

BRIEF SUMMARY OF THE INVENTION

[0006] The present ~~disclosureinvention~~ provides methods and compositions for extracting nucleic acids from biological samples by using divalent ions, proteases, detergents, and low pH conditions.

5 [0007] In ~~one a first aspect, the present inventionthere is provided~~ a methods for extraction of a target nucleic acid from a biological sample comprising cells or microorganisms, the method comprising:

(a) adding a transition metal salt, an alkaline earth metal salt, or combination thereof to the biological sample in an amount sufficient to inactivate nucleases in the sample;

10 (b) adding an extraction solution to the biological sample in an amount sufficient to lyse the cells or microorganisms, thereby forming a lysate;

(c) adding an acidic buffer to the lysate to an amount sufficient to reduce the pH of the lysate to between pH 1 to pH 5; and

(d) separating the target nucleic acid from the lysate to extract the target
15 nucleic acid from the biological sample.

[0008] In ~~another aspectone embodiment~~, the methods of the ~~disclosureinvention~~ further comprise performing a nucleic acid amplification following step (d). The nucleic acid amplification is carried out using PCR in some embodiments.

20 [0009] In one ~~embodimentaspect~~, the methods of the ~~disclosureinvention~~ further comprise detecting an amplified nucleic acid product generated from the nucleic acid amplification.

[0010] In some ~~embodimentaspects~~, step (a) and step (b) are performed simultaneously.

[0011] In some ~~embodimentaspects~~, the biological sample is whole blood, serum, plasma, sputum, saliva, urine, stool, cells, or microorganisms.

25 [0012] In some ~~embodimentaspects~~, the transition metal salt is selected from the group consisting of a manganese salt or a zinc salt.

[0013] In some ~~embodimentaspects~~, the alkaline earth metal salt is selected from the group consisting of a magnesium salt or a calcium salt.

[0014] In some ~~embodimentaspects~~, the target nucleic acid is RNA. In some embodiments, the target nucleic acid is viral RNA.

[0015] In some embodimentaspects, the target nucleic acid is DNA. In some embodiments, the target nucleic acid is viral DNA.

[0016] In some embodimentaspects, the acidic buffer is citric acid buffer or acetic acid buffer. In some embodiments, the pH of the lysate after carrying out step (c) (*i.e.* following addition of the acidic buffer) is in the acidic range between about pH 1 to about pH 5.

[0017] In some embodimentaspects, the extraction solution comprises proteinase K, detergent, or a combination thereof.

[0018] In some embodimentaspects, the detergent is a cationic or non-ionic detergent. In some embodiments, the cationic detergent is cetrimonium bromide. In some embodiments, the non-ionic detergent is Triton X-100. In some embodiments, the non-ionic detergent is NP-40. In other embodiments, the detergent is a block copolymer (e.g., pluronic solution).

[0019] In some embodimentaspects, for example when the sample comprises Hepatitis B virus, the methods of the disclosureinvention further comprise a step of heating the biological sample prior to separating the target nucleic acid from the lysate. In a typical embodiment, the biological sample is heated to a temperature between about 50°C and about 80°C, usually between about 65°C and about 75°C. The step of heating can be carried out for about 30 seconds to about 90 seconds or for about 50 seconds to about 70 seconds. In some embodiments, the heating step is carried out after the addition of the extraction solution and prior to the addition of the acidic buffer.

[0020] In some embodimentaspects, step (d) is conducted by adding magnetic particles to the lysate in step (c); separating the magnetic microparticles from the lysate using magnetic microparticle separation; adding a wash solution to the magnetic microparticles, and adding an elution buffer to the magnetic microparticles to elute the nucleic acid.

[0021] In one embodimentaspect, the disclosureinvention provides a method for detecting an amplified nucleic acid product in a sample comprising a target nucleic acid and a nuclease, the method comprising:

(a) adding a transition metal salt, an alkaline earth metal salt, or combination thereof to the sample in an amount sufficient to inactivate the nuclease in the sample;

(b) adding an acidic buffer to the sample in an amount sufficient to inactivate the nuclease in the sample;

(c) performing a nucleic acid amplification reaction to produce the amplified nucleic acid product from the target nucleic acid;

(d) detecting the amplified nucleic acid product.

