CLAIMS

1. A method comprising:

a) contacting a first dilution sample with a first aptamer, wherein a first aptamer affinity complex is formed by the interaction of the first aptamer with its target molecule if the target molecule is present in the first dilution sample;

b) contacting a second dilution sample with a second aptamer, wherein a second aptamer affinity complex is formed by the interaction of the second aptamer with its target molecule if the target molecule is present in the second dilution sample;

~~c) incubating the first and second dilution samples separately to allow aptamer affinity complex formation;~~

~~d) transferring the first dilution sample with the first aptamer affinity complex to a first mixture, wherein the first aptamer affinity complex is captured on a solid support in the first mixture;~~

~~e) after step d), transferring the second dilution sample to the first mixture to form a second mixture, wherein the second aptamer affinity complex of the second dilution is captured on a solid support in the second mixture;~~

~~f) detecting for the presence of or determining the level of the first aptamer and second aptamer of the first and second aptamer affinity complexes, or the presence or amount of one or more first and second aptamer affinity complexes;~~

~~wherein, the first dilution and the second dilution are different dilutions of the same test sample; and~~

~~wherein the method further comprises~~c) contacting a third dilution sample with a third aptamer, wherein a third aptamer affinity complex is formed by the interaction of the third aptamer with its target molecule if the target molecule is present in the third dilution sample~~.~~;

d) incubating the first, second, and third dilution samples separately to allow aptamer affinity complex formation, wherein each of the first aptamer affinity complex, the second aptamer affinity complex, and the third aptamer affinity complex are immobilized on separate first solid supports;

e) releasing and capturing the first aptamer affinity complex on a second solid support;

f) after step e), releasing and capturing the second aptamer affinity complex on the second solid support;

g) after step f), releasing and capturing the third aptamer affinity complex on the second solid support;

h) detecting for the presence of or determining the level of the first aptamer, the second aptamer, and the third aptamer of the first, second, and third aptamer affinity complexes, or the presence or amount of the first, second, and third aptamer affinity complexes;

wherein, the first dilution and the second dilution are different dilutions of the same test sample.

2. The method of claim 1, wherein the test sample is selected from plasma, serum, urine, whole blood, leukocytes, peripheral blood mononuclear cells, huffy coat, sputum, tears, mucus, nasal washes, nasal aspirate, semen, saliva, peritoneal washings, ascites, cystic fluid, meningeal fluid, amniotic fluid, glandular fluid, lymph fluid, nipple aspirate, bronchial aspirate, bronchial brushing, synovial fluid, joint aspirate, organ secretions, cells, a cellular extract, and cerebrospinal fluid.

3. The method of claim 1, wherein the first and second aptamer-target molecule affinity complexes are non-covalent complexes.

4. The method of claim 1, wherein the target molecule is selected from a protein, a peptide, a carbohydrate, a polysaccharide, a glycoprotein, a hormone, a receptor, an antigen, an antibody, a vims, a bacteria, a metabolite, a cofactor, an inhibitor, a drug, a dye, a nutrient, a growth factor, a cell and a tissue.

5. The method of claim 1, wherein the first dilution is a dilution of the test sample of from 0.001% to 0.009% (or is 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008% or 0.009%) or is from 0.002% to 0.008% or is from 0.003% to 0.007% or is about 0.005%, and the second dilution is a dilution of the test sample of from 0.01% to 1% (or is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1%) or is from 0.1% to 0.8% or is from 0.2% to 0.75% or is about 0.5%.

6. The method of claim 1 , wherein the first dilution is a dilution of the test sample of from 0.001% to 0.009% (or 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008% or 0.009%) or is from 0.002% to 0.008%, or is from 0.003% to 0.007% or is about 0.005%; and the second dilution is a dilution of the test sample of from 5% to 39% (or is 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38% or 39%), or is from 15% to 30%, or is from 15% to 25%, or is about 20%.

7. The method of claim 1, wherein the first dilution is a dilution of the test sample of from 0.01% to 1% (or is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1%) or is from 0.1% to 0.8%, or is from 0.2% to 0.75%, or is about 0.5%; and the second dilution is a dilution of the test sample of from 5% to 39% (or is 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38% or 39%), or is from 15% to 30%, or is from 15% to 25%, or is about 20%.

8. The method of claim 1, wherein the first dilution is a dilution of the test sample of from 0.01% to 1% (or is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1%) or is from 0.1% to 0.8%, or is from 0.2% to 0.75%, or is about 0.5%; and the second dilution is a dilution of the test sample of from 0.001% to 0.009% (or is 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008% or 0.009%) or is from 0.002% to 0.008%, or is from 0.003% to 0.007%, or is about 0.005%.

9. The method of claim 1 , wherein the first dilution is a dilution of the test sample of from 5% to 39% (or is 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38% or 39%), or is from 15% to 30%, or is from 15% to 25%, or is about 20%, and the second dilution is a dilution of the test sample of from 0.01% to 1% (or is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1%) or is from 0.1% to 0.8%, or is from 0.2% to 0.75%, or is about 0.5%.

