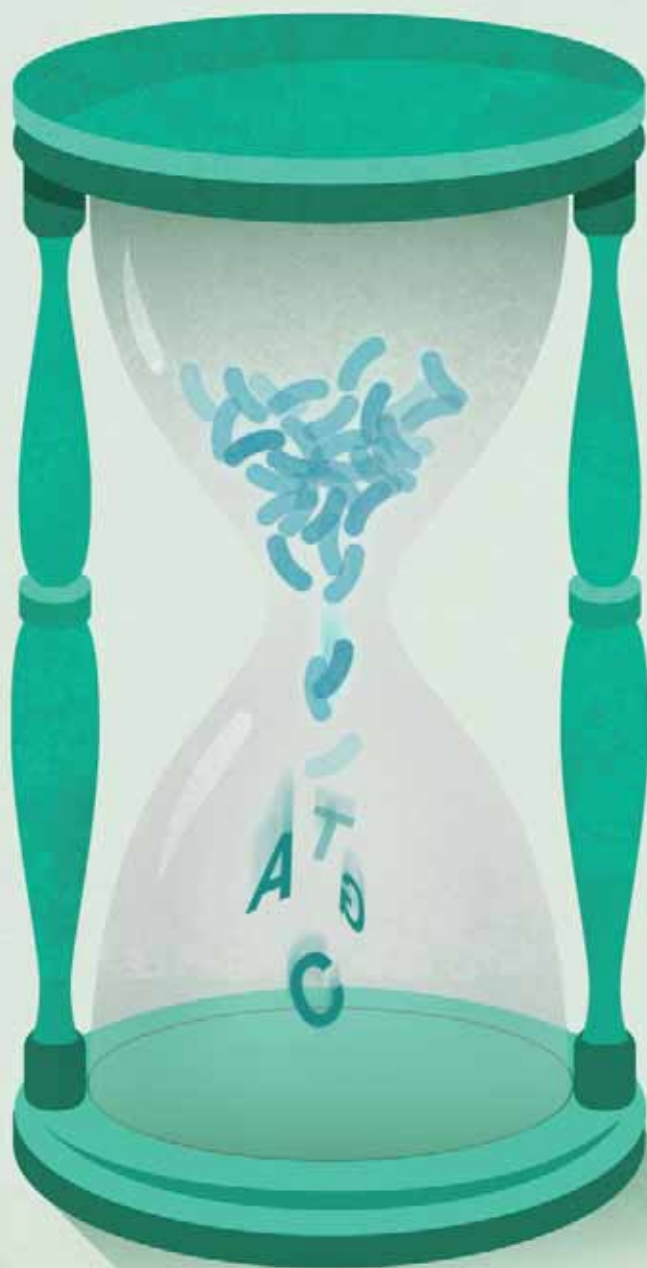# NOVES TECNOLOGIES PER AL DIAGNÒSTIC MOLECULAR DE LA SÈPSIA

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2015

RESULTS AND DISCUSSION

1. Overall of PCR/ESI–MS versions, study design and data analysis

1.1. Versions of the PCR/ESI–MS technology evaluated

Since its original design by Ibis Biosciences, the PCR/ESI–MS technology has been continuously evolving, leading to several evaluations of the different PCR/ESI–MS versions during the development of this thesis.

The first commercially available version was the IBIS T–5000, which allowed an automated analysis of specimens and was intended to be used in health and industry settings; it provided highly sensitive detection without requiring a highly trained operator [115].

With the incorporation of Ibis Biosciences into the Abbott group, the system has been upgraded on several occasions:

- PLEX–ID: in 2009, our group performed a pilot evaluation of this version obtaining promising results and identifying some critical points to be improved (**Appendix III**, **Poster 1**). A second evaluation of the technology using the PLEX–ID version was carried out in 2011 (**Article I**).
- IRIDICA: recently, this newer version was developed with improvements focused on the analysis of direct clinical specimens. One of the principal improvements was increased sensitivity due to a higher volume of blood utilized (5 mL) Thus, a third evaluation using this version was performed during 2014 (**Article II**).

The main differences between the PLEX–ID and the IRIDICA versions are depicted below (**Table 11** and **Figura 20**).

Table 11. Technical characteristics of the PLEX-ID and the IRIDICA versions of the PCR/ESI-MS technology

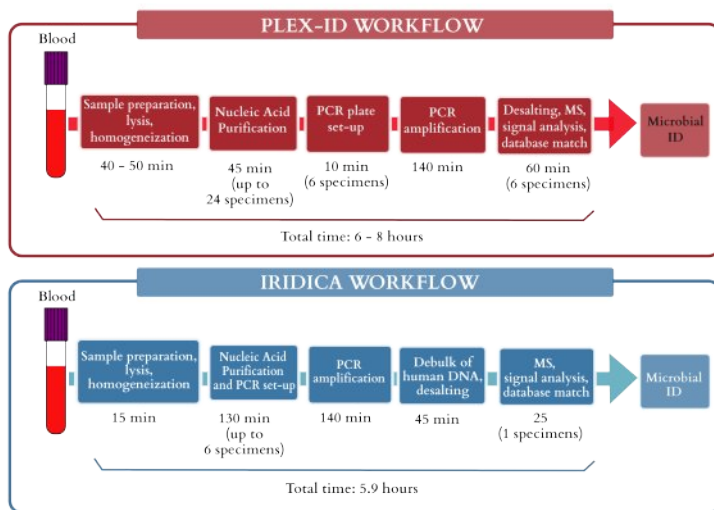|	PLEX-ID	IRIDICA
Volume of whole blood analyzed	1.25 mL	5 mL
Samples per run of nucleic acid extraction	1 – 24 (24-well plate format, manual dispensation of reagents and specimens)	1–6 (ready-to-use individual reagent cartridges)
Minimum number of samples during MS analysis	6 (96-well plate)	1 (one individual 16-well strip per specimen)
Pre-analytical analysis equipment	4 (mechanical lysis, magnetic nucleic acid extraction, fluid handler, thermocyler)	3 (mechanical lysis, magnetic nucleic acid extraction, thermocyler)
Analytic equipment	1 large instrument (desalting and MS in the same instrument)fluid handler, thermocyler)	2 bench-top instruments (separation of desalting and MS)extraction, thermocyler)
Time-to-result	6 h	5 – 6 h

