background.

[0040] Referring now to FIG. 4-FIG. 5, patient urine samples ($n = 42$) obtained from the Clinical Trials in Organ Transplantation (CTOT)-19 trial were tested for CXCL9 using exemplary embodiments of ImmunoMag-CRISPR LFA. ImmunoMag-CRISPR LFA samples with T/C ratio ≥ 0.9 , corresponding to HRP-based ELISA at least 200 pg/mL CXCL9, were classified as positive, while T/C ratio < 0.35 , corresponding to HRP-based ELISA < 100 pg/mL (LOD value), were classified as negative. Intermediate ranges in the two assays were classified as borderline positive. Based on these cutoff ranges, 11 samples in ImmunoMag-CRISPR LFA were classified as positive and 12 samples in HRP-based ELISA were classified as positive. The extra positive with HRP-based ELISA was sample 20, which had T/C = 0.85 determined by

ImmunoMag-CRISPR LFA and 220 pg/mL CXCL9 determined by HRP-ELISA, both values near the positive cutoff values for the two methods (see FIG. 5).

[0041] The increased sensitivity of the ImmunoMag-CRISPR LFA embodiments consistent with the present disclosure compared with HRP-ELISA is evident in the detectable ($> \text{LOD}$) signal for the former versus the latter. Specifically, for $0.20 < \text{T/C} < 0.35$, five samples had detectable levels of CXCL9. These all correspond to $\text{CXCL9} < 100 \text{ pg/mL}$, and hence, undetectable as being below LOD for the HRP-ELISA assay. Interestingly, for the borderline positive samples, only five are detected by HRP-ELISA compared to 11 for ImmunoMag-CRISPR LFA. Without wishing to be bound by theory, this suggests that the ImmunoMag-CRISPR LFA has a greater dynamic range and can better distinguish between borderline positive and negative urinary CXCL9 levels.

[0042] Referring now to FIG. 6, broadly similar results were obtained for CXCL10 detection. Based on comparison of ImmunoMag-CRISPR LFA consistent with embodiments of the present disclosure and HRP-ELISA for CXCL10, the former had 22 samples showing $> \text{LOD}$ detectable signal compared with 17 for the latter, once again reflecting the increased sensitivity. In aggregate, these results suggest that the systems and methods of the embodiments of the present disclosure have the potential for very early-stage identification, e.g., of conditions that are indicators of worsening injury in kidney transplant recipients.

[0043] Referring now to FIG. 7A, some embodiments of the present disclosure are directed to a method 700 for detecting one or more target biomarkers in a sample. As discussed above, in some embodiments, the one or more target biomarkers include a urinary biomarker. In some embodiments, the urinary biomarker includes CXCL9, CXCL10, or combinations thereof.

[0044] In some embodiments, at 702, a sample from a patient is obtained. In some embodiments, the sample is obtained 702 from a human patient. In some embodiments, at 704, a first composition is prepared. In some embodiments, the first composition includes a plurality of magnetic particles, e.g., magnetic beads, complexed with one or more first biomarker antibodies, and further a plurality of second biomarker antibodies complexed with one or more DNA barcodes. As discussed above, while this embodiment describes a first composition where the first biomarker antibody-containing complexes and second biomarker antibody-containing complexes are both present, the present disclosure is not intended to be limiting in this regard, as the second biomarker antibody-containing complexes can be prepared in a separate composition. In some embodiments, the first biomarker antibody includes one or more first heavy chain variable regions and one or more first light chain variable regions, e.g., VHCDR1, VHCDR2,

VHCDR3, VLCDR1, VLCDR2, VLCDR3, etc., configured to bind to target biomarkers. In some embodiments, the second biomarker antibody is structured to bind the same target biomarker as the first biomarker antibody. In some embodiments, the second biomarker antibody includes one or more second heavy chain variable regions and one or more second light chain variable regions, e.g., VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, VLCDR3, etc., configured to bind to the target biomarkers. In some embodiments, the first biomarker antibody and the second biomarker antibody have sufficient sequence identity to display the same or substantially the same binding affinity for the target biomarkers. In some embodiments, the first heavy chain variable regions and the second heavy chain variable regions have above about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity. In some embodiments, the first light chain variable regions and the second light chain variable regions have above about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity.