10. The method of claim 1, wherein the first dilution is a dilution of the test sample of from 5% to 39% (or is 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38% or 39%), or is from 15% to 30%, or is from 15% to 25%, or is about 20%, and the second dilution is a dilution of the test sample of from 0.001% to 0.009% (or is 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008% or 0.009%) or is from 0.002% to 0.008%, or is from 0.003% to 0.007%, or is about 0.005%.

11. The method of claim 1-, wherein the detecting for the presence or the determining of the level of the ~~dissociated~~ first ~~and~~aptamer, second ~~capture reagents~~aptamer, and third aptamer of the first, second, and third aptamer affinity complexes, or the presence or amount of the first, second, and third aptamer affinity complexes is performed by PCR, mass spectrometry, nucleic acid sequencing, next-generation sequencing (NGS) or hybridization.

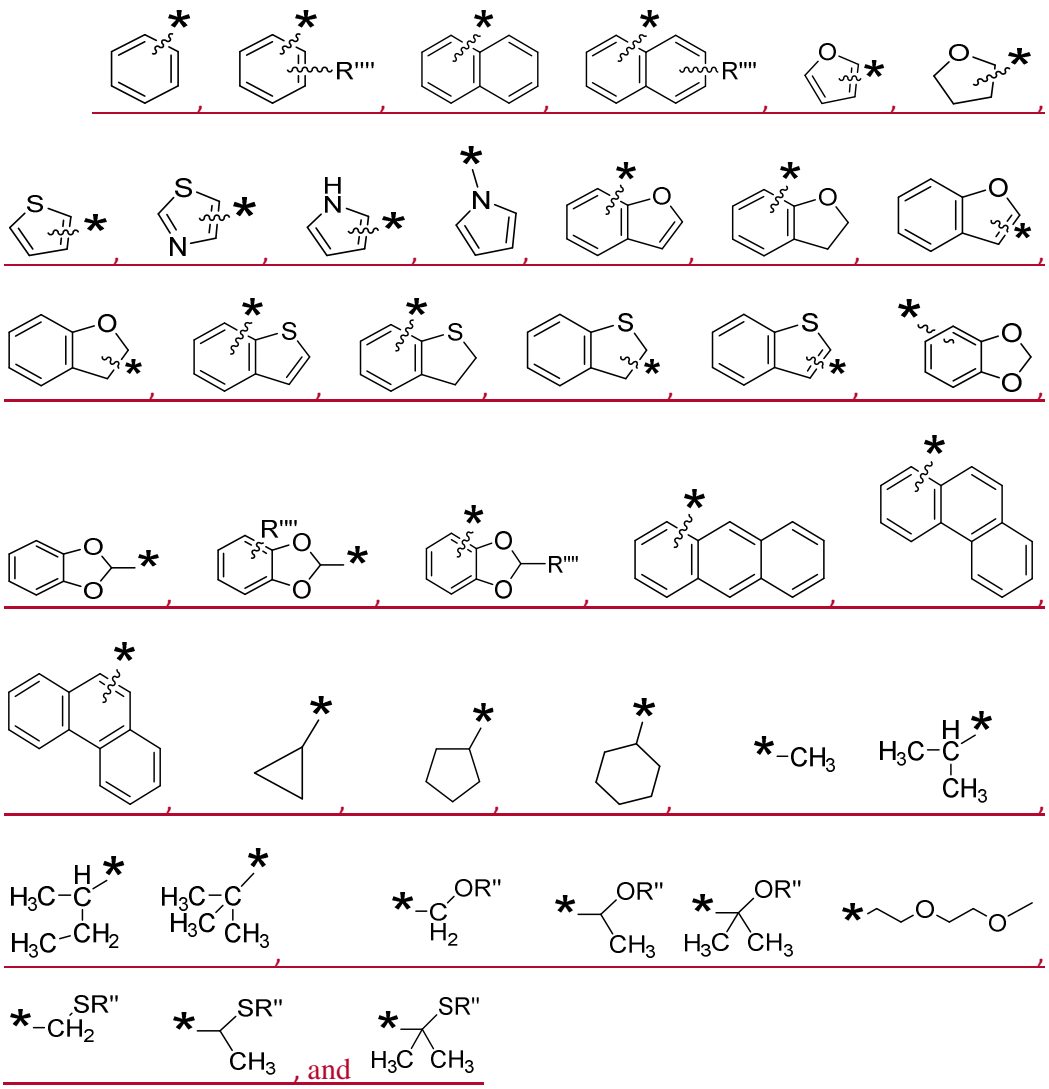
12. The method of claim 1, wherein the first aptamer and/or the second ~~ap tamer~~aptamer, independently, comprises at least one 5-position modified pyrimidine.

13. The method of claim 12, wherein the at least one 5-positon modified pyrimidine comprises a linker at the 5 -position of the pyrimidine and a moiety attached to the linker.

14. The method of claim 13, wherein the linker is selected from amide linker, a carbonyl linker, a propynyl linker, an alkyne linker, an ester linker, a urea linker, a carbamate linker, a guanidine linker, an amidine linker, a sulfoxide linker, and a sulfone linker.

