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Research article

Ultrasensitive qPCR platform for rapid detection of bacterial contamination of raw biological samples at the point of care

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ABSTRACT

Contamination of cell cultures can result in a significant loss of precious biological material, particularly in long-term processes including amplification of chimeric antigen receptors (CAR)-T cells and differentiation of patient-derived stem cells, for therapeutic purposes. Bacterial contamination can also lead to more complex conditions such as sepsis which can cause morbidity and mortality, despite strict controls and good laboratory/manufacturing practices in the manipulation of complex biological samples such as blood used in autologous and allogeneic stem cells transplantation.

The current standard method to identify biological risk is the set-up of microbial cultures, which can be time consuming with the likelihood of wasting large amounts of reagents in the event of contamination.

Real-Time Polymerase Chain Reaction (qPCR) is a molecular method able to detect biological agents in a highly sensitive and specific way and in a short time. However, qPCR assays require complex DNA/RNA purification steps and expensive benchtop instruments, which may not always be available.

This paper reports an extraction-free and low-volume protocol for qPCR in a standard instrument, which has been demonstrated to be effective on both Gram-positive (Gram+) and Gram-negative (Gram-) bacteria. Detection has been obtained from spiked cell culture samples, reaching a limit of detection (LOD) of 1 colony forming unit (CFU)/ml. To demonstrate the high potential of this optimized procedure, the same samples were also tested on a Point-Of-Care platform, which includes a cartridge with micro-chambers and a compact instrument, capable of performing qPCR with the same efficiency. *Staphylococcus aureus* (Gram+) was selected as the target for a proof of concept, achieving a LOD of 1 CFU/ml also on the portable device. The availability of these results paves the way for a simplified protocol for DNA extraction and amplification.

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1. Introduction

Mammalian/eucaryotic cell culture models and the use of clinical-grade bioreactors are on the rise in biomedical research and applications. Bioreactors provide biomimetic nutrients and stimuli to boost cellular growth, differentiation and tissue repair. Examples include T-cell expansion or tissue engineering bioreactors and lab-on-a-chip systems [1,2]. At the same time, an increasing number of clinical treatments (e.g. for injury recovery, arthrosis, chimeric CAR-T cells immunotherapy or many types of leukemia) involves the use of stem cells, administered by infusion or injection. Therefore, good laboratory/manufacturing practices and constant control of the cell lines are critical to prevent bacterial contamination [3], as well as expansion processes.

Bacteria contamination of blood and long-term cell cultures is a big threat in transfusion medicine as the risk of sepsis is significant and can heavily compromise the health of the receiving patient [4]. Peripheral blood should be strictly controlled for microbial presence, because of its use in both autologous and allogeneic transplantation.

Handling samples can be a source of contamination both prior to and following practices such as cryopreservation.

Bacterial contamination and resulting sepsis/infections remain the main cause of morbidity and mortality besides transfusion-related acute lung injury (TRALI) and transfusion-associated sepsis (TAS) [5].

Standard detection protocols require that blood components be stored for approximately 36–48 h to allow for the proliferation of contaminant microorganisms. Then, 8 ml of sample are inoculated into specific bottles for bacterial culture (BacT/ALERT 3D Automated Microbiology Detection System) and incubated at a temperature of 36 $\pm$ 0.5 °C for a period of 7 days. The sensitivity of this assay is directly proportional to the bacterial load in the sample volume analyzed. The metabolic activity of microorganisms is detected using a colorimetric assay with a patented technology: bacteria produce carbon dioxide in the culture broth that will reach the optical sensor located at the bottom of the bottle. Because of the change in pH, a color change will occur [6].

While this and other similar methods are highly standardized in current practice to identify the presence of bacteria, they show many disadvantages. Firstly, 7 days of waiting time and a large sample volume.

In the case of agar-based bacteria cultures, during sterilization processes, the interaction between agar and phosphate leads to the production of hydrogen peroxide that slows down the growth of bacteria [7] thus further prolonging the time needed for a response. Such a long delay is likely the major negative aspect of these standard procedures. For instance, having the results about contamination after one week means that in case of positive results the entire culture must be discarded, and with it all the efforts to collect T lymphocytes from patients, modify them to become CAR-T, select them and maintain the culture in the meantime.