Fig. 20. Workflow diagram of the PLEX-ID and the IRIDICA versions of the PCR/ESI-MS technology.

1.2. Study design

The design of the two evaluation studies (PLEX–ID and IRIDICA) is depicted in **Figure 21**, at the end of this section. In both studies, the results obtained with the PCR/ESI–MS technology were compared with those obtained by the conventional microbiological methods (blood culture, biochemical identification and antibiotic susceptibility testing), which constitutes the gold standard methodology for the microbiological diagnosis of sepsis. A short description of the methodology used can be found below.

- **Conventional microbiological methods.** For each adult patient, a set of two blood cultures, including two aerobic and one anaerobic blood culture bottles, were inoculated with up to 10 mL of blood each. The blood culture bottles were then incubated in the BactT/Alert (bioMériuex, Marseille–L'Etoile, France; PLEX–ID evaluation; **Article I**) or Bactec 9240 blood culture system (Becton Dickinson, Franklin Lakes, NJ, USA; IRIDICA evaluation, **Article II**) for up to 5 days. The identification and susceptibility testing of the microorganisms were achieved using the Vitek-2 Compact system (BioMérieux, Marseille–L'Étoile, France) directly from positive blood culture bottles after performing a Gram stain and a concentration procedure. Conventional cultures were also performed, following standard microbiological methods for identification and antibiotic susceptibility testing (disc diffusion and minimum inhibitory concentration methods) as required.

- **Specimen processing with PCR/ESI–MS technology.** In brief, whole blood samples (1.250 mL for PLEX–ID and 5 mL for IRIDICA) were lysed using chemical agents and a bead mill homogenizer. After DNA extraction, PCR amplification was performed using the BAC BSI Assay. This assay includes 18 primer pairs per specimen designed to amplify variable

fragments from a broad range of bacteria and *Candida* spp., as well as four antibiotic resistance genes: *mec*A (resistance to methicillin), *van*A and *van*B (resistance to vancomycin) and bla_{KPC} (resistance to carbapenems). After DNA amplification, PCR products were transferred to the "purification instrument" and finally to the ESI–MS instrument. The final identification was obtained from clinical specimens in 6–8 h (PLEX-ID) or 6 h (IRIDICA) (including all the above-mentioned steps). For the PLEX-ID evaluation, DNA was extracted at the Microbiology Service (HUGTiP) and further testing was performed at Ibis Biosciences, while for the IRIDICA evaluation all sample processing steps were performed at Ibis Biosciences (on both occasions, through a Short-term Fellowship for international stays, SEIMC).

- **Data interpretation.** The results obtained with the PCR/ESI–MS technology were compared with two different gold standards. Firstly, the results were analyzed considering the blood culture as gold standard. When discrepancies between these methods were found, the clinical significance of the discrepant results was determined by comparison with the constructed **"clinical infection criterion"** gold standard. For this purpose, a clinical microbiologist together with a clinician were asked to retrospectively evaluate the discrepant results obtained by PCR/ESI–MS and to interpret them in the same way as the blood culture results are customarily evaluated. Thus, the clinical records of the patients were reviewed in order to identify the diagnosed focus of infection, as well as the results of cultures from other specimens (i.e. microorganisms detected only by PCR/ESI–MS were considered true positives when the same microorganism had been isolated from a culture from another specimen type reflecting the focus of infection or supported by the nature of the underlying infection).

Since polymicrobial detections are not uncommon in blood-stream infections, the results obtained by PCR/ESI–MS technology and blood culture were compared at two levels using the two aforementioned gold standards: 1) **by microorganism:** a direct comparison for each microorganism isolated by conventional methods vs. the same microorganism detected by the molecular method, taking into consideration all microorganisms identified; and 2) **by specimen:** for each specimen with a single detection. Matched positive or negative results from each method were recorded. In the latter case, specimens with polymicrobial detections were excluded, as they could not be properly classified (i.e. the two methods aligned with some but not all microorganisms identified).
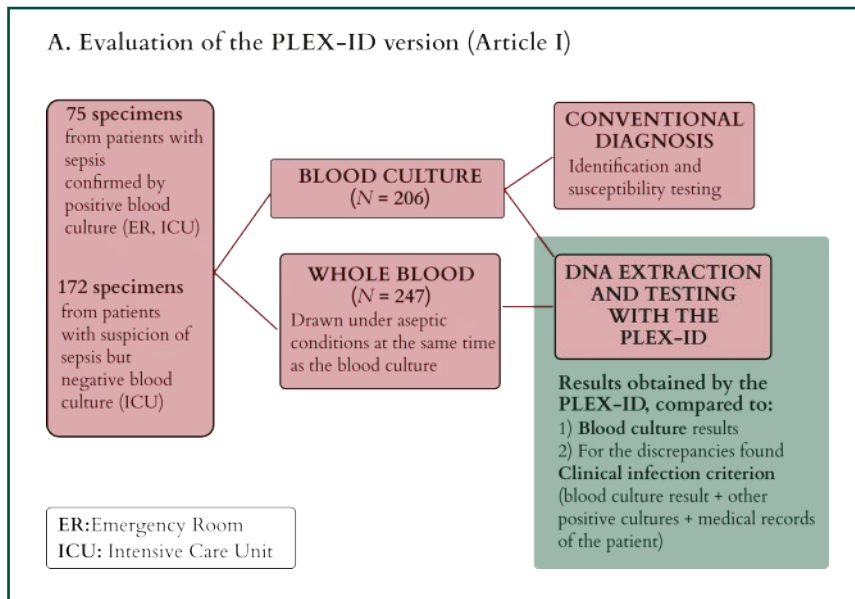