15. The method of claim 13, wherein the moiety is a hydrophobic moiety.

16. The method of claim 15, wherein the moiety is selected from ~~the moieties of Groups I, II, III, IV, V, VII, VIII, IX, XI, XII, XIII, XV and XVI of Figure 1.~~:



, and

wherein * denotes the point of attachment of the R' group to a linker, and wherein R'' and R'''' are, independently, selected from a halogen (-F, -Cl, -Br, -I); nitrile (-CN); -NH_2; -OH; branched lower alkyl (C1-C20); linear lower alkyl (C1-C20); primary amide; secondary amide; tertiary amide; and alkoxy group.

17. The method of claim 15, wherein the moiety is selected from a naphthyl moiety, a benzyl moiety, a fluorobenzyl moiety, a tyrosyl moiety, an indole moiety a morpholino moiety, an isobutyl moiety, a 3,4-methylenedioxy benzyl moiety, a benzothiophenyl moiety, and a benzofuranyl moiety.

18. The method of claim 12, wherein the pyrimidine of the 5-position modified pyrimidine is a uridine, cytidine or thymidine.

~~19. The method of claim 18, wherein the third dilution sample is incubated separately from the first and second dilution samples to allow aptamer affinity complex formation of the third aptamer with its target molecule.~~

~~20. The method of 19, further comprising transferring the third dilution sample to the second mixture to form a third mixture, wherein the third aptamer affinity complex of the third dilution is captured on a solid support in the third mixture.~~

~~21. The method of claim 20, further comprising detecting for the presence of or determining the level of the third aptamer of the third aptamer affinity complex, or the presence or amount of the third aptamer affinity complex;~~

~~The method of claim 18~~

~~22.~~19. The method of claim 1, wherein the third dilution is a different dilution from the first dilution and the second dilution of the same test sample.

~~23.~~20. The method of claim ~~18~~1, wherein the third dilution is a dilution of the test sample selected from 5% to 39% (or is 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38% or 39%), from 15% to 30%, from 15% to 25%, about 20%; from 0.01% to 1% (or 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1%), from 0.1% to 0.8%, from 0.2% to 0.75%, about 0.5%; and from 0.001% to 0.009% (or 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008% or 0.009%), or from 0.002% to 0.008%, from 0.003% to 0.007%, about 0.005%.

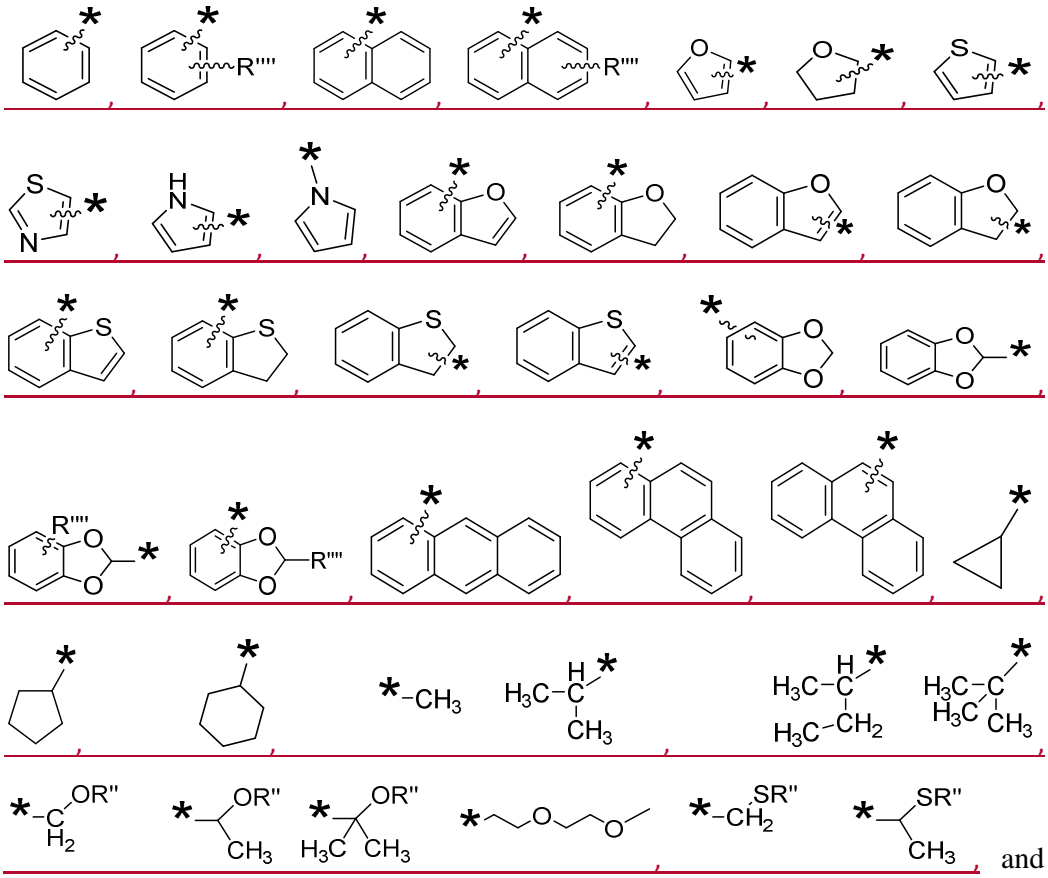
~~24.~~21. The method of claim ~~18~~1, wherein the third aptamer comprises at least one 5 -position modified pyrimidine.

