developing improved DNA extraction compositions and methods that are particularly suitable for extracting DNA from urine samples.

SUMMARY

[006] The present disclosure provides compositions for storing a urine sample obtained from a subject. In one aspect, the present disclosure refers to a composition for storing a urine sample obtained from a subject, wherein the composition comprises a pH buffer, a chelating agent, and a surfactant, wherein the pH buffer is configured to adjust a pH of the composition to within 5.0 to 6.5, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA) and has a concentration of 10 to 25 mmol/L, and wherein the surfactant is sodium docecyl sulfate (SDS) and has a concentration of 5% to 10% (m/v). In some examples, the compositions comprise, comprise essentially of, or consist of a pH buffer, a chelating agent, and a surfactant.

[007] In some examples, the pH buffer is configured to adjust a pH of the composition to within a preselected range. In some examples, the pH buffer comprises acetic acid and a salt of acetic acid. In some examples, the preselected range of pH is about 5.0 to 6.5. In some examples, the pH of the composition is about 6.0.

[008] In some examples, the salt of acetic acid is sodium acetate. In some examples, the sodium acetate has a concentration of about 0.5 to 1.0 mol/L, for example, about 0.5-0.7 mol/L, or about 0.6-0.7 mol/L.

[009] In some examples, the chelating agent is an aminopolycarboxylic acid. In some examples, chelating agent is ethylenediaminetetraacetic acid (EDTA). In some examples, the EDTA has a concentration of about 10 to 20 mmol/L, for example, about 15-20 mmol/L, about 16-20 mmol/L or about 16-18 mmol/L.

[010] In some examples, the surfactant is an anionic surfactant. In some examples, the anionic surfactant is a salt of dodecyl hydrogen sulfate. In some examples, the salt is a sodium salt, and the anionic surfactant is sodium docecyl sulfate (SDS). In some examples, the SDS has a concentration of about 5% to 10% (m/v), for example, about 5%-8%, about 5%-7%, about 6%-8%, or about 6%-7%.

[011] In some examples, the composition does not contain a preservative, a cell fixative, or a formaldehyde quencher.

[012] The present disclosure also provides a processed urine sample. In another aspect, the present disclosure refers to a processed urine sample for storage, wherein the processed urine sample comprises a urine sample collected from a subject, a pH buffer, a chelating agent, and a surfactant, wherein the pH buffer is configured to adjust a pH of the processed urine sample to within 5.0 to 6.5, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA) and has a concentration of 1 to 2.5 mmol/L, wherein the surfactant is sodium docecyl sulfate (SDS) and has a concentration of 0.5% to 1.5% (m/v). The urine sample can be used for DNA extraction right away, or after being stored. In some examples, the processed urine sample comprises a urine sample collected from a subject, a pH buffer, a chelating agent, and a surfactant. In some examples, the pH buffer is configured to adjust a pH of the composition to within a preselected range. In some examples, the pH buffer comprises acetic acid and a salt of acetic acid. In some examples, the salt of acetic acid is sodium acetate.

[013] In some examples, the preselected range of pH is about 5.0 to 6.5. In some examples, the pH of the composition is about 6.0. In some examples, the sodium acetate in the processed urine sample has a concentration of about 0.05 to 0.1 mol/L, for example, about 0.05-0.07 mol/L, or about 0.06-0.07 mol/L. In some examples, the chelating agent is an aminopolycarboxylic acid. In some examples, the chelating agent is ethylenediaminetetraacetic acid (EDTA). In some examples, the EDTA has a concentration of about 1 to 2.5 mmol/L, for example, about 1.5-2 mmol/L, about 1.6-2 mmol/L or about 1.6-1.8 mmol/L. In some examples, the surfactant is an anionic surfactant. In some examples, the anionic surfactant is a salt of dodecyl hydrogen sulfate. In some examples, the salt is a sodium salt, and the anionic surfactant is sodium docecyl sulfate (SDS). In some examples, the SDS has a concentration of about 0.5% to 1.5% (m/v), for example, about 0.5%-0.8%, about 0.5%-0.7%, about 0.6%-0.8%, or about 0.6%-0.7%. In some examples, the processed urine sample does not contain a preservative, a cell fixative, or a formaldehyde quencher.

[014] The present disclosure further provides methods for producing a processed urine sample for storage. In another aspect, the present disclosure refers to a method for producing a processed urine sample for storage, comprising mixing a urine sample collected from a subject with the composition as disclosed herein. In some examples, the methods comprise mixing a urine sample collected from a subject with a pH buffer, a chelating agent, and a surfactant, or with a composition of the present disclosure as described herein.

