

DTECT™: Guide-Directed Endonuclease Cleavage for Confirmatory-Grade Isothermal Amplification

A technical reference on how Biomeme's isothermal chemistry closes the specificity gap between isothermal nucleic acid amplification and real-time PCR

1. Introduction

Nucleic acid amplification tests (NAATs) are the confirmatory standard for identifying an organism from its genetic sequence. NAATs may be broadly classified into two categories: isothermal amplification technologies and polymerase chain reaction (PCR) technologies. PCR-based NAATs require repeated cycles of precisely controlled heating and cooling, whereas isothermal technologies drive exponential amplification at a single continuous temperature. PCR-based technologies are considered the standard for accuracy and sensitivity but require the use of expensive instrumentation and complex sample preparation workflows. Isothermal technologies hold the speed and instrument simplicity advantage but have several technical drawbacks, including assay complexity and compromised sensitivity. Previously, adopting one or the other has required end-users to accept this technical compromise.

This document explains how the chemistry behind DTECT (Differential Targeted Endonuclease Cleavage Technology) eliminates this compromise. Biomeme has developed the DTECT isothermal amplification system for the Biomeme/5™ platform, though DTECT is also broadly compatible with standard laboratory qPCR equipment. We describe how DTECT overcomes the specific, published failure modes of the established isothermal technologies to achieve sensitivity and specificity that is equivalent to PCR. Additionally, we present performance data supporting our claim that a DTECT result is equivalent in analytical sensitivity to a laboratory real-time PCR result on the same target.

2. Background

Every isothermal amplification chemistry in wide use solves the same problem the same way: it replaces thermal nucleic acid denaturation with an enzyme system, then relies on 3' extensible primers to amplify a specific target. This single design decision is the origin of the field's persistent weakness. Primers that must both find the target and prime extension can prime each other, prime the wrong site, or produce a non-primed amplification product. Published studies of LAMP, SDA, NASBA and EXPAR each describe non-specific or target-independent amplification as a defining limitation of the method [1–3].

DTECT separates the two jobs. A guide oligonucleotide binds the target, but it cannot be extended: its 3' end is deliberately blocked. What the guide does instead is deliver a Type IIS restriction endonuclease recognition site to a position of the assay designer's choosing, so the enzyme cuts *inside the target sequence itself*. The target — not the oligonucleotide — becomes the extendable 3' end that a strand-displacing polymerase elongates.

The structural consequence

In DTECT, the background amplification is defeated because the target is provided a 'bypass' of the rate limiting step for amplification. The primer-extension artifacts/background in nicking-enzyme and strand-displacement chemistries are outcompeted by the targeted amplification; much like in PCR. The blocked 3' guide oligonucleotides cannot trigger on-target or off target extension.

The practical results follow from that. A DTECT reaction uses two oligonucleotides (extension blocked 'guides') and a molecular beacon per target rather than the four to six primers LAMP requires. This allows for multiplexing where LAMPs complexity largely does not. It reaches time-to-detection in the range of three to eight minutes rather than the 20 minutes to two hours typical of the established isothermal methods. It amplifies DNA and RNA with equivalent kinetics. In a side-by-side comparison of *Bacillus anthracis* amplification, crude lysate directly added to DTECT reactions matched the sensitivity of column-purified DNA added to laboratory real-time PCR reactions.

3. The category, and the compromise inside it

A nucleic acid amplification test identifies an organism by copying a defined stretch of its genome until the amplified copies are detectable. Because identification rests on sequence rather than on a physical or immunological signature, NAATs are what field doctrine and clinical practice treat as confirmatory. Immunoassays and mass-spectrometric methods detect; NAATs identify.

Real-time PCR earns that standing through thermodynamic control. Each cycle heats the reaction to about 95 °C to separate (melt) DNA double helix, then drops to an annealing temperature chosen so that only well-matched primers stay bound. The thermal melt step is the bottleneck applied 30 to 40 times, and the cost of applying it is a thermal block, the power to drive it, and the 60 to 120 minutes it takes to run.

Isothermal amplification removes the thermal cycler by using enzymes to open the DNA double helix. Different enzymes can do the job such as a recombinase, a helicase, a nicking endonuclease, or the displacement activity of the polymerase itself.