Figure 21.A. Study design of the clinical evaluations of the PLEX-ID version.
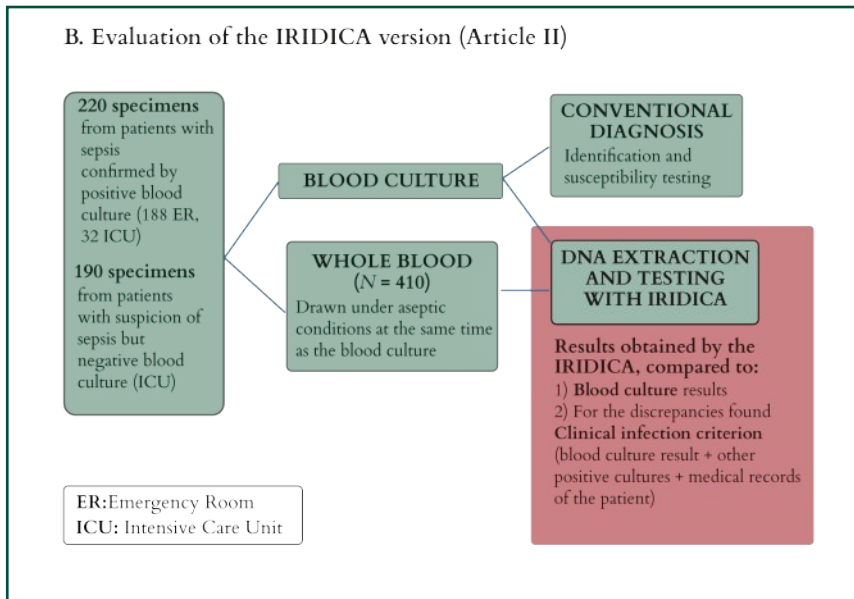
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Figure 21.B. Study design of the clinical evaluations of the IRIDICA version.

2. Determination of the system capability for the detection and identification of a wide-range of pathogens (Articles I and II)

Sepsis may be caused by a wide diversity of pathogens, mainly bacteria and fungi. However, the identification of all microorganisms in a single diagnostic assay may be challenging. In order to assess the ability of the PCR/ESI-MS technology for identification of a wide range of pathogens, whole blood specimens with a paired blood culture positive for a high diversity of sepsis-related pathogens, were tested. For this purpose, a total of 75 positive blood culture aliquots and 295 whole blood specimens (75 by PLEX-ID and 220 by IRIDICA) with a paired positive blood culture were analyzed. For this analysis, only microorganisms detected by the PCR/ESI-MS with clinical significance were included. For the PLEX-ID study, a total of 21 different species of microorganisms were isolated by both conventional methods and by PLEX-ID (**Table 3, Article** I). Among them, 20 species were concordant while one *Hafnia alvei* was isolated only by culture and two *Clostridium* spp. were detected only through the molecular method. From whole blood specimens, a total of 44 different species were isolated by conventional methods and the PCR/ESI-MS identified a total of 35 (**Table 3, Article I and Table 2, Article** II). Interestingly, PCR/ESI-MS (IRIDICA version) detected four species of microorganisms that were not isolated by culture: *Prevotella* spp. ($N = 1$), *Mycoplasma hominis* ($N = 1$), *Mycobacterium simiae* ($N = 2$) and fungi not identified (potentially *Aspergillus* spp., $N = 2$). However, the PCR/ESI-MS did not detect the following nine species, corresponding to 10 sepsis cases: *Enterococcus gallinarum* ($N = 2$), *E. casseliflavus* ($N = 1$), *Clostridium* spp. ($N = 1$), *Bacillus* spp. ($N = 1$), *Lactobacillus* spp. ($N = 1$), *Hafnia alvei* ($N = 1$), *Candida glabrata* ($N = 1$), *C. lusitanie* ($N = 1$) and *C. famata* ($N = 1$).

In **Figure 22**, a Venn diagram representing the concordance of specimens identified by PCR/ESI-MS compared to conventional methods is depicted.
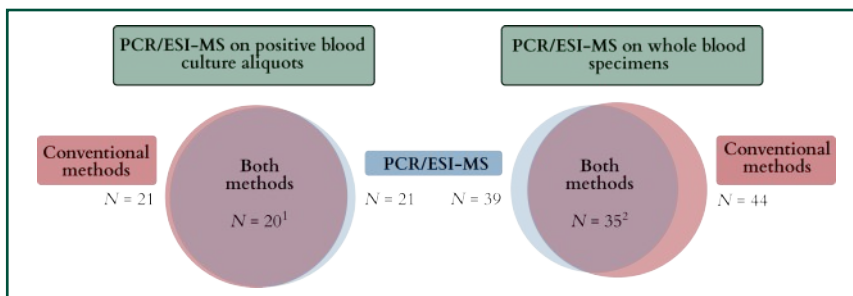
Figure 22. Venn's diagram depicting the concordance of species identified between conventional methods and PCR/ESI-MS technology depending on the specimen analyzed.

