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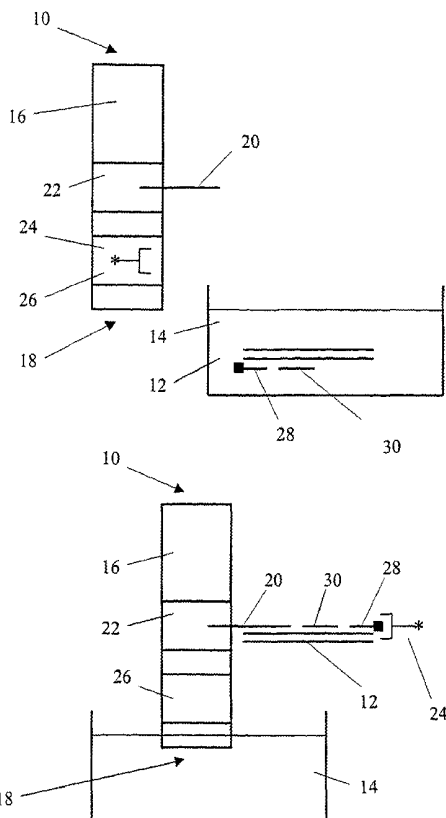
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(54) Title: IMPROVED STABILITY OF HYBRIDISATION INTERACTIONS IN DIPSTICK ASSAYS



(57) Abstract: Use of probes with non complementary nucleotides in dipstick assays to test for the presence of a target nucleic acid in a sample solution is described. The probes have greater affinity for target nucleic acid and can be used in capture and/or detection of target nucleic acid. The sensitivity of target nucleic acid detection is thereby increased. Dipsticks and kits are also described.



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Improved Stability of Hybridisation Interactions in
Dipstick Assays

5 The present invention relates to enhanced nucleic acid detection by dipsticks. Dipsticks of the invention are used to detect the presence of a target nucleic acid in a sample solution, for example to identify whether a patient is infected with a disease causing microorganism such as *Chlamydia trachomatis*.

10 Some conventional tests for detecting the presence of a target nucleic acid in a sample solution rely on amplification of the target nucleic acid using the polymerase chain reaction (PCR). Whilst this reaction allows detection of small quantities of target nucleic acid, it can
15 take several hours before a result is obtained. This can be a significant disadvantage because it is often desired to obtain the result as quickly as possible, for example, to keep patient waiting times to a minimum. Further disadvantages of such methods are the requirement for
20 expensive specialist equipment to perform the reaction and the relatively high cost of the reagents.

In contrast, dipsticks detect unamplified target nucleic acid without the requirement for any specialist equipment and the results can be obtained much more rapidly than PCR-
25 based methods. Patients can then be treated in the same visit. This is particularly advantageous where the patient is unlikely to, or cannot, return at a later date.

In a typical conventional dipstick described in US 5,310,650, a single stranded DNA capture probe is

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immobilised on a nitrocellulose filter at a capture zone remote from one end of the filter (the contact end). Part of the sequence of the capture probe is complementary to the sequence of a first region of the target nucleic acid to be detected. A labelled single stranded DNA detection probe is immobilised on the nitrocellulose filter at a probe zone located between the capture zone and the contact end of the filter. The detection probe has sequence complementary to the sequence of a second region (distinct from the first region) of the target nucleic acid.

To detect target DNA in a sample solution thought to contain target DNA, the contact end of the nitrocellulose filter is contacted with the sample solution. The sample solution wicks up the filter by capillary action and passes the probe zone and the capture zone. As the sample solution passes the probe zone, it mobilises the detection probe and causes it to rise with the sample solution towards the capture zone. Mobilised detection probe can then hybridise to the second region of any target DNA present in the sample solution.

When the hybridised detection probe and target DNA arrive at the capture zone, the first region of the target DNA can hybridise to the immobilised capture probe. A ternary complex is thereby formed between the target nucleic acid, the capture probe and the labelled detection probe. Presence of label at the capture zone, therefore, indicates that target DNA is present in the sample solution.

With a second type of conventional dipstick, the labelled DNA detection probe is not immobilised on the nitrocellulose filter. Instead the detection probe is added to the sample solution under conditions allowing hybridisation of the

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detection probe to any target nucleic acid in the sample solution. The contact end of the nitrocellulose filter is then contacted with the sample solution and as the sample solution wicks up the dipstick, target nucleic acid which is hybridised to the detection probe is captured at the capture zone by the capture probe.

It has been found, however, that the sensitivity of nucleic acid detection by conventional dipsticks can be low. If the target nucleic acid is double stranded, the sensitivity of dipstick detection can be particularly low. Consequently, the presence of target nucleic acid in a sample solution can sometimes be undetected. It is desired, therefore, to improve the sensitivity of target nucleic acid detection by dipsticks.

According to a first aspect of the invention there is provided a dipstick for testing for the presence of target nucleic acid in a sample solution which comprises:
a chromatographic strip having a contact end for contacting the sample solution; and
a capture probe immobilised at a capture zone remote from the contact end, the capture probe being capable of hybridising to the target nucleic acid or to a hook capture probe bound to the target nucleic acid, wherein one or both ends of the capture probe are coupled to one or more nucleotides, preferably at least three nucleotides, which do not hybridise to the target nucleic acid when the capture probe has hybridised to the target nucleic acid or which do not hybridise to the hook capture probe when the capture probe has hybridised to the hook capture probe.

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There is also provided according to the first aspect of the invention a kit for testing for the presence of target nucleic acid in a sample solution which comprises a dipstick according to the invention and a detection probe capable of
5 hybridising to the target nucleic acid thereby allowing detection of target nucleic acid utilising the detection probe.

The term 'chromatographic strip' is used herein to mean any porous strip of material capable of transporting a solution by capillarity.

10 The detection probe may instead be releasably immobilised at a probe zone located between the contact end and the capture zone of the dipstick.

One or both ends of the detection probe may be coupled to one or more nucleotides, preferably at least three
15 nucleotides, which do not hybridise to the target nucleic acid when the detection probe has hybridised to the target nucleic acid.

According to a second aspect of the invention there is provided a dipstick for testing for the presence of target
20 nucleic acid in a sample solution which comprises:

a chromatographic strip having a contact end for contacting the sample solution;

a capture moiety, immobilised at a capture zone remote from the contact end, the capture moiety being capable of binding
25 directly or indirectly to the target nucleic acid; and

a detection probe, releasably immobilised at a probe zone located between the contact end and the capture zone, the detection probe being capable of hybridising to the target nucleic acid to allow detection of the target nucleic acid

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utilising the detection probe, wherein one or both ends of the detection probe are coupled to one or more nucleotides, preferably at least three nucleotides, which do not hybridise to the target nucleic acid when the detection probe has hybridised to the target nucleic acid.

According to the invention there is further provided a kit for testing for the presence of target nucleic acid in a sample solution which comprises:

a dipstick according to the second aspect of the invention in which the capture moiety is capable of binding to a capture ligand coupled to a capture probe bound to the target nucleic acid; and

a capture probe capable of hybridising to the target nucleic acid or to a hook capture probe bound to the target nucleic acid wherein the capture probe is coupled to a capture ligand which can be bound by the capture moiety.

One or both ends of the capture probe may be coupled to one or more nucleotides, preferably at least three nucleotides, which do not hybridise to the target nucleic acid when the capture probe has hybridised to the target nucleic acid or which do not hybridise to the hook capture probe when the capture probe has hybridised to the hook capture probe.

There is also provided according to the invention a kit for testing for the presence of target nucleic acid in a sample solution which comprises:

i) a dipstick comprising a chromatographic strip having a contact end for contacting the sample solution and a capture probe capable of hybridising to the target nucleic acid, or to a hook capture probe bound to the target nucleic acid,

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the capture probe being immobilised at a capture zone of the chromatographic strip remote from the contact end; and

ii) a detection probe capable of hybridising to the target nucleic acid thereby allowing detection of the target nucleic acid utilising the detection probe, wherein one or both ends of the detection probe are coupled to one or more nucleotides, preferably at least three nucleotides, which do not hybridise to the target nucleic acid when the detection probe has hybridised to the target nucleic acid.