The compromise is measurable in deployed systems. In a clinical evaluation and meta-analysis published in *eLife*, a widely deployed point-of-care isothermal platform based on nicking enzyme amplification showed a pooled sensitivity of 82% (95% CI 67–91%) against 98% (95% CI 96–99%) for RT-PCR [4]. That gap, not the absence of a thermal cycler, is what has kept isothermal chemistry in the screening tier.

4. Where the established isothermal chemistries break

The table below summarizes the principal isothermal chemistries as characterized in the peer-reviewed literature. The right-hand column reports the limitations identified by a body of peer-review literature.

Chemistry	Oligos per target	Temp.	Enzymes	Limitations reported in the literature
LAMP	4–6 primers across 6–8 target regions	60–65 °C	1 strand-displacing polymerase	“Complex primer design” and “susceptibility to false positives” [1]; false positives arise from mispriming and non-specific primer interaction [2]; multiplexing in one tube is “a significant limitation” because more primer sets raise the risk of primer dimers and non-specific product [5].
RPA	2 primers (+ probe)	37–42 °C	Recombinase, SSB protein, polymerase	Stringent reaction conditions; products require specialized detection; kits are expensive; no FDA-approved assay to date [1]. Primer selection remains largely empirical.
SDA	2–4 primers, including bumper primers	30–50 °C	Restriction endonuclease, exo-deficient polymerase	“Prone to false positives”; the low reaction temperature increases non-specific primer binding; limited to short targets [1].
NEAR / EXPAR	Short templates plus nicking site	≈55 °C	Nicking endonuclease, strand-displacing polymerase	“Limited by the high levels of non-specific amplification,” which lowers both specificity and sensitivity; background arises from template–polymerase interaction <i>independent of the target</i> and is sequence-dependent [3].
HDA	2 primers	≈37–65 °C	Helicase, SSB protein, polymerase	Requires a coordinated multi-enzyme system; limited commercial assay menu; Limited by the high levels of non-specific amplification, which lowers both specificity and sensitivity [1].
NASBA / TMA	2 primers	≈41 °C	Reverse transcriptase, RNase H, T7 RNA polymerase	Requires optimizing three enzymes; “false-positives more common than false-negatives”; kits expensive; “not ideal for DNA targets” [1].
RCA / MDA	2 primers (or random hexamers)	23–60 °C	Phi29 polymerase	“Only works with circular templates” and requires a ligation step for linear targets; no RCA diagnostic kit has reached the market [1].
DTECT	2 guide oligos + 1 molecular beacon	58°C	Strand-displacing polymerase, site-specific nicking endonuclease (+ RT for RNA)	See sections 4–5. The guide cannot be extended, so primer-initiated background has no pathway; the recognition site is supplied by the guide, so it is not constrained by target sequence.

a. Oligonucleotides that can prime anything they touch

In every chemistry above, the oligonucleotide that confers specificity is also the oligonucleotide the polymerase extends. Any hybridization event whether that be to the target, to another primer, to an unrelated sequence, or in EXPAR's case to nothing sequence-specific at all — can therefore initiate a product. Once started, that product amplifies with the same exponential kinetics as the real one, and because isothermal reactions never return to a stringent annealing temperature, that amplification is sustained. This is why LAMP assays fail from mispriming [2], why SDA is “prone to false positives” at its operating temperature [1], and why EXPAR's background is target-independent [3].

b. A primer-design burden that limits multiplexing

LAMP needs four to six primers recognizing six to eight regions of a single target. Adding a second target roughly doubles the oligonucleotide population in the tube, and every added primer is another opportunity for a dimer that amplifies. The 2024 review of LAMP is explicit that single-tube multiplexing is “another significant limitation” for exactly this reason [5]. A chemistry that needs six oligonucleotides per target cannot reach the panel breadth that biothreat identification and host-response gene expression require.