1. Coagulase–negative staphylococci (CoNS) (**Table 3, Article** I) corresponded to *Staphylococcus epidermidis* and *S. hominis*.

2. CoNS (**Table 2, Article** II) corresponded to *S. epidermidis, S. haemolyticus* and *S. hominis. Enterococcus* spp. corresponded to *E. faecalis* and *E. faecium.*

Compared with other molecular assays (**Appendix** I, **Tables 1 and 2**), PCR/ESI-MS was able to detect a total of 7 species that were not included in other assays (neither those for diagnosis of bloodstream infections from positive blood cultures nor whole blood). Those pathogens, which corresponded to 11 cases of sepsis, were *Fusobacterium* spp. ($N = 3$), *Prevotella* spp. ($N = 1$), *Granulicatella* spp. ($N = 1$), *Streptococcus gallolyticus* ($N = 2$), *Elisabethkingia meningoseptica* ($N = 1$), *Mycobacterius simiae* ($N = 2$) and *Mycoplasma hominis* ($N = 1$).

By design, the technology is able to differentiate among > 750 pathogens (bacteria and *Candida* spp.). Through our studies, we have demonstrated the detection of the most frequent and relevant sepsis–associated pathogens and also those more uncommon although clinically relevant microorganisms. Thus, those cases in which the PCR/ESI-MS was not able to detect some microorganisms were most likely due to low concentration of bacteria in whole blood (resulting in the absence of bacterial DNA in the tested aliquot) or insufficient amplification of product to be detected.

3. Determination of the system capability for the detection and identification of pathogens from positive blood cultures

This study was performed with the PLEX–ID version in order to:

1) Assess the potential of the technology for the detection and identification of a wide range of sepsis-related pathogens (discussed above) and
2) Compare the diagnostic yield obtained from blood cultures and whole blood.

As expected, the PLEX–ID showed very high agreement in the detection and identification of pathogens from positive blood cultures (analysis by microorganism), with an overall agreement of 92.0 % (**Table 13** of this section and **Table 1A, Article 1**). A total of 5 clinically relevant microorganisms were detected only through the molecular method and therefore, when compared with the clinical infection criterion, the overall agreement rose to 94.2 % (**Tables 1A and 5, Article 1**).

Table 13. Agreement between microorganisms isolated by conventional methods.

		Blood culture gold standard			Clinical infection criterion		
		Positive	Negative	Total	Positive	Negative	Total
PLEX–ID	Positive	78	11	89	83	6	89
	Negative	7	128	135	7	128	135
	Total	85	139	224	90	134	224
Agreement (%)		91.8[a]	92.1[b]	92.0[c]	92.2[a]	95.5[b]	94.2[c]

a. Positive agreement; b. Negative agreement; c. Overall agreement

These results are consistent with the data obtained during the pilot study, where 93 (90.3 %) of the 103 microorganisms identified by conventional methods were also detected by PCR/ESI-MS (45 different species identified, **Appendix III, Poster** 1).

A total of 14 specimens were polymicrobial by either or both methods. Six out of the seven microorganisms missed by the PLEX-ID corresponded to polymicrobial specimens (**Table 2, Article** I). The parameters of analytical performance were calculated including only the monomicrobial specimens (analysis by specimen) and were as follows: 96.7 % sensitivity, 97.7 % specificity, 95.2 % positive predictive value (PPV) and 98.5 % negative predictive value (NPV; **Table 4A, Article** I). Given that the PLEX-ID identified a clinically significant microorganism in a negative blood culture (**Table 5, Article** I), those values rose to 96.8 %, 98.5 %, 96.8 % and 98.5 %, respectively, when comparing with the clinical infection criterion.

As shown in **Table 7,** the sensitivity and specificity of the PLEX-ID is comparable to other molecular assays used for the diagnosis of bloodstream infection from positive blood cultures (sensitivity ranging from 80 % to 100 % and specificity ranging from 92.6 - 100 %). However, the time-to-result of the other assays is significantly shorter (Film Array: 1 h; Verigene: 2.5 h; Prove-it Sepsis: 3 h) in comparison with the PLEX-ID (6 h).

Recently, another mass spectrometry-based technology has been adapted to the microbiological diagnosis. A Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) approach has been developed to obtain the protein spectrum of microbial pathogens from blood culture [87, 120, 121]. This methodology also permits the identification of a wide variety of pathogens. E. J. Kaleta *et al.* [122] compared the PCR/ESI-MS technology with the MALDI-TOF approach from positive blood culture specimens where a highly accurate identification at species level was achieved with both methodologies (95.2 % and

94.3 %, respectively). Whereas, in that study, the MALDI–TOF analysis was performed from isolated microorganisms, this technology currently allows microbiologists to identify pathogens directly from positive blood cultures [85, 89]. Thus, the time-to-result is drastically different, as MALDI–TOF requires around 20 minutes for achieving microbial identification whereas the PLEX–ID technology, being based on PCR amplification, needs around 6 hours. Nowadays, the specific assay for the identification of pathogens from positive blood cultures by PCR/ESI–MS technology has been discontinued, even while the most promising feature of this technology is its ability to detect microorganisms from direct clinical specimens.