There is also provided according to the invention a kit for testing for the presence of target nucleic acid in a sample solution which comprises:

i) a dipstick comprising a chromatographic strip having a contact end for contacting the sample solution and a capture moiety immobilised at a capture zone of the chromatographic strip remote from the contact end, the capture moiety being capable of binding directly or indirectly to the target nucleic acid;

ii) a capture probe capable of hybridising to the target nucleic acid, or to a hook capture probe bound to the target nucleic acid, wherein the capture probe is coupled to a capture ligand which can be bound by the capture moiety; and

iii) a detection probe capable of hybridising to the target nucleic acid, wherein one or both ends of the detection probe are coupled to one or more nucleotides, preferably at least three nucleotides, which do not hybridise to the target nucleic acid when the detection probe has hybridised to the target nucleic acid.

There is also provided according to the invention a kit for testing for the presence of target nucleic acid in a sample solution which comprises:

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- i) a dipstick comprising a chromatographic strip having a contact end for contacting the sample solution and a capture moiety immobilised at a capture zone of the chromatographic strip remote from the contact end;
- 5 ii) a capture probe capable of hybridising to the target nucleic acid, or to a hook capture probe bound to the target nucleic acid, wherein the capture probe is coupled to a capture ligand which can be bound by the capture moiety and one or both ends of the capture probe are coupled to one or
- 10 more nucleotides, preferably at least three nucleotides, which do not hybridise to the target nucleic acid when the capture probe has hybridised to the target nucleic acid; and
- iii) a detection probe capable of hybridising to the target nucleic acid thereby allowing detection of the target
- 15 nucleic acid utilising the detection probe.

Dipsticks and kits of the invention may be used in methods for testing for the presence of target nucleic acid in a sample solution in which a detection probe capable of hybridising to the target nucleic acid is incubated with the

20 sample solution under conditions for hybridisation of the detection probe to target nucleic acid. The contact end of the dipstick is contacted with the sample solution allowing sample solution to move up the dipstick by capillary action. Target nucleic acid hybridised to the detection probe in the

25 sample solution can then be captured by the capture probe at the capture zone. The presence of target nucleic acid in the sample solution is then indicated by the presence of the detection probe at the capture zone.

However, where the detection probe is releasably immobilised

30 to the dipstick, for example at a probe zone between the contact end and the capture zone of the chromatographic

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strip, the contact end of the dipstick is contacted with the sample solution so that sample solution wicks up the dipstick by capillary action. As the sample solution passes the probe zone of the dipstick it mobilises the detection probe so that the detection probe can hybridise to target nucleic acid in the sample solution and move with the target nucleic acid to the capture zone. Target nucleic acid hybridised to the detection probe is captured by the capture probe as the sample solution passes the capture zone. The presence of target nucleic acid in the sample solution is then indicated by the presence of the detection probe at the capture zone.

The capture moiety may be capable of binding directly or indirectly to the target nucleic acid by base pairing or non base pairing interaction. For example, the capture moiety may comprise a capture probe capable of hybridising directly to the target nucleic acid. Alternatively, the capture moiety may comprise a capture probe capable of hybridising to a hook capture probe bound to the target nucleic acid.

The capture moiety may be capable of binding to a capture ligand coupled to a capture probe bound to the target nucleic acid, thereby allowing indirect binding of the capture moiety to the target nucleic acid. For example the capture moiety may be an antibody or antibody fragment. If the capture probe is coupled to a capture ligand, the capture probe may be linked to a capture probe spacer which is linked to the capture ligand to space the capture ligand from the capture probe.

The hook capture probe can be added to the sample solution so that it can bind to target nucleic acid in the sample

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solution and be captured by the capture probe as the sample solution wicks up the dipstick by capillary action.

The capture probe, the hook capture probe and the detection probe may each comprise at least one nucleic acid or nucleic acid analogue. Where a probe comprises more than one nucleic acid or nucleic acid analogue, they are preferably hybridised together.

The detection probe may be coupled to a label thereby allowing direct detection of target nucleic acid at the capture zone. Alternatively the detection probe may be coupled to a detection ligand allowing indirect detection of target nucleic acid using a detection ligand binding moiety. Examples of suitable labels include textile dyes, metal sol such as colloidal gold, and coloured particles such as coloured latex particles. Such labels can be coupled directly to the detection probe or, if the detection probe is coupled to a detection ligand, to the detection ligand binding moiety.

Examples of suitable capture or detection ligands include biotin (captured or detected for example by an anti-biotin antibody, avidin, streptavidin or a derivative thereof), fluorescein (captured or detected for example by an anti-fluorescein antibody) and 2,4-dinitrophenol (DNP) (captured or detected for example by an anti-DNP antibody).

The detection probe may comprise a universal detection probe which is capable of hybridising to a hook detection probe bound to the target nucleic acid. The universal detection probe may be linked to a label or a detection ligand thereby allowing detection of the detection probe.

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It will be appreciated that kits of the invention may further comprise any reagent required for the kit to be used to detect target nucleic acid in a sample solution. For example, kits of the invention which comprise a detection
5 probe coupled to a detection ligand may further comprise a detection ligand binding moiety.

The detection ligand binding moiety may comprise an antibody or antibody fragment, or a non antibody. For example, if the detection ligand comprises biotin the detection ligand
10 binding moiety may comprise an anti-biotin antibody, streptavidin, avidin or a derivative thereof which retains biotin binding activity. Preferably the detection ligand binding moiety is labelled thereby allowing indirect detection of target nucleic acid utilising the detection
15 probe and the detection ligand binding moiety.

It will be understood that the invention relates to use of detection probes and/or capture probes linked to one or more 'non pairing' nucleotides and that the detection probe or capture probe may be immobilised to the dipstick or
20 incubated with the sample solution depending on the method of detection of target nucleic acid.

Use of non pairing nucleotides comprising nucleobases in accordance with the invention is thought to be particularly effective because the nucleotide or nucleobase is able to
25 form stacking interactions with the base pairs formed between the capture probe or the detection probe and the target nucleic acid. Formation of the stacking interactions is thought to enhance the hybridisation of the capture probe or the detection probe to the target nucleic acid thereby
30 improving the efficiency of capture or detection of the target nucleic acid at the capture zone of the dipstick.

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Where appropriate, dipsticks and kits of the invention may be used in the following types of dipstick assay to test for the presence of a target nucleic acid in a sample solution:

- 1) A dipstick is provided which comprises a chromatographic strip having a contact end and a capture probe immobilised at a capture zone remote from the contact end, the capture probe being capable of hybridising to the target nucleic acid. A detection probe is contacted with the sample solution under conditions for hybridisation of the detection probe to the target nucleic acid. The sample solution is contacted with the contact end of the dipstick to cause sample solution to move by capillary action to the capture zone, thereby allowing target nucleic acid and the detection probe to move with the sample solution to the capture zone, and target nucleic acid to be captured at the capture zone. Detection probe can then be detected for at the capture zone. The presence of detection probe at the capture zone indicates that target nucleic acid was present in the sample solution.
- 20 In a variation of this assay, the detection probe may be releasably immobilised to the dipstick between the contact end and the capture zone instead of being separate from the dipstick. When the contact end of the dipstick is contacted with the sample solution causing the sample solution to move by capillary action to the capture zone, the detection probe is released into the sample solution so that released detection probe can hybridise to target nucleic acid in the sample solution as it moves to the capture zone.

In further variations of this assay, the detection probe may be separate from the sample solution and contacted with the

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capture zone of the dipstick. This will usually be done after the contact end of the dipstick has been contacted with the sample solution. The detection probe may be contacted directly with the capture zone, or the detection
5 probe may be in a separate probe solution which is contacted with the contact end of the dipstick to cause the probe solution to move by capillary action to the capture zone.

2) A dipstick is provided which comprises a chromatographic strip having a contact end and a capture moiety immobilised
10 at a capture zone remote from the contact end, the capture moiety being capable of binding a capture probe hybridised to the target nucleic acid. The capture probe is contacted with the sample solution under conditions for hybridisation of the capture probe to the target nucleic acid. The sample
15 solution is contacted with the contact end of the dipstick to cause sample solution to move by capillary action to the capture zone, thereby allowing target nucleic acid and the capture probe to move with the sample solution to the capture zone, and target nucleic acid to be captured at the
20 capture zone by binding of the capture moiety to the capture probe. Target nucleic acid can then be detected for at the capture zone. Target nucleic acid may be detected using a detection probe as described for assay (1). The detection probe may be added to the sample solution with the capture
25 probe or separately from the capture probe (in any order). Alternatively the detection probe may be releasably immobilised to the dipstick between the contact end and the capture zone, or may be contacted separately with the capture zone as described for assay (1).