[0045] In some embodiments, the DNA barcodes include one or more CRISPR/Cas recognition sites. In some embodiments, the one or more CRISPR/Cas recognition sites include a Cas9 recognition site, a Cas12a recognition site, a Cas13a recognition site, or combinations thereof. In some embodiments, the one or more CRISPR/Cas recognition sites include one or more Cas12a recognition sites.

[0046] In some embodiments, at 706, a second composition is prepared. In some embodiments, the second composition includes a plurality of Cas-RNA complexes. As discussed above, the Cas-RNA complexes are active when bound with the DNA barcodes and inactive when not bound with the one or more DNA barcodes. In some embodiments, the Cas-RNA complexes include a Cas9/crRNA complex, a Cas12a/crRNA complex, a Cas13a/crRNA complex, or combinations thereof.

[0047] In some embodiments, at 708, the sample is contacted with the first composition. As discussed above, upon contacting 708, the first biomarker antibodies and the second biomarker antibodies bind with the target biomarkers in the sample to form an amount of sandwich structure complexes with biomarker bound between the first biomarker antibody and the second biomarker antibody. In some embodiments, at 710, a magnetic field is applied to the sample to initiate movement of the magnetic particles, and thus bound target biomarkers, therein. In some embodiments, at 712, the sandwich structure complexes migrate via the magnetic field to form a first portion of the sample enriched for the sandwich structure complexes relative to a second portion of the sample, e.g., a depleted portion.

[0048] In some embodiments, at 714, the first portion of the sample, enriched for magnetic particles and thus target biomarkers, is contacted with the second composition to bind the DNA barcodes with the Cas-RNA complexes. In some embodiments, at 716, the first portion of the sample is contacted with one or more reporter complexes as well. As discussed above, in some embodiments, the reporter complexes include a control portion and a test portion. In some
5 embodiments, the one or more reporter complexes include an FB reporter, FQ reporter, or combinations thereof. In some embodiments, at 718, the test portion is cleaved from the control portion of the one or more reporter complexes via the active Cas-RNA complexes. In some embodiments, at 720, an amount of test portion cleaved from the reporter complexes is detected.

10 [0049] Referring now to FIG. 7B, in some embodiments of detecting 720 an amount of test portion cleaved from the reporter complexes, an amount of the first portion, e.g., the enriched portion, is administered 720A to a lateral flow assay test strip. As discussed above, in some embodiments, the lateral flow assay test strip includes a control line configured to bind the control portion of the reporter and a test line configured to bind the test portion of the reporter.
15 In some embodiments, the lateral flow assay test strip is a commercially available paper-based strip including a streptavidin-coated control line and an anti-rabbit antibody-coated test line. In some embodiments, at 720B, the first portion is migrated along the lateral flow assay test strip. In some embodiments, at 720C, the control portion of reporter complexes is bound at the control line. In some embodiments, at 720D, an amount of test portion cleaved from the reporter
20 complexes is bound at the test line.

[0050] Some embodiments of the present disclosure are directed to a biomarker assay kit. In some embodiments, the biomarker assay kit includes a plurality of magnetic particle-antibody complexes. In some embodiments, the magnetic particle-antibody complexes include a first biomarker antibody structured to bind to one or more target biomarkers, e.g., urinary biomarkers
25 such as CXCL9, CXCL10, or combinations thereof. In some embodiments, the magnetic particles include magnetic beads. In some embodiments, the biomarker assay kit includes a plurality of oligonucleotide-antibody complexes. In some embodiments, the oligonucleotide-antibody complexes include one or more DNA barcodes and a second biomarker antibody structured to bind to the target biomarkers. In some embodiments, the DNA barcodes include
30 one or more CRISPR/Cas recognition sites. In some embodiments, the one or more CRISPR/Cas recognition sites include a Cas9 recognition site, a Cas12a recognition site, a Cas13a recognition site, or combinations thereof. In some embodiments, the one or more CRISPR/Cas recognition sites include one or more Cas12a recognition sites.