4. Determination of the capability PCR/ESI–MS for the detection of pathogens from whole blood (Articles I and II)

The desirable goal when developing molecular assays for the diagnosis of sepsis is the ability to analyze the patient's blood directly, avoiding time-consuming culturing. This would anticipate the diagnosis up to 40 hours in terms of using conventional methods. However, as afore-mentioned, working with whole blood can be difficult due to the po-tential inhibitors, the high amount of human DNA and the low bacterial load present in this specimen type. In order to overcome these issues, several strategies such as increasing the volume of blood ana-lyzed, performing a previous depletion of the human DNA or enriching the bacterial DNA have been developed, as discussed below.

4.1. Overall agreement of microorganisms between PCR/ESI–MS and conventional methods dependent on the version of the technology

Firstly, a direct comparison of the microorganisms isolated by culture versus the same microorganisms detected by the molecular method was performed (analysis by microorganism). The results obtained through both versions of the PCR/ESI-MS technology in comparison with the blood culture gold standard are depicted in **Table 14.**

Although the overall agreement between either of the two versions of the technology and the blood culture were comparable, the positive agreement was clearly higher for the IRIDICA version (the differences in the negative agreement will be discussed in the next section). As ob-served in **Table 11** and in **Appendix II**, Article 3, the main improve-ment of the IRIDICA version with respect to PLEX-ID is the increase in the volume of blood analyzed (from 1.250 to 5 mL). Although the results are not directly comparable because a different set of specimens was used in each of the two studies, the analysis of a larger volume of

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blood in the IRIDICA version seems to have been decisive in the increase of the sensitivity of the technology.

Table 14. Performance of the PCR/ESI-MS for the detection of microorganisms from whole blood according to the version used in comparison of conventional methods (blood culture gold standard).

		PLEX-ID			IRIDICA		
		Positive	Negative	Total	Positive	Negative	Total
PCR/ ESI-MS	Positive	37	25	62	176	80	256
	Negative	48	152	200	64	143	207
	Total	85	177	262	240	223	463
Agreement (%)		43.5[a]	85.9[b]	72.1[c]	73.3[a]	64.1[b]	68.9[c]

a. Positive agreement; b. Negative agreement; c. Overall agreement

The intrinsic relationship between the volume of blood analyzed and the sensitivity of the molecular methods for detecting the pathogens' DNA has been previously evaluated and the majority of the studies agree in the fact that using a higher volume of blood increases sensitivity [111, 123 – 125]. However, it should be kept in mind that a large amount of human DNA may hamper the detection of microbial DNA. Regarding the influence of high levels of human DNA on microbial detection, A. Bacconi *et al.* [114] demonstrated that IRIDICA performance was not hampered by the presence of up to 4.0×10^7 white blood cells/mL. Additionally, in our studies no significant difference in white blood cell count was found between PLEX-ID or IRIDICA-negative and –positive specimens (PLEX-ID: 13.9 *vs.* 12×10^6 cells/mL; IRIDICA: 11.9 vs. 11.6×10^6 cells/mL). Alternatively, other molecular assays have implemented strategies in order to enrich microbial DNA and are used in some commercially available assay for the molecular diagnosis of bloodstream infections. SepsiTest (Molzym, Bremen, Germany) uses a method that includes the degradation of the human DNA before the lysis and purification of the microbial DNA [96]. The same strategy is used by the Magicplex (Seegene, Seoul, Korea) [99, 108] as the DNA extraction is also performed using Molzym reagents. In the case of Vyoo

(SIRS-Lab, Jena, Germany) [105], an electrophoresis column that specifically binds the microbial DNA is used. The IRIDICA system has overcome this issue by designing a highly robust PCR, with variation in the amounts of primers and polymerase used in comparison with the PLEX-ID version. Additionally, during the purification step, the microbial DNA is positively selected by the size of the particles used to bind the DNA and thus, it is enriched just before the analysis [114].

Furthermore, the volume of blood analyzed may be a critical factor when assessing the sensitivity of molecular methods compared with blood culture. Usually, molecular methods analyze from 1 to 5 mL of blood whereas blood culture tests a minimum of 30 mL. Due to the low bacterial load (< 10 CFU/mL), this difference in the analyzed volume may lead to suboptimal sensitivities of molecular methods compared with culture. In our studies, the etiological agents of the bloodstream infection were not detected by the molecular method in a total of 48 cases for the PLEX-ID and 64 cases for the IRIDICA version. Additionally, the software used for the analysis (for both PLEX-ID and IRIDICA versions) could also have contributed to those results. This software has specific thresholds for reporting different microorganisms (3 - 10 genomes/well for most pathogenic bacteria, and 10 genomes/well for those microorganisms that can also be found as skin contaminants). Detections below this threshold are not reported in order to increase the assay specificity. However, this could lead to false negative results in certain cases. For the PLEX-ID study, in nine whole blood specimens with a positive paired blood culture, PCR/ESI–MS achieved correct identifications but were not reported as they were under the aforementioned threshold. These being three CoNS (coagulase-negative staphylococci) and the others *Escherichia coli* ($N = 2$), *Enterococcus faecalis* ($N = 1$), *Serratia* spp. ($N = 1$), *Bacteroides* spp. ($N = 1$) and *Candida albicans* ($N = 1$). For the IRIDICA study, four microorganisms isolated by blood culture were also detected but were not reported as they were below those levels as well (*Staphylococcus epidermidis,* $N = 1$; *Bacillus* spp., $N = 1$; *Bacteroides* spp., $N = 1$; *Lactobacillus* spp., $N = 1$).