Nucleic acid tests (NAT) offer a promising alternative to bacterial culture. In particular, Real-Time Polymerase Chain Reaction (qPCR) is a molecular biology method able to detect pathogens with high sensitivity and specificity from small amounts of sample and giving results in a shorter time than culture methods [8].

Amplification of specific portions of bacterial genes occurs through the chain reaction activity of the enzyme Taq polymerase and real-time detection of amplified DNA is associated with increasing fluorescence. The fluorescence is detected at the end of each amplification cycle and its intensity indicates the quantity of DNA amplicons reported at that time [9].

Standard protocols for qPCR assays need purified samples with related steps of sample manipulation/extraction and a reaction volume of at least 20 μl which can be problematic when the sample is limited or comes from “precious material” as in the above-mentioned bioreactors for CAR-T cells production. Moreover, qPCR reagents are usually expensive, encouraging the development of low-cost methods with comparable efficiency [10,11]. At the same time, since 2020, with the spreading of the SARS-CoV-2 pandemics, the interest in Point-of-Care (POC) devices has increased [12]. They are miniaturized systems for mass screening which can be faster, cheaper and with competitive sensitivity if compared to standard devices [13,14]. One of the main goals of POCs is to be easy-to-use [15], requiring no specialized or heavily trained personnel. Several examples of POC setups to detect microbial contamination from biological fluids have been proposed, based on protein assays and molecular tests [16,17].

In the present work, a qPCR approach was applied to successfully screen bioreactors and mammalian cell cultures for the presence of bacteria. To simulate the contamination expected in a bioreactor, cell culture media was spiked with two frequently identified external contaminants, namely *Staphylococcus aureus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative).

The Gram-positive (Gram+) *S. aureus* is typically identified by standard bacteriological methods, showing several morphotypes of mucous colonies and a high antibiotic resistance rate in persistent infections [18]. It lacks an outer membrane, but it is encapsulated by a cell-wall made of several layers of peptidoglycan. *P. aeruginosa*, classified as Gram-negative (Gram-), is a multi-drug resistant opportunistic pathogen, causing acute or chronic infections. It can be lethal in immunocompromised individuals with chronic obstructive pulmonary disease (COPD), cystic fibrosis, cancer, traumas, burns, sepsis, and ventilator-associated pneumonia (VAP) [19]. It is surrounded by an outer membrane containing lipopolysaccharide and only a few layers of peptidoglycan.

The choice of the two microorganisms is based on the intent to demonstrate that the optimized protocol for extraction and reduced analysis volume is effective on both Gram+ and Gram– bacteria.

Once flasks with immortalized cell cultures were intentionally contaminated, serial dilutions of culture media were prepared and treated. Usually, established protocols expose cells to lysis buffer containing non-ionic detergents, then include centrifugation steps to collect supernatant and purification procedures for nucleic acids (for example in phenol or chloroform) starting from hundreds of microliters of raw sample[a] [20]

The novelty of this work lies in the reduced volume of specimen used compared with standard protocols and in the possibility of avoiding the DNA extraction step. In the procedure described in this paper, the release of cell content in the medium is achieved through hypotonic stress induced to bacteria. Then, to accentuate this effect, a thermal treatment before the amplification step is also carried out directly in the thermocycler. The whole procedure has been optimized to operate in a final volume of 5 μl starting from less than 1 μl of raw sample. We also demonstrated that our specifically developed qPCR protocol works on a non-standard device as well,

identifying a strategy to obtain an on-field assay, which avoids time and reagents loss during sample preparation and performs the entire process in around 1 h. Our approach involves the use of a POC device (Q3 by STMicroelectronics) connected to a laptop and the comparison with a benchtop standard machine (CFX96 by Bio-Rad), proving the efficiency of the reaction down to 1 CFU/ml also in the case of Gram + bacteria. A scheme reporting the rationale of the experimental work is reported in Fig. 1.

The results obtained demonstrate that such an optimized protocol and the necessary diffusion of innovative POC devices could bring enormous advantages, especially when avoiding time-consuming practices or preserving the sample volume is a constraining factor.

2. Materials and methods

2.1. Biological materials and reagents

The bacterial cell cultures of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were supplied by the Department of General Microbiology of the University of Salento in Lecce, Italy.

