ULTRA-SENSITIVE PATHOGEN DNA EXTRACTION FROM WHOLE BLOOD

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Background and Objective

Traditional DNA extraction procedures for blood are lengthy and cumbersome. **SwiftX™ extraction** kits work **fundamentally different** than conventional DNA extraction kits:

Cell capture and **Reverse purification** enable rapid and user-friendly extraction of DNA:

- ✓ Modification of the cell capturing buffer for capture of microbial and fungal cells with magnetic Beads directly from whole blood
- ✓ Unlike conventional purification methods reverse purification binds impurities - leading to purified DNA/RNA.



We aim to provide a **robust and sensitive extraction tool** with the following characteristics:

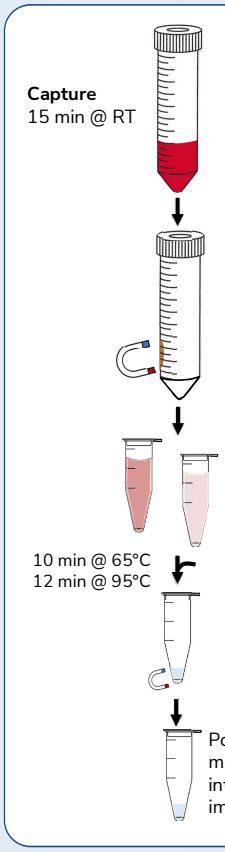
- **Replacing blood culture** by a direct ultra-sensitive bacterial and fungal DNA extraction down to <10 CFU/mL
- **Large volumes**: from 400µL up to 10 mL citrate, EDTA, fresh or frozen whole blood (no heparin)
- **Reduction of time to result by several hours**: 45 min total prep time
- **Easy to use, automatable process** (e.g. on **SwiftXtractor™ SL**)
- **Enables faster detection and response** to bacteremia and fungemia conditions saving lives
- **No hazardous reagents**

Workflow

DIRECT CELL CAPTURE
Magnetic bead-based binding of pathogens directly from whole blood

HEAT-ASSISTED CELL LYSIS
Heat-assisted cell lysis that is typical for all SwiftX™ extraction kits. Use of Proteinase K leads to an improved rupture of pathogens.

REVERSE PURIFICATION
Unlike conventional purification methods, reverse purification binds impurities - leading to purified nucleic acids.



Capture
15 min @ RT
1 vol whole blood (10mL)
+0.6 vol Buffer FBC (6mL)
+60µl Beads A

Magnetic separation (**2min**)
Redisperse in 1mL Buffer FBW
Transfer to 1.5mL/2mL Tube

Perform two washes with Buffer FBW (1mL)

Lysis
10 min @ 65°C
12 min @ 95°C
76µL Buffer FBL
+4µL PK

Post lysis, place **1min** on the magnet and transfer supernatant into a storage tube or process imediately with NAAT (qPCR,..)

qPCR validation

Total time of extraction ~45 min

Validation results

	CFU/mL		CFU/mL
E. coli *Gram-neg. tested with LDT at Xpedite using 10µL sample + 10µL Mastermix*	1000 100 10 **3** 0	***P. aeruginosa*** *Gram-neg. tested with LDT at Xpedite using 10µL sample + 10µL Mastermix*	1000 100 **10** 0
S. aureus *Gram-pos. tested with LDT at Xpedite using 10µL sample + 10µL Mastermix*	1000 100 **30** **10** 0	***S. pneumoniae*** *Gram-neg. tested with LDT at Xpedite using 10µL sample + 10µL Mastermix*	1000 **100** **10** 0
K. pneumoniae *Gram-neg. tested with LDT at Xpedite using 4µL sample + 16µL Mastermix*	1000 100 **10** 0	***E. faecalis*** *Gram-pos. tested with LDT at Xpedite' partner using 10µL sample + 10µL Mastermix*	500 50 **5** 0
C. albicans *Fungi tested with LDT at Xpedite using 4µL sample + 16µL Mastermix*	1000 **100** **10** 0		

LDT = Laboratory Developed Test

The initial validation has been performed using blood from at least 4 healthy donors, which was spiked with the respective pathogens.

The qPCR assays used for the validation are not optimized for most sensitive detection of the pathogens. All assays detect single-copy genes.

*Using a **multicopy target and optimized PCR mixes** may further increase the sensitivity of the workflow, potentially reaching **sensitivities <10 cfu/mL of whole blood**.*

Conclusion & Outlook

We present a breakthrough in sepsis diagnostics: a **scalable, rapid DNA extraction method designed to process 10 mL of blood with sensitivity on par with blood culture**. This method represents a major step towards real-time detection of bloodstream infections, enabling earlier intervention and improved patient survival.

The protocol consistently recovered pathogen DNA from blood samples containing <10^2 CFU/mL, matching or exceeding the analytical sensitivity of blood culture.

The extraction workflow was designed for a direct integration into a rapid, automated, and large-volume processing device (**SwiftXtractor™ SL**). This has the potential to transform the extraction workflow into a culture-free diagnostic solution, significantly accelerating time to actionable results and strengthening its clinical utility in acute care settings.



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