30 In a variation of assay (2), the capture probe instead of being mixed with the sample solution, may be releasably

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immobilised to the dipstick between the contact end and the capture zone. When the contact end of the dipstick is contacted with the sample solution causing the sample solution to move by capillary action to the capture zone, the capture probe is released into the sample solution so that released capture probe is released into the sample solution so that released capture probe can hybridise to target nucleic acid in the sample solution as it moves to the capture zone. Target nucleic acid may be detected for using a detection probe which may be contacted with the sample solution, releasably immobilised to the dipstick between the contact end and the capture zone, or contacted separately with the capture zone.

In a further variation of assay (2), the capture probe may be contacted with the capture zone before, (or exceptionally, at the same time as) the sample solution reaches the capture zone by capillary action. This will allow the capture probe to be bound by the capture moiety at the capture zone so that target nucleic acid may be captured. The capture probe may be in a separate capture probe solution which is contacted separately with the capture zone by directly applying it to the capture zone, or by contacting the capture probe solution with the contact end of the dipstick to cause the capture probe to move by capillary action to the capture zone. Subsequent contact of the contact end of the dipstick with the sample solution will allow target nucleic acid reaching the capture zone by capillary action to be captured there. Again, target nucleic acid may be detected for using a detection probe which may be contacted with the sample solution, releasably immobilised to the dipstick between the contact end and the capture zone, or contacted separately with the capture zone.

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As an alternative to use of a detection probe in assay (2), the target nucleic acid may be labelled directly in the sample solution, for example by covalent attachment of a label to the target nucleic acid. This may be achieved by
5 contact of a precursor label with the sample solution and incubation of the sample solution and precursor label under conditions for covalent attachment of the label to target nucleic acid.

The capture moiety of assay (2) may be a universal capture
10 probe capable of hybridising to the capture probe, or the capture moiety may be capable of binding by non base pairing interaction to the capture probe. For example, when the capture probe comprises one or more capture ligands, the capture moiety is a capture ligand binding moiety.

15 Where the dipstick assay uses more than one probe capable of hybridising to the target nucleic acid it is preferred that all the probes are added to the sample solution and that hybridisation is carried out in a single step. This simplifies the assay, making it easier and quicker to
20 perform. It has been found that the sensitivity of detection of target nucleic acid using a one step hybridisation assay is about equal to the sensitivity of detection when hybridisation is carried out in multiple steps. Multiple step hybridisation may be carried out by
25 sequential hybridisation of the different probes to the target nucleic acid in the sample solution, or by contacting the dipstick with different solutions each containing a different probe. Usually, the latter method of multiple step hybridisation will involve washing the dipstick between
30 each contact with a different probe solution. Whilst there may be circumstances in which multiple step hybridisation is

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preferred, it will be appreciated that the simpler and quicker format of one step hybridisation will usually be preferred.

It is most preferred that the sample solution is of suitable composition to allow the hybridisation reactions to take place in a single hybridisation step and also to allow non base pairing interactions to take place (for example between a detection ligand and a detection ligand binding moiety and between a capture ligand and a capture ligand binding moiety) and transport a complex comprising target nucleic acid and one or more hybridised probes and (optionally) ligand binding moieties by capillary action up the dipstick. Using such a sample solution, it will be appreciated that the hybridisation reactions can then be carried out in a single step, and any ligand-ligand binding moiety interactions can take place, before the sample solution is contacted directly with the contact end of the dipstick (without the need to first dilute or alter the sample solution). Ligand-ligand binding moiety interactions can additionally or alternatively take place on the dipstick if desired as the sample solution travels to the capture zone. Simple and rapid dipstick detection of target nucleic acid is thereby facilitated.

We have found that such results are achieved with sample solutions comprising a standard hybridisation buffer (such as SSPE buffer or Tris buffer) with salt, detergent and a blocking protein such as BSA or powdered milk. The sensitivity of detection of target nucleic acid using such assays has been found to be about equal to or better than that of other dipstick assays.

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According to the invention there is also provided a probe comprising sequence which is complementary to sequence of a target nucleic acid, thereby enabling the probe to hybridise to the target nucleic acid in a sample solution, and
5 sequence which is non complementary to sequence of the target nucleic acid, wherein the non complementary sequence is at an end of the probe sequence and the non complementary sequence is not bound by components of the sample solution when the probe is hybridised to the target nucleic acid in
10 the sample solution.

Preferably the probe is a nucleic acid probe. However, the probe may be a nucleic acid analogue such as protein nucleic acid (PNA).

Preferably the non complementary sequence is at least three
15 nucleotides long, more preferably at least six nucleotides long.

Preferably the complementary sequence is 10-100 nucleotides long, more preferably 20-35 nucleotides long.

Preferably the non complementary sequence is at both ends of
20 the probe sequence.

Preferably a label or ligand is coupled to the probe. Preferably the label or ligand is covalently linked to the end of the non complementary sequence furthest from the complementary sequence.

25 There is also provided according to the invention use of a probe of the invention in a dipstick assay to test for the presence of a target nucleic acid in a sample solution.

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There is further provided according to the invention use of a dipstick or kit of the invention in a dipstick assay to test for the presence of a target nucleic acid in a sample solution.

5 The term "dipstick assay" is used here to mean any assay using a dipstick in which sample solution is contacted with the dipstick to cause sample solution to move by capillary action to a capture zone of the dipstick thereby allowing target nucleic acid in the sample solution to be captured
10 and detected at the capture zone, capture and/or detection of the target nucleic acid involving hybridisation of a capture/detection probe to the target nucleic acid.

Embodiments of the invention are now described by way of example with reference to the accompanying drawings in
15 which:

Figure 1 shows the chemical structure of non protein components suitable as components of the capture probe spacer or the detection probe spacer of the invention;
Figure 2 shows detection of *Chlamydia trachomatis* target
20 nucleic acid using an embodiment of the invention;
Figure 3 shows the experimental setup for Example 1;
Figure 4 shows the experimental setup for Example 2;
Figure 5 shows the experimental setup for Example 3;
Figure 6 shows the experimental setup for Example 4;
25 Figure 7 shows the experimental setup for Example 6;
Figure 8 shows the experimental setup for Example 7; and
Figure 9 shows the structure of detection probes used in the examples.

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The examples relate to detection of a DNA fragment of *Chlamydia trachomatis* (CT) cryptic plasmid DNA. CT is one of the most common causes of sexually transmitted disease. CT infections can cause infertility and, during pregnancy, can result in spontaneous abortion, still birth or postpartum endometritis. In neonates, CT infection can cause blindness and chronic respiratory disease. Approximately 10% of infected men and upto 70% of infected women do not show symptoms of CT infection. Consequently, accurate diagnosis of CT infection is important so that early treatment of the disease can be initiated.

In the examples, a dipstick 10 is used to try to detect single or double stranded CT target nucleic acid 12 in a sample solution 14 (see Figure 2). The dipstick 10 comprises a strip of nitrocellulose 16 having a contact end 18 for contacting the sample solution 14 and a capture probe 20 immobilised at a capture zone 22 of the nitrocellulose strip 16 remote from the contact end 18. An anti-biotin antibody-dye conjugate 24 (or an anti-fluorescein antibody-dye conjugate in example 5) is releasably immobilised at a conjugate zone 26 of the nitrocellulose strip located between the contact end 18 and the capture zone 22. The capture probe 20 is capable of hybridising to a first region of one strand (the first strand) of the target nucleic acid 12.

The sample solution 14 is prepared by spiking 1ml urine with *Chlamydia trachomatis* bacteria then spinning the urine at 15K rpm for 30 minutes. The pellets are resuspended in 100µl standard hybridisation buffer (including a blocking agent such as casein or BSA). A detection probe 28 capable of hybridising to the target nucleic acid is then added

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(together with a helper probe capable of hybridising to the target nucleic acid adjacent the region recognised by the detection probe and/or the capture probe if used). The detection probe 28 is coupled to biotin (or to fluorescein in example 5) (using methods well known to those of skill in the art). The sample solution 14 is then heated to 100°C for 7 minutes and cooled.