For each of them, a colony of cells from solid medium was resuspended in RPMI 1640 1× without L-glutamine (Corning - 5040CV) added with 10% of Fetal Bovine Serum (Sigma-Aldrich - F7524), 1% of L-Glutamine (Sigma-Aldrich solution - G7513), 1% Sodium pyruvate solution (Sigma-Aldrich - S8636) and 1% Penicillin-Streptomycin (Sigma-Aldrich - P0781).

Cells were quantified at the cell counting chamber, using an inverted EVOS XL microscope from Life Technologies (Thermo Fisher) at a magnification of 40×. We assumed that the number of bacterial cells/ml counted by optical evaluation in the media of contaminated flasks corresponds to the number of CFU/ml.

Starting from a known concentration of bacterial cells of 2000 CFU/ml, serial dilutions at 1000 CFU/ml, 500 CFU/ml, 100 CFU/ml, 50 CFU/ml, 10 CFU/ml, 5 CFU/ml and 1 CFU/ml were prepared in a sample solution consisting of 20% cell culture medium RPMI 1640 complete and 80% deionized water to induce hypoosmotic stress to bacteria cell as preliminary step to avoid nucleic acid extraction. The experiments were performed with all the dilutions. For qPCR experiments, Ingenetix kits, BactoReal Staphylococcus spp. (DVEB03711) and BactoReal *Pseudomonas aeruginosa* (RTGM906) have been used. The bacterial targets in the amplification process is the 23S rRNA gene for *Staphylococcus aureus* and the 16S rRNA gene for *Pseudomonas aeruginosa* which are detected by an oligonucleotide TaqMan probe labelled with 6-carboxyfluorescein (FAM) fluorescent dye, emitting at around 530 nm. Also included in the kits is another TaqMan probe labelled with the VIC dye, emitting at around 554 nm as internal positive control. A negative control was always included in the experimental setup.

2.2. Sample treatment and qPCR parameters

In the qPCR platforms tested (BioRad CFX96 and Q3 portable device), the reaction mix had a final volume of 5 µl, in which the amounts of individual components were readjusted proportionally, in comparison to the standard protocol suggested for 20 µl. Specifically, for both kits and a single reaction, 2.5 µl of DNA Reaction mix; 0.25 µl of Assay Mix (from commercial kit); 0.25 µl Internal Positive Control; 0.75 µl of water; 1.25 µl of spiked sample (obtained with the abovementioned procedure) were added. The DNA reaction mix and Internal Positive control are provided together with the commercial kits. Table 1 provides details about the optimized volumes of reagents and samples used to develop the new protocol.

The TaqMan probes used are labelled with two fluorophores: a reporter at the 5′ end (6-carboxyfluorescein [FAM] or VIC) and a quencher (6-carboxy-tetramethylrhodamine [TAMRA]) at the 3' end. When the probe is intact and not involved in nuclease activity, the two fluorophores are close enough that the fluorescence emitted by the reporter is absorbed by the quencher and no signal is

Fig. 1. Scheme of the optimization process of a time- and reagent-saving protocol for qPCR described in the following section.

Table 1
Volumes of sample and reagents necessary for standard protocol and newly optimized protocol.

Reagents	Standard protocol	New optimized protocol
Sample	5 μl (from extraction steps)	1.25 (0.25 raw sample + 1 μl water)
DNA reaction mix (2×)	10 μl	2.5 μl
Assay Mix solution	1 μl	0.25 μl
Internal positive control	1 μl	0.25 μl
Water	3 μl	0.75 μl
Total volume	**20 μl**	**5 μl**

emitted. When the probe is bound to the target, Taq polymerase cleaves the probe away from the quencher. In this way, the fluorescence emitted by the reporter can be revealed by the detector, allowing monitoring of PCR amplification and real-time quantification of the gene of interest [21].

To minimize sample pretreatment, in this paper a protocol to release cell content without the use of chemical reagent has been developed, exploiting the combination of hypotonic lysis and thermal treatment.