[0051] In some embodiments, the first biomarker antibody includes one or more first heavy chain variable regions and one or more first light chain variable regions, e.g., VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, VLCDR3, etc., configured to bind to target biomarkers. In some embodiments, the second biomarker antibody is structured to bind the same target biomarker as the first biomarker antibody. In some embodiments, the second biomarker antibody includes one or more second heavy chain variable regions and one or more second light chain variable regions, e.g., VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, VLCDR3, etc., configured to bind to the target biomarkers. In some embodiments, the first biomarker antibody and the second biomarker antibody have sufficient sequence identity to display the same or substantially the same binding affinity for the target biomarkers. In some embodiments, the first heavy chain variable regions and the second heavy chain variable regions have above about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity. In some embodiments, the first light chain variable regions and the second light chain variable regions have above about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity.

[0052] In some embodiments, the biomarker assay kit includes one or more reporter complexes. In some embodiments, the reporter complexes include a control portion and a test portion. In some embodiments, the one or more reporter complexes include an FB reporter, an FQ reporter, or combinations thereof.

[0053] In some embodiments, the biomarker assay kit includes a plurality of Cas-RNA complexes. As discussed above, in some embodiments, the Cas-RNA complexes are configured to bind to the DNA barcodes, be active when bound to the DNA barcodes and inactive when not bound to the DNA barcodes, and cleave the control portion of the reporter complexes from the test portion of the reporter complexes when active. In some embodiments, the Cas-RNA complexes include a Cas9/crRNA complex, a Cas12a/crRNA complex, a Cas13a/crRNA complex, or combinations thereof. In some embodiments, the biomarker assay kit includes a lateral flow assay test strip, a microfluidic apparatus, or combinations thereof to help detect target biomarkers in a sample. In some embodiments, the lateral flow assay test strip includes a control line configured to bind the control portion of the reporter and a test line configured to bind the test portion of the reporter. In some embodiments, the lateral flow assay test strip is a commercially available paper-based strip including a streptavidin-coated control line and an anti-rabbit antibody-coated test line.

[0054] Embodiments of the present disclosure include magnetic enrichment of a patient sample, e.g., a urine sample, for the one or more biomarkers. In this exemplary embodiment, first biomarker antibodies on magnetic beads bind with a target biomarker, which is further bound by the second biomarker antibody/DNA barcode complex, e.g., a detection antibody-streptavidin (SA)-DNA barcode complex formed by the antigen-antibody interaction, to form sandwich structure complexes. A magnetic field is then applied to form a portion of the sample enriched for the biomarkers relative to the remainder of the sample, which is then separated from the remainder of the sample. Cas-RNA complexes bind to and are activated by the DNA barcodes as discussed above. Subsequent contact with reporter complexes results in cleavage of the reporter complexes to control portions and test portions. The control and test portions are then detected, e.g., reaction products from the ImmunoMag-CRISPR assay are applied to a paper-based strip such as a commercially available LFA strip. In some embodiments, the signal, e.g., from the strip, is detectable with the human eye, camera, etc., or combinations thereof.