[015] The present disclosure further provides methods for storing a urine sample collected from a subject. In another aspect, the present disclosure refers to a method for storing a urine sample collected from a subject, comprising mixing the urine sample collected from the subject with a pH buffer, a chelating agent, and a surfactant to produce a urine sample ready for storage, wherein the pH buffer is configured to adjust a pH of the urine sample ready for storage to within 5.0 to 6.5, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA) and has a concentration of 1 to 2.5 mmol/L in the urine sample ready for storage, wherein the surfactant is sodium docecyl sulfate (SDS) and has a concentration of 0.5% to 1.5% (m/v) in the urine sample ready for storage. In some examples, the methods comprise mixing the urine sample collected from the subject with a pH buffer, a chelating agent, and a surfactant, or with a composition of the present disclosure as described herein to produce a urine sample ready for storage. In some examples, the pH buffer, the chelating agent, and the surfactant are provided in a mixture before they are mixed with the urine sample collected from the subject, such as a composition of the present disclosure as described herein. In some examples, the urine sample collected from the subject contains cells of the subject and at least one viral pathogen, and both the cells and the viral pathogen are lysed after the urine sample is ready for storage. In some examples, the viral pathogen is a Human papillomavirus (HPV). In some examples, comprising storing the urine sample ready for storage at a predetermined temperature, such as 4°C, -20°C, -80°C, or at room temperature. In some examples, DNA content in the urine sample is stable after a 15-day to 30-day storage time. In some examples, DNA content in the urine sample is stable after a 1-week to 2-week storage time.

[016] The present disclosure further provides methods for detecting the presence or absence of one or more analytes in a urine sample collected from a subject. In another aspect, the present disclosure

[029] In some examples, steps (3), (4), and/or (5) in the methods for DNA extraction comprise using a magnetic frame or an automatic nucleic acid extraction instrument.

[030] The present disclosure provides methods for detecting the presence or absence of an analyte in a urine sample collected from a subject. In another aspect, the present disclosure refers to a method for detecting the presence or absence of an analyte in a urine sample collected from a subject, wherein the method comprises: (1) using a processed urine sample of any one of claims 16 to 30; and (2) extracting DNA from the processed urine sample, comprising: (a) contacting the urine sample with magnetic nanoparticles and a protease to produce a pre-treated urine sample; (b) lysing the pre-treated urine sample obtained in step (a) in a lysis solution to produce a lysed urine sample; (c) washing the magnetic nanoparticles obtained in step (b) with a first washing buffer; (d) washing the magnetic nanoparticles obtained in step (c) with a second washing buffer; (e) collecting magnetic nanoparticles in the urine sample obtained in step (d); and (f) washing off DNA from the collected magnetic nanoparticles obtained in step (e) with an elution buffer to obtain extract DNA. In some examples, the methods comprises using DNA extracted from the urine sample using a kit or a collection of composition as described herein. In some examples, the analyte is a virus, such as a HPV. In some examples, the detection of the analyte comprises detecting DNA of the virus.

[031] The present disclosure provides methods for detecting the presence or absence of an analyte in a urine sample collected from a subject. In some examples, the methods comprise using a processed urine sample as described herein. In some examples, the methods further comprise extracting DNA from the processed urine sample. In some examples, the step of extracting DNA from a sample comprises (a) contacting the urine sample with magnetic nanoparticles and a protease to produce a pre-treated urine sample; (b) lysing the pre-treated urine sample obtained in step (a) in a lysis solution to produce a lysed urine sample; (c) washing the magnetic nanoparticles containing DNA from urine samples obtained in step (b) with a first washing buffer; (d) washing the magnetic nanoparticles containing DNA from urine samples obtained in step (c) with a second washing buffer; (e) washing off DNA from the magnetic nanoparticles collected in step (d) with a elution buffer to obtain the extracted DNA. In some examples, the lysis solution, the magnetic nanoparticles, the first washing buffer, the second washing buffer, the elution buffer, the protease are those described in the present disclosure herein.

[032] In some examples, the methods for detecting the presence or absence of an analyte in a urine sample of a subject further comprises treating the subject based on the presence or absence of the analyte in the urine sample.

[033] The present disclosure further provides methods for extracting DNA from a urine sample of a subject. In some examples, the methods comprise using a kit as described herein. In some examples, the methods comprise: (1) contacting the urine sample with magnetic nanoparticles and a protease to

CLAIMS

1. A composition for storing a urine sample obtained from a subject, wherein the composition comprises a pH buffer, a chelating agent, and a surfactant, wherein the pH buffer is configured to adjust a pH of the composition to within 5.0 to 6.5, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA) and has a concentration of 10 to 25 mmol/L, and wherein the surfactant is sodium docecyl sulfate (SDS) and has a concentration of 5% to 10% (m/v).

2. The composition of claim 1, wherein the pH buffer comprises acetic acid and a salt of acetic acid.

3. The composition of claim 2, wherein the salt of acetic acid is sodium acetate.

4. The composition of claim 1, wherein the pH of the composition is 6.0.

5. The composition of claim 3, wherein the sodium acetate has a concentration of 0.5 to 1.0 mol/L.

6. The composition of claim 1, wherein the composition does not contain a preservative, a cell fixative, or a formaldehyde quencher.

