**Figure 2 | Fabrication and functionalization of MEMS nanofluidic sensors embedded in a plastic injection molded capsule.** (A) Picture of the top and bottom view of the open capsule holder in which the sensors are embedded. An RFID tag is placed in the capsule lid to communicate with the device. The sample is deposited onto a membrane that filters the blood and drives the sample by capillary action to the entry of the biosensors. (B) Conceptual drawing of the sensors (top panel) top view showing the microfluidic entry channels (on the left side) and the connected nano-scaled channel (orange area) that is biofunctionalized to capture PSP. The sample flows from the left to the right through passive capillary action triggered by the high surface-to-volume ratio of the hydrophilic reservoir. Magnification on the functionalized nanochannel (side view, not to scale, bottom panel). The sensing surface area is specifically modified to immobilize capture molecules, while the surrounding surfaces are coated with a hydrophilic polymer to prevent non-specific adsorption of biomolecules.
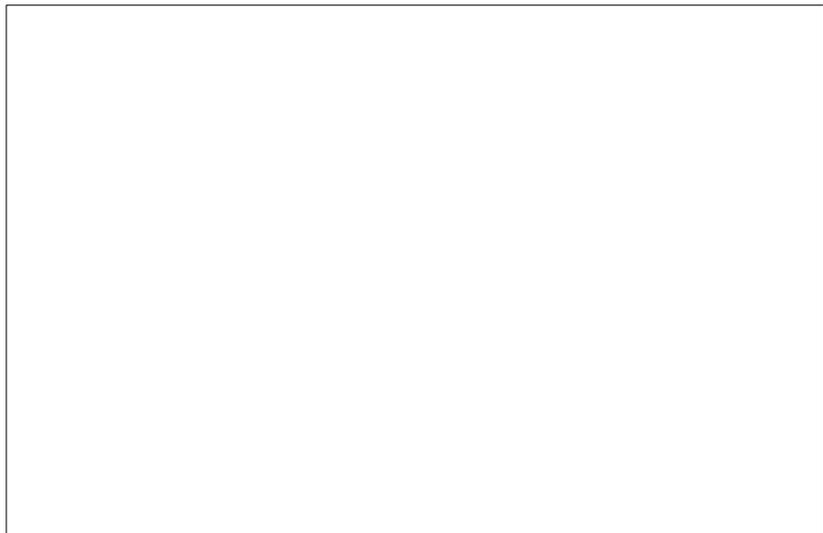
Passive capillary forces drive the sample through the system. This single-use capsule is then introduced into the tabletop size reader (the 'abioSCOPE'), which automatically analyzes the signal. It takes no more than 5 minutes for the results to be displayed on the large touchscreen of the device.

In agreement with the nanofluidic principles published in several academic works[19, 20] and confirmed for several analytes in the abioSCOPE device[21], a near 100% analyte capture efficiency is observed in the sensors with analyte capture profiles showing a dose-dependent signal increase (Figure 3).

PSP Capture Profile

Legend:
- 7.5 ng/ml
- 25 ng/ml
- 75 ng/ml
- 125 ng/ml
- 250 ng/ml
- 400 ng/ml
- 800 ng/ml
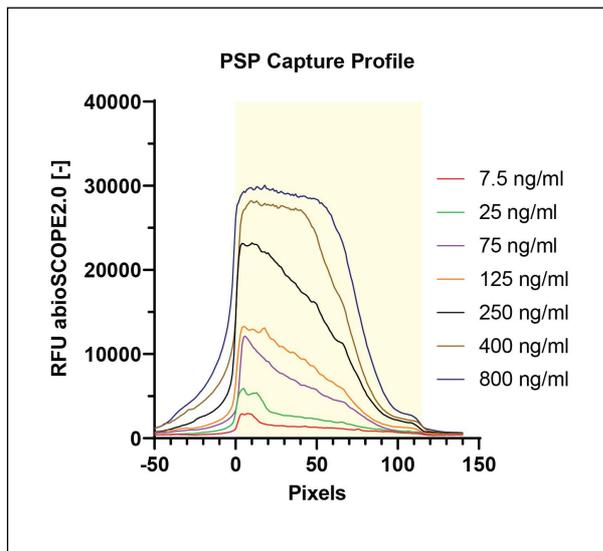
Figure 3 | Highly efficient capture of analyte thanks to nanofluidics. Average of the relative fluorescent unit (RFU) values are plotted as a function of the position in the nanochannel, expressed in pixels, for different concentrations of PSP. The yellow area delineates the boundaries of the sensing area. Data are from one sensor per dose, representative of 15 replicates.