15 EXAMPLES

[0055] EnGen® Lba Cas12a (Cpf1) and buffer for Cas12a were purchased from New England Biolabs (Ipswich, MA). Oligonucleotides with biotin modification for DNA barcode preparation, CRISPR RNA (crRNA), FQ reporter, and FB reporter for Cas12a were synthesized by Integrated DNA Technologies (Coralville, IA) (see Table 1 below). Dynabeads™ MyOne™ with carboxylic acid, Dynabeads™ M-280 with streptavidin and streptavidin were purchased from Thermo Fisher Scientific (Waltham, MA). Polystyrene based magnetic particles were purchased from Magsphere (Pasadena, CA). 1-Ethyl-3-(3-(dimethylamino)propyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), 2-(N-morpholino) ethanesulfonic acid (MES), phosphate buffered saline (PBS) and Tween 20 were purchased from Sigma-Aldrich (St. Louis, MO). Streptavidin-conjugated HRP, substrate reagents for HRP activity, recombinant human CXCL9 and CXCL10, and their capture and detection antibodies were purchased from R&D Systems (Minneapolis, MN). Synthetic urine was purchased from Medica Corporation (Bedford, MA). Lateral flow assay kit was purchased from Milenia Biotec GmbH (Gießen, Germany). All solutions were prepared with purified water by a Milli-Q purification system from Millipore (Burlington, MA).

Oligonucleotide Name	Sequence (5' → 3')
DNA barcode-F	/biotin/TAAAGTTGCTTTGCACAACATGGGGGATCATGTAACCTGTCGCCCT
DNA barcode-R	/biotin/AGGGCGACAAGTTACATGATCCCCCATGTTGTGCAAAGCAACTTTA
Fluorophore-quencher (FQ) reporter	/56-FAM/TTATT/3IABKFQ/
Fluorophore-biotin (FB) reporter	/56-FAM/TTATT/biotin/

Table 1: Oligonucleotides used in exemplary embodiments of the present disclosure.

[0056] Preparation of antibody-conjugated magnetic beads. Three types of magnetic beads were used: carboxylate functionalized, streptavidin-coated, and polystyrene-coated. Antibody was immobilized onto the carboxylate functionalized magnetic beads using EDC/NHS chemistry. Carboxylate functionalized magnetic beads (2 mg) were mixed with EDC (25 mg/mL) and NHS (25 mg/mL) in MES buffer (50 mM, pH 6.0) under slow tilt rotation at room temperature for 30 minutes, followed by washing with MES buffer by magnetic capture and resuspension. The capture antibody in PBS (1 mL, 200 µg/mL) was added to EDC/NHS-treated magnetic beads. The capture antibody was also added to non-treated polystyrene-coated magnetic beads. In the case of streptavidin-coated magnetic beads, biotinylated capture antibody was used. The mixtures were then incubated under slow tilt rotation at room temperature for 30 min, and at 4°C overnight. To block each mixture, 1 mL of BSA solution in PBS was added and incubated under slow tilt rotation at room temperature for another 30 minutes, followed by washing 3-times with washing buffer (PBS with Tween 20) by magnetic capture and resuspension. Antibody loading on each magnetic bead was estimated. In brief, the protein concentrations of the prepared capture antibody solution and the supernatants from three PBS washes were determined by using a Pierce Micro-BCA protein assay kit from Thermo Fisher Scientific. The amount of antibody immobilized onto each magnetic bead was calculated by subtracting the total protein determined in the supernatants from the initial protein concentration in the antibody solution.

[0057] Magnetic bead-based immunoassays for urinary biomarker detection. Two magnetic bead-based immunoassays consistent with embodiments of the present disclosure were prepared: fluorescence-based ImmunoMag-CRISPR assay and ImmunoMag-CRISPR LFA for urinary biomarker detection. First, 1 mL of CXCL9 or CXCL10 solution in synthetic urine was added to each mixture and incubated under slow tilt rotation at room temperature for 20 minutes,

followed by washing 3-times with washing buffer by magnetic capture and resuspension. A detection antibody-HRP or DNA barcode complex was prepared by assembly with streptavidin. One milliliter of detection antibody-streptavidin-HRP or DNA barcode complexes was then added to each mixture and incubated under slow tilt rotation at room temperature for 20 minutes, followed by washing 3-times with washing buffer by magnetic capture and resuspension. After concentrating the mixture to 50 μ L by magnetic capture of the beads, the resulting material was stored at 4°C until use. HRP activity of a detection antibody-HRP complex was measured to characterize and configure the magnetic bead-based immunoassay. After adding substrate solution for HRP (1 mM of 3,3',5,5'-tetramethylbenzidine and 3 mM of H₂O₂), the samples were incubated at room temperature for 30 minutes. The absorbance was then measured at 655 nm. Experiments were performed in triplicate, and standard deviations were indicated.