Hypotonic lysis is a method that can be used for simple disruption of microbial cells exposed to a surrounding environment with significantly lower osmotic strength than the osmolarity of standard culture medium. The hypotonic environment induces the penetration of water into cells, disrupting their integrity and allowing the release of the intracellular cell content [22]. Since hypoosmotic treatment alone may not be efficient enough to obtain the complete release of cell content, a thermal treatment into the hypotonic environment was added to the protocol. This step is effective on cell membrane and cell wall disaggregation and useful to destroy inhibitors of the reaction, reducing variability between replicates and false negatives [23]. Thermal treatment was performed directly in the qPCR reaction tube/well as a preliminary step to amplification cycles. This "on-board" thermal treatment at 95 °C for 5 min prior to qPCR allowed the complete elimination of nucleic acid extraction steps. Thus, the optimized thermal protocol for sample pre-treatment and qPCR amplification includes the following steps: 95 °C for 5 min, 50 °C for 120 s, 95 °C for 20 s, 95 °C for 5 s and 60 °C for 60 s. The last two steps were repeated for 45 cycles, acquiring FAM and VIC fluorescence at 60 °C at the end of each cycle (Fig. S1B in Supplementary Information file reports the "Q3-Plus V2 Suite" software interface). Each experiment was performed at least three times for each concentration.

2.3. Devices and instrumentations

The qPCR was run in parallel using the CFX96 system from Bio-Rad and the POC Q3 miniature device from STMicroelectronics. Each experiment was repeated at least three times and the average results were compared and plotted.

The Q3 system is a small device (L 14 cm x P 7 cm x H 8.5 cm) for on-chip qPCR that is easy to use and performs as well as standard commercial devices [24]. Amplification reactions take place in disposable cartridges, obtained by a microelectronic fabrication process (described in the SI file) with six microchambers holding a volume of 5 μl volume each. The chambers are aligned with a silicon substrate that integrates a temperature sensor and microheaters that allowa fast ramp for heating (15 °C/s) and cooling (7 °C/s). The wells are prefilled with wax which, once heated, prevents sample evaporation during thermocycling. This system works in an automated manner and the operator only has to fill the chambers with a standard micropipette and set the software parameters for thermal cycling and fluorescence acquisition. The graphical interface of the "Q3-Plus V2 Suite" software is reported in Fig. S1A in Supplementary Information file. The integrated CMOS photodetector records fluorescence frames at the end of each cycle. There are three possible excitation wavelengths (FAM, VIC and SYBR Green spectrum). A short movie of fluorescence acquisition is reported in the SI file (Video SI1).

2.4. Data analysis

For both qPCR instruments, the results were evaluated in terms of threshold cycle (Ct) values. First, the software for each device fits the raw fluorescence data. While the fitting algorithm for the Bio-Rad CFX96 software is not disclosed, the "Q3-Plus V2 Suite" software uses a five-parameter sigmoidal fitting model. Then, the Ct value for a fitted qPCR curve is defined as the cycle – i.e. the moment during the analysis – when the curve crosses a certain fluorescence threshold. This value is typically set slightly above the background, to always lie in the exponential portion of the curves. For both instruments, the threshold was left at the automatic value.

For the final data analysis, the Ct values of all the replicates were averaged, and their standard deviation was calculated.

3. Results

The aim of this work is to provide a new qPCR protocol for the detection of cell cultures contamination using a time-saving protocol that skips the steps of sample purification/DNA extraction and uses a very small amount of specimen. This method was proven to be effective on both the Bio-Rad's CFX96 standard benchtop instruments and the Q3 portable platform, meeting two challenging goals at the same time: the possibility to use minimal volumes of samples/reagents and to provide an affordable protocol for *on-field* assays, that requires minimal manipulation and low-cost instrumentation. As a proof of concept, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, two bacteria belonging to the Gram+ and Gram– groups respectively, were selected to demonstrate the efficiency of the

developed protocol. Both are common bacteria responsible for a wide range of diseases and, in some cases, for nosocomial infections [25].

3.1. Evaluation of the extraction-free protocol on standard instrument

Samples for qPCR experiments were prepared in a controlled environment after deliberately contaminating cell culture flasks with a known concentration of *S. aureus and P. aeruginosa.* Serial dilutions were prepared from known suspensions and samples were treated as described in the Materials and Methods Section prior to analysis. The qPCR thermal protocol was modified from that provided by the commercial kit manufacturer of the to include a preamplification step of 5 min at 95 °C. Such a step enhances the hypotonic effect and allows the cell membrane/cell wall to disaggregate, favoring the release of DNA into the reaction mix. 45 amplification cycles were then performed and fluorescence was recorded at the end of each cycle.

In Fig. 2, amplification curves obtained from reactions performed on a standard system (BioRad CFX96 machine) using samples taken directly from cell cultures contaminated by *S.aureus* (Fig. 2A) and *P. aeruginosa* (Fig. 2B) are reported. The analysis was conducted with n = 3 samples per concentration.