c. A dependence on purified input

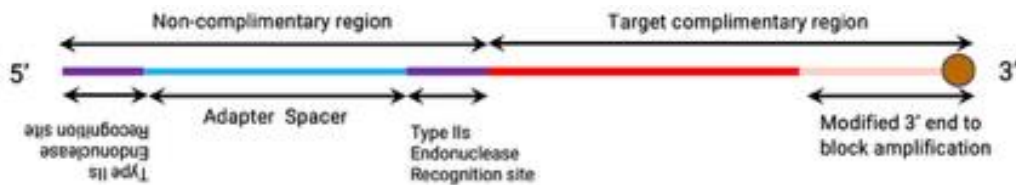
Most amplification chemistries assume extracted, purified nucleic acid, because inhibitors in blood, urine, soil and transport media interfere with the enzymes. Purification is where point-of-need workflows lose their time advantage and where operator error can decrease utility and effectiveness. A chemistry robust enough to run on crude lysate is contributing to diagnostic function in a way that the reaction-time figure alone does not capture.

5. DTECT: what the mechanism actually does

DTECT is a strand-displacement chemistry, but the way it creates the extendable end is different from every method in section 3. It uses a guide oligonucleotide that integrates an *exogenous* endonuclease recognition site to the target locus — a site that does not need to occur naturally in the target sequence, and does not need to be anywhere near where the cut lands.

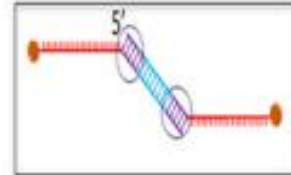
Type IIS enzymes cut at a fixed distance from their recognition sequence rather than within it. In DTECT that distance is four to six bases, and it is aimed into the target-complementary region. The assay designer therefore chooses where in the target the cut lands, independent of whether the target happens to contain a usable restriction site. The enzymes named in the published application include the site-specific nicking endonucleases N.BstNBI, N.Bst9I and N.BspD6I [6].

a. Anatomy of a DTECT™ guide oligonucleotide

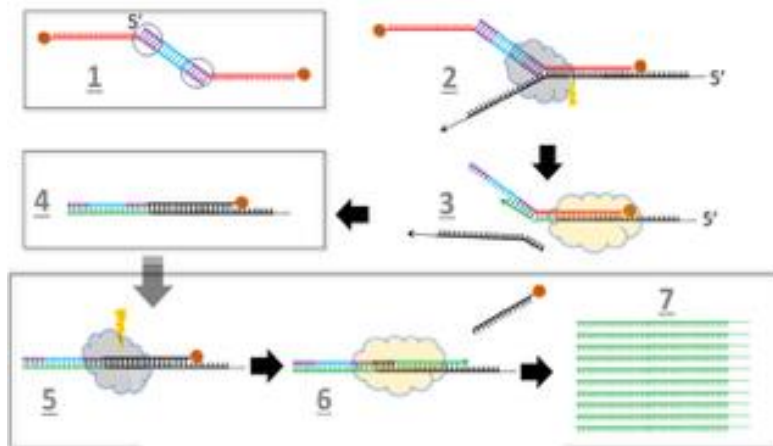


DTECT™ Guide Oligos

- NOT extendable, blocked by addition of 2'-O-Methyl RNA bases at the 3' end of the oligo
- Self complementary – hybridize to form structure on right with two Type IIs Endonuclease sites pointing in opposite directions (circled)
- Cleavage is 4-6 bases downstream from recognition site in target complimentary region.
- Allows for specific placement of endonuclease site to desired target.



b. DTECT™ chemistry mechanism



1. Binding of two guide molecules produces a duplex DNA to allow endonuclease recognition (in purple and circled).

2. Recruitment of endonuclease (gray blob) and target sequence hybridization prepares target DNA template for strand displacement through endonucleolytic cleavage of target strand.

3. The newly formed 3' end is then recognized by DNA polymerase (orange blob) that promotes strand displacement and strand extension (new products in green).

4. Initial product of strand extension.

5 – 6. Product of 4 then serves as template for a new round of strand displacement and extension.