~~25.~~22. The method of claim ~~24~~21, wherein the at least one 5-positon modified pyrimidine comprises a linker at the 5 -position of the pyrimidine and a moiety attached to the linker.

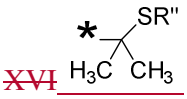
~~26.~~23. The method of claim ~~25~~22, wherein the linker is selected from amide linker, a carbonyl linker, a propynyl linker, an alkyne linker, an ester linker, a urea linker, a carbamate linker, a guanidine linker, an amidine linker, a sulfoxide linker, and a sulfone linker.

~~27.~~24. The method of claim ~~25~~22, wherein the moiety is a hydrophobic moiety.

25. The method of claim ~~27~~24, wherein the moiety is selected from the moieties ~~of Groups I, II, III, IV, V, VII, VIII, IX, XI, XII, XIII, XV~~:



and



~~XVI~~

~~28.~~ wherein * denotes the point of attachment of ~~Figure 1~~the R' group to a linker, and wherein R" and R"" are, independently, selected from a halogen (-F, -Cl, -Br, -I); nitrile (-CN); -NH_2; -OH; branched lower alkyl (C1-C20); linear lower alkyl (C1-C20); primary amide; secondary amide; tertiary amide; and alkoxy group.

~~29.~~26. The method of claim ~~27~~24, wherein the moiety is selected from a naphthyl moiety, a benzyl moiety, a fluorobenzyl moiety, a tyrosyl moiety, an indole moiety a morpholino moiety , an isobutyl moiety, a 3,4-methylenedioxy benzyl moiety, a benzothiophenyl moiety, and a benzofuranyl moiety.

~~30.~~27. The method of claim ~~24~~21, wherein the pyrimidine of the 5 -position modified pyrimidine is a uridine, cytidine or thymidine.

~~31.~~28. A method comprising:

a) contacting a first capture reagent with a first dilution to form a first mixture ~~and,~~ a second capture reagent with a second dilution to form a second mixture, and a third capture reagent with a third dilution for form a third dilution mixture; wherein each of the first ~~and,~~ second, and third capture reagents are each immobilized on a solid

support, and wherein each of the first ~~and,~~ second, and third capture reagents have affinity for a different target molecule;

b) incubating the first mixture, the second mixture, and the ~~second~~third mixture separately, wherein a first capture reagent-target molecule affinity complex is formed in the first mixture if the target molecule to which the first capture reagent has affinity for is present in the first mixture, wherein a second capture reagent-target molecule affinity complex is formed in the second mixture if the target molecule to which the second capture reagent has affinity for is present in the second mixture~~;~~, wherein a third capture reagent-target molecule affinity complex is formed in the third mixture if the target molecule to which the third capture reagent has affinity for is present in the third mixture;

c) sequentially releasing and combining the affinity complexes in a fourth mixture in an order selected from (i) the first capture reagent-target molecule affinity complex, followed by the second capture reagent-target molecule affinity complex ~~and,~~ followed by the third capture reagent-target molecule affinity complex; (ii) the second capture reagent-target molecule affinity complex, followed the first capture reagent-target molecule affinity complex~~;~~, followed by the third capture reagent-target molecule affinity complex; (iii) the first capture reagent-target molecule affinity complex, followed by the third capture reagent-target molecule affinity complex, followed by the second capture reagent-target molecule affinity complex; (iv) the second capture reagent-target molecule affinity complex, followed by the third capture reagent-target molecule affinity complex, followed by the first capture reagent-target molecule affinity complex; (v) the third capture reagent-target molecule affinity complex, followed by the first capture reagent-target molecule affinity complex, followed by the second capture reagent-target molecule affinity complex; and (vi) the third capture reagent-target molecule affinity complex, followed by the second capture reagent-target molecule affinity complex, followed by the first capture reagent-target molecule affinity complex;

d) attaching a first tag to the target molecule of the first ~~and,~~ second, and third capture reagent--target molecule affinity complexes;

e) contacting the tagged first ~~and,~~ second, and third capture reagent-target molecule affinity complexes to one or more solid supports such that the tag immobilizes the first ~~and,~~ second, and third capture reagent-target molecule affinity complexes to the one or more ~~one~~ solid supports;

f) dissociating the capture reagents from the capture reagent-target molecule affinity complexes;

g) detecting for the presence of or determining the level of the dissociated capture reagents;

wherein, the first dilution ~~and,~~ the second dilution ~~-~~, and the third dilution are different dilutions of a test sample~~; and~~.

~~wherein the method further comprises contacting a third capture reagent with a third dilution to form a third mixture, wherein the third capture reagent is immobilized on a~~

~~solid support, and wherein the third capture reagent has affinity for a different target molecule than the target molecules of the first and second capture reagents.~~

~~32.~~29. The method of claim ~~31~~28, wherein the test sample is selected from plasma, serum, urine, whole blood, leukocytes, peripheral blood mononuclear cells, huffy coat, sputum, tears, mucus, nasal washes, nasal aspirate, semen, saliva, peritoneal washings, ascites, cystic fluid, meningeal fluid, amniotic fluid, glandular fluid, lymph fluid, nipple aspirate, bronchial aspirate, bronchial brushing, synovial fluid, joint aspirate, organ secretions, cells, a cellular extract, and cerebrospinal fluid.

