NEW SAMPLE PREPARATION METHOD ENABLING PCR-BASED PATHOGEN IDENTIFICATION DIRECTLY FROM BLOOD TO ACCELERATE BLOOD STREAM INFECTION (BSI) DIAGNOSIS

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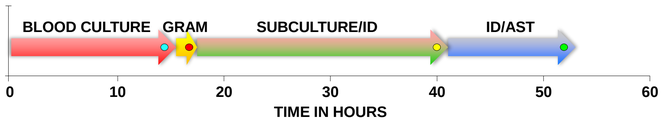
SUMMARY

Blood stream infection (BSI) are serious infections where treatment accuracy and rapidity drastically impact patient outcome. Currently, a Blood Cultures (BC) step taking up to 24 hours or more, followed by an additional subculture step is needed before pathogen identification, because direct diagnostic tests from blood are not sensitive or fast enough.



FibroTrap™ is a fibrinogen-based technology able to concentrate bacteria and yeasts from whole blood in about one hour. Our hypothesis is that processing blood samples through FibroTrap™ would enable PCR-based identification or microbiological methods to detect these pathogens directly from blood without a BC step.
After preliminary investigation of the performance of FibroTrap™ on spiked blood from healthy volunteers and from septic patients, we have studied the capacity of FibroTrap™ to capture microorganisms in clinical settings.
The objective of this clinical verification was to compare the performance of FibroTrap™ followed by PCR-based pathogen identification to standard BC-based diagnostic methods on blood samples from patients with BSI. The concordance between the standard BC-based identification and identification by PCR after FibroTrap™ has been calculated on 1281 patients for 7 of the most prevalent species in BSI (*E. coli, S. aureus, S. epidermidis, K. pneumoniae, S. pneumoniae, P. aeruginosa, and C. parapsilosis*).

BACKGROUND

FibroTrap™, a fibrinogen-based technology able to concentrate bacteria and yeasts from whole blood in one hour

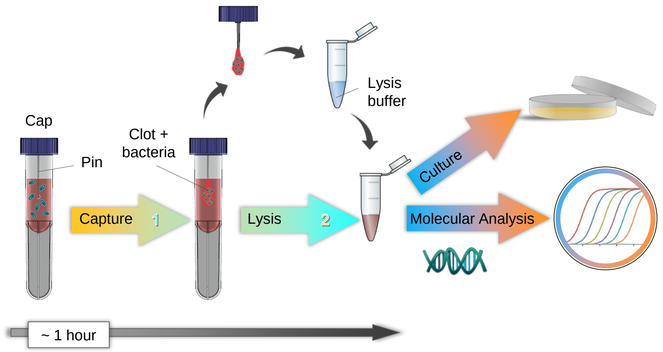


Figure 1. FibroTrap™ technology. FibroTrap™ allows the efficient concentration of microorganisms from 5 ml blood samples.
The whole process consists of two simple steps. 1) capture: the microorganisms are trapped into a small clot that is anchoring around a pin structure in a single reaction 2) lysis: the clot is lysed to release the microorganisms in a small volume of liquid.

METHODS

Performance of FibroTrap™ capture

The minimun concentration of bacteria (CFU/mL) detected by FibroTrap™ has been determined using a PCR readout for 5 bacterial species (Table A). Serial CFU dilutions for each strain were spiked in 5 ml of blood from healthy donors in order to determine the minimal dilution that can be detected by PCR following FibroTrap™. The number of CFU in the initial 5 ml of blood has been determined by plating and the number of genome copies has been determined by PCR. All the PCR assays were developed and optimized specifically for the study. The lowest concentration detected is the lowest concentration that has been positively detected by PCR after FibroTrap™ in all the replicates tested.

To evaluate the performance of FibroTrap™ in blood from patients with BSI, 500, 50 and 5 and 0 CFU of 2 strains of *S. aureus* (listed in Table B) were spiked in 5 ml of blood from patients presenting clinical signs and symptoms of BSI. The microbiological culture readout was used for these experiments. In brief, the number of CFU in the initial inoculum, the supernatant and the clot, were determined by plating on blood agar plates and the % of recovery was calculated as: (number of CFU in the clot)/(number of CFU in the supernatant + number of CFU in the clot) x100.