Results on the Bio-Rad CFX96 system showed an LOD of 1 CFU/ml, below which no sample showed an amplification curve.

The calculated mean and standard deviation of the Ct values for each concentration are shown in Table 2.

These values were obtained by analyzing the specimens shortly after the preparation of the spiked samples, observing an increase in the Ct values as the bacterial concentration decreased, as expected and as shown in the graphs in Fig. 3 related to the amplification of *S. aureus* (Fig. 3A) and *P. aeruginosa* (Fig. 3B).

The ability to achieve such a low limit of detection with such a small amount of sample, highlights that the established protocol works well even without any extraction step preliminary to amplification reactions and simply includes a very rapid thermal treatment directly on the qPCR instrument.

An internal positive control provided with the *Ingenetix kits* was always amplified and detected on the VIC channel at around 554 nm, confirming that the amplification occurred properly.

It should be noted that the protocol is effective for both Gram+ and Gram– bacteria, indicating that such a procedure is not hindered by the bacterial cell wall.

3.2. POC device results

To test the efficiency of the optimized extraction-free protocol in minimal volume analysis, the qPCR assay was also performed on the Q3 instrument, combining the simplified sample preparation method with the use of a portable instrument. The amplification of the Gram + *S. aureus* was performed in a total reaction volume of 5 μl as a proof of concept. The Q3 device, a compact and portable instrument connected to a PC, was chosen to demonstrate the efficiency of the optimized extraction-free protocol for qPCR and the availability of a tool for the on-field identification of contaminations from minimal volumes of biological solutions. To transfer the protocol to the portable platform, the two highest and the three lowest concentrations tested in the benchtop instrument were selected.

Fig. 4 shows the graphical user interface of the Q3 software during the final data analysis is shown. The amplification curves of 1000 CFU/ml of *S. aureus* are reported in blue; the green curves refer to the internal positive control provided with the *Bactoreal kits* (Ingenetix).

Table 3 reports the average Ct values for qPCR detection of *S. aureus* in the Q3 portable platform. It can be seen that the average Ct value is slightly higher (few amplification cycles) when compared to the Ct values obtained for the same concentrations in the Bio-Rad

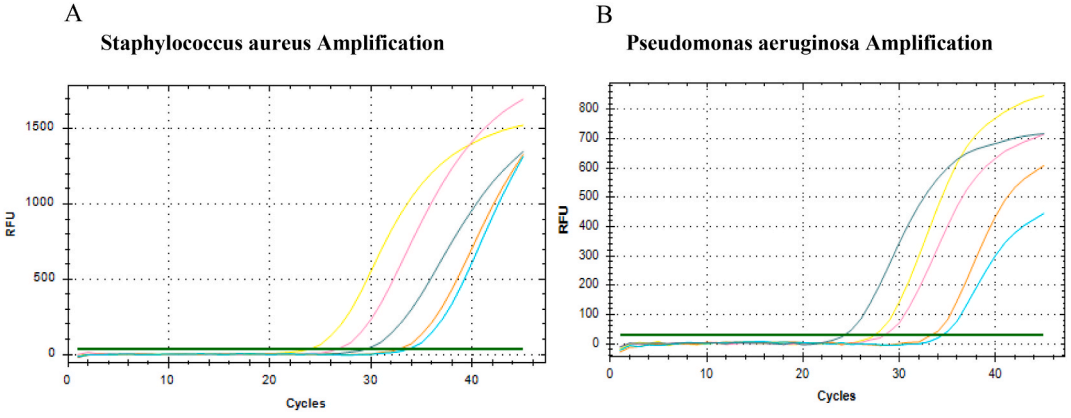

Fig. 2. Amplification plot obtained on standard benchtop instrument using a FAM fluorophore probe for detection of 23S rRNA gene of *Staphylococcus aureus* (**A**) and 16S rRNA gene of *Pseudomonas aeruginosa* (**B**) at different concentrations per ml: 2000 CFU/ml (yellow line), 1000 CFU/ml (pink line), 5 CFU/ml (orange line), 1 CFU/ml (sky blue line) and positive control (green line). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Average Ct value (and related standard deviation) for *Staphylococcus aureus* and *Pseudomonas aeruginosa* obtained on CFX96 at different concentrations. "CTRL POS": positive control provided with the commercial kits.