~~33.~~30. The method of claim ~~31~~28, wherein the first ~~and~~, second, and third capture reagent-target protein affinity complexes are non-covalent complexes.

~~34.~~31. The method of claim ~~31~~28, wherein the first capture reagent ~~and~~, the second capture reagent, and the third capture reagent are, independently, selected from an aptamer or an antibody.

~~35.~~32. The method of claim ~~31~~28, wherein the target molecule is selected from a protein, a peptide, a carbohydrate, a polysaccharide, a glycoprotein, a hormone, a receptor, an antigen, an antibody, a vims, a bacteria, a metabolite, a cofactor, an inhibitor, a drug, a dye, a nutrient, a growth factor, a cell and a tissue.

~~36.~~33. The method of claim ~~31~~28, wherein the first dilution is a dilution of the test sample of from 0.001% to 0.009% (or is 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008% or 0.009%) or is from 0.002% to 0.008% or is from 0.003% to 0.007% or is about 0.005%, and the second dilution is a dilution of the test sample of from 0.01% to 1% (or is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1%) or is from 0.1% to 0.8% or is from 0.2% to 0.75% or is about 0.5% and the third dilution is a dilution of the test sample of from 5% to 39% or is 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38% or 39%, or is from 15% to 30%, or is from 15% to 25%, or is 20%.

~~37.~~34. The method of claim ~~31~~28, wherein the first dilution is a dilution of the test sample of from 0.001% to 0.009% (or 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008% or 0.009%) or is from 0.002% to 0.008%, or is from 0.003% to 0.007% or is about 0.005%; and the second dilution is a dilution of the test sample of from 5% to 39% (or is 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38% or 39%), or is from 15% to 30%, or is from 15% to 25%, or is about 20~~%.~~%; and the third dilution is a dilution of the test sample of from 0.01% to 1% or is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1% or is from 0.1% to 0.8% or is from 0.2% to 0.75% or is 0.5%..

38.35. The method of claim 3128, wherein the first dilution is a dilution of the test sample of from 0.01% to 1% (or is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1%) or is from 0.1% to 0.8%, or is from 0.2% to 0.75%, or is about 0.5%; and the second dilution is a dilution of the test sample of from 5% to 39% (or is 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38% or 39%), or is from 15% to 30%, or is from 15% to 25%, or is about 20%; and the third dilution is a dilution of the test sample of from 0.001% to 0.009% or 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008% or 0.009% or is from 0.002% to 0.008%, or is from 0.003% to 0.007% or is 0.005%.

39.36. The method of claim 3128, wherein the first dilution is a dilution of the test sample of from 0.01% to 1% (or is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1%) or is from 0.1% to 0.8%, or is from 0.2% to 0.75%, or is about 0.5%; and the second dilution is a dilution of the test sample of from 0.001% to 0.009% (or is 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008% or 0.009%) or is from 0.002% to 0.008%, or is from 0.003% to 0.007%, or is about 0.005%; and the third dilution is a dilution of the test sample of from 5% to 39% or is 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38% or 39%, or is from 15% to 30%, or is from 15% to 25%, or is 20%.

40.37. The method of claim 3128, wherein the first dilution is a dilution of the test sample of from 5% to 39% (or is 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38% or 39%), or is from 15% to 30%, or is from 15% to 25%, or is about 20%, and the second dilution is a dilution of the test sample of from 0.01% to 1% (or is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1%) or is from 0.1% to 0.8%, or is from 0.2% to 0.75%, or is about 0.5%; and the third dilution is a dilution of the test sample of from 0.001% to 0.009% or 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008% or 0.009% or is from 0.002% to 0.008%, or is from 0.003% to 0.007% or is 0.005%.

41.38. The method of claim 3128, wherein the first dilution is a dilution of the test sample of from 5% to 39% (or is 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38% or 39%), or is from 15% to 30%, or is from 15% to 25%, or is about 20%, and the second dilution is a dilution of the test sample of from 0.001% to 0.009% (or is 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008% or 0.009%) or is from 0.002% to 0.008%, or is from 0.003% to 0.007%, or is about 0.005%; and the third dilution is a dilution of the test sample of from 0.01% to 1% or is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%,

0.6%, 0.7%, 0.8%, 0.9% or 1% or is from 0.1% to 0.8% or is from 0.2% to 0.75% or is 0.5%.

42.39. The method of claim 3128, wherein the detecting for the presence or the determining of the level of the dissociated first and, second, and third capture reagents is performed by PCR, mass spectrometry, nucleic acid sequencing, next-generation sequencing (NGS) or hybridization.

43. The method of claim 31, further comprising incubating the third mixture separately from the first mixture and the second mixture, wherein a third capture reagent-target molecule affinity complex is formed in the third mixture if the target molecule to which the third capture reagent has affinity for is present in the third mixture.