4.2. Clinical significance of the microorganisms detected solely by the molecular method

The use of blood culture as gold standard has several limitations for the assessment of molecular methods. First of all, its positivity rate is rather low, with only about 10 % of all blood cultures being positive [80, 81]. This method also shows a decreased sensitivity for the detection of fastidious or slow-growing microorganisms in addition to when the patient is already under antibiotic treatment at the time of specimen collection [54, 77, 78]. Therefore, in certain cases, the molecular methods may detect microorganisms that have not grown in blood culture (overcalls).

In both studies, the PCR/ESI-MS technology detected microorganisms that were not isolated by culture (in 25 cases by the PLEX-ID and in 80 cases by IRIDICA). The interpretation of those discrepancies (culture negative *vs.* PCR positive) is challenging, as the PCR results may be due to true detections, the presence of non-viable or unculturable microorganisms or contamination. Given that blood culture −the current gold standard for the diagnosis of sepsis in the clinical microbiology laboratory− is not sensitive enough, the clinical records of the patients as well as the results from other cultures were used in order to discern if those results were clinically significant. This approach has already been described by other authors [54, 113, 126, 127]. The microorganisms detected only by PCR/ESI-MS are listed in **Table 6 (Article i)** and in **S1 and S2 tables (Article ii).** These tables include the clinical interpretation of the PCR/ESI-MS detection. As observed in **Figure 23,** in both studies the proportion of clinically significant microorganisms detected only by the molecular method (overcalls) are similar (PLEX-ID, 52 % ($N = 13$); IRIDICA, 51.2 % ($N = 41$).

On the other hand, the detection of potentially pathogenic microorganisms for which no clinical explanation for its detection could be found varied from 8.8 % (IRIDICA, $N = 7$) to 16.0 % (PLEX-ID, $N = 4$).

Finally, 32 – 40 % of the detections were easily classified as contaminants (skin or environmental; PLEX-ID, $N = 8$; IRIDICA, $N = 32$).

Skin or environmental contaminants can also be found in a small percentage of blood cultures due to insufficient aseptic practices during extraction (up to 5 % of blood cultures in our setting). It should also be kept in mind that for both studies, those patients in which a skin contaminant had been identified by blood culture were excluded from the study. These results reinforce the fact that the results obtained with molecular methods, as for the blood culture, should be interpreted by the clinician in light of the observed clinical signs and symptoms.
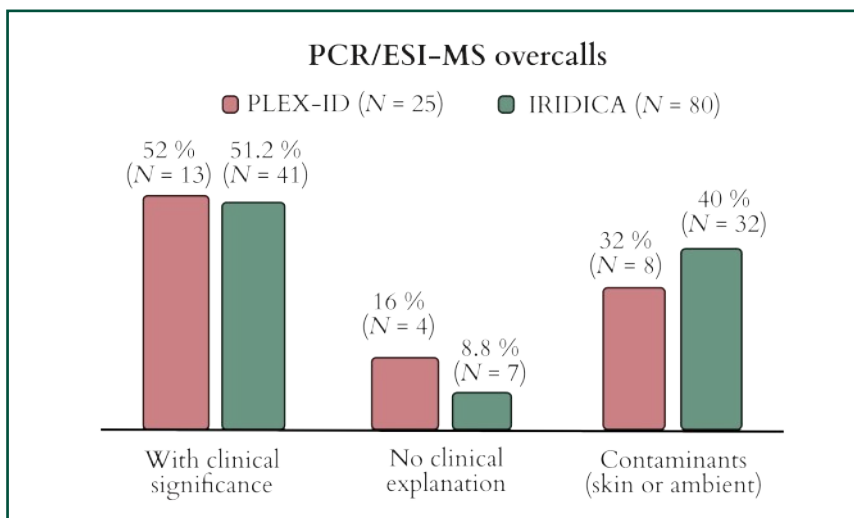

Figure 23. Distribution of the PCR/ESI-MS overcalls depending on the version evaluated.

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4.3. Overall agreement between PCR/ESI–MS and conventional methods using the clinical infection criterion according to the technology version by microorganism

After reviewing all the discrepancies found, the agreement between the molecular methods and conventional methods using the clinical infection criterion was subsequently evaluated.

Table 15. Performance of the PCR/ESI–MS in comparison with blood culture or the clinical infection criterion for the detection of clinically-significant microorganisms from whole blood according to the version used.