	S. aureus	*P. aeruginosa*
CFU/ml	**Average Ct**	**Average Ct**
2000	22.86 ± 1.17	27.08 ± 2.07
1000	23.74 ± 1.41	27.10 ± 1.04
500	23.56 ± 2.49	28.89 ± 0.14
100	27.39 ± 0.73	29.75 ± 0.12
50	28.51 ± 0.34	30.12 ± 1.17
10	31.20 ± 0.74	30.04 ± 0.23
5	32.60 ± 1.38	33.96 ± 1.14
1	33.90 ± 1.50	33.87 ± 1.51
CTRL POS	30.19 ± 0.72	25.43 ± 2.40

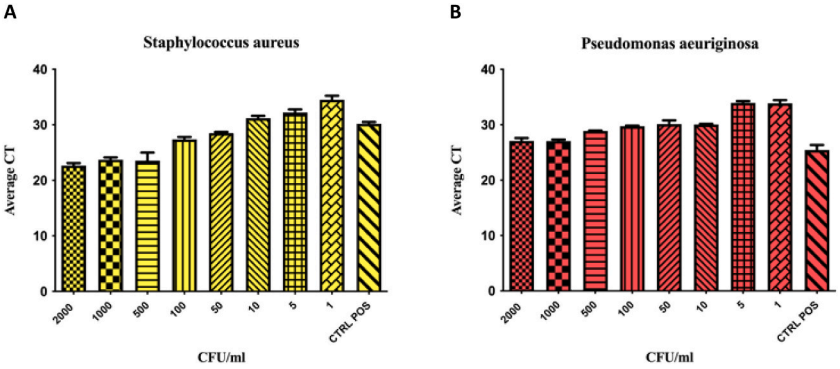

Fig. 3. Histograms reporting the mean Ct value and standard deviation obtained in standard instrumentation (CFX96) in relation to the concentration of both *S. aureus* (A) and *P. aeruginosa* (B) from 2000 CFU/ml to 1 CFU/ml "CTRL POS": positive control provided with the commercial kits.

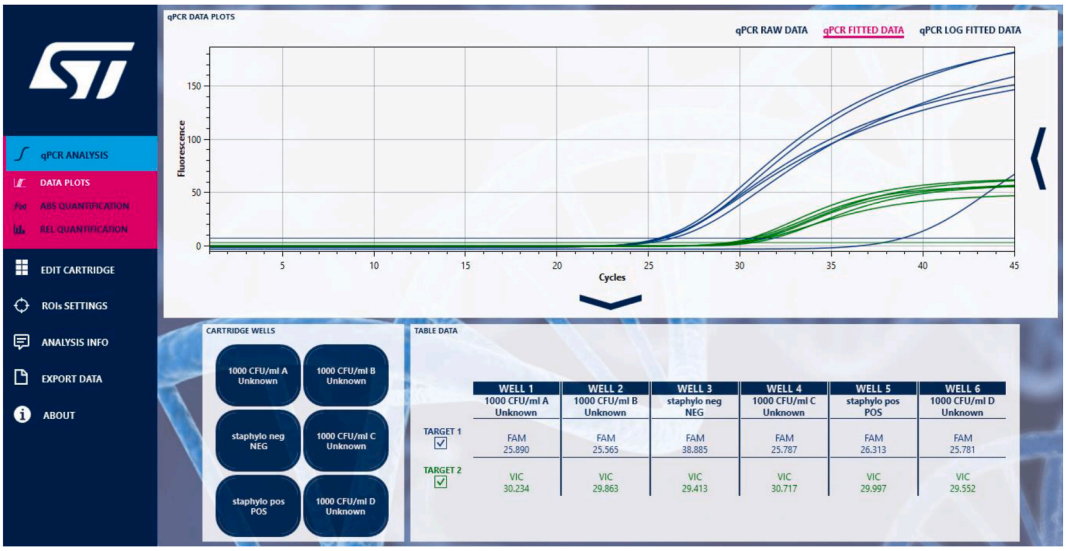

Fig. 4. Graphic interface of Q3 software during data analysis. On the left, a menu for setting parameters of qPCR analysis, cartridge information, ROIs tuning, analysis info, and export data. In the upper section, the amplification curves for 23S rRNA gene of *Staphylococcus aureus* at 1000 CFU/ml, plus positive and negative control (FAM signal, blue curves) are reported. In green, the amplification curves of *BactoReal* internal positive control (VIC signal) are plotted. In the bottom section, the Ct values for FAM and VIC signals associated with the respective position in the cartridge are shown. Well 3 contains negative control. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Average Ct and standard deviation of Ct values for *S. aureus* at different concentrations in Q3 device compared with CFX96 device. "CTRL POS": positive control provided with the commercial kit.