[0058] The traditional HRP-based ELISA and the plate-based Immuno-CRISPR assay in the form of LFA without using magnetic beads, i.e., Immuno-CRISPR LFA, were performed. Sandwich assemblies with capture antibody, antigen, and detection antibody-DNA barcode complex were prepared in a 96-well plate as previously reported. The HRP-based ELISA was tested by measuring HRP activity. After adding substrate solution for HRP (1 mM of 3,3',5,5'-tetramethylbenzidine and 3 mM of H₂O₂), the samples were incubated at room temperature for 30 minutes. The absorbance was then measured at 655 nm. Total assay time for the HRP-based ELISA was around 4 hours. Experiments were performed in triplicate, and standard deviations were indicated.

[0059] Fluorescence-based assay in solution using FQ reporter. To test the exemplary embodiments of the ImmunoMag-CRISPR assay with a detection antibody-streptavidin-DNA barcode complex, *trans*-cleavage activity of the Cas12a complex was measured using an FQ reporter-based assay. Briefly, the Cas12a complex was pre-assembled by mixing Cas12a (1 μ M) and crRNA (1.2 μ M) in the buffer for Cas12a (50 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl₂ and 100 μ g/ml recombinant albumin) at room temperature for 30 minutes. The FQ reporter (1 μ M) was then mixed with the diluted Cas12a complex (0.5 μ M). After adding the prepared magnetic bead solutions (50 μ L) to the reaction cocktails (50 μ L), the mixtures were incubated at room temperature for 30 minutes. The fluorescence signal from excitation at $\lambda_{\text{ex}} = 485$ nm and emission at $\lambda_{\text{em}} = 530$ nm was measured using a plate reader (SpectraMax M5, San Jose, CA). Total assay time from magnetic enrichment for the fluorescence-based ImmunoMag-CRISPR assay was around 90 minutes. Experiments were performed in triplicate, and standard deviations were indicated. The LOD was calculated from

each immunoassay-based standard curve by determining the concentration with a fluorescence signal consistent with $2\sqrt{2}$ times the standard deviation of the blank.

5 [0060] LFA using FB reporter. To test the ImmunoMag-CRISPR LFA (consistent with embodiments of the present disclosure) and Immuno-CRISPR LFA, the *trans*-cleavage activity of the Cas12a complex was also measured using a LFA and with an FB reporter. Reaction cocktails with Cas12a complex (0.5 μ M) and FB reporter (1 μ M) were prepared in the same way as for the fluorescence-based assay. After adding the prepared magnetic bead solutions (50 μ L) to the reaction cocktails (50 μ L), the mixtures were incubated at room temperature for 30 minutes. For the plate-based Immuno-CRISPR LFA, reaction cocktails (50 μ L) were then added to the prepared wells, and incubated at room temperature for 30 minutes. Fifty microliters of the resulting solution were mixed with 50 μ L of the assay buffer from the lateral flow assay kit and transferred to another 96-well plate. Then, a test strip was placed upright and removed from the well after 3 minutes. Total assay time from magnetic enrichment for the fluorescence-based ImmunoMag-CRISPR assay was around 93 minutes.