		PLEX-ID (Article I)					
		Blood culture gold standard			Clinical infection criterion		
		Positive	Negative	Total	Positive	Negative	Total
PCR/ ESI–MS	Positive	37	25	**62**	50	12	**62**
	Negative	48	152	**200**	48	152	**200**
	Total	**85**	**177**	**262**	**98**	**164**	**262**
Agreement (%)		43.5[a]	85.9[b]	72.1[c]	51[a]	92.7[b]	77.1[c]

		IRIDICA (Article II)					
		Blood culture gold standard			Clinical infection criterion		
		Positive	Negative	Total	Positive	Negative	Total
PCR/ ESI–MS	Positive	176	80	**256**	217	39	**256**
	Negative	64	143	**207**	64	143	**207**
	Total	**240**	**223**	**463**	**240**	**182**	**463**
Agreement (%)		73.3[a]	64.1[b]	68.9[c]	77.2[a]	78.6[b]	77.8[c]

a. Positive agreement; b. Negative agreement; c. Overall agreement

As expected, the PCR/ESI–MS technology had an improved positive agreement when compared with the clinical infection criterion than when compared to blood culture (51 % vs. 77.2 %), due to the detection

of clinically relevant microorganisms not isolated by culture. The clinical explanation for each additional microorganism can be found in **Table 6 (Article I)** and **S1 Table (Article II)**. Also, a brief summary of the most common causes of the PCR/ESI-MS overcalls is depicted in **Table 16.** As anticipated, most of the specimens in which microorganisms with clinical significance were detected only by the molecular method came from patients under antimicrobial therapy. Other microorganisms (such as anaerobes) or slow-growing microorganisms (such as *Candida* spp. and fungi) were also detected by the PCR/ESI-MS technology. Some other cases, mainly found in the IRIDICA evaluation, came from sepsis of abdominal origin. This type of sepsis is usually polymicrobial due to microorganisms from the gut. In polymicrobial infections, especially when the implied microorganisms show different growth dynamics, or are present at markedly different bacterial loads, the detection of all microorganisms may be challenging.

Table 16. Brief summary depicting the main possible reasons for the clinically relevant PCR/ESI-MS overcalls.

	No. of microorganisms detected by PCR/ESI-MS and not by blood culture	
	PLEX-ID (N = 13)	IRIDICA (N = 41)
ATB treatment	10 microorganisms detected from 9 patients under ATB treatment	30 microorganisms detected from 23 patients under ATB treatment
Sepsis of abdominal origin (potentially polymicrobial)	2 microorganisms	9 microorganisms
Anaerobes, slow growing or unculturable microorganisms detected	*Candida albicans* (N = 1)	11 microorganisms: - *Fusobacterium nucleatum* (N = 2) - *Prevotella* spp., (N = 1) - *C. albicans*, (N = 2) - *C. tropicalis*, (N = 1) - Fungi no identified, (N = 2) (Probable *Aspergillus* spp.) - *Mycobacterium simiae*, (N = 2) - *Mycoplasma hominis*, (N = 1)

4.4. Polymicrobial infections and performance of the PCR/ESI–MS by specimen dependent on the version and the gold standard used.

Polymicrobial infections with clinical significance were detected by either one or both methods in 11 (14.7 %) out of the 75 cases with a positive result for the PLEX-ID version **(Table 2, Article i)** or in 28 out of 245 (11.4 %) specimens with a positive result for the IRIDICA version **(S3 Table, Article ii).** Although in the aforementioned tables the detailed list of the polymicrobial infections can be found, **Table 17** summarizes the different situations encountered.

Table 17. Summary of the different situations encountered for the polymicrobial infections.

	PLEX-ID *N* (%)	IRIDICA *N* (%)
Blood culture and PCR/ESI–MS (concordant ID)	1 (9.1)	8 (28.6)
Blood culture detected more microorganisms than PCR/ESI–MS	4 (36.4)	5 (17.9)
PCR/ESI–MS detected more microorganisms than blood culture	3 (27.3)	5 (17.9)
Blood culture and PCR/ESI–MS detected different microorganisms	1 (9.1)	3 (10.7)
Only by blood culture	2 (18.2)	2 (7.1)
Only by PCR/ESI–MS	0	5 (17.9)
Total number of polymicrobial infections	11	28

Given that both methods agreed in some but not all the microorganisms identified, and since it was not possible to classify them properly (i.e. both methods agreed in some but not all microorganisms identified), these variant polymicrobial samples were excluded in order to calculate the overall agreement of both technologies by specimen.

As observed in **Table 18,** the overall agreement with conventional methods was similar for both versions of the PCR/ESI–MS technology. However, the positive agreement, which indicates the sensitivity of the technology, was clearly improved with the IRIDICA version.

Table 18. Performance of the PCR/ESI–MS by specimen depending on the version and the gold standard used.

		PLEX–ID (Article I)					
		Blood culture gold standard			Clinical infection criterion		
		Positive	Negative	Total	Positive	Negative	Total
PCR/ ESI–MS	Positive	27	20	**47**	37	10	**47**
	Negative	37	152	**189**	37	152	**200**
	Total	**64**	**172**	**236**	**74**	**162**	**236**
Agreement (%)		42.2[a]	88.4[b]	75.8[c]	50[a]	93.8[b]	80.1[c]

		IRIDICA (Article II)					
		Blood culture gold standard			Clinical infection criterion		
		Positive	Negative	Total	Positive	Negative	Total
PCR/ ESI–MS	Positive	148	39	**187**	166	21	**187**
	Negative	50	143	**193**	50	143	**193**
	Total	**198**	**182**	**380**	**216**	**164**	**380**
Agreement (%)		74.8[a]	78.6[b]	76.6[c]	76.8[a]	87.2[b]	81.3[c]

a. Positive agreement; b. Negative agreement; c. Overall agreement

The PLEX–ID was also evaluated for the diagnosis of bloodstream infections from whole blood by T. Laffler *et al.* [126]. In this study, 464 whole blood specimens with a positive paired blood culture and 442 with a paired negative blood culture result were tested. The overall agreement between blood culture followed by biochemical identification against the PLEX–ID version was 78.6 %, which is comparable to the 75.8 % obtained in our study.