	S. aureus	
	Q3 results	**CFX96 results**
CFU/ml	**Average Ct**	**Average Ct**
2000	22.98 ± 0.28	22.86 ± 1.17
1000	25.71 ± 0.10	23.74 ± 1.41
10	35.61 ± 0.54	31.20 ± 0.74
5	37.39 ± 0.71	32.60 ± 1.38
1	38.07 ± 2.51	33.90 ± 1.50
CTRL POS	28.09 ± 0.37	30.19 ± 0.72

CFX96 instrument (histogram in Fig. 5). This could be due to a different time required for thermal variation between denaturation and annealing/extension steps, which is faster in Q3 due to the smaller dimensions of the cartridge, but also to a different data analysis. In fact, going from raw fluorescence data to the final Ct value requires at least two algorithms– the first for raw data fitting, and the second for Ct calculation – which are most likely different between the two instruments, as the algorithms implemented in the CFX96 software are not disclosed. On both devices the trend of Ct values among different samples increases as concentrations decrease, as expected. These results indicate the reliability of the portable instrument tested, which achieve the same detection limits as a standard instrument.

This result, combined with the simplified protocol for Gram + detection without nucleic acids extraction and the low volume of sample volume (0.25 μl), confirms the advantage of such a developed assay when being in shortness of sample or when repeated assays are required.

4. Discussion and conclusions

Sepsis and its progression to septic shock are potentially life-threatening conditions, representing the most common cause of death in Intensive Care Units. The physiological response to infection can become dangerous for the body's own tissues and this dramatic cascade of events culminates in a sharp drop in blood pressure, leading to severe organ damage and ultimately death.

In this and several other contexts, such as the use of blood bags for transfusion or the amplification of CAR-T cells in bioreactors, the ability to take advantage of rapid and simple screening methods for bacterial contamination could be crucial. Reliable methods to identify bacteria or viruses from small amount of samples could save time, reagents, costs and human lives without compromising their availability.

Bacterial cell culture is currently the gold standard method for identifying contamination; however it often fails to detect low concentrations of bacteria or spores, resulting in false negative outcomes [26]. Also, standard culture assays require several days for analysis. qPCR overcomes the disadvantages of cell culture methods and minimizes the risk of false negatives. In addition, by conducting the analysis directly on the test sample and avoiding DNA extraction, the processing time, cost, labour and risk of contamination [27] are significantly reduced. Furthermore, by eliminating the need for manual bacteria DNA isolation [28], efficiency of the process could be dramatically improved both in terms of time required (less than 2 h) and potential sample loss and it allows a higher number of samples to be processed than standard methods.

In this paper, a new protocol for the extraction-free detection of bacteria by qPCR from minimal raw sample volumes has been optimized. This was achieved by directly diluting cell culture media in deionized water and adding an on-board pre-amplification step prior to the qPCR thermal protocol. Results are similar for both Gram+ (*S. aureus*) and Gram– (*P. aeruginosa*) bacteria, reaching an LOD of 1 CFU/ml. The newly developed assay is particularly suitable for on-field diagnostics as it works directly from minimal volumes of raw samples. This last aspect has been demonstrated by validating the analysis on both a standard benchtop instrument and a portable device, with a reaction volume of 5 μl. As a proof of concept, to translate the protocol to the portable platform, tests were performed under the most challenging conditions, demonstrating the feasibility of the assay on Gram + *S. aureus* bacterium and improving the LODs obtained by standard methods [29]. The portable instrument tested requires only a laptop computer with software to operate in all settings, including limited resources contexts. Since the volume of raw sample is minimal and the sample pretreatment includes only a very little quantity of deionized water, lyophilized reagents including the qPCR mix could, in principle, be provided in the cartridge, allowing the analysis to be performed in any environment.