Clinical verification

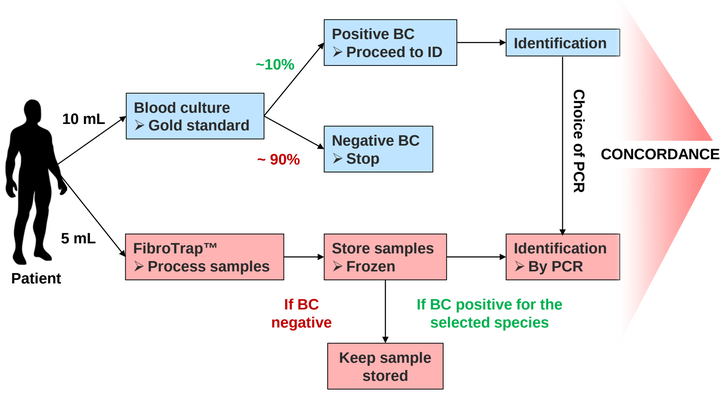


Figure 2. Clinical verification study design. Blood samples from patients suspected to have a bacteremia were collected for the standard BC-based diagnostic and for the FibroTrap™ procedure (2 samples of 5 ml from each arm). All the FibroTrap™ samples were processed through FibroTrap™ within 24h from collection by following the FibroTrap™ procedure (FT processed samples) and stored until BC samples analysis and results. The FT processed samples from patients with positive BC for the microorganisms listed in Table C were analyzed using a targeted PCR specific for the organism identified. The FT processed samples from patients with negative BC were not analyzed in this study.
The overall concordance % between the FT processed sample PCR results and the BC results have been calculated by patient and by species (Table C).

Control of contaminations

From sample collection to PCR assay, several reagents and manipulations steps could be the source of environmental contamination, in particular with *S. epidermidis* a very common contaminant in BC. To evaluate the risk of contamination with *S. epidermidis*, 40 FT processed samples from 20 different healthy volunteers (2 FT processed samples per volunteer) were analyzed with a PCR targeting this organism.

RESULTS

FibroTrap™ performance on spiked blood

For all bacteria tested, the lowest bacterial charge detected following the FibroTrap™ protocol in spiked blood from healthy volunteers was lower than 3 CFU/mL (Table A). During the assays, the number of genome copies of *S. aureus*, *K. pneumoniae*, *S. epidermidis* detected after FibroTrap™ were higher than those in the inoculums, indicating that bacteria were able to grow during the FibroTrap™ process.

With spiked blood from patients presenting clinical symptoms of BSI, the recovery rate by culture for the two *S. aureus* strains tested was >90% for all the concentrations tested (Table B). This recovery rate was equivalent to the recovery rate obtained with blood from healthy volunteers (Data not shown).

A

Species	Strains	# assays	Lowest concentration
S. aureus	ATCC 27660	2	2* CFU/mL
S. pneumoniae	ATCC 49619	2	2* CFU/mL
E. coli	ATCC 25922	2	1 CFU/mL
K. pneumoniae	HUG #13932019	2	2* CFU/mL
S. epidermidis	HUG #47	2	1* CFU/mL

* Not tested below the lowest concentration detected

B

Species	Strains	Spiked CFUs	# assays	% Recovery
S. aureus	ATCC 27660	353-788	9	94.4
S. aureus	ATCC 27660	35-50	9	97.7
S. aureus	ATCC 27660	8-14	16	96.9
S. aureus	MW2	352-450	6	93.6
S. aureus	MW2	36-57	11	93.1
S. aureus	MW2	7-10	14	97

Clinical verification & control of contaminations

Blood cultures from a total of 1281 patients from the CHU de Québec-Université Laval (Québec City, Québec, Canada) have been analyzed. 105 (8.2%) patients had positive identification according to BC-based standard diagnostic methods. 48 (3.8%) patients presented an infection with an organism listed in the protocol (in bold in Table C). 57 (4.4%) patients had a positive BC for an organism that was not listed in the protocol.

The overall % of concordance of FibroTrap™ identification with the standard BC method is 85.4% (Table C). All the species tested have been efficiently detected. This study also demonstrated that the FibroTrap™ method is compatible with common medications such as anticoagulants, antiplatelets, antipyretics, and cancer treatments (Table D).

Out of the 40 FT processed samples from 20 healthy volunteers, *S. epidermidis* was weakly detected by PCR in only 1 sample (only 1 FT sample out of 2 FT samples from the same blood volunteer), indicating that under controlled sterile conditions, the rate of *S. epidermidis* contamination is very low (Data not shown).

C

Species	# patients with positive ID-BC	# patients with positive ID -FibroTrap™ PCR	% concordance
S. aureus	**13**	**12**	**12/13**
S. pneumoniae	**4**	**4**	**4/4**
E. coli	**20**	**17**	**17/20**
K. pneumoniae	**5**	**3**	**3/5**
S. epidermidis	**4**	**3**	**3/4**
P. aeruginosa	**1**	**1**	**1/1**
C. parapsilosis	**1**	**1**	**1/1**
Total	**48 (a)**	**41 (b)**	**85.4 %**

(a) One patient was positive for both *E. coli* and *K. pneumoniae*
(b) Only *K. pneumoniae* was detected by PCR after FibtroTrap™ for the patient with both *E. coli* and *K. pneumoniae*

D

Treatments	# FT samples	% FT samples with clot formation
Anticoagulants	1 377	97.9
Antibiotics	1 550	99
Antiplatelets	997	98.1
Antipyretics	3 417	98.5
Cancer treatments	574	97.5
No treatment	357	97.8

CONCLUSIONS:

- For all bacteria tested, the lowest bacterial charge detected by PCR following FibroTrap™ was lower than 3 CFU/mL
- FibroTrap™ associated with PCR readout allows the detection of all the species tested with a high concordance to the current blood culture-based diagnostic methods
- Common treatments did not affect FibroTrap™ performance

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