44. The method of claim 43, further comprising sequentially releasing and combining the third capture reagent-target molecule affinity with the first and second capture reagent-target molecule affinity complexes into the fourth mixture in an order selected from (i) the first capture reagent-target molecule affinity complex, followed by the second capture reagent- target molecule affinity complex, followed by the third capture reagent-target molecule affinity complex; (ii) the first capture reagent-target molecule affinity complex, followed by the third capture reagent-target molecule affinity complex, followed by the second capture reagent- target molecule affinity complex; (iii) the second capture reagent-target molecule affinity complex, followed by the third capture reagent-target molecule affinity complex, followed by the first capture reagent-target molecule affinity complex; (iv) the second capture reagent-target molecule affinity complex, followed by the first capture reagent-target molecule affinity complex, followed by the third capture reagent-target molecule affinity complex; (v) the third capture reagent-target molecule affinity complex, followed by the first capture reagent-target molecule affinity complex, followed by the second capture reagent-target molecule affinity complex; and (vi) the third capture reagent-target molecule affinity complex, followed by the second capture reagent-target molecule affinity complex, followed by the first capture reagent- target molecule affinity complex.

45. The method of claim 43, wherein the third dilution is a different dilution from the first dilution and the second dilution of the same test sample.

46. The method of claim 43, wherein the third dilution is a dilution of the test sample selected from 5% to 39% (or is 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38% or 39%), from 15% to 30%, from 15% to 25%, about 20%; from 0.01% to 1% (or 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1%), from 0.1% to 0.8%, from 0.2% to 0.75%, about 0.5%; and from 0.001% to 0.009% (or 0.001%, 0.002%, 0.003%,

~~0.004%, 0.005%, 0.006%, 0.007%, 0.008% or 0.009%), or from 0.002% to 0.008%, from 0.003% to 0.007%, about 0.005%.~~

~~47. The method of claim 44, further comprising detecting for the presence of or determining the level of the third aptamer of the third aptamer affinity complex, or the presence or amount of the third aptamer affinity complex.~~

~~48.~~40. ~~The method of claims 31 to 47,~~ wherein the aptamer comprises at least one 5-position modified pyrimidine.

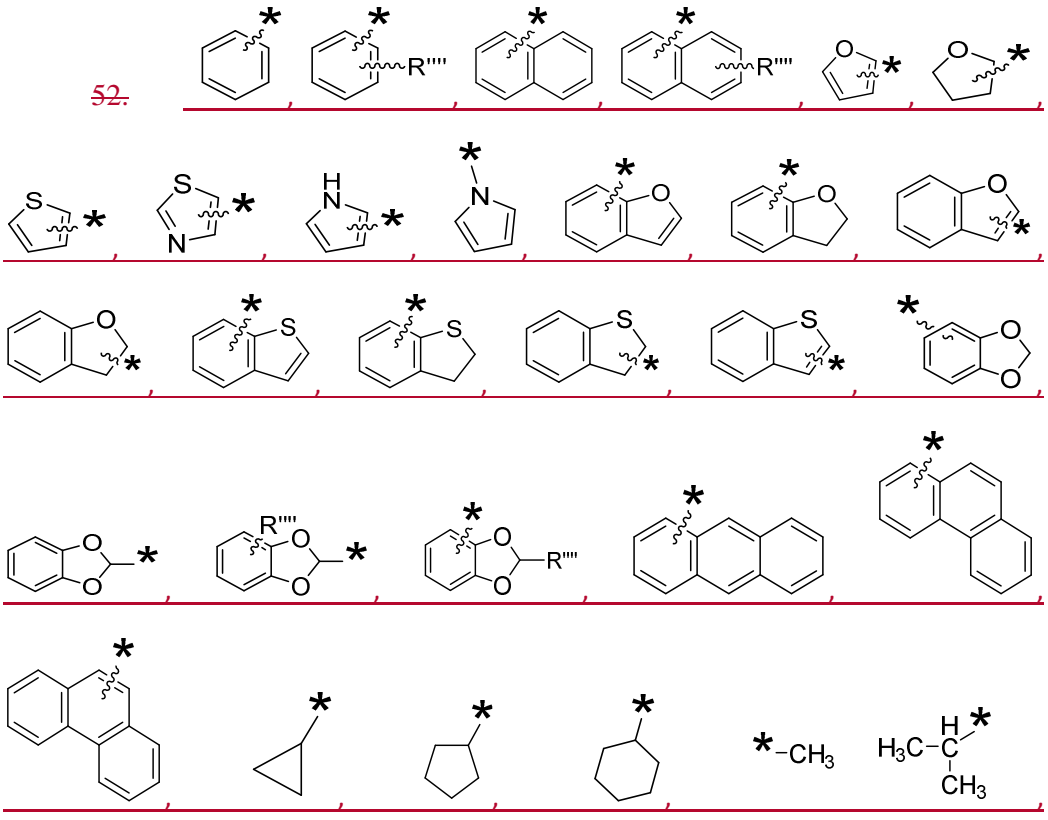
~~49.~~41. The method of claim ~~48~~40, wherein the at least one 5-positon modified pyrimidine comprises a linker at the 5 -position of the pyrimidine and a moiety attached to the linker.