[0021a] In a second aspect, there is provided a method for detecting an amplified nucleic acid product in a sample comprising a target nucleic acid and nucleases, the method comprising:

(a) adding a transition metal salt, an alkaline earth metal salt, or combination thereof to the sample in an amount sufficient to inactivate the nucleases in the sample;

(b) adding an acidic buffer to the sample in an amount sufficient to reduce the pH of the sample to less than pH 5;

(c) separating the target nucleic acid from the sample;

(d) performing a nucleic acid amplification reaction to produce the amplified nucleic acid product from the target nucleic acid;

(e) detecting the amplified nucleic acid product.

[0022] In one embodiment, step (a) and step (b) are performed simultaneously.

[0023] In one embodiment, the transition metal salt is selected from the group consisting of a manganese salt or a zinc salt. In one embodiment, the alkaline earth metal salt is selected from the group consisting of a magnesium salt or a calcium salt.

[0024] In some embodiments, the acidic buffer is citric acid buffer or acetic acid buffer.

[0025] In some embodiments, the nucleic acid amplification reaction is a PCR process.

[0026] In some embodiments, the target nucleic acid is RNA. In some embodiments, the target nucleic acid is viral RNA.

[0027] In some embodiments, the target nucleic acid is DNA. In some embodiments, the target nucleic acid is viral DNA.

[0028] In one embodiment, the invention provides a kit for extracting nucleic acid from a biological sample comprising nucleic acids, nucleases and inhibitors comprising:

(a) an extraction solution;

(b) a transition metal salt solution, an alkaline earth metal salt solution, or combination thereof;

(c) an acidic buffer, and

(d) a wash solution.

[0028a] In a third aspect, there is provided use of a kit for extracting nucleic acid from a biological sample comprising nucleic acids, nucleases and inhibitors comprising:

(a) an extraction solution;

(b) a transition metal salt solution, an alkaline earth metal salt solution, or combination thereof;

(c) an acidic buffer, and

(d) a wash solution;

in a method according to the first aspect.

[0029] In some embodiments, the extraction solution comprises proteinase K, detergent, or a combination thereof.

[0030] In some embodiments, the transition metal salt is selected from the group consisting of a manganese salt or a zinc salt.

[0031] In some embodiments, the alkaline earth metal salt is selected from the group consisting of a magnesium salt or a calcium salt.

[0032] In some embodiments, the acidic buffer is citric acid buffer or acetic acid buffer.

[0033] In some embodiments, the wash solution is trifluoroacetic acid solution or hydrochloric acid solution.

[0034] Other objects, features, and advantages of the present invention will be apparent to one of skill in the art from the following detailed description and figures.

WHAT IS CLAIMED IS:

1 1. A method for extraction of a target nucleic acid from a biological
2 sample comprising cells or microorganisms, the method comprising:
3 (a) adding a transition metal salt, an alkaline earth metal salt, or combination
4 thereof to the biological sample in an amount sufficient to inactivate nucleases in the sample;
5 (b) adding an extraction solution to the biological sample in an amount
6 sufficient to lyse the cells or microorganisms, thereby forming a lysate;
7 (c) adding an acidic buffer to the lysate to an amount sufficient to reduce the
8 pH of the lysate; and
9 (d) separating the target nucleic acid from the lysate to extract the target
10 nucleic acid from the biological sample.

1 2. The method of claim 1, further comprises performing a nucleic acid
2 amplification following step (d).

1 3. The method of claim 2, wherein nucleic acid amplification is PCR.

1 4. The method of claim 2 or 3, further comprising detecting an amplified
2 nucleic acid product generated from the nucleic acid amplification.

1 5. The method of any one of claims 1 to 4, wherein step (a) and step (b)
2 are performed simultaneously.

1 6. The method of any one of claims 1 to 5, wherein the biological sample
2 is whole blood, serum, plasma, sputum, saliva, stool, or tissue.

1 7. The method of any one of claims 1 to 6, wherein the transition metal
2 salt is selected from the group consisting of a manganese salt or a zinc salt.

1 8. The method of any one of claims 1 to 7, wherein the alkaline earth
2 metal salt is selected from the group consisting of a magnesium salt or a calcium salt.

1 9. The method of any one of claims 1 to 8, wherein the target nucleic acid
2 is RNA.

1 10. The method of claim 9, wherein the target nucleic acid is viral RNA.

1 11. The method of any one of claims 1 to 8, wherein the target nucleic acid
2 is DNA.

1 12. The method of any one of claims 1 to 11, wherein the acidic buffer is
2 citric acid buffer or acetic acid buffer.

1 13. The method of any one of claims 1 to 12, wherein the pH of the lysate
2 after carrying out step (c) is in the acidic range between pH 1 to pH 5.