The contact end 18 of the dipstick 10 is then contacted with the sample solution 14. The sample solution 14 and any target nucleic acid 12 hybridised to the detection probe 28 moves up the dipstick 10 by capillary action. As the sample solution 14 passes the conjugate zone 26, it mobilises the anti-biotin antibody-dye conjugate 24. Released anti-biotin antibody-dye conjugate 24 can then bind to the biotin coupled to the detection probe 28 hybridised to the target nucleic acid 12.

Complex formed between the anti-biotin antibody-dye conjugate 24, the detection probe 28 and the target nucleic acid 12 then moves up the dipstick 10 to the capture zone 22 where the target nucleic acid of the complex can hybridise to the immobilised capture probe 20. The capture probe 20 is immobilised at the capture zone 22 in such a way that it cannot be mobilised by the sample solution 14 as it moves past the capture zone 22. Consequently, the complex bound to the capture probe remains in the capture zone and can be detected by the presence of the dye of the anti-biotin antibody-dye conjugate at the capture zone.

If there is no CT target nucleic acid in the sample solution, the detection probe 28 cannot be captured at the capture zone 22 and so no dye is visible at the capture

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zone. If there is CT target nucleic acid in the sample solution, but insufficient amounts of the target nucleic acid can be captured at the capture zone the presence of the target nucleic acid in the sample solution will not be
5 detected.

It has been found that the sensitivity of detection of target nucleic acid can be reduced if the distance between the region of the target nucleic acid to which the capture probe hybridises and the region to which the detection probe
10 hybridises is less than 26 nucleotides. Thus, it is preferred that the distance between these regions is at least 26 nucleotides and preferably at least 200 nucleotides.

The capture of target nucleic acid described above is
15 referred to as direct probe capture in the examples below. The strength of the detection of the target nucleic acid in the examples is recorded on a scale of 0 to 5, with 5 representing the strongest detection, and 0 no detection.

The sequences of the probes used in the following examples
20 are:

SEQ ID No1: 5' TGC AAC TCT TGG TGG TAG ACT TTG C
SEQ ID No2: 5' GCG CAC AGA CGA TCT ATT TTT TGC A
SEQ ID No3: 5' CGG GCG ATT TGC CTT AAC CCC ACC A
SEQ ID No4: 5' CCA AGC TTA AGA CTT CAG AGG AGC G
SEQ ID No5: 5' CAT GCG TTT CCA ATA GGA TTC TTG G
SEQ ID No6: 5' CAC AGT CAG AAA TTG GAG TGC TGG C
SEQ ID No7: 5' CTT GCT GCT CGA ACT TGT TTA GTA C
SEQ ID No8: 5' AGA AGT CTT GGC AGA GGA AAC TTT T
SEQ ID No9: 5' CTA GAA TTA GAT TAT GAT TTA AAA GGG
SEQ ID No10: 5' TTC ATA TCC AAG GAC AAT AGA CCA A
SEQ ID No11: 5' TGA TCT ACA AGT ATG TTT GTT GAG T

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SEQ ID No 12: 5' TGC ATA ATA ACT TCG AAT AAG GAG AAG
 SEQ ID No 13: 5' TCC CTC GTG ATA TAA CCT ATC CG
 SEQ ID No 14: 5' CAG GTT GTT AAC AGG ATA GCA CGC
 SEQ ID No 15: 5' CTC GTT CCG AAA TAG AAA ATC GCA
 SEQ ID No 16: 5' GGT AAA GCT CTG ATA TTT GAA GAC
 SEQ ID No 17: 5' CTG AGG CAG CTT GCT AAT TAT GAG T

The structures of the detection probes in the examples described below are shown schematically in Figure 9.

Example 1

Experimental Setup

5 Capture format: direct probe capture (cp) Seq ID No 14 immobilised on the dipstick;
 Detection probe (dp): Seq ID No 13 or Seq ID No 15 with biotin coupled directly to the 5'-end, or by a 3 nucleotide (N_3), 6 nucleotide (N_6), S, or SS spacer, or Seq ID No 13
 10 with biotin coupled directly to the 3'-end. 10^{12} copies of each.
 Detection format: anti-biotin antibody-dye conjugate;
 Target DNA: 73 or 76 nucleotide single stranded DNA fragments at 5×10^{11} - 10^{10} copies.

15 Results

dp Seq ID No 13

Copies of target: 5×10^{11} 10^{11} 5×10^{10} 10^{10}

	<u>Detection probe</u>	<u>Signal strength</u>			
	dp-B 5'	3.5	3.0	2.0	1.0
20	dp- N_3 -B 5'	4.5	3.5	3.0	1.5
	dp- N_6 -B 5'	5.0	4.0	3.0	2.0
	dp-S-B 5'	4.0	3.0	2.0	1.0
	dp-SS-B 5'	4.5	3.5	2.5	1.0
	3' B-dp	5.0	3.5	3.0	2.0

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dp Seq ID No 15Copies of target: 5x10¹¹ 10¹¹ 5x10¹⁰Detection probe Signal strength

	dp-B 5'	3.5	2.0	1.0
5	dp-N ₃ -B 5'	4.0	3.0	2.0
	dp-N ₆ -B 5'	4.5	3.0	2.0
	dp-S-B 5'	4.0	2.5	1.5
	dp-SS-B 5'	4.0	2.5	1.5

These results show:

10 The sensitivity of target nucleic acid detection using detection probes with biotin coupled to the 5'-end by a N₆, N₃, SS or S spacer was higher than the sensitivity using a detection probe with biotin coupled directly to the 5'-end.

15 The N₃ and N₆ spacers were better than the S and SS spacers.

 It is preferred that the biotin (or other detection ligand) is coupled to one end of the detection probe. In the complex formed when the capture probe and the detection probe are hybridised to the target nucleic acid at the capture zone then the biotin (or other detection ligand) can be at the end of the detection probe proximal to the region of the target nucleic acid which hybridises to the capture probe (internally orientated) or, preferably, at the end of the detection probe distal to the region of the target nucleic acid which hybridises to the capture probe (externally orientated). If no spacer is used to couple the detection ligand to the detection probe, then the sensitivity of detection of target nucleic acid is generally higher if the detection ligand is externally orientated.

20 Consequently, the detection probe is usually chosen so that

25 the detection ligand is externally orientated.

30

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However, in this example, the sensitivity of detection using a detection probe with externally orientated biotin coupled directly to the 3'-end of the detection probe was as good as the sensitivity using a detection probe with internally orientated biotin coupled to the 5'-end of the detection probe by a six nucleotide spacer. Thus, when spacers are used in accordance with the invention, the detection probe does not have to be chosen so that the detection ligand is externally orientated in the complex captured at the capture zone.

Example 2:Experimental Setup

Capture format: direct probe capture (cp) Seq ID No 14 immobilised on dipstick membrane;

15 Detection probe: detection probe (dp) Seq ID No 13, Seq ID No 15 and Seq ID No 16 with biotin coupled to the 5'-end by a 3 nucleotide (N_3), 6 nucleotide (N_6), S or SS spacer. 10^{12} copies of each.

Detection format: anti-biotin antibody-dye conjugate;

20 Target DNA: 214 bp double stranded DNA fragments at 10^{11} - 10^{10} copies.

Results

Copies of target:		10^{11}	5×10^{10}	10^{10}
<u>Detection probe</u>		<u>Signal Strength</u>		
25	dp13-B + dp15-B + dp16-B	1.5	1.0	0.0
	dp13- N_3 -B + dp15- N_3 -B + dp16- N_3 -B	3.0	2.0	0.5
	dp13- N_6 -B + dp15- N_6 -B + dp16- N_6 -B	4.5	3.0	1.0
	dp13-S-B + dp15-S-B + dp16-S-B	3.0	2.0	<0.5
	dp13-SS-B + dp15-SS-B + dp16-SS-B	3.5	3.0	0.5
30	dp13 non-lab + dp15- N_6 -B + dp16- N_6 -B	2.5	1.5	0.5

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These results show:

The sensitivity of detection of double stranded target nucleic acid was improved more than five-fold using N₃, N₆, S or SS spacers.

5 The sensitivity of detection was higher with the N₆ and SS spacers than with the N₃ and S spacers, indicating that the length of the spacer is important for improved sensitivity of detection.

10 The sensitivity of detection was higher with the N₆ spacer than the SS spacer, despite the fact that these spacers are of equivalent length, indicating that the physicochemical properties of the spacer are important for improved sensitivity of detection.