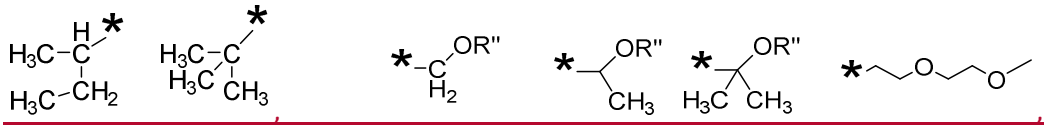
~~50.~~42. The method of claim ~~49~~41, wherein the linker is selected from amide linker, a carbonyl linker, a propynyl linker, an alkyne linker, an ester linker, a urea linker, a carbamate linker, a guanidine linker, an amidine linker, a sulfoxide linker, and a sulfone linker.

~~51.~~43. The method of claim ~~49~~41, wherein the moiety is a hydrophobic moiety.

44. The method of claim ~~51~~43, wherein the moiety is selected from the moieties ~~of Groups I, II, III, IV, V, VII, VIII, IX, XI, XII, XIII, XV~~:

~~52.~~







, and ~~XVI of Figure 1.~~

~~53. The method of claim 51,~~ wherein ~~the moiety is selected from a naphthyl moiety, a benzyl moiety, a fluorobenzyl moiety, a tyrosyl moiety, an indole moiety a morpholino moiety, an isobutyl moiety, a 3,4-methylenedioxy benzyl moiety, a benzothiophenyl moiety~~* denotes the point of attachment of the R' group to a linker, and ~~a benzofuranyl moiety.~~

~~54. The method of claim 48,~~ wherein ~~the pyrimidine of the 5-position modified pyrimidine is a uridine, cytidine or thymidine.~~

~~55. A method comprising:~~

~~a) contacting a first capture reagent with a first dilution to form a first mixture, a second capture reagent with a second dilution to form a second mixture,~~R" and ~~a third capture reagent with a third dilution to form a third dilution mixture, wherein each of the first, second, and third capture reagents are each immobilized on a solid support, and wherein each of the first, second and third capture reagents have affinity for a different target molecule;~~

~~b) incubating the first mixture, second mixture and third mixture separately, wherein a first capture reagent-target molecule affinity complex is formed in the first mixture if the target molecule to which the first capture reagent has affinity for is present in the first mixture, wherein a second capture reagent-target molecule affinity complex is formed in the second mixture if the target molecule to which the second capture reagent has affinity for is present in the second mixture, and wherein a third capture reagent-target molecule affinity complex is formed in the third mixture if the target molecule to which the third capture reagent has affinity for is present in the third mixture;~~

~~c) sequentially releasing and combining the affinity complexes in a forth mixture in an order selected from (i) the first capture reagent-target molecule affinity complex, followed by the second capture reagent-target molecule affinity complex, followed by the third capture reagent-target molecule affinity complex; (ii) the first capture reagent-target molecule affinity complex, followed by the third capture reagent-target molecule affinity complex, followed by the second capture reagent-target molecule affinity complex; (iii) the second capture reagent-target molecule affinity complex, followed by the third capture reagent-target molecule affinity complex, followed by the first capture reagent-target molecule affinity complex; (iv) the second capture reagent-target molecule affinity complex, followed by the first capture reagent-target molecule affinity complex, followed by the third capture reagent- target molecule affinity complex; (v) the third capture reagent-target molecule affinity complex, followed by the first capture reagent-target molecule affinity complex, followed by~~

the second capture reagent-target molecule affinity complex; and (vi) the third capture reagent–target molecule affinity complex, followed by the second capture reagent–target molecule affinity complex, followed by the first capture reagent-target molecule affinity complex;

d) attaching a first tag to the target molecule of the first, second, and third capture reagent–target molecule affinity complexes;

e) contacting the tagged first, second, and third capture reagent-target molecule affinity complexes to one or more solid supports such that the tag immobilizes the first, second and third capture reagent-target molecule affinity complexes to the one or more one solid supports;

f) dissociating the capture reagents from the capture reagent-target molecule affinity complexes;

g) detecting for the presence of or determining the level of the dissociated capture reagents;

wherein, the first dilution, the second dilution, and third dilution are different dilutions of a test sample.

56. The method of claim 55, wherein the test sample is selected from plasma, serum, urine, whole blood, leukocytes, peripheral blood mononuclear cells, buffy coat, sputum, tears, mucus, nasal washes, nasal aspirate, semen, saliva, peritoneal washings, ascites, cystic fluid, meningeal fluid, amniotic fluid, glandular fluid, lymph fluid, nipple aspirate, bronchial aspirate, bronchial brushing, synovial fluid, joint aspirate, organ secretions, cells, a cellular extract, and cerebrospinal fluid.

57. The method of claim 55, wherein the first, second and third capture reagent-target protein affinity complexes are non-covalent complexes.

58. The method of claim 55, wherein the first capture reagent, the second capture reagent and the third capture reagentR"" are, independently, selected from an aptamer or an antibodya halogen (-F, -Cl, -Br, -I); nitrile (-CN); -NH_2; -OH; branched lower alkyl (C1-C20); linear lower alkyl (C1-C20); primary amide; secondary amide; tertiary amide; and alkoxy group.

59. The method of claim 55, wherein the target molecule is selected from a protein, a peptide, a carbohydrate, a polysaccharide, a glycoprotein, a hormone, a receptor, an antigen, an antibody, a virus, a bacteria, a metabolite, a cofactor, an inhibitor, a drug, a dye, a nutrient, a growth factor, a cell and a tissue.

