INSTRUCTIONS FOR USE

FOR PROFESSIONAL USE

Reagent Pack 1

REF **P561**

Reagent Pack 2

REF **P562**

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IVD

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Section **Page**

1. Intended Use

Cognitor® Minus is an *in vitro* diagnostic product designed for use within the hospital microbiology laboratory for confirming the absence of bacteria and fungi in human blood culture specimens from routine clinical blood culture bottles that are negative at the time of sampling for the Cognitor® Minus test and have had more than 12 hr incubation in an automated blood culture cabinet.

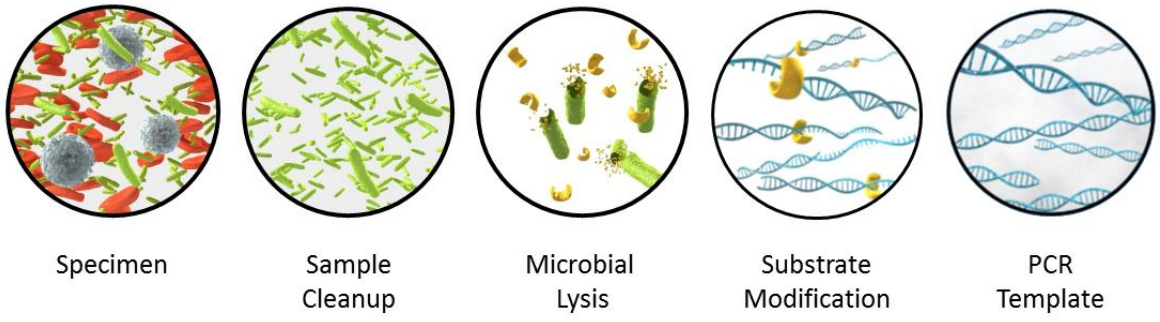
For professional use only. Cognitor® Minus should be used in conjunction with clinical presentation and/or other established tests as an aid in the management of patients with suspected sepsis and other bacterial/fungal blood stream infections.

2. Indication for Use

For the testing of blood culture bottle specimens from patients suspected of bacteraemia or fungaemia.

3. Principle of the Test

ETGA® (Enzymatic Template Generation & Amplification) is a technology that detects the activity of nucleic acid modifying enzymes from micro-organisms. The Cognitor® Minus test utilises ETGA® technology to detect micro-organisms by measuring microbial DNA polymerase activity. This means that Cognitor® Minus can be used to universally detect any micro-organism because DNA polymerase activity is common to all living things. Cognitor® Minus does not identify micro-organisms.



When bacterial or fungal cells die, enzymes such as DNA polymerase are rapidly lost to the surrounding media. The diagram above shows how the Cognitor® Minus test detects living organisms by separating intact (viable) organisms from the specimen and neutralising background levels of enzyme activity. Following microbial lysis, DNA polymerase activity is then detected using a proprietary synthetic DNA substrate that can be modified by DNA polymerase. Cognitor® Minus does not detect microbial or human DNA or RNA. The amount of modified Cognitor® Minus substrate is indicative of microbial DNA polymerase activity and can be measured by quantitative/real-time PCR (qPCR), so when the amount of modified substrate exceeds a threshold level, the result is “positive”. Because Cognitor® Minus has been designed to provide a high negative predictive value, ‘positive’ results cannot be reliably reported as due to infection and these results are reported as ”not determined”.

4. Cognitor® Minus Reagents

Reagent Pack 1 - Product Code: P561

Made up of 1 box containing reagent bottles and tubes, each box sufficient for 6 tests and 2 controls. Each box contains:

Contents	Colour	Quantity
Reagent A Saponin, Tween 20, sodium chloride	White cap	4 mL (1 x 15 mL bottle)
Reagent B <0.1% sodium hydroxide	White cap	15 mL (1 x 15 mL bottle)
Reagent C Ammonium sulphate, magnesium sulphate heptahydrate, potassium chloride, Tris-HCI pH 8.0	White cap	12 mL (1 x 15 mL bottle)
Sample Tubes Empty	White cap	1 pouch containing; 8 x 1.5 mL vials
Beadmill Tubes Glass beads	Yellow cap	1 pouch containing; 8 x 1.5 mL vials

Reagent Pack 2 - Product Code: P562

Made up of 1 foil pouch containing vials sufficient for 6 tests and 2 controls. Each pouch contains:

Contents	Colour	Quantity
Lysis Mix (LM) Bovine serum albumin, Triton X-100, Tween 20, magnesium sulphate heptahydrate, potassium chloride, dNTPs (A,G,C,T), Cognitor® substrate, internal positive control oligonucleotide, Tris-HCl, EDTA	Green cap	500 µL (1 x 1.5 mL vial)
Positive Control (PC) Glycerol, Tris-HCI pH 7.4, dithiothreitol, DNA polymerase I	Red cap	200 µL (1 x 0.5 mL vial)
Master Mix (MM) PCR components, Cognitor® oligonucleotides, buffer	Blue cap	270 µL (1 x 1.5 mL vial)

5. Materials/Equipment required but not provided

a. Specialised equipment

Product	Supplier	Website	Product Code
SmartCycler® including SmartCycler®centrifuge	Cepheid Inc.	www.cepheid.com	Various models available
Disruptor Genie®	Scientific Industries	www.scientificindustries.com	#SI-D268 or #SI-DD68
Disruptor Genie® tube holder assembly for screw-top tubes	Scientific Industries	www.scientificindustries.com	#0A-0563-011

b. General laboratory equipment required for the test

Item
Suitable blood culture sample extraction device
Laminar airflow (LAF) or PCR cabinet
Microcentrifuge (with 16 spaces, capable of 7300 x *g*)
Microtube rack
Two heater blocks for microtubes capable of temperatures of 37°C and 95°C
Unused blood culture bottle selected from the appropriate types listed in Section 6
Sterile pipette tips (20 µL, 200 µL, 1 mL) and corresponding pipettes
Sterile 3 mL luer lock syringes
Laboratory tissue paper
Alcohol wipes
Timers (1 for up to 6 tests, 2 for up to 14 tests)
Biological waste receptacle

6. Prior to starting the assay

The term 'specimen' used below refers to a blood culture sample for testing and 'sample' refers to either a blood culture sample or a control. A blood culture sample for testing comprises 0.5 mL of fluid from each of the blood culture bottles in a 2-bottle (aerobic/anaerobic) set or 1 mL of fluid from a 1-bottle set (e.g. a paediatric bottle).

a. Identify the number of blood culture bottle sets that are to be tested. Blood culture bottles must be negative according to automated blood culture at the time of sampling for Cognitor® Minus testing and have had more than 12 hr incubation in an automated blood culture cabinet. In all cases an appropriate automated blood culture system and culture bottle type must have been used.

   The appropriate automated blood culture systems are:
   bioMérieux BacT/ALERT® 3D System
   BD BACTEC™ FX Blood Culture System
   BD BACTEC™ 9000 Blood Culture System

   The appropriate culture bottle types are:
   bioMérieux BacT/ALERT® SA bottles (PC: 259789)
   bioMérieux BacT/ALERT® SN bottles (PC: 259790)
   bioMérieux BacT/ALERT® FA Plus bottles (PC: 410851)
   bioMérieux BacT/ALERT® FN Plus bottles (PC: 410852)
   bioMérieux BacT/ALERT® PF Plus bottles (PC: 410853)
   BD BACTEC™ Plus Aerobic/F Medium (PC: 442192)
   BD BACTEC™ Plus Anaerobic/F Medium (PC: 42193)
   BD BACTEC™ PEDS PLUS™/F Medium (PC: 42194)

b. Use of qPCR systems that have not been validated for this test is not recommended.

   Validated qPCR systems are:
   Cepheid SmartCycler® including SmartCycler®centrifuge

c. Reagent Pack 1 is a card box containing reagent bottles and tubes which is shipped at ambient temperature but should be stored at 2-8°C. Reagent Pack 2 consists of a single foil pouch, shipped on dry ice and stored at -20°C. Once opened a pouch, bottle or tube must not be reused. One Reagent Pack 1 and one Reagent Pack 2 together contain sufficient reagents to carry out 8 tests (6 specimens with two controls). If two Reagent Pack 1 (from the same batch) and two Reagent Pack 2 (from the same batch) are opened, it is possible to test 14 specimens simultaneously with 2 controls. No more than 14 specimens with 2 controls should be tested at any one time by a single operator.

d. Prepare and log-in to the PCR system and software following the procedures described by the manufacturer.

e. Wipe down the surfaces of the Laminar Airflow (LAF) or PCR cabinet to be used with a suitable disinfectant. We recommend 70% isopropyl alcohol (IPA).