15 The sensitivity of detection using only two detection probes each coupled at the 5'-end to biotin by an N₆ spacer (dp13 non-lab + dp15-N₆-B + dp16-N₆-B), in which the biotin is internally orientated in the complex captured by the capture probe, was greater than the sensitivity of detection using three detection probes each coupled at the 5'-end
20 directly to biotin (dp13-B + dp15-B + dp16-B) in which the biotin of one detection probe (dp13-B) is externally orientated in the complex captured by the capture probe.

Conclusions from examples 1 and 2

25 The sensitivity of target nucleic acid detection is increased by using a spacer to couple the detection ligand to the detection probe.

Longer spacers are better than shorter spacers.

30 Spacers of equivalent length but with different physicochemical properties had different effects on the sensitivity of detection. In particular, spacers in which the non protein component consists only of nucleotides are

- 25 -

better than spacers which include non nucleotide components.
Possible explanations for this are:

1. Nucleotide spacers may improve the sensitivity of detection by enhancing hybridisation of the detection probe to the target nucleic acid. The nucleotides of these spacers are not expected to base pair to nucleotides of the target nucleic acid when the detection probe hybridises to the target nucleic acid. The nucleobases of these nucleotides may form stacking interactions with the base pairs formed when the detection probe hybridises to the target nucleic acid. These stacking interactions may enhance the stability of the hybrid formed between the target nucleic acid and the detection probe, thereby enhancing the sensitivity of detection of target nucleic acid.
2. Nucleotide spacers may be more rigid than the S and SS spacers. The ribose rings of the nucleotide spacers are expected to provide much more rigidity than the polyethylene glycol groups of the S and SS spacers. This greater rigidity might increase the availability of the detection ligand coupled to the nucleotide spacer for interaction with the detection ligand binding moiety.
3. Polarity differences between the nucleotide and non nucleotide spacers may cause differences in the sensitivity of detection.

Example 3

Experimental Setup

Capture format: direct probe capture (cp) Seq ID No 10 immobilised on the dipstick;

Detection probe: detection probe (dp) Seq ID No 13 coupled to biotin at the 5'-end either directly or by a N_6 , SS, (dS)₆, (SC₃)₆ or SN₃SN₃S spacer. 10¹² copies of each.

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Detection format: anti-biotin antibody-dye conjugate;
 Helper probes: SEQ ID No 5 and SEQ ID No 6 adjacent to SEQ
 ID No 10; SEQ ID No 1 and SEQ ID No 2 adjacent to SEQ ID No
 13 at 10^{12} copies;

5 Target DNA: 872 bp double stranded DNA fragment at 2×10^{11} -
 5×10^{10} copies.

Results

Copies of target:		2×10^{11}	5×10^{10}
	<u>Detection probe</u>	<u>Signal</u>	<u>strength</u>
10	dp-B	<1.0	0.0
	dp -N ₆ -B	2.0	0.5
	dp -SS-B	1.0	0.0
	dp - (dS) ₆ -B	1.0	0.0
	dp - (SC ₃) ₆ -B	1.0	0.0
15	dp -SN ₃ SN ₃ S-B	1.5	0.0

These results show:

The SS, (dS)₆, and (SC₃)₆ spacers are of equivalent
 length and had a similar effect on enhancing the sensitivity
 of detection of target nucleic acid despite their structural
 20 differences and properties.

The N₆ spacer is of equivalent length to the SS, (dS)₆,
 and (SC₃)₆ spacers. However, the N₆ spacer had the greatest
 effect on improving the sensitivity of detection of target
 nucleic acid.

25 The sensitivity of target nucleic acid detection was
 greater using the N₆ spacer than the SN₃SN₃S spacer (the
 longest spacer tested). A possible explanation for this
 could be that the S monomer reduces or eliminates stacking
 interaction between the nucleobases of the N₃ components of
 30 the spacer and the base pairs formed between the detection

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probe and the target nucleic acid. This data supports the conclusion that stacking interactions between unpaired nucleobases of the spacer and the duplex formed between the target nucleic acid and the detection probe are important in enhancing the sensitivity of detection of target nucleic acid.

Example 4

Spacers with different physicochemical properties and lengths were evaluated by the dipstick test and by dot blot analysis. Dot blot analysis enables the efficiency of the interaction of the anti-biotin antibody with the biotin coupled to the detection probe to be analysed in the absence of hybridisation of the detection probe to the target nucleic acid. 5×10^8 - 5×10^{11} copies of detection probes coupled to biotin by different spacers were spotted at different places on a positively charged nylon membrane and UV cross-linked to the membrane. The membrane was then incubated with an anti-biotin antibody coupled to alkaline phosphatase (capable of converting a Nitro Blue Tetrazolium/5-Bromo-4-Chloro-3-Indolyl Phosphate (NBT/BCIP) chromogenic substrate), washed, and incubated with the NBT/BCIP chromogenic substrate, and the membrane was observed to see if any colour formed at the capture zone.

Experimental setup for dipstick test

Capture format: direct probe capture (cp) Seq ID No 14 immobilised on the dipstick;
Detection probe: detection probe (dp) Seq ID No 13 coupled to biotin at the 5'-end either directly or by a nucleotide or non-nucleotide spacer. 10^{12} copies of each.
Detection format: anti-biotin antibody-dye conjugate;

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Helper probes: SEQ ID No 2 and SEQ ID No 3 adjacent to SEQ ID No 14 at 10^{12} copies;

Target DNA: 416 bp double stranded DNA fragment at 5×10^{10} - 5×10^9 copies.

5 Results

copies of target: 5×10^{10} 5×10^9

	<u>Detection probe</u>	<u>Signal</u>	<u>strength</u>
	dp-B ^{5'}	3.5	0.5
	dp-N ₃ -B ^{5'}	4.5	1.5
10	dp-N ₄ -B ^{5'}	4.5	1.0
	dp-N ₅ -B ^{5'}	4.5	1.0
	dp-N ₆ -B ^{5'}	5.0	1.5
	dp-(dS) ₆ -B ^{5'}	4.0	0.5
	dp-S-B ^{5'}	3.5	0.0
15	dp-SS-B ^{5'}	4.0	0.0
	dp-SSS-B ^{5'}	4.5	0.0
	dp-SSSS-B ^{5'}	4.0	0.0
	dp-S N ₃ S N ₃ S-B ^{5'}	4.5	0.5

Experimental setup for dot-blot analysis

20 Detection probes: detection probe Seq ID No 13 coupled to biotin at the 5'-end, either directly or by a nucleotide or non-nucleotide spacer.

Detection format: anti-biotin antibody coupled to alkaline phosphatase, detection by NBT/BSIP chromogenic substrate.

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Results

	Spacer	Detection limit
	without	5.0 x E11
	N ₃	5.0 x E10
5	N ₄	5.0 x E10
	N ₅	5.0 x E10
	N ₆	2.5 x E10
	(dS) ₆	2.5 x E10
	SN ₃ SN ₃ S	2.5 x E9
10	SSSS	2.5 x E9
	SSS	5.0 x E9
	SS	2.5 x E10
	S	5.0 x E11

The results of the dipstick test show:

15 There is no significant difference in the sensitivity of target nucleic acid detection using detection probes with three, four or five nucleotide spacers.

 The sensitivity of detection with a six nucleotide spacer is marginally better than 3-5 nucleotide spacers.

20 The sensitivity of detection with spacers consisting only of nucleotides was better than with spacers which include non-nucleotides. For example the N₃ spacer was better than the longest spacer SN₃SN₃S, equivalent to 15 nucleotides in length.

25 The (dS)₆ spacer is slightly better than the SS spacer.

The results of the dot-blot test show:

 The sensitivity of detection was highest with the longest spacers (SSSS and SN₃SN₃S).

30 The sensitivity of detection using equivalent length spacers (N₆, (dS)₆ and SS) with different physicochemical properties was similar.

- 30 -

Conclusions from examples 3 and 4

The dS component has a similar structure to a nucleotide. Both have a ribose residue, expected to provide rigidity. However, the nucleotide has a nucleobase which is not present in the dS component. The different effect of the (dS)₆ spacer on the sensitivity of target nucleic acid detection compared to the N₆ spacer suggests that the greater effect on the improvement of sensitivity of detection using a nucleotide spacer is explained principally by the presence of the nucleobases which do not base pair with the target nucleic acid.