2.2. Assay Calibration

From the signal output of the different PSP doses, a calibration curve is generated using a standard 5 parameter logistic (5-PL) fitting strategy to maximize accuracy and precision in the clinically relevant range of the assay (Figure 4).
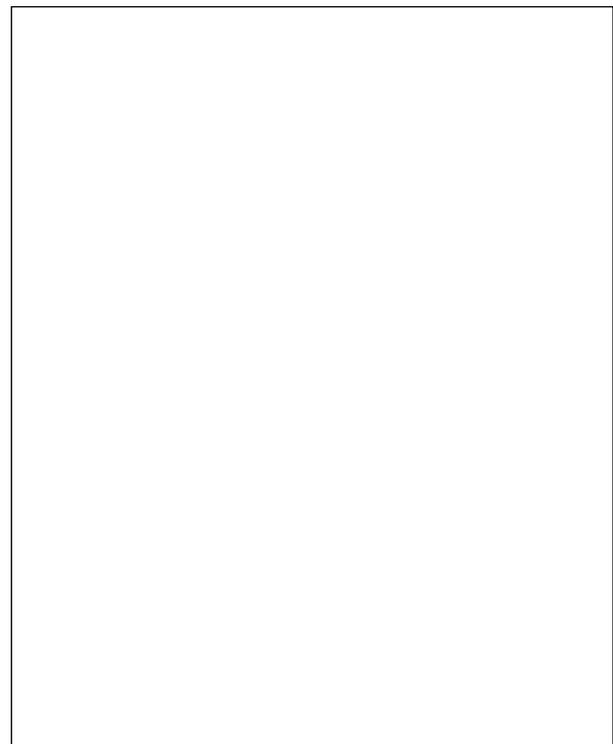
Figure 4 | Semi-log graph representing a dose-response calibration curve for PSP in the abioSCOPE device. Top panel shows the fit of the dose responses; lower panel shows the percentage recovery back to the expected values, for each individual dose. Each calibrator dose was measured 3 times and data points correspond to the mean of replicates. RFU: relative fluorescent unit, PSP: pancreatic stone protein.

The average imprecision in the range 20 to 600 ng/ml was 6.71% with a mean percentage recovery of 99.98% (ranging from 96.5% – 103.1%)

2.3. Linearity and Assay Range

The linearity of the PSP test in the abioSCOPE was demonstrated through a dilution-recovery analysis from a pool of six human sera with clinically elevated PSP levels that was diluted in PSP-depleted serum. The PSP assay was found to be linear from the lower limit of 20 ng/ml to an upper limit of 591 ng/ml (Figure 5).
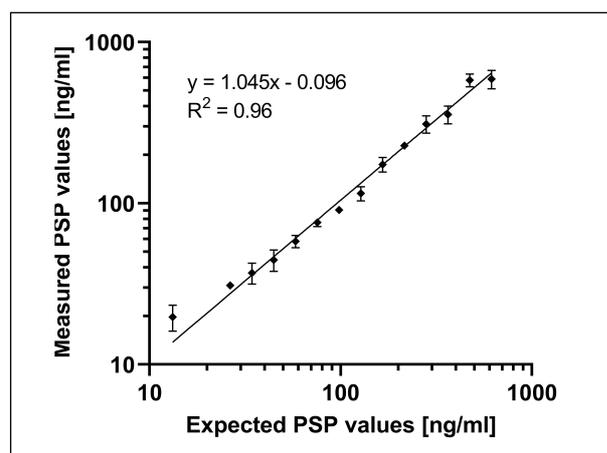
Order	Coef. Symbol	Coef. Value	R^2
First	b_0	-0.096	0.96
	b_1	1.045	
Second	b_0	-11.198	0.98
	b_1	1.204	
	b_3	$3 \cdot 10^{-4}$	
Third	b_0	20.521	0.98
	b_1	0.418	
	b_3	$32 \cdot 10^{-4}$	
	b_4	$-4 \cdot 10^{-6}$	

Figure 5 | Linearity of the PSP assay on the abioSCOPE device. The top graph displays the linear regression fit and equation of the average of triplicates measured per dilution. Data are shown as the PSP value measured in abioSCOPE [ng/ml] against the expected value [ng/ml]. Standard deviation error bars displayed per dose. The table displays the first, second and third polynomial fits of the data, showing no significant improvement of the R^2 between the first, second and third polynomials, thus supporting the claim that the assay is linear across the range of 20 - 591 ng/ml. PSP: Pancreatic stone protein.