15 [0061] Photographs of LFA strips were then taken using a smartphone to quantify the concentrations of target proteins on the control and test lines using ImageJ software (NIH). Briefly, rectangular regions in the control line, test line and background were selected, inverted, and converted into a 32-bit image, followed by measuring its average grey scale intensity. The intensities of test and control lines were background subtracted, followed by estimating the ratio of the test to control line signal intensities (T/C ratio). The LOD was calculated from the standard curve of each LFA system generated by plotting T/C ratio against the CXCL9 or CXCL10 concentrations.

[0062] Evaluation of clinical samples. As discussed above, 42 urine samples from kidney transplant recipients participating in the Clinical Trials in Organ Transplantation (CTOT)-19 trial were tested and analyzed by a lateral flow assay according to embodiments of the present disclosure. The CTOT urine samples were obtained at the time of kidney allograft biopsies as per study protocol, and the sample collection was approved by institutional review boards (IRBs) at participating centers. The samples were aliquoted and stored at -20°C. These isolated urines were used as received and without pretreatment. The urine samples were de-identified and tested blinded to biopsy diagnosis. All samples collected also were analyzed for urinary CXCL9 and CXCL10 using sandwich ELISA as published for comparison.

[0063] Methods and systems of the present disclosure are advantageous to provide target biomarker assays utilizing antibody-DNA barcode complexes, e.g., including multiple Cas12a recognition sites, with magnetic enrichment of the target biomarkers via antibody-magnetic particle complexes to identify those biomarkers in a sample from a patient. Magnetic enrichment
5 can be followed by activating trans-cleavage activity of Cas-RNA complexes, e.g., Cas12a/crRNA, to cleave collateral reporters, e.g., FAM-biotin, without a preamplification step. These systems and methods advantageously enable improved, LFA-compatible, naked-eye detection of urinary biomarkers.

[0064] A demonstration of the detection sensitivity for urinary CXCL9 and CXCL10 by
10 systems and methods consistent with embodiments of the present disclosure was evaluated and compared to traditional ELISA and LFA without magnetic enrichment. Lateral flow assays consistent with the embodiments of the present disclosure were then used to evaluate 42 clinical urine samples by visual inspection and quantification of urinary biomarkers, establishing performance on patient samples.

[0065] The LOD for solution-phase CXCL9 using assays consistent with embodiments
15 of the present disclosure was 0.6 pg/mL, which is a 20-fold improvement over traditional well plate-based ELISA. Further, an LOD of 18 pg/mL was demonstrated via naked-eye detection in the lateral flow assays, and 11 positive, 11 borderline positive, and 20 negative urinary samples from kidney transplant recipients were determined through a simple visual comparison of the test
20 line and the control line of a test strip. Importantly, of the 20 clinically negative samples, five samples had detectable levels of CXCL9 (above LOD on the lateral flow assay). Similar results were obtained for CXCL10. This suggests that very early stage CXCL9 and CXCL10 levels can be detected using the embodiments of the present disclosure, and has the potential to be extended to a wide range of POCTs for highly sensitive biomarker detection.

[0066] Therefore, embodiments of the present disclosure provide for a rapid, selective,
25 and highly sensitive detection methodology for point-of-care testing, e.g., of CXCL9 and CXCL10. This technique can be further combined with smartphone-driven lateral flow assay quantification and adapted to patient point-of-use home testing in future clinical analyses. Given the opportunity for POCT, when coupled with a smartphone-based intensity measurement
30 system, simple home-based measurements by patients are enabled for alerting their physicians, e.g., by kidney transplant patients, spanning several months to assess time-based increases in their urinary chemokine levels.

[0067] Although the invention has been described and illustrated with respect to exemplary embodiments thereof, it should be understood by those skilled in the art that the foregoing and various other changes, omissions and additions may be made therein and thereto, without parting from the spirit and scope of the present invention.