The composition of the spacer appears to be more important than its length (compare the results for dp-N₃-B^{5'} and dp-SN₃SN₃S-B^{5'} in the dipstick test of example 4).

The sensitivity of detection using a (dS)₆ spacer was greater than with an SS spacer. These spacers are of equivalent length. This suggests that the physicochemical properties of the spacer, such as rigidity or polarity, may also have an effect on the improvement of sensitivity of detection by the spacer.

The dot blot analysis of example 4 shows that the length of the spacer is important for the availability of the biotin to the anti-biotin antibody. The sensitivity of biotin recognition by the anti-biotin antibody was similar whether the biotin was coupled to the immobilised probe by an N₆, (dS)₆ or SS spacer. However, the effect of these spacers on the sensitivity of detection in the dipstick test of example 4 was different. This suggests that the composition of the spacer is more important in the hybridisation of the detection probe to the target nucleic acid than in the recognition of the biotin by the anti-biotin antibody. The length of the spacer appears to be more important than the composition of the spacer for recognition

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of the biotin (or other detection ligand) by the anti-biotin antibody (or other detection ligand binding moiety).

Example 5

Experimental Setup

- 5 Capture format: direct probe capture (cp) Seq ID No 14 coupled to BSA immobilised to the dipstick. The capture probe is coupled to the BSA either directly or by a six nucleotide spacer;
- 10 Detection probe: detection probe (dp) Seq ID No 13 coupled to fluorescein. 10^{12} copies.
- Detection format: anti-fluorescein antibody-dye conjugate;
- Target DNA: 73 nt or 76 nt single stranded DNA fragments at 10^{11} copies.

Result:

- 15 Copies of target: 10^{11}
- | <u>capture probe</u> | <u>signal strength</u> |
|---------------------------------|------------------------|
| cp-BSA-dipstick | 4.0 |
| cp-N ₆ -BSA-dipstick | 5.0 |

Example 6

20 Experimental Setup

- Capture format: direct probe capture (cp) Seq ID No 14 coupled at the 5'-end to BSA immobilised on the dipstick. The capture probe is coupled to the BSA by an N₆ or SN₃SN₃S spacer;
- 25 Detection probes: detection probes (dp) Seq ID No 7, 8, 9, 10, 11, 12, 13, 15, 16 and 17 coupled to biotin. 10^{12} copies of each;
- Detection format: anti-biotin antibody-dye conjugate;

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Target : 872 bp double stranded DNA at 10^{11} ~ 2.5×10^9 copies.

Result

Copies of target:		10^{11}	2.5×10^{10}	10^{10}	5×10^9	2.5×10^9
<u>Capture probe</u>		<u>signal strength</u>				
5	cp-SN ₃ SN ₃ S-BSA-dipstick	4.0	3.5	2.5	1.0	0.0
	cp-N ₆ -BSA-dipstick	4.5	4.0	3.0	1.5	0.0

Example 7

Experimental Setup

Capture format: direct probe capture (cp) Seq ID No 15
 10 coupled to BSA immobilised on the dipstick. The capture probe is coupled to the BSA by an N₆ spacer;

Helper probes: SEQ ID No 3 and SEQ ID No 4 adjacent to SEQ ID No 15;

Detection probe: In this example, the detection probe
 15 comprises a hook probe and a universal probe. The hook probe has sequence corresponding to Seq ID No 17 (capable of hybridising to the target nucleic acid) and sequence complementary to the sequence of a universal probe. The universal probe is coupled to a textile dye by an N₆ or SN₃SN₆ spacer. There are 10^{12} copies of the hook probe.

Target: 872 bp double stranded DNA at 10^{11} and 10^{10} copies.

Result

Copies of target:		10^{11}	10^{10}
<u>Capture probe</u>		<u>signal</u>	<u>strength</u>
25	Universal probe-SN ₃ SN ₆ -Dye	2.0	0.0
	Universal probe-N ₆ -Dye	2.0	0.5

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Conclusions from examples 5, 6, and 7

Use of spacers comprising a protein immobilised to the dipstick and a non protein to couple the capture probe to the immobilised protein (examples 5 and 6), or use of non
5 protein spacers to couple the label to the detection probe (example 7) improve the sensitivity of detection of target nucleic acid.

The sensitivity of nucleic acid detection was highest when the non protein component of the spacer consisted
10 entirely of nucleotides.

The sensitivity of detection of single and double stranded target nucleic acid by dipsticks has been found to be significantly improved by the use of non pairing nucleotides in accordance with the invention. The sensitivity of
15 detection of double stranded circular target nucleic acid can also be increased by use of non pairing nucleotides in accordance with the invention.

Single stranded target nucleic acid is known to form secondary structure by means of intramolecular base pairing
20 interactions. Such secondary structure can inhibit binding of the capture probe and the detection probe to the target nucleic acid. Consequently, the region of the target nucleic acid to which the detection probe and capture probe bind is often chosen as a region predicted to be substantially free
25 of secondary structure.

The improved sensitivity of detection of double stranded target nucleic acid achieved by use of non pairing nucleotides in accordance with the invention means that the sensitivity of detection of single stranded target nucleic
30 acid using a capture probe and/or a detection probe which

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recognises a region of the target nucleic acid involved in secondary structure will also be improved. An advantage of this is that the capture probe and/or detection probe may not have to be chosen based on predictions of the secondary structure formed by the target nucleic acid, thus
5 simplifying the choice of capture and detection probe.

Conventional methods of dipstick detection are thought to be very poor at detecting circular double stranded target nucleic acid. Consequently, such target nucleic acid is usually treated with an enzyme that linearises the double
10 stranded target before the target is detected. Improved detection of circular double stranded target nucleic acid using non pairing nucleotides in accordance with the invention means that linearisation of the target nucleic
15 acid may not be required, thus simplifying the detection methods.

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Figure Legends

Figure 1 - Design of Spacer

B=biotin coupled to a linker

Figure 3

5 310 - cp
 320 - dp
 330 - target

Figure 4

 450 - dp Seq ID No 13
10 460 - cp Seq ID No 14
 470 - dp Seq ID No 15
 480 - dp Seq ID No 16

Figure 5

 510 - cp
15 520 - dp
 540 - helper probes

Figure 6

 610 - cp
 620 - dp
20 640 - hp

Figure 7

710 - cp-N₆-BSA or cp-SN₃-SN₃-S-BSA

Figure 8

 810 - cp
25 821 - detection hook
 822 - Universal probe-N₆-Dye or Universal probe-SN₃-SN₆- Dye
 840 - helper probes

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Figure 9

—	represents nucleic acid of the detection probe
B	represents biotin coupled to a linker
x	represents the number of nucleotides
5 y	represents the number of hexaethyleneglycol phosphate monomers

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Claims

1. A dipstick for testing for the presence of target nucleic acid in a sample solution which comprises:
a chromatographic strip having a contact end for contacting
5 the sample solution; and
a capture probe immobilised at a capture zone remote from the contact end, the capture probe being capable of hybridising to the target nucleic acid or to a hook capture probe bound to the target nucleic acid, wherein one or both
10 ends of the capture probe are coupled to one or more nucleotides, preferably at least three nucleotides, which do not hybridise to the target nucleic acid when the capture probe has hybridised to the target nucleic acid or which do not hybridise to the hook capture probe when the capture
15 probe has hybridised to the hook capture probe.
2. A dipstick according to claim 1 which further comprises a detection probe, releasably immobilised at a probe zone located between the contact end and the capture zone, the detection probe being capable of hybridising to the target
20 nucleic acid to allow detection of the target nucleic acid utilising the detection probe.
3. A dipstick according to claim 2 in which one or both ends of the detection probe are coupled to one or more nucleotides, preferably at least three nucleotides, which do
25 not hybridise to the target nucleic acid when the detection probe has hybridised to the target nucleic acid.
4. A dipstick for testing for the presence of target nucleic acid in a sample solution which comprises:
a chromatographic strip having a contact end for contacting

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the sample solution;

a capture moiety, immobilised at a capture zone remote from the contact end, the capture moiety being capable of binding directly or indirectly to the target nucleic acid; and

- 5 a detection probe, releasably immobilised at a probe zone located between the contact end and the capture zone, the detection probe being capable of hybridising to the target nucleic acid to allow detection of the target nucleic acid utilising the detection probe.