7. A processed urine sample for storage, wherein the processed urine sample comprises a urine sample collected from a subject, a pH buffer, a chelating agent, and a surfactant, wherein the pH buffer is configured to adjust a pH of the processed urine sample to within 5.0 to 6.5, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA) and has a concentration of 1 to 2.5 mmol/L, wherein the surfactant is sodium docecyl sulfate (SDS) and has a concentration of 0.5% to 1.5% (m/v).

8. The processed urine sample for storage of claim 7, wherein the pH buffer comprises acetic acid and a salt of acetic acid.

9. The processed urine sample for storage of claim 8, wherein the salt of acetic acid is sodium acetate.

10. The processed urine sample for storage of claim 7, wherein the pH of the processed urine sample is 6.0.

11. The processed urine sample for storage of claim 9, wherein the sodium acetate in the processed urine sample has a concentration of 0.05 to 0.1 mol/L.

12. The processed urine sample of claim 7, wherein the processed urine sample does not contain a preservative, a cell fixative, or a formaldehyde quencher.

13. A method for producing a processed urine sample for storage, comprising mixing a urine sample collected from a subject with the composition of any one of claims 1 to 6.

14. The method of claim 13, wherein the pH buffer comprises acetic acid and a sodium acetate.

15. The method of claim 14, wherein the sodium acetate in the processed urine sample has a concentration of 0.05 to 0.1 mol/L.

16. The method of claim 13, wherein the EDTA in the processed urine sample has a concentration of 1 to 2.5 mmol/L.

17. The method of claim 13, wherein the SDS in the processed urine sample has a concentration of 0.5% to 1.5% (m/v).

18. A method for storing a urine sample collected from a subject, comprising mixing the urine sample collected from the subject with a pH buffer, a chelating agent, and a surfactant to produce a urine sample ready for storage, wherein the pH buffer is configured to adjust a pH of the urine sample ready for storage to within 5.0 to 6.5, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA) and has a concentration of 1 to 2.5 mmol/L in the

urine sample ready for storage, wherein the surfactant is sodium docecyl sulfate (SDS) and has a concentration of 0.5% to 1.5% (m/v) in the urine sample ready for storage.

19. The method of claim 18, wherein the pH buffer, the chelating agent, and the surfactant are provided in a mixture before they are mixed with the urine sample collected from the subject.

20. The method of claim 18, wherein the pH buffer comprises acetic acid and a salt of acetic acid.

21. The method of claim 20, wherein the salt of acetic acid is sodium acetate.

22. The method of claim 18, wherein the pH of the urine sample ready for storage is 6.0.

23. The method of claim 21, wherein the sodium acetate in the urine sample ready for storage has a concentration of 0.05 to 0.1 mol/L.

24. The method of claim 18, wherein the urine sample ready for storage does not contain a preservative, a cell fixative, or a formaldehyde quencher.

25. The method of claim 18, wherein the urine sample collected from the subject contains cells of the subject and at least one viral pathogen, and both the cells and the viral pathogen are lysed after the urine sample is ready for storage.

26. The method of claim 25, wherein the viral pathogen is a Human papillomavirus (HPV).

27. The method of claim 18, comprising storing the urine sample ready for storage at 4°C.

28. The method of claim 18, comprising storing the urine sample ready for storage at room temperature.

29. The method of claim 27, wherein DNA content in the urine sample is stable after a 15-day to 30-day storage time.

30. The method of claim 28, wherein DNA content in the urine sample is stable after a 1-week to 2-week storage time.

31. A method for detecting the presence or absence of one or more analytes in a urine sample collected from a subject, wherein the method comprises using a processed urine sample of any one of claims 7 to 12.

32. The method of claim 31, wherein the analyte is a virus.

33. The method of claim 32, wherein the virus is a HPV.

34. The method of claim 32, wherein the detection of the analyte comprises detecting DNA of the virus.

35. A method for detecting the presence or absence of an analyte in a urine sample collected from a subject, wherein the method comprises:

(1) using a processed urine sample of any one of claims 7 to 12; and

(2) extracting DNA from the processed urine sample, comprising:

(a) contacting the urine sample with magnetic nanoparticles and a protease to produce a pre-treated urine sample;

(b) lysing the pre-treated urine sample obtained in step (a) in a lysis solution to produce a lysed urine sample;

(c) washing the magnetic nanoparticles obtained in step (b) with a first washing buffer;

(d) washing the magnetic nanoparticles obtained in step (c) with a second washing buffer;

(e) collecting magnetic nanoparticles in the urine sample obtained in step (d); and

(f) washing off DNA from the collected magnetic nanoparticles obtained in step (e) with an elution buffer to obtain extract DNA.

36. The method of claim 35, wherein the lysis solution comprises guanidinium isothiocyanate, Triton X−100, Tris-HCl, EDTA, and isopropanol,

wherein the guanidinium isothiocyanate has a concentration of 1 to 2 M;

wherein the Triton X 100 has a concentration of 1 to 2%;

wherein the Tris-HCl has a concentration of 5 to 10 mM and the lysis solution has a pH of 6-7;

wherein the EDTA has a concentration of 3 to 5 mM;

and

wherein the isopropanol has a volume of 50% to 80% (v/v) of the lysis solution.