CLAIMS

What is claimed is:

1. A method for detecting one or more target biomarkers in a sample, comprising:
 - obtaining a sample from a patient;
 - 5 preparing a first composition, the first composition comprising:
 - a plurality of magnetic beads complexed with one or more first biomarker antibodies, the first biomarker antibodies configured to bind to the one or more target biomarkers; and
 - 10 a plurality of second biomarker antibodies complexed with one or more DNA barcodes, the second biomarker antibodies configured to bind to the one or more target biomarkers, and the DNA barcodes including one or more CRISPR/Cas recognition sites,
 - 15 preparing a second composition, the second composition including a plurality of Cas-RNA complexes, wherein the Cas-RNA complexes are active when bound with the DNA barcodes and inactive when not bound with the one or more DNA barcodes;
 - 20 contacting the sample with the first composition to bind the first biomarker antibodies and the second biomarker antibodies with the target biomarkers in the sample to form an amount of sandwich structure complexes with biomarker bound between the first biomarker antibody and the second biomarker antibody;
 - applying a magnetic field to the sample;
 - migrating via the magnetic field the sandwich structure complexes to form a first portion of the sample enriched for the sandwich structure complexes relative to a second portion of the sample;
 - 25 contacting the first portion of the sample with the second composition to bind the DNA barcodes with the Cas-RNA complexes;
 - contacting the first portion of the sample with one or more reporter complexes, wherein the reporter complexes include a control portion and a test portion;

cleaving the test portion from the control portion of the one or more reporter complexes via the active Cas-RNA complexes from the first portion of the sample; and

detecting an amount of test portion cleaved from the reporter complexes.

- 5 2. The method according to claim 1, wherein detecting an amount of test portion cleaved from the reporter complexes comprises:

administering an amount of the first portion to a lateral flow assay test strip including a control line configured to bind the control portion of the reporter and a test line configured to bind the test portion of the reporter;

- 10 migrating the first portion along the lateral flow assay test strip;

binding the control portion of reporter complexes at the control line; and

binding the amount of test portion cleaved from the reporter complexes at the test line.

- 15 3. The method according to claim 2, wherein the lateral flow assay test strip is a commercially available paper-based strip including a streptavidin-coated control line and an anti-rabbit antibody-coated test line.

4. The method according to claim 1, wherein the one or more CRISPR/Cas recognition sites include a Cas9 recognition site, a Cas12a recognition site, a Cas13a recognition site, or combinations thereof.

- 20 5. The method according to claim 1, wherein the Cas-RNA complexes include a Cas9/crRNA complex, a Cas12a/crRNA complex, a Cas13a/crRNA complex, or combinations thereof.

- 25 6. The method according to claim 1, wherein the one or more reporter complexes include a fluorophore-biotin (FB) reporter, fluorophore-quencher (FQ) reporter, or combinations thereof.

7. The method according to claim 1, wherein the one or more target biomarkers include a urinary biomarker.

8. The method according to claim 7, wherein the urinary biomarker includes chemokine ligand 9 (CXCL9), chemokine ligand 10 (CXCL10), or combinations thereof.
9. A biomarker assay kit, comprising:
- 5 a plurality of magnetic particle-antibody complexes, the magnetic particle-antibody complexes including a first biomarker antibody structured to bind to one or more target biomarkers;
- 10 a plurality of oligonucleotide-antibody complexes, the oligonucleotide-antibody complexes including one or more DNA barcodes and a second biomarker antibody structured to bind to the target biomarkers, wherein the DNA barcodes include one or more CRISPR/Cas recognition sites;
- one or more reporter complexes, wherein the reporter complexes include a control portion and a test portion; and
- a plurality of Cas-RNA complexes, wherein the Cas-RNA complexes are configured to:
- 15 bind to the DNA barcodes;
- be active when bound to the DNA barcodes and inactive when not bound to the DNA barcodes; and
- cleave the control portion of the reporter complexes from the test portion of the reporter complexes when active.
- 20 10. The kit according to claim 9, wherein the magnetic particles include magnetic beads.
11. The kit according to claim 9, further comprising a lateral flow assay test strip, a microfluidic apparatus, or combinations thereof.
12. The kit according to claim 11, wherein the lateral flow assay test strip includes a control line configured to bind the control portion of the reporter and a test line configured to
- 25 bind the test portion of the reporter.
13. The kit according to claim 9, wherein the first biomarker antibody includes first heavy chain variable regions and first light chain variable regions, and the second biomarker

antibody includes second heavy chain variable regions and second light chain variable regions, wherein:

the first heavy chain variable regions and the second heavy chain variable regions have above about 85% sequence identity; and