- 10 5. A dipstick according to claim 4 in which the capture moiety is capable of binding to a capture ligand coupled to a capture probe bound to the target nucleic acid.

6. A kit for testing for the presence of target nucleic acid in a sample solution which comprises:

- 15 a dipstick according to claim 1; and
a detection probe capable of hybridising to the target nucleic acid thereby allowing detection of target nucleic acid utilising the detection probe.

- 20 7. A kit according to claim 6 in which one or both ends of the detection probe are coupled to one or more nucleotides, preferably at least three nucleotides, which do not hybridise to the target nucleic acid when the detection probe has hybridised to the target nucleic acid.

- 25 8. A kit for testing for the presence of target nucleic acid in a sample solution which comprises:

a dipstick according to claim 5; and
a capture probe capable of hybridising to the target nucleic acid or to a hook capture probe bound to the target nucleic acid, wherein the capture probe is coupled to a capture

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ligand which can be bound by the capture moiety.

9. A kit according to claim 8 in which one or both ends of the capture probe are coupled to one or more nucleotides, preferably at least three nucleotides, which do not
5 hybridise to the target nucleic acid when the capture probe has hybridised to the target nucleic acid or which do not hybridise to the hook capture probe when the capture probe has hybridised to the hook capture probe.

10. A kit for testing for the presence of target nucleic
10 acid in a sample solution which comprises:

i) a dipstick comprising a chromatographic strip having a contact end for contacting the sample solution and a capture probe capable of hybridising to the target nucleic acid, or to a hook capture probe bound to the target nucleic acid,
15 the capture probe being immobilised at a capture zone of the chromatographic strip remote from the contact end; and
ii) a detection probe capable of hybridising to the target nucleic acid thereby allowing detection of the target nucleic acid utilising the detection probe, wherein one or
20 both ends of the detection probe are coupled to one or more nucleotides, preferably at least three nucleotides, which do not hybridise to the target nucleic acid when the detection probe has hybridised to the target nucleic acid.

11. A kit for testing for the presence of target nucleic
25 acid in a sample solution which comprises:

i) a dipstick comprising a chromatographic strip having a contact end for contacting the sample solution and a capture moiety immobilised at a capture zone of the chromatographic strip remote from the contact end, the capture moiety being

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capable of binding directly or indirectly to the target nucleic acid;

- ii) a capture probe capable of hybridising to the target nucleic acid, or to a hook capture probe bound to the target nucleic acid, wherein the capture probe is coupled to a capture ligand which can be bound by the capture moiety; and
- iii) a detection probe capable of hybridising to the target nucleic acid, wherein one or both ends of the detection probe are coupled to one or more nucleotides, preferably at least three nucleotides, which do not hybridise to the target nucleic acid when the detection probe has hybridised to the target nucleic acid.

12. A kit for testing for the presence of target nucleic acid in a sample solution which comprises:

- i) a dipstick comprising a chromatographic strip having a contact end for contacting the sample solution and a capture moiety immobilised at a capture zone of the chromatographic strip remote from the contact end;
- ii) a capture probe capable of hybridising to the target nucleic acid, or to a hook capture probe bound to the target nucleic acid, wherein the capture probe is coupled to a capture ligand which can be bound by the capture moiety and one or both ends of the capture probe are coupled to one or more nucleotides, preferably at least three nucleotides, which do not hybridise to the target nucleic acid when the capture probe has hybridised to the target nucleic acid; and
- iii) a detection probe capable of hybridising to the target nucleic acid thereby allowing detection of the target nucleic acid utilising the detection probe.

- 13. A probe comprising sequence which is complementary to sequence of a target nucleic acid, thereby enabling the

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probe to hybridise to the target nucleic acid in a sample solution, and sequence which is non complementary to sequence of the target nucleic acid, wherein the non complementary sequence is at an end of the probe sequence
5 and the non complementary sequence is not bound by components of the sample solution when the probe is hybridised to the target nucleic acid in the sample solution.

14. A probe according to claim 13 which is a nucleic acid
10 probe.

15. A probe according to claim 14 wherein the non complementary sequence is at least three nucleotides long.

16. A probe according to claim 14 wherein the non complementary sequence is at least six nucleotides long.

15 17. A probe according to any of claims 14 to 16 wherein the complementary sequence is 10-100 nucleotides long.

16. A probe according to any of claims 13 to 15 wherein a label or ligand is coupled to the probe.

17. A probe according to claim 16 wherein the label or
20 ligand is covalently linked to the end of the non complementary sequence furthest from the complementary sequence.

18. Use of a probe according to any of claims 13 to 17 in a dipstick assay to test for the presence of a target
25 nucleic acid in a sample solution.

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19. Use of a dipstick or kit according to any of claims 1 to 12 in a dipstick assay to test for the presence of a target nucleic acid in a sample solution.

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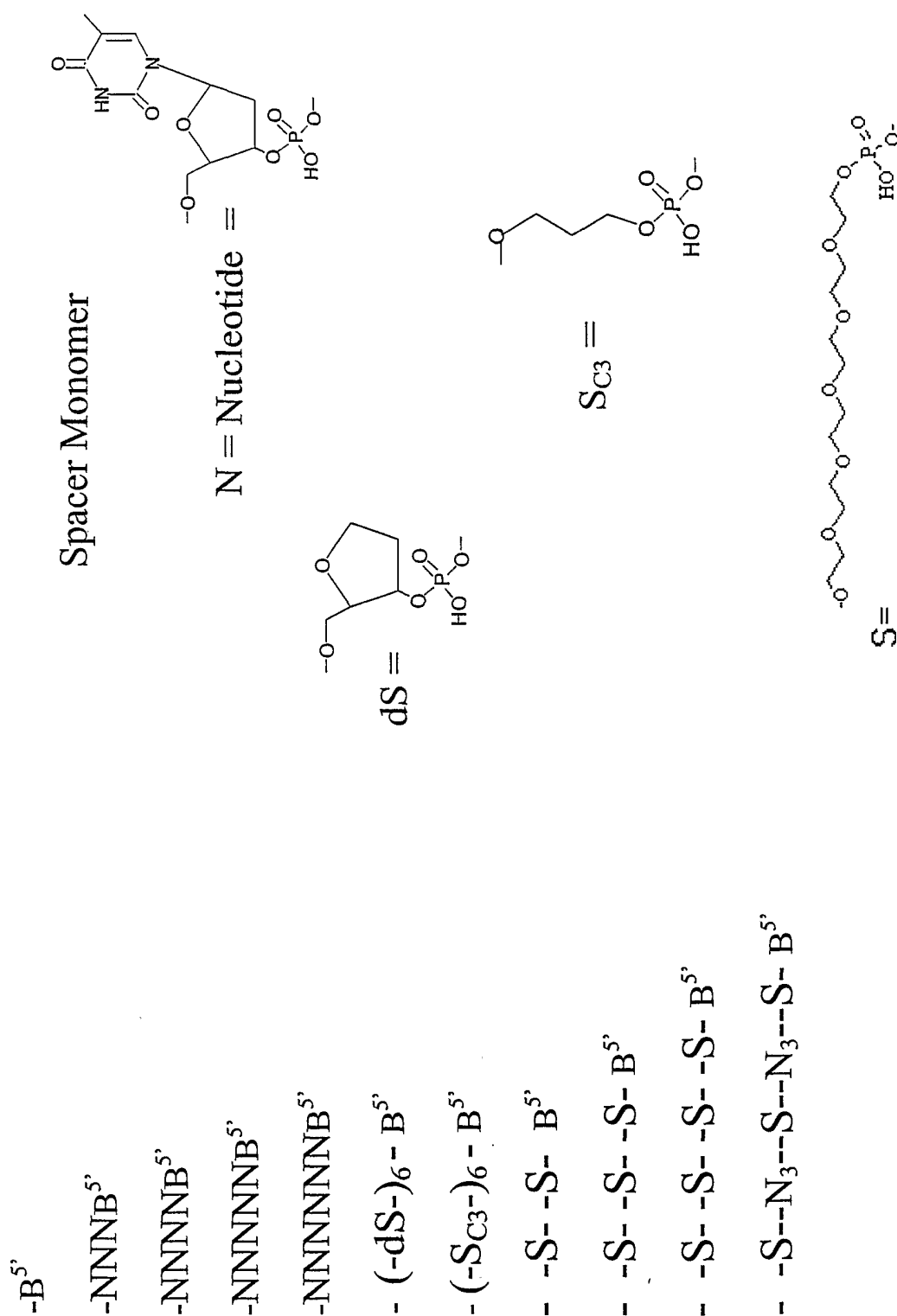