7. Newly synthesized products can serve as templates for the complementary strand guide to repeat the process.

Blue/Purple = Guide molecule complementary sequence

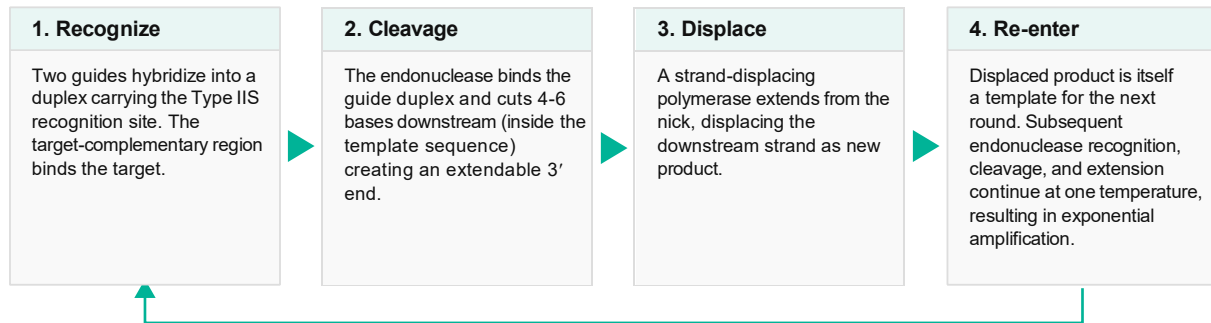
Purple = Recognition site for endonuclease

Red = Complementary to the target template

Orange circle = Block to prevent adventitious DNA extension

c. The cycle

Recognition, cleavage, strand displacement and re-entry, all at one temperature. Products of extension serve as templates for further rounds, which is what makes the gain exponential rather than linear.



One further element makes the cycle directional. The endonuclease cuts the guide's target-binding region only after it cuts the target sequence — the “differential” in Differential Targeted Endonuclease Cleavage. The published application also describes modifying the adapter region — with 2'-O-methoxyethyl bases, locked nucleic acids, peptide nucleic acids or phosphorothioate linkages — to accelerate or decelerate launch, which is how the kinetics of individual assays in a multiplex are tuned into the same detection window [6].

d. What is in the tube

Two enzymes and two oligonucleotides per target, plus a molecular beacon for detection; a reverse transcriptase and primer are added for RNA targets. The complete formulation lyophilizes for room-temperature shelf stability. For comparison, LAMP requires four to six primers per target and NASBA requires three enzymes.

6. Why the mechanism removes the failure modes

The argument of this document is not that DTECT is better optimized than the alternatives. It is that the three failure modes in section 3 are consequences of a shared design decision, and DTECT does not make that decision.

Failure mode	Why it happens elsewhere	What DTECT does instead
Primer-initiated background	The specificity oligonucleotide is also the extendable one, so any hybridization event — or in EXPAR, none at all — can start an amplifiable product [3].	The guide's 3' end is blocked to prevent polymerase extension. It is designed to be unextendable. The amplification is achieved through cleavage within the target, so a detectable amplicon requires target to be present.
Restriction-site dependence	SDA must introduce a hemi-modified recognition site through the primer and is "limited to small targets" [1]; the chemistry constrains where the reaction can be aimed.	The recognition site travels on the guide in a non-complementary region. Cleavage lands 4–6 bases into the target-complementary region, so the cut site is chosen by design rather than found in the sequence.
Multiplex ceiling	LAMP's 4–6 primers per target compound into primer-dimer and non-specific amplification as targets are added [5].	Two guides and one beacon per target. Triplex and tetraplex reactions have been run directly in whole blood and in swab matrix (section 6); the Biomeme/5 instrument provides five optical channels across twelve reaction wells.
Non-specific readout	Intercalating dyes and turbidity report nucleic acid amplification; NOT target specific amplification — which is why LAMP false positives are hard to detect at the point of use [2].	Detection is by molecular beacon, a sequence-specific probe. As with hydrolysis probes in real-time PCR, the probe is an independent confirmation of identity layered on top of the guide's specificity.
Purified-input dependence	Inhibitors in blood, urine and environmental matrices interfere with amplification enzymes, so most workflows require extraction.	The SampleDirect two-buffer system (lysis, then recovery) prepares nucleic acids in under a minute with no purification step, and the published application describes a detergent-solubilizer-cyclodextrin composition that stabilizes the polymerase and suppresses nuclease activity in crude lysate [7].

7. Performance

The figures in this section are Biomeme internal development data unless a reference is given. Reaction time is reported as time-to-detection (Td), the isothermal analogue of a PCR quantitation cycle: the point at which fluorescence crosses threshold.

a. Speed, reproducibility and linearity

A single-plex assay against the human IFI44L transcript, run as a dilution series from 25,000 to 250 copies per reaction, gives the following. DNA and *in vitro* transcribed RNA were run in parallel on the same target.