5 the first light chain variable regions and the second light chain variable regions have above about 85% sequence identity.

14. The kit according to claim 9, wherein the one or more CRISPR/Cas recognition sites include a Cas9 recognition site, a Cas12a recognition site, a Cas13a recognition site, or combinations thereof.

10 15. The kit according to claim 9, wherein the Cas-RNA complexes include a Cas9/crRNA complex, a Cas12a/crRNA complex, a Cas13a/crRNA complex, or combinations thereof.

16. The kit according to claim 9, wherein the one or more reporter complexes include a fluorophore-biotin (FB) reporter, fluorophore-quencher (FQ) reporter, or combinations thereof.

15 17. The kit according to claim 9, wherein the one or more target biomarkers include a urinary biomarker.

18. The kit according to claim 9, wherein the urinary biomarker includes chemokine ligand 9 (CXCL9), chemokine ligand 10 (CXCL10), or combinations thereof.

19. A method for detecting one or more target biomarkers in a urinary sample, comprising:

20 obtaining a urinary sample from a patient;

preparing a plurality of magnetic particle-antibody complexes, the magnetic particle-antibody complexes including a first biomarker antibody structured to bind to one or more target biomarkers;

25 preparing a plurality of oligonucleotide-antibody complexes, the oligonucleotide-antibody complexes including one or more DNA barcodes and a second biomarker antibody structured to bind to the one or more target biomarkers, wherein the DNA barcodes include one or more CRISPR/Cas recognition sites;

preparing one or more reporter complexes, wherein the one or more reporter complexes include a control portion and a test portion;

preparing a plurality of Cas-RNA complexes, wherein the Cas-RNA complexes are configured to:

5 bind to the one or more DNA barcodes;

 be active when bound to the one or more DNA barcodes and inactive when not bound to the DNA barcodes; and

 cleave the control portion of the reporter complexes from the test portion of the reporter complexes when active,

10 contacting the sample with an amount of the magnetic particle-antibody complexes and an amount of the oligonucleotide-antibody complexes to form an amount of sandwich structure complexes with biomarker from the urinary sample bound between first biomarker antibody and second biomarker antibody;

 applying a magnetic field to the sample to form an enriched portion of the sample and a depleted portion of the sample, the enriched portion having an elevated concentration of the sandwich structure complexes relative to the depleted portion;

15 contacting the enriched portion with the Cas-RNA complexes and the one or more reporter complexes to form a test urinary sample;

20 cleaving the test portion from the control portion of the one or more reporter complexes via active Cas-RNA complexes in the test urinary sample;

 administering the test urinary sample to a lateral flow assay test strip including a control line configured to bind the control portion of the reporter and a test line configured to bind the test portion of the reporter; and

25 detecting an amount of test portion bound to the test line.

20. The method according to claim 19, wherein:

 the one or more CRISPR/Cas recognition sites include a Cas9 recognition site, a Cas12a recognition site, a Cas13a recognition site, or combinations thereof;

the Cas-RNA complexes include a Cas9/crRNA complex, a Cas12a/crRNA complex, a Cas13a/crRNA complex, or combinations thereof;

the one or more reporter complexes include a fluorophore-biotin (FB) reporter, fluorophore-quencher (FQ) reporter, or combinations thereof; and

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the one or more urinary biomarkers include chemokine ligand 9 (CXCL9), chemokine ligand 10 (CXCL10), or combinations thereof.