Figure 1

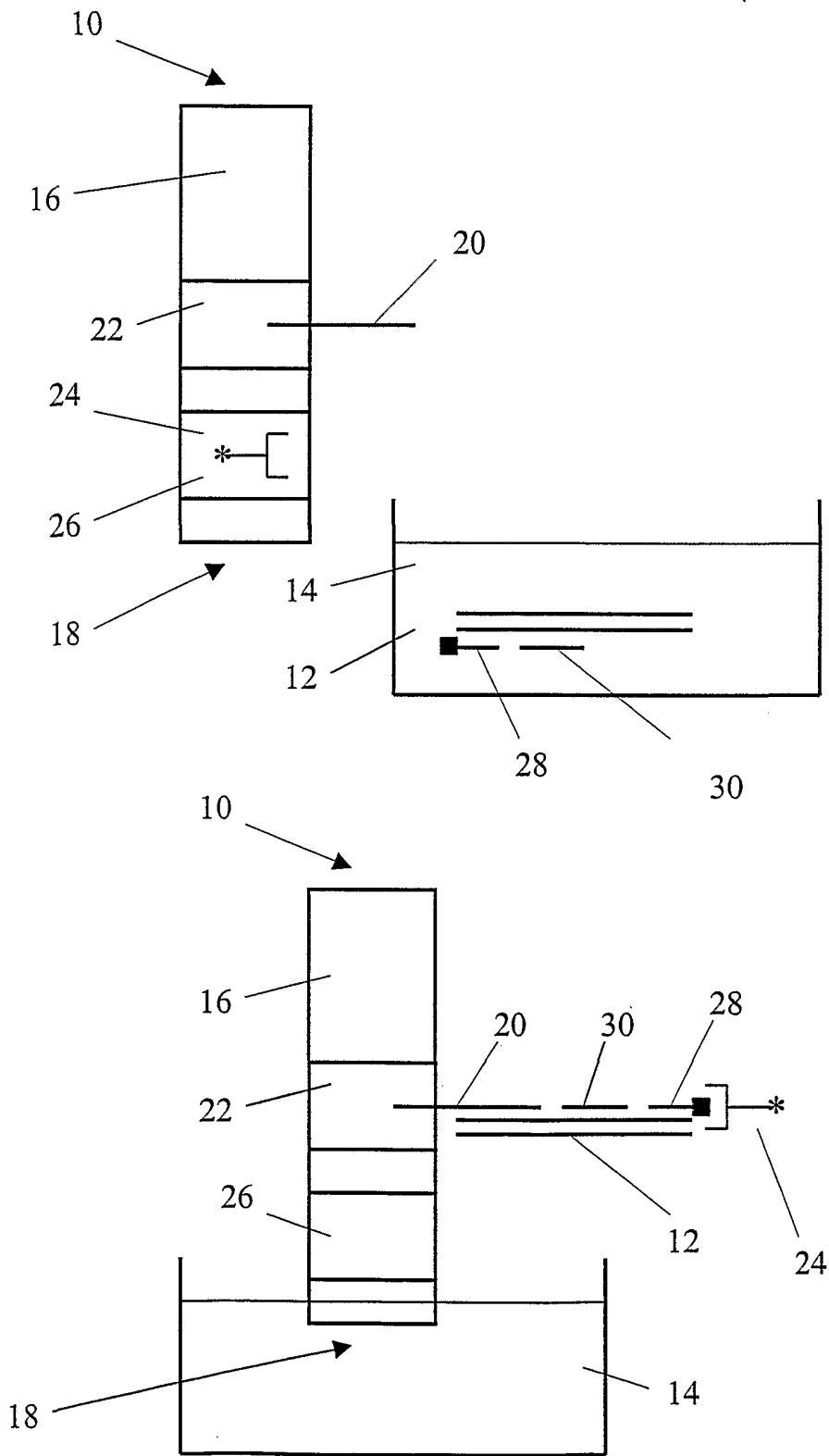


Figure 2

Figure 3

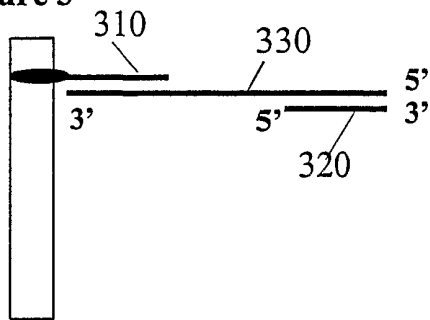


Figure 4

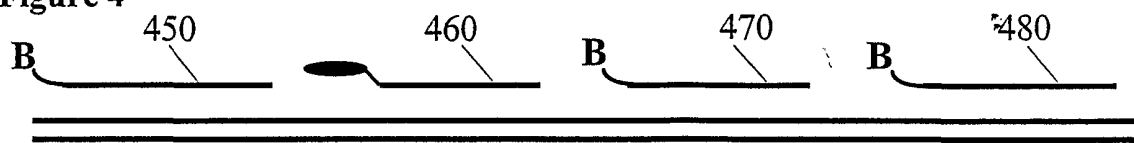


Figure 5

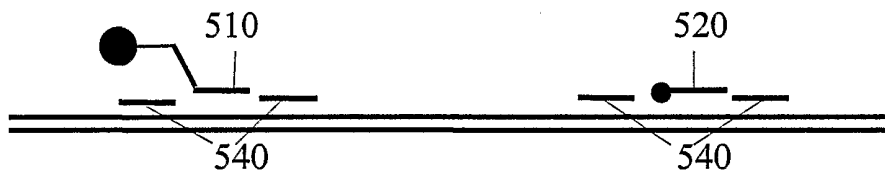


Figure 6



Figure 7

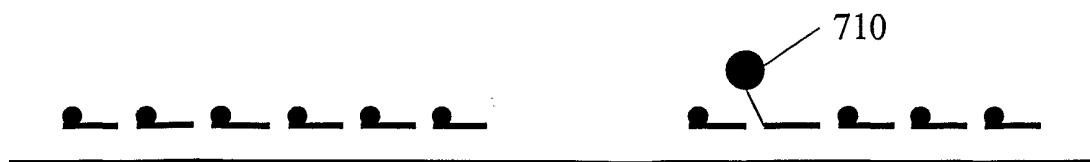


Figure 8

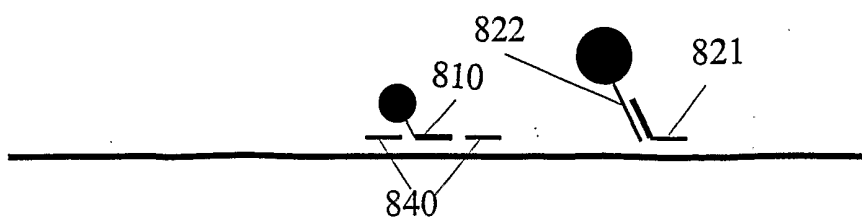


Figure 9

<u>Detection Probe</u> <u>Name</u>	<u>Structure</u>
dp-B ^{5'}	$\begin{array}{ccc} 5' & & 3' \\ B & \text{-----} & \end{array}$
dp-(N) _x -B ^{5'}	$\begin{array}{ccc} 5' & & 3' \\ B-(N)_x & \text{-----} & \end{array}$
dp-(S) _y -B ^{5'}	$\begin{array}{ccc} 5' & & 3' \\ B-(S)_y & \text{-----} & \end{array}$
^{3'} B-dp	$\begin{array}{ccc} 5' & & 3' \\ \text{-----} & & B- \end{array}$
dp-(dS) ₆ -B ^{5'}	$\begin{array}{ccc} 5' & & 3' \\ B-(dS)_6 & \text{-----} & \end{array}$
dp-(SC ₃) ₆ -B ^{5'}	$\begin{array}{ccc} 5' & & 3' \\ B-(SC_3)_6 & \text{-----} & \end{array}$
dp-SN ₃ SN ₃ S-B ^{5'}	$\begin{array}{ccc} & & 5' & & 3' \\ B-S-N-N-N-S-N-N-N-S & \text{-----} & \end{array}$