f. Prepare the reagents:
    i. Identify how many blood culture sets are to be tested in a single batch. For 1-6 blood culture sets, remove 1 x Reagent Pack 1, and 1 x Reagent Pack 2 from storage. For 7-14 blood culture sets, remove 2 x Reagent Pack 1 and 2 x Reagent Pack 2 from storage.
    ii. Open Reagent Pack 2 and place the contents in a microtube rack in a refrigerator and allow to thaw. This will take between 30-60 min and will normally be complete by the time the reagents are required in the protocol.
    iii. Open Reagent Pack 1 and place **Reagent A**, **Reagent B** and **Reagent C** to one side. Place **Sample Tubes** and **Beadmill Tubes** in a Microtube rack at room temperature. Label 1 **Sample Tube** and 1 **Beadmill Tube** with a unique identifier for each blood culture set. Save 2 **Sample Tubes** and 2 **Beadmill Tubes** for the test controls. **Beadmill Tubes** should be labelled on the side of the tube to prevent labels being rubbed off during bead milling and then put to one side, away from the sample processing area.
    iv. Label 1 **Sample Tube** and 1 **Beadmill Tube** for the positive control.
    v. Label 1 **Sample Tube** and 1 **Beadmill Tube** for the negative control.

7. Sample Collection and Preparation

Remove blood culture bottles from the laboratory's automated blood culture system following the procedure described by the system manufacturer. Ensure only bottles registering NEGATIVE on the automated blood culture system at the time of sampling for the Cognitor® Minus test are tested. Blood culture bottles must be inverted 5 times to ensure a good suspension of the micro-organisms. Aseptically remove a 0.5 mL specimen from each of the aerobic and anaerobic bottle in a blood culture set, using a suitable extraction device, and place both specimens together into a single Sample Tube from Pack 1 to create 1mL of sample or aseptically remove a 1 mL specimen from a single paediatric bottle, using a suitable extraction device, and then transfer to a single labelled Sample Tube from Pack 1. This should be done using good aseptic technique to prevent contamination. Repeat for the remaining blood culture bottles to be tested. Promptly return blood culture bottles to the laboratory's automated blood culture system for continued testing following the procedure described by the system manufacturer. Collected samples should be tested using Cognitor® Minus test as soon as possible and should not be stored for later testing.

8. Assay Procedure

a. Transfer 1 mL of media from an unused standard blood culture bottle (selected from the appropriate bottle types listed in Section 6) to each of the 2 control **Sample Tubes** (see Quality Control - Section 12).

b. Add 330 µL of **Reagent A** to each **Sample Tube** then tighten the cap and invert the tube 5 times. Start timer after addition of **Reagent A** to the final sample. Allow all **Sample Tubes** to incubate at room temperature for 15 min.

c. Place all **Sample Tubes** in a microcentrifuge and centrifuge for 3 min at 7300 x *g*. During centrifugation, fold 2 pieces of clean laboratory tissue paper and place flat on a suitable shallow tray that can be autoclaved or disinfected.

d. After centrifugation, remove each **Sample Tube** in turn from the centrifuge and place in a microtube rack. Taking the first **Sample Tube,** remove cap and decant supernatant into a clinical waste receptacle. Then, keeping the tube inverted, gently dab the tube rim on a clean area of the prepared tissue paper to soak away residual supernatant. Tighten the cap and replace the tube in the microtube rack.

e. Note the following protocol alternatives for up to 8 samples (i.e. up to 6 specimens with 2 controls) and for more than 8 samples;

   i. For up to 8 samples; process each **Sample Tube** before moving on to the next. Using a sterile pipette tip transfer 750 µL of **Reagent B** to a **Sample Tube** and pipette up and down 10 times to resuspend any pellet. Tighten the cap and invert the tube 3 times. Repeat for each **Sample Tube**. After inverting the last **Sample Tube**, start a laboratory timer set for 5 min.
   ii. For more than 8 samples; process the first 8 **Sample Tubes** as in i and start a laboratory timer set for 5 min. Then repeat as above for the remaining 8 samples and after inverting the last **Sample Tube**, start a second laboratory timer set for 5 min.

f. After 5 min has elapsed on the laboratory timer, transfer 500 µL of **Reagent C** to the first **Sample Tube**. Replace and tighten the cap and invert the tube 3 times. Repeat for each **Sample Tube**. If processing more than 8 samples (see step (e) ii), the first set of 8 samples should be mixed with **Reagent C** first, and then the second set should be processed after completion of the 5 min incubation timed by the second timer.