60. The method of claim 55, wherein the detecting for the presence or the determining of the level of the dissociated first and second capture reagents is performed by PCR, mass spectrometry, nucleic acid sequencing, next-generation sequencing (NGS) or hybridization.

61. The method of claim 58, wherein the aptamer comprises at least one 5-position modified pyrimidine.

62. The method of claim 61, wherein the at least one 5-positon modified pyrimidine comprises a linker at the 5-position of the pyrimidine and a moiety attached to the linker.

63. The method of claim 62, wherein the linker is selected from amide linker, a carbonyl linker, a propynyl linker, an alkyne linker, an ester linker, a urea linker, a carbamate linker, a guanidine linker, an amidine linker, a sulfoxide linker, and a sulfone linker.

64. The method of claim 62, wherein the moiety is a hydrophobic moiety.

65. The method of claim 64, wherein the moiety is selected from the moieties of Groups I, II, III, IV, V, VII, VIII, IX, XI, XII, XIII, XV and XVI of Figure 1.

66.45. The method of claim 64The method of claim 43, wherein the moiety is selected from a naphthyl moiety, a benzyl moiety, a fluorobenzyl moiety, a tyrosyl moiety, an indole moiety a morpholino moiety, an isobutyl moiety, a 3,4-methylenedioxy benzyl moiety, a benzothiophenyl moiety, and a benzofuranyl moiety.

67.46. The method of claim 6140, wherein the pyrimidine of the 5--position modified pyrimidine is a uridine, cytidine or thymidine.

68. The method of claim 55, wherein the first dilution is a dilution of the test sample of from 0.001% to 0.009% (or is 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008% or 0.009%) or is from 0.002% to 0.008% or is from 0.003% to 0.007% or is about 0.005%, the second dilution is a dilution of the test sample of from 0.01% to 1% (or is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1%) or is from 0.1% to 0.8% or is from 0.2% to 0.75% or is about 0.5%; and the third dilution is a dilution of the test sample of from 5% to 39% (or is 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38% or 39%), or is from 15% to 30%, or is from 15% to 25%, or is about 20%.

69. The method of claim 55, wherein the first dilution is a dilution of the test sample of from 0.001% to 0.009% (or 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008% or 0.009%) or is from 0.002% to 0.008%, or is from 0.003% to 0.007% or is about 0.005%; the second dilution is a dilution of the test sample of from 5% to 39% (or is 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38% or 39%), or is from 15% to 30%, or is from 15% to 25%, or is about 20%; and the third dilution is a dilution of the test sample of from 0.01% to 1% (or is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1%) or is from 0.1% to 0.8% or is from 0.2% to 0.75% or is about 0.5%.

~~70. The method of claim 55, wherein the first dilution is a dilution of the test sample of from 0.01% to 1% (or is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1%) or is from 0.1% to 0.8%, or is from 0.2% to 0.75%, or is about 0.5%; the second dilution is a dilution of the test sample of from 5% to 39% (or is 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38% or 39%), or is from 15% to 30%, or is from 15% to 25%, or is about 20%; and the third dilution is a dilution of the test sample of from 0.001% to 0.009% (or 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008% or 0.009%) or is from 0.002% to 0.008%, or is from 0.003% to 0.007% or is about 0.005%.~~

~~71. The method of claim 55, wherein the first dilution is a dilution of the test sample of from 0.01% to 1% (or is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1%) or is from 0.1% to 0.8%, or is from 0.2% to 0.75%, or is about 0.5%; the second dilution is a dilution of the test sample of from 0.001% to 0.009% (or is 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008% or 0.009%) or is from 0.002% to 0.008%, or is from 0.003% to 0.007%, or is about 0.005%; and the third dilution is a dilution of the test sample of from 5% to 39% (or is 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38% or 39%), or is from 15% to 30%, or is from 15% to 25%, or is about 20%.~~

~~72. The method of claim 55, wherein the first dilution is a dilution of the test sample of from 5% to 39% (or is 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38% or 39%), or is from 15% to 30%, or is from 15% to 25%, or is about 20%, the second dilution is a dilution of the test sample of from 0.01% to 1% (or is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1%) or is from 0.1% to 0.8%, or is from 0.2% to 0.75%, or is about 0.5%; and the third dilution is a dilution of the test sample of from 0.001% to 0.009% (or 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008% or 0.009%) or is from 0.002% to 0.008%, or is from 0.003% to 0.007% or is about 0.005%.~~

~~The method of claim 55, wherein the first dilution is a dilution of the test sample of from 5% to 39% (or is 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38% or 39%), or is from 15% to 30%, or is from 15% to 25%, or is about 20%; the second dilution is a dilution of the test sample of from 0.001% to 0.009% (or is 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008% or 0.009%) or is from 0.002% to 0.008%, or is from 0.003% to 0.007%, or is about 0.005%; and the third dilution is a dilution of the test sample of from 0.01% to 1% (or is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.6%, 0.7%,~~

~~0.8%, 0.9% or 1%) or is from 0.1% to 0.8% or is from 0.2% to 0.75% or is about 0.5%.~~

~~73.~~