2.4. Precision Testing

The precision of the PSP assay on the abioSCOPE device was estimated in a between-run study that included 10 replicates covering 3 doses of PSP (low, intermediate and high) as measured on the same device, same day, same operator and same product lot. The average imprecision of the test was 8.9% across three doses (Table 1). The device-to-device and lot-to-lot imprecision was then evaluated with a limited number of

replicates. The measurements indicate a good comparability of test results across devices and lots (Table 2).

PSP Dose [ng/ml]	N [-]	Mean [ng/ml]	SD [-]	CV [%]
Low	10	49.5	2.3	7.2
Intermediate	10	110.8	10.8	8.0
High	10	176.8	25.4	11.5
Average imprecision				8.9

Table 1 | Between-run imprecision of the PSP assay on the abioSCOPE device. The average between-run imprecision, calculated as the mean coefficient of variation issued from 10 replicates obtained on a same device with a same lot, was 7.2% for a low dose sample, 8.0% for an intermediate dose sample and 11.5% for a high dose sample. PSP: Pancreatic stone protein, N: Number of replicates, SD: Standard deviation, CV: Coefficient of variation.

	Sample #1 (low)			Sample #2 (intermediate)		
	Mean [ng/ml]	SD [-]	CV (%)	Mean [ng/ml]	SD [-]	CV (%)
Device-to-device	73.8	2.5	3.5	118.8	3.9	3.3
Lot-to-lot	76	3.1	4.1	112.6	8.8	7.9
Total imprecision	75.7	9.1	12.0	113.5	17.1	20.1

Table 2 | Device-to-device and lot-to-lot imprecision of the PSP assay on the abioSCOPE device. Two PSP doses (Sample #1 and Sample #2) were measured in triplicate on four devices and two product lots, for a total of 24 datapoints. Device-to-device and lot-to-lot imprecisions were computed as the ratio, expressed in percent (coefficient of variation), of the standard deviation of the replicates by the mean value of the replicates (either by taking the devices as a variable or the lots). Total imprecision is computed as the ratio, expressed in percent (coefficient of variation), of the standard deviation of all replicates for a given dose by the average value of these replicates. CV: coefficient of variation; SD: standard deviation.

2.5. Interference Testing

The PSP assay was evaluated for endogenous substances known to potentially interfere with results of immunoassays. At a PSP concentration of 400 ng/ml, negligible biases in test results were observed when clinically elevated concentrations of human anti-mouse antibodies or rheumatoid factors were present (Table 3). No high dose Hook effect was observed at clinically extreme PSP concentrations (5000 ng/ml was the highest dose tested).

Tested substance	Highest tested doses	PSP [ng/ml]	Bias [%]
HAMA[1]	651 ng/ml	400	1.8
Rheumatoid factor	1164 ng/ml	400	4.0
High dose PSP	5000 ng/ml	5000	Reported as "> ULOD"[2]

Table 3 | Interferences caused by HAMA and RF, as well as high PSP doses. The bias is computed as the difference in the results between the control sample (without the interferent) and the test sample (contains the interferent), expressed in percent. Bias exceeding 10% was considered as relevant interference with the test results. Triplicates were tested for HAMA, Rheumatoid factor and the high dose of PSP. [1] Human anti-mouse antibodies; [2] ULOD: upper limit of detection.

2.6. The PSP test on the abioSCOPE device allows for an early diagnosis of sepsis

In a landmark paper comparing several emerging sepsis biomarkers in an unselected ICU and high-dependency care population, Llewelyn and colleagues found that PSP was the best marker to distinguish sepsis from non-infectious inflammation [22] (Table 4). In the past years several other independent clinical trials have evaluated the diagnostic accuracy of PSP versus reference biomarkers in critically ill patients and confirmed Llewelyn's findings (reviewed in [11]).

| Marker	Cut off	Sensitivity	Specificity	PPV	NPV
PSP	30 ng/ml	90%	83%	86%	87%
PCT	1.0 ng/ml	71%	82%	83%	71%
sCD25	2.5 ng/ml	83%	83%	85%	81%
HBP	50 ng/ml	78%	36%	59%	59%
IL-6	0.2 ng/ml	68%	68%	71%	65%
IL-8	0.08 ng/ml	78%	63%	71%	71%
IL-1β	0.001 ng/ml	61%	88%	86%	69%

Table 4 | Biomarker performance in distinguishing sepsis (n=87) from infection without an infective aetiolgy (n=75) in patients admitted to the ICU or high-dependency care unit. ICU = intensive care unit; PPV= positive predictive value; NPV = negative predictive value; PSP = pancreatic stone protein; PCT = procalcitonin; sCD25 = soluble cluster of differentiation 25; HBP = heparin binding protein; IL = interleukin. Data reproduced from [22].