Differences between the Q3 instrument and standard qPCR equipment depend firstly on the costs of the different components. The CFX96 benchtop instrument is more expensive but allows 96 contemporary analysis in ordinary plasticware and tubes. Conversely, the Q3 platform has a low costs for the instrument (about 1/5th of the benchtop instrument cost, or less; it could even be rented for free, according to the business model) but requires proprietary disposable cartridges that allow performing 6 tests per run for a single excitation wavelength. Considering that usually at least a negative and a positive control have to be set up, this reduces the number of samples to be tested to 4 per single excitation wavelength. This drawback, which is significant for large-scale screening, becomes negligible when qPCR analysis has to be performed at a POC site, where the advantage of having a quick analysis without recurring to a clinical laboratory or to skilled personnel far outweighs the limitations. With respect to other features, such as accuracy or limits of detection, the two systems are comparable, achieving the same performance even with reduced sample volumes when reported

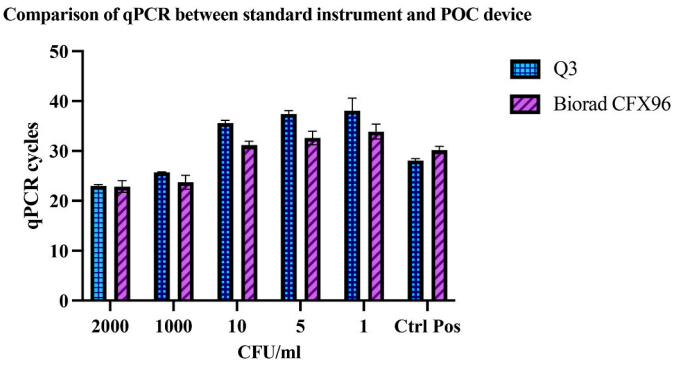

Fig. 5. Histogram showing the comparison of average Ct values and standard deviation at different *S. aureus* concentrations on the Q3 device and on the benchtop CFX96 instrument. *y* values report the number of PCR cycles, *x* values report the concentrations tested. "Ctrl Pos": positive control provided with the commercial kits.

according to standard protocols. The ability to work with a few microliters, needed in the portable platform and transferred to the benchtop instrument, is an example due to the validation of the assay. In fact, the volume of reaction mix required per test is usually about 20 μl. It is clear therefore, that running a complete and validated set of tests (96) on a standard platform will require a much larger amount of reagents than on the portable platform. In addition, the proportional amount of sample will be saved. Table 4 highlights these strengths and other limitations of the methods.

In conclusion, the protocol defined and tested in this paper maximizes the ability to rapidly, efficiently and accurately identify contamination *on-site* and shows great potential for further development as a standard technique for easy and rapid detection of bacteria.

Author contribution statement

Valeria Garzarelli: Performed the experiments; Wrote the paper.

Maria Serena Chiriacò: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Marco Cereda; Francesco Ferrara: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Giuseppe Gigli: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data included in article/supplementary material/referenced in article.

Additional information

Supplementary content related to this article has been published online at [URL].

Table 4
Comparison among standard bacteria cultures, standard instrument for qPCR tests, qPCR on Point-Of-Care device in terms of costs, quantity of reagents, affordability of the analysis.

Method	Advantages	Limitations
Bacteria cell cultures	• Low costs • Established procedures approved by law.	• At least 5–7 days to obtain results • Need for big instruments (incubators/shackers)
Standard sample treatment on standard qPCR instrument	• Established procedures approved by law. • Effective for large-scale tests (96 tests/run) • Possibility to perform duplicates • Low cost for disposable plasticwares	• High starting volume of sample (hundreds of microliters to be purified) • 20 μl of reaction mix/qPCR test (at least 15 μl of reagents) • High costs for benchtop instrument
New protocol for sample treatment on POC qPCR device	• Portability and Point-Of-Care usability • 5 μl of reaction mix/qPCR test (4.75 μl of reagents) • Rapid test for decision-making • Low costs for portable instruments	• No high-throughput analysis • 6 samples/run • Disposable cartridges costs higher than plasticwares

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16229.

a. https://www.ingenetix.com/wp-content/uploads/2021/10/BactoReal-Kit-Staphylococcus_Saureus_IFU_IVD_v1-0en-1.pdf
b. https://www.luminultra.com/blog/converting-qpcr-results-to-resemble-culture-method-results/

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