1 14. The method any one of claims 1 to 13, wherein the extraction solution
2 comprises proteinase K, detergent, or a combination thereof.

1 15. The method of claim 14, wherein the detergent is cationic or non-ionic
2 detergent.

1 16. The method of claim 15, wherein the cationic detergent is cetrimonium
2 bromide.

1 17. The method of claim 15, wherein the non-ionic detergent is Triton X-
2 100.

1 18. The method of any one of claims 1 to 17, wherein step (d) is conducted
2 by adding magnetic particles to the lysate in step (c); separating the magnetic microparticles
3 from the lysate using magnetic microparticle separation; adding a wash solution to the
4 magnetic microparticles, and adding an elution buffer to the magnetic microparticles to elute
5 the nucleic acid.

1 19. A method for detecting an amplified nucleic acid product in a sample
2 comprising a target nucleic acid and nucleases, the method comprising:

3 (a) adding a transition metal salt, an alkaline earth metal salt, or combination
4 thereof to the sample in an amount sufficient to inactivate the nucleases in the sample;

5 (b) adding an acidic buffer to the sample in an amount sufficient to an amount
6 sufficient to reduce the pH of the lysate;

7 (c) performing a nucleic acid amplification reaction to produce the amplified
8 nucleic acid product from the target nucleic acid;

9 (d) detecting the amplified nucleic acid product.

1 **20.** The method of claim **19**, wherein step (a) and step (b) are performed
2 simultaneously.

1 **21.** The method of claim **19** or **20**, wherein the transition metal salt is
2 selected from the group consisting of a manganese salt or a zinc salt.

1 **22.** The method of any one of claims **19** to **21**, wherein the alkaline earth
2 metal salt is selected from the group consisting of a magnesium salt or a calcium salt.

1 **23.** The method of any one of claims **19** to **22**, wherein the acidic buffer is
2 citric acid buffer or acetic acid buffer.

1 **24.** The method of any one of claims **19** to **23**, wherein the nucleic acid
2 amplification reaction is a PCR process.

1 **25.** The method of any one of claims **19** to **24**, wherein the target nucleic
2 acid is RNA.

1 **26.** The method of claim **25**, wherein the target nucleic acid is viral RNA.

1 **27.** The method of any one of claims **19** to **25**, wherein the target nucleic
2 acid is DNA, optionally viral DNA.

1 **28.** A kit for extracting a nucleic acid from a biological sample comprising
2 nucleic acids, nucleases and inhibitors comprising:

3 (a) an extraction solution;

4 (b) a transition metal salt solution, an alkaline earth metal salt solution, or
5 combination thereof;

6 (c) an acidic buffer, and

7 (d) a wash solution.

1 **29.** The kit of claim **28**, wherein the extraction solution comprises
2 proteinase K, a detergent, or a combination thereof.

1 **30.** The kit of claim **28** or **29**, wherein the transition metal salt is selected
2 from the group consisting of a manganese salt or a zinc salt.

1 **31.** The kit of any one of claims **28** to **30**, wherein the alkaline earth metal
2 salt is selected from the group consisting of a magnesium salt or a calcium salt.

1 **32.** The kit of any one of claim **28 to 31**, wherein the acidic buffer is citric
2 acid buffer or acetic acid buffer.

1 **33.** The kit of any one of claim **28 to 32**, wherein the wash solution is
2 trifluoroacetic acid solution or hydrochloric acid solution.

1 **34.** A method for extraction of a target Hepatitis B viral nucleic acid from
2 a biological sample comprising Hepatitis B virus, the method comprising:

3 (a) adding a transition metal salt, an alkaline earth metal salt, or combination
4 thereof to the biological sample in an amount sufficient to inactivate nucleases in the sample;

5 (b) adding an extraction solution to the biological sample in an amount
6 sufficient to lyse the cells or microorganisms, thereby forming a lysate;

7 (c) adding an acidic buffer to the lysate to an amount sufficient to reduce the
8 pH of the lysate;

9 (d) heating the sample; and

10 (e) separating the target nucleic acid from the lysate to extract the target
11 nucleic acid from the biological sample

1 **35.** The method of claim **34**, wherein the biological sample is heated to a
2 temperature between about 50°C and about 80°C.

1 **36.** The method of claim **35**, wherein the biological sample is heated to a
2 temperature between about 65°C and about 75°C.

1 **37.** The method of claim **34**, wherein the step of heating is carried out for
2 about 30 seconds to about 90 seconds.