More interestingly, Abionic Researchers have recently demonstrated in ICU patients, that the kinetics of PSP during sepsis make it highly valuable in aiding clinicians in identifying this condition earlier than the current standard of care. To illustrate this, two patient case reports are summarized in Figure 5. The kinetics of PSP and PCT were measured on a daily basis and are shown together with the moment the clinicians first identified an ongoing infection. In both patients, the concentration of PSP was raised before the clinical diagnosis of an infection, suggesting that clinicians may have acted differently if they had had access to the data. Of note, the PCT in patient #1 increased much later than the PSP did, while in patient #2, the PCT was non-specifically high upon ICU admission and did not increase during the infectious event, supporting the better specificity of PSP over PCT.

Abionic is currently engaged with World-renowned Sepsis Experts and leading institutions in an ambitious clinical program that aims at demonstrating the value of this approach in various clinical situations and on diverse patient populations.
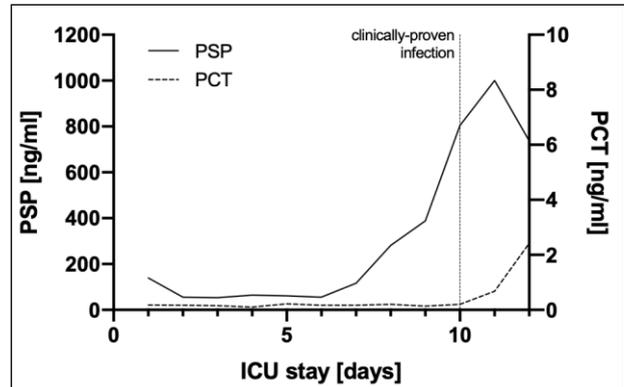
Case report #1 | A 71-year-old patient admitted in ICU for a brain trauma, put under invasive mechanical ventilation from day 1. No microbiological culture ordered until day 10, when a clinically documented infection was identified. The patient developed a sepsis on day 12 and died that day.
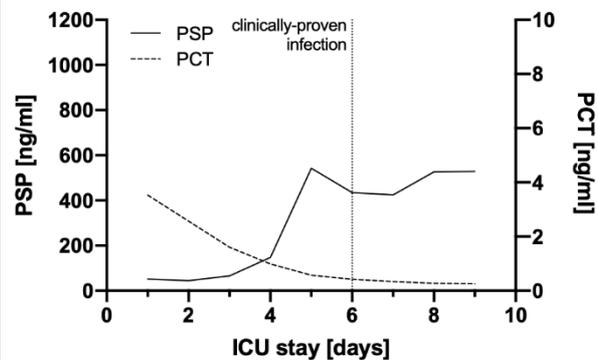
Case report #2 | A 54-year-old patient admitted to ICU following a hemorrhagic shock and put immediately under invasive mechanical ventilation. An infection was identified on day 6. PCT was high at admission and decreased during the onset of the infection, while the PSP was unaffected by the hemorrhagic shock and raised with the onset of the infection.

Figure 6 | Early identification of infection and sepsis by real-time monitoring of PSP at the bedside. The graph depicts the PSP values (left axis) and the PCT values (right axis), measured daily. Clinicians were blinded to the PSP and PCT results. PSP = pancreatic stone protein; PCT = procalcitonin; ICU = intensive care unit.

3. Conclusion

Sepsis is a medical emergency where every hour that passes is critical. Physicians will benefit greatly from any solutions that will aid them in identifying sepsis earlier, thus substantially improving the chances of patient survival. With this in mind, the combination of the abioSCOPE, the fastest technology for immunoassay and a highly sensitive and specific biomarker, PSP, is well positioned to achieve this ambitious but important mission.

Key Messages

- PSP is a reliable, highly specific and sensitive biomarker for the early diagnosis of sepsis.
- Early data suggest that PSP raises earlier than PCT and CRP in case of infection and sepsis.
- Besides the diagnostic accuracy of PSP for sepsis, its capacity to correctly predict sepsis complications such as in-hospital mortality, makes it a valuable prognostic marker in the ICU setting.
- PSP is secreted from the pancreas in response to remote infection-induced organ dysfunction and participates in the activation of neutrophil granulocytes.
- The PSP nanofluidic immunoassay on the abioSCOPE device pioneers a new paradigm for sepsis diagnosis and clinical management.
- The abioSCOPE has been designed to meet all the requirements of an extensive use in hospital settings. The analytical performances of the PSP test on the abioSCOPE device are in line with the clinical needs.
- Altogether, the data presented here support the disruptive potential of PSP to reduce the burden of sepsis worldwide.
- Abionic is currently preparing a technical file supporting product registration due for Q4-2019.

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