Copies per reaction	25,000	2,500	1,250	625	250
DNA — mean Td (min)	3.39	3.80	3.96	4.12	4.23
DNA — %CV	0.77	2.44	2.55	2.33	4.35
RNA — mean Td (min)	3.73	4.14	4.24	4.43	4.80
RNA — %CV	1.82	1.41	1.55	2.40	3.94

Three properties matter more than the headline speed. Td tracks copy number monotonically across two orders of magnitude, so the reaction is quantitative in the same sense a PCR quantitation cycle is. Coefficients of variation stay below 5% across the range. And RNA performs within about half a minute of DNA on the same target, which is not generally true of chemistries that bolt reverse transcription onto a DNA method.

b. Limit of detection, confirmed by an independent laboratory

Preliminary limits of detection for six biowarfare-agent assays were determined internally and then submitted to an independent U.S. government reference laboratory for confirmatory testing: 30 replicates at the stated LOD plus three negative controls per assay, with the LOD confirmed if 95% or more of replicates were positive.

Assay	Target level (GE/reaction)	Td (mean $\pm$ SD, min)	Replicates positive	Confirmed
BA	10	5.4 $\pm$ 0.9	97%	Yes
BTK	10	10.0 $\pm$ 0.8	100%	Yes
FT	10	10.6 $\pm$ 2.3	73%	No — repeat recommended at higher input
PA	10	8.8 $\pm$ 1.0	100%	Yes
RIC1	5	6.0 $\pm$ 0.4	100%	Yes
YP	5	5.2 $\pm$ 0.1	100%	Yes

Five of six assays were confirmed at 10 genome equivalents per reaction or better. The sixth was not, and is reported here as it was reported to Biomeme. Single-digit genome equivalents per reaction is the range in which laboratory real-time PCR operates.

c. Multiplexing in unpurified matrix

A triplex RNA assay against three human transcripts (IL1RN, MCTP1 and 18S rRNA) run directly in 6.25% venous whole blood in SampleDirect buffer, with no purification step, gave mean Td values of 3.53, 3.53 and 3.97 minutes with total %CV of 4.16, 3.60 and 4.07 respectively across sixteen replicates. A tetraplex assay against *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis* and an RNase P control amplified all four targets down to 80 CFU/mL with Td between four and eight minutes.

d. Matrix tolerance

- Whole blood.** RNase P was detected robustly in reactions containing 22.5% whole blood — roughly four times the concentration Biomeme uses in routine assays, run to establish headroom rather than as an operating point.
- Spores.** A biowarfare triplex amplified from washed *Bacillus anthracis* Sterne spores lysed directly in SampleDirect buffer. The expected absence of capB signal, which sits on the pXO2 plasmid the attenuated Sterne strain lacks, was observed — an internal specificity control as well as a sensitivity result.
- Urine and swab matrix.** *N. gonorrhoeae* was detected from vaginal swab matrix spiked at 4 CFU/mL and from male urine spiked at 1 CFU/mL, at levels comparable to currently cleared point-of-care platforms.

e. Lyophilized formulation

The complete DTECT formulation has been lyophilized to a room-temperature shelf-stable assay. Against an endogenous whole-blood RNA target, wet chemistry gave a total mean Td of 2.83 minutes (%CV 2.40, n = 18) and lyophilized chemistry gave 2.93 minutes (%CV 1.61, n = 24). Reproducibility was equivalent; the small offset in Td is the only difference.

8. The head-to-head with real-time PCR

The comparison that matters is not chemistry against chemistry in isolation but workflow against workflow, because the purification step is part of what a laboratory PCR result costs. Biomeme ran a dilution series of *B. anthracis* Sterne two ways: column-purified genomic DNA into laboratory real-time PCR on a benchtop instrument, and crude SampleDirect lysate of the same material into DTECT on a portable Biomeme instrument.

Result

Sensitivity of SampleDirect plus DTECT was on par with column purification plus real-time PCR across the dilution series — with no extraction step, on a battery-powered instrument, in a fraction of the time.