1 **38.** The method of claim **37**, wherein the step of heating is carried out for
2 about 50 seconds to about 70 seconds.

CLAIMS

1. A method for extraction of a target nucleic acid from a biological sample comprising cells or microorganisms, the method comprising:
 - 5 (a) adding a transition metal salt, an alkaline earth metal salt, or combination thereof to the biological sample in an amount sufficient to inactivate nucleases in the sample;
 - (b) adding an extraction solution to the biological sample in an amount sufficient to lyse the cells or microorganisms, thereby forming a lysate;
 - 10 (c) adding an acidic buffer to the lysate to an amount sufficient to reduce the pH of the lysate to between pH 1 to pH 5; and
 - (d) separating the target nucleic acid from the lysate to extract the target nucleic acid from the biological sample.
- 15 2. The method of claim 1, further comprising performing a nucleic acid amplification, optionally using PCR, following step (d) and detecting the amplified nucleic acid product.
3. The method of any one of claims 1 to 2, wherein the biological sample is whole blood, serum, plasma, sputum, saliva, stool, or tissue.
- 20 4. The method of any one of claims 1 to 3, wherein the target nucleic acid is DNA, optionally viral DNA.
5. The method any one of claims 1 to 4, wherein the extraction solution comprises proteinase K, detergent, or a combination thereof.
- 25 6. The method of claim 5, wherein the detergent is cationic detergent, optionally cetrimonium bromide.
- 30 7. The method of claim 5, wherein the detergent is non-ionic detergent, optionally Triton X-100.
8. The method of any one of claims 1 to 7, wherein step (d) is conducted by adding magnetic particles to the lysate in step (c); separating the magnetic microparticles from the

lysate using magnetic microparticle separation; adding a wash solution to the magnetic microparticles, and adding an elution buffer to the magnetic microparticles to elute the nucleic acid.

- 5 9. A method for detecting an amplified nucleic acid product in a sample comprising a target nucleic acid and nucleases, the method comprising:
- (a) adding a transition metal salt, an alkaline earth metal salt, or combination thereof to the sample in an amount sufficient to inactivate the nucleases in the sample;
- (b) adding an acidic buffer to the sample in an amount sufficient to reduce the pH of the
10 sample to less than pH 5;
- (c) separating the target nucleic acid from the sample;
- (d) performing a nucleic acid amplification reaction to produce the amplified nucleic acid product from the target nucleic acid;
- (e) detecting the amplified nucleic acid product.
- 15
10. The method of claim 9, wherein the nucleic acid amplification reaction is a PCR process and/or wherein the target nucleic acid is DNA, optionally viral DNA.
11. The method of any one of claims 1-10, wherein:
- 20 (a) step (a) and step (b) are performed simultaneously; and/or
- (b) the transition metal salt is selected from the group consisting of a manganese salt or a zinc salt; and/or
- (c) the alkaline earth metal salt is selected from the group consisting of a magnesium salt or a calcium salt; and/or
- 25 (d) the acidic buffer is citric acid buffer or acetic acid buffer; and/or
- (e) the target nucleic acid is RNA optionally viral RNA.
12. Use of a kit for extracting a nucleic acid from a biological sample comprising nucleic acids, nucleases and inhibitors comprising:
- 30 (a) an extraction solution;
- (b) a transition metal salt solution, an alkaline earth metal salt solution, or combination thereof;
- (c) an acidic buffer, and
- (d) a wash solution;

in a method according to claim 1.

13. The use of claim 12, wherein

- 5 (a) the extraction solution comprises proteinase K, a detergent, or a combination thereof and/or
- (b) the transition metal salt is selected from the group consisting of a manganese salt or a zinc salt and/or
- (c) the alkaline earth metal salt is selected from the group consisting of a magnesium salt or a calcium salt; and/or
- 10 (d) the acidic buffer is citric acid buffer or acetic acid buffer; and/or
- (e) the wash solution is trifluoroacetic acid solution or hydrochloric acid solution.

14. The method of any one of claims 1-11 wherein the target nucleic acid is Hepatitis B viral nucleic acid and the biological sample comprises Hepatitis B virus, and wherein the
15 method further comprises heating the sample between steps (c) and (d).

15. The method of claim 15, wherein:

- 20 (a) the biological sample is heated to a temperature between 50°C and 80°C, optionally wherein the biological sample is heated to a temperature between 65°C and 75°C; and/or
- (b) the step of heating is carried out for 30 seconds to 90 seconds, optionally wherein the step of heating is carried out for 50 seconds to 70 seconds.