g. Place all **Sample Tubes** in a microcentrifuge and centrifuge for 3 min at 7300 x *g*.

h. After centrifugation, remove each **Sample Tube** in turn from the centrifuge and place in a microtube rack. Taking the first **Sample Tube,** remove cap and decant supernatant into a clinical waste receptacle. Then, keeping the tube inverted, gently dab the tube rim on a clean area of the prepared tissue paper to soak away residual supernatant. Tighten the cap and replace the tube in the microtube rack.

i. Using a sterile pipette tip, taking each **Sample Tube** in turn, add 500 µL of **Reagent C** and resuspend any pellet by pipetting up and down 10 times. Without changing the pipette tip, immediately transfer the entire sample to the corresponding labelled **Beadmill Tube**. Discard the **Sample Tube** and tighten the **Beadmill Tube** cap. Repeat for each **Sample Tube.**

j. Place all **Beadmill Tubes** in a microcentrifuge and centrifuge for 3 min at 7300 x *g*.

k. During centrifugation;
   i. Remove the **Lysis Mix (LM)** from the refrigerator. Shake the tube downwards to move any liquid caught in the tube cap.
   ii. If using more than one pack of reagents, combine the contents of each aliquot of **Lysis Mix (LM)** in a single tube.

l. Remove each **Beadmill Tube** from the centrifuge and without disturbing the glass bead pellet, carefully remove the clear supernatant from each tube and discard using a 1 mL pipette with a fresh tip for each sample. Replace and tighten the yellow cap, and place the sample in a Microtube rack.

m. Using a sterile pipette tip, dispense 50 µL of **Lysis Mix (LM)** in to each **Beadmill Tube**. Change tips between each sample.

n. Remove the **Positive Control (PC)** from the refrigerator, shaking the tube downwards to move any liquid caught in the tube cap. Then add 10 µL of the **Positive Control (PC)** to the positive control tube ONLY. Replace yellow cap and tighten the tube.

o. Place each **Beadmill Tube** in the Disruptor Genie and run for 6 min at 2800 rpm to grind the samples by bead-milling.

p. After bead-milling, transfer all **Beadmill Tubes** to a 37°C heating block and leave for 20 min.

q. Transfer all **Beadmill Tubes** to a 95°C heating block and leave for 5 min.

r. During the 95°C incubation:
    i. Remove the qPCR **Master Mix (MM)** from the refrigerator, shake the tube downwards to remove any liquid from the cap and place inside a sanitised laminar airflow (LAF) or PCR cabinet.
    ii. Prepare a PCR tube or well of a 96-well PCR plate for each sample by placing in an appropriate rack inside the LAF or PCR cabinet.
    iii. Dispense 27 µL of **Master Mix (MM)** into each PCR tube/well in preparation for each of the samples. The PCR tubes may remain uncapped for the duration between preparation and addition of sample provided they remain under sterile laminar airflow.
    iv. Prepare the PCR system software following the procedures described by the manufacturer. Cycling conditions and programming parameters for validated PCR systems are available from Momentum Bioscience or your local approved supplier. Data analysis information can be found in Section 16.

s. After completion of the 95°C incubation, allow the **Beadmill Tubes** to cool at room temperature for at least 1 min.

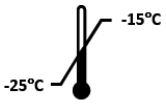
t. Place all **Beadmill Tubes** in a microcentrifuge and centrifuge for 30 sec at 7300 x *g*. Samples can be stored at this point for up to 24 hr at 2-8°C.

u. Place all **Beadmill Tubes** in a Microtube rack and take them to the LAF or PCR cabinet. Transfer 3 µL from each **Beadmill Tube** to a separate PCR tube/plate well containing the **Master Mix (MM)**. Close the PCR tube lid, or seal the plate, as required.

v. If required, centrifuge the PCR tube or plate to collect the reaction mixture at the bottom of the tube/plate well. Place PCR tube/plate in PCR machine and start the run.

9. Reagent Storage

Reagent Pack 1 should be stored at 2°C to 8°C.



Reagent Pack 2 should be stored frozen at -25°C to -15°C.

10. Precautions and Disposal of Reagents

a. Do not use the Pack 2 reagents if they are thawed on receipt. **Please contact Momentum Bioscience or your distributor if this has occurred for replacement reagents.**

b. Do not freeze Reagent Pack 1. These reagents are transported at ambient but should be stored at 2°C to 8°C on receipt.

c. Wear suitable protective clothing, gloves and eye/face protection.

d. All samples should be treated as a biohazard.

e. All contaminated plasticware and reagents should be treated as clinical waste and disposed of according to local Health & Safety procedures.

f. Unused reagents should be disposed of according to local Health & Safety procedures.