This is the claim the rest of the document supports, and it is worth stating precisely what it does and does not assert. It asserts analytical sensitivity parity on the assays tested, measured against a purified-input laboratory reference method. It does not assert clinical sensitivity and specificity parity across a validated menu — real-time PCR has three decades of published clinical evaluation behind it and DTECT does not. Section 10 sets out what is still outstanding.

9. Quantitation without a standard curve

Quantitation has been a recurring weakness of isothermal methods: without discrete cycles there is no clearly defined cycle count. Biomeme's published application WO 2025/207576 describes a competitive approach in which a reference nucleic acid of known quantity is co-amplified in the same reaction and competes with the target for shared reagents [8]. The target's reaction launch time then varies inversely with its abundance relative to the reference, and relative quantitation falls out of the same reaction that performs detection — with no standard curve generated at the time of testing. The application reports multiplexing across four to seven targets and limits of detection in the range of one to ten target molecules.

10. Where DTECT amplifies

DTECT is the amplification chemistry of the Biomeme/5 platform, the fifth generation of Biomeme's field molecular systems and the first to use isothermal rather than PCR chemistry. Biomeme's third and fourth generation instruments —the Franklin™ three9 and three9 ISP — are real-time PCR systems and remain in service; the distinction matters when reading specifications, because DTECT chemistry is back compatible; but prior PCR assays are not compatible with the new Biomeme/5™ isothermal platform as it does not thermocycle.

Biomeme/5	
Chemistry	DTECT isothermal amplification
Optics	Five color channels across twelve reaction wells
Time to result	5-15 minutes depending on assay and protocol
Workflow	Fully automated sample-to-answer; single-use cartridge with onboard fluidics and reaction chambers
Sample input	Environmental swabs and buffer (RUO); whole blood, venous or fingerstick (IVD)
Environmental	Water and dust ingress protected
Connectivity	USB-C, BLE, Wi-Fi, ATAK
Reagent storage	Room-temperature shelf-stable lyophilized cartridges

Current published specifications are maintained at biomeme.com/platforms/biomeme-5.

11. Limits of the present evidence

A technical document that only reports favorable results is not a technical document. The following are the boundaries of what the data above supports.

- **No published head-to-head against LAMP, RPA or NEAR.** The comparison in sections 3 and 5 is a mechanistic argument supported by each method's own published limitations. It is not a bench comparison run under common conditions, and it should not be read as one.
- **Sensitivity parity is demonstrated per assay, not per platform.** The *B. anthracis* comparison and the six-assay LOD confirmation establish parity on those targets. Each new assay carries its own verification burden.
- **One of six assays did not confirm.** The FT assay returned 22 of 30 replicates positive at 10 genome equivalents per reaction and a repeat at higher input was recommended.
- **Clinical validation is in progress, not complete.** DTECT assays are for research use unless stated otherwise. The host-response bacterial/viral panel is on a regulatory pathway; no DTECT assay is cleared at the time of writing.
- **Development data, not a validation report.** Section 6 reports development-stage results with the replicate counts stated. Controlled verification and validation data will be reported separately.

12. Patented technology

The DTECT™ and SampleDirect™ systems are the subject of the following published international patent applications, all assigned to Biomeme, Inc. A complete list of granted patents is published at biomeme.com/patents.

Publication	Title	Priority	What it covers
WO 2025/184067 A1	Methods and compositions for processing and amplification of nucleic acids	26 Feb 2024	The core DTECT mechanism: a guide complex that recruits a Type IIS restriction enzyme to cut both the target and the guide at differential rates, generating an extendable 3' end within the target; modified adapter regions that tune reaction kinetics.
WO 2025/207576 A1	Methods and compositions for amplification and analysis of nucleic acids	25 Mar 2024	Relative quantitation by competitive co-amplification of a reference nucleic acid, removing the need to generate a standard curve at the time of testing; multiplexed isothermal detection.
WO 2025/049920 A1	Methods and compositions for sample processing	1 Sep 2023	The SampleDirect chemistry: a detergent, solubilizer and cyclodextrin composition that lyses difficult matrices while stabilizing amplification enzymes and suppressing nuclease activity, allowing crude lysate to be amplified directly.

References

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