11. Equipment Procedure and Maintenance

Follow all procedures and maintenance protocols as described by equipment manufacturer.

12. Quality Control

The analytical interpretation of the data also assesses the negative and positive control results (aliquots of uninoculated blood culture media). Failure of either of the control tests indicates that there has been a problem with the running of the test; if either negative or positive control is **INVALID** this should be noted and results not reported. In addition to these positive and negative controls, an internal positive control (IPC) molecule is included within the **Lysis Mix (LM)** to validate the PCR reaction.

13. Troubleshooting

Problem	Cause	Solution
Pellet difficult to fully resuspend in **Reagent B**	Blood cell debris	Resuspend pellet as fully as possible with 10 tip-mixes. Continue protocol even if not fully resuspended as the next step will break pellet down further. Poorly resuspended pellet may increase levels of background DNA polymerase activity. *Hint: Aspirate by placing pipette tip close to the pellet, taking care to ensure the pipette tip (or aspiration process) does not cause the liquid to overflow.*
Pellet difficult to fully resuspend in **Reagent C**	Blood cell debris	Resuspend pellet as fully as possible with 10 tip-mixes. Continue protocol even if not fully resuspended. Complete dispersal of the pellet is not critical at this stage.
Labels on top of **Beadmill Tube** rub off	Lid of Disruptor Genie rubbing cap of **Beadmill Tubes**	Label side of tube.
Supernatant appears cloudy after 95°C step	Detergent in lysis reagent reaches cloud point	Allow samples to cool to room temperature prior to PCR set up. Cloudiness does not affect PCR, but may affect ability to see surface of the pellet and remove a sample of the supernatant.
Low C_t value for negative control	Contamination of reagents or broth sample	Check expiry date of reagents and broth. Repeat test. Use good aseptic technique for removal of broth from bottle. Avoid touching rims of bottles. Avoid leaving bottles and samples uncapped in an open laboratory. Use LAF cabinet.
PCR run fails	Power disruption/ system failure	If less than 24 hr old, and stored at 2-8°C, original sample lysates can be re-used (from step t in the assay procedure). Repeat PCR run (from step u) using the same processed samples and a new Reagent Pack 2.
PCR controls fail	**Positive Control (PC)** not added to positive control. Negative control contaminated.	Repeat test.
Run Fail	Controls failed	Check that controls have been set up properly and that sterile broth was used. Repeat the test.
Test Fail	PCR inhibition/loss of substrate/ carryover of contaminants from preparation process	Ensure that vials are inverted after resuspending sample pellet in **Reagent B**. Ensure that as much residual supernatant is removed by dabbing centrifuged samples on to the tissue where indicated in the procedure. Ensure that specimen was negative according to automated blood culture at the time of testing.
Increased occurrence of apparent discrepant results	Increased incidence of control failures	Contact product supplier/distributor.

14. Performance Characteristics

A performance evaluation was conducted over a 3 month period at two UK NHS acute hospitals. Data was collected from a broad range of patients within the catchment area of the two hospitals. The specification for sample collection time was more than 12 hr incubation in the automated blood culture cabinet.

Results were compared with the final result at 5 days from the automated blood culture system. The study data was reviewed and verified by the Chief Clinical Investigator for the study.

A total of 1497 sample sets were evaluated. Samples that failed to provide a reportable result due to failure of either the positive control, negative control or internal positive control were removed. The 1457 remaining sample results were further analysed.

		Blood Culture	
		Negative	Positive
Cognitor Minus	Negative	1291	7[2]
	Not Determined[1]	131[3]	28

[1] The **Cognitor® Minus** test has been optimised to deliver a rapid negative result with a high negative predictive value for clinically relevant organisms. It does not report a **POSITIVE** result. Non-negative results are reported as **NOT DETERMINED**.

[2] Six sample sets negative by the **Cognitor® Minus** test, and positive by blood culture were identified as contaminants following clinical review and were reclassified as true negatives.

[3] 91 of the 131 sample sets reported above as "not determined" by the Cognitor® Minus test, and negative by automated blood culture after 5 days, were identified as likely clinical positives following clinical review.

The study data provides a negative predictive value (NPV) for the Cognitor® Minus test of 99.5% versus the result after 5 days incubation in the automated blood culture incubator.

15. Interpretation of Results

The Cognitor® Minus test has been optimised to deliver a rapid negative result with a high negative predictive value for clinically relevant organisms. **A positive result does not necessarily indicate the presence of micro-organisms in the specimen**. Therefore **POSITIVE** results should be considered **NOT DETERMINED.**

Each validated PCR system reports results in differing ways based on the cycle threshold (C_t) value obtained by the qPCR amplification reaction. Details for each validated PCR system are:

Cepheid SmartCycler®
NEGATIVE indicates that the specimen does not contain a living organism **NOT DETERMINED** a positive result does not indicate that a sample contains a microorganism, only that the result was not determined **UNRESOLVED** or **INVALID** indicates either an individual test failure or a run failure

16. Data Analysis

Data analysis parameters for validated real time PCR system are:

Cepheid SmartCycler®
• The test sample is analysed in the FAM channel at an emission wavelength of 520 nm • The IPC is analysed in the TxR channel at 616 nm • For each sample, a Crossing Threshold (C_t) is calculated in both the FAM and TxR channels • In the FAM channel, **NEGATIVE** samples will have a C_t value of >43.5 units or 0 and samples that are **NOT DETERMINED** will have a C_t value of ≤43.5 units. **UNRESOLVED** samples will show a C_t value of 0 (and 0 in the TxR channel). • In the TxR channel, **NEGATIVE** samples will have a C_t value of <45.0 units. The C_t value in the TxR channel is not relevant for samples classified as **NOT DETERMINED** but could have a C_t value of <45.0 or 0. **UNRESOLVED** samples will show a C_t value of 0 (and 0 in the FAM channel). • Controls: In the FAM channel the accepted range for the Positive Control is ≤43.5 C_t and for the Negative PCR control is >43.5. C_t. In the TxR channel, the C_t value for the Positive Control is not relevant, but must be <45.0 C_t units for the Negative control. • **INVALID** runs contain control samples that fall outside the control criteria rendering the entire run **INVALID** and not reportable.

17. Detection

The following organisms have been detected by **Cognitor® Minus** test

Acinetobacter baumanii	*Enterobacter aerogenes*	*Peptostreptococcus prevotii*
Acinetobacter junii/johnsonii	*Enterococcus faecalis*	*Peptostreptococcus tetradius*
Aspergillus fumigatus	*Escherichia coli*	*Propionibacterium acnes*
Bacillus subtilis	*Flavimonas oryzihabitans*	*Proteus mirabilis*
Bacteroides fragilis	*Gemella moribillorum*	*Pseudomonas aeuruginosa*
Borrelia burgdorferi	*H.actinomycetemcomitans*	*Rhodococcus sp*
Campylobacter jejuni	*Haemophilus influenzae*	*Salmonella enteritidis*
Candida albicans	*Haemophilus parainfluenzae*	*Serratia marcescens*
Candida glabrata	*Helicobacter pylori*	*Staphylococcus aureus*
Candida parapsilosis	*Kingella kingae*	*Staphylococcus epidermidis*
Cardiobacterium hominis	*Klebsiella pneumoniae*	*Streptococcus agalacticae*
Clostridium perfringens	*Listeria monocytogenes*	*Streptococcus mitis*
Corynebacterium urealyticum	*Micrococcus luteus*	*Streptococcus pneumoniae*
Cryptococcus neoformans	*Mycobacterium intracellulare*	*Streptococcus pyogenes*
Eikenella corrodens	*Neisseria meningitidis*	

18. Limitations of the Test

- Samples must be incubated using an appropriate blood culture system and bottle type.
- Use of qPCR systems that have not been validated for this test is not recommended.
- The performance of this test has not been evaluated for sample types other than human blood culture specimens.
- Samples must be negative at the time of testing.
- Samples must have incubated for at least 12 hr in an automated blood culture cabinet.
- For professional use only.

19. Interfering Substances

The following substances were found not to interfere with the Cognitor® Minus test:

- Ampicillin
- Gentamicin
- Ciprofloxacin
- Erythromycin
- Vancomycin
- Tetracycline
- Chloramphenicol
- Warfarin
- Caffeine
- Paracetamol
- Ibuprofen
- White blood cells

Aspirin and ascorbic acid in blood at levels of 4.34 mmol/L and 342 µmol/L, respectively, were suspected of causing an increased chance of a “not determined” result from negative blood cultures.

20. Cross Reactivity

- No cross-reactivity has been identified, to date, for this test.