For the IRIDICA version, two other studies have been performed. A. Baconni *et al.* [114] tested 311 whole blood specimens (18 with a positive paired blood culture and 293 with a negative blood culture result) and described a sensitivity ranging from 83 % to 91 % compared with conventional methods. In the RADICAL study, a multicenter observational study performed in 2014 (including eight ICUs from six European countries), a total of 609 whole blood specimens from 543 patients admitted to the ICU were included and the sensitivity was 81 % [128].

These results obtained with the IRIDICA version are comparable to those obtained in our study, demonstrating the robustness of this diagnostic method. However, differences in the characteristics of the patients (e.g., clinical condition, age, antimicrobial treatment at the time of the blood draw), the microorganisms isolated, the number of blood culture bottles inoculated, and the volume of blood drawn for culture may result in slight differences between studies [129].

5. IRIDICA performance in ICU patients *versus* ER patients.

Given that the IRIDICA version is intended to be used for the diagnosis of infectious diseases in the critically ill patient, a sub-analysis including only those specimens from patients admitted to the ICU was performed. While only specimens with a paired positive blood culture were from ER patients, specimens from ICU patients were consecutively collected (including one specimen per patient and sepsis episode with either positive or negative paired blood culture); this enabled us to calculate the parameters of analytical performance in the ICU setting.

As observed in **Table 19 (Table 3, Article II),** in the analysis by microorganism, the sensitivity, specificity, PPV and NPV of IRIDICA in comparison with blood culture were 78.4 %, 70.8 %, 33 % and 95 % respectively. When the discrepancies found were evaluated using the clinical infection criterion **(S1 Table, Article II),** those values rose to 89 %, 86.1 %, 73.9 % and 95 % respectively. A total of 14 polymicrobial infections by either or both methods were excluded in order to perform the analysis by specimen **(S3 Table, Article II).** In those terms, the sensitivity and specificity compared with blood culture were 83.3 % and 78.6 %, respectively, and then rose to 90.5 % and 87.2 % when considering the clinical infection criterion.

The results obtained during the RADICAL study (direct comparison by microorganism between IRIDICA results to those obtained by blood culture) were more consistent with those obtained in our study when a similar comparison was made: 81 % sensitivity, 69 % specificity, 24 % PPV and 97 % NPV for the RADICAL study vs. 78.4 %, 70.8 %, 33 % and 95 % in ours.

For the discrepancies found between methods, the RADICAL study performed a replicate testing in order to determine if the pathogen identified by IRIDICA was a true or false detection. However, as it was

not possible to test a replicate in all cases that were IRIDICA positive and culture negative, the parameters of analytical performance were not recalculated taking into account the results of the replicate tests performed. Thus, the values obtained in our study in comparison with the clinical infection criterion cannot be compared with the RADICAL study.

Table 19. Performance of the IRIDICA system in ICU patients.

		By microorganism					
		Blood culture gold standard			Clinical infection criterion		
		Positive	Negative	Total	Positive	Negative	Total
IRIDICA	Positive	29	59	88	65	23	88
	Negative	8	143	151	8	143	151
	Total	37	202	239	73	166	239
Agreement (%)		78.4[a]	70.8[b]	72[c]	89[a]	86.1[b]	80.1[c]

		By specimen					
		Blood culture gold standard			Clinical infection criterion		
		Positive	Negative	Total	Positive	Negative	Total
IRIDICA	Positive	20	39	59	38	21	59
	Negative	4	143	147	4	143	147
	Total	24	182	206	42	164	206
Agreement (%)		83.3[a]	78.6[b]	79.1[c]	90.5[a]	87.2[b]	87.9[c]

a. Positive agreement; b. Negative agreement; c. Overall agreement

As for the ER group, only patients with a positive blood culture were included. From the 203 microorganisms isolated by culture, 147 were correctly detected by IRIDICA. Thus, the positive agreement by microorganism in comparison with blood culture was 72.4 %. A total of 5 microorganisms with clinical significance were detected by IRIDICA only, giving a positive agreement of 73.2 % when the clinical infection

criterion was used. When analyzed by specimen, the positive agreement was 72.7 % while comparing it to either blood culture or clinical infection criterion (128 matched detections out of 176 monomicrobial infections).

IRIDICA performed particularly well in the subgroup of ICU patients compared to the clinical infection criterion gold standard; sensitivity was 89 % in the analysis by microorganism vs. 73.1 % in the ER (p = 0.005) and 90.5 % in the analysis by specimen vs. 73.6 % in the ER (p = 0.02). The varied performance of IRIDICA in the ICU and ER settings could be explained, at least in part, by the inherent characteristics of the patients admitted to the ICU. These patients are severely ill and suffering from underlying pathologies that may increase the risk of developing sepsis. They also have a major risk of suffering from nosocomial infections due to the use of several intravascular devices, such as catheters. Furthermore, patients staying at the ICU setting for a long period of time may suffer from immunological impairment. All these factors may be related to the presence of higher bacterial loads (a significantly higher number of genomes per well was observed in ICU patients compared with those from the ER). Interestingly, the agreement between IRIDICA and the clinical infection criterion was higher than that of blood culture (κ = 0.711 vs. κ = 0.315), which points to the presence of clinically relevant microorganisms detected only by molecular method (73.3 % of these patients were under antimicrobial therapy). Finally, IRIDICA showed a NPV of 95 % in patients admitted to the ICU, indicating that this technology could be useful for ruling out infection in this setting when the clinical suspicion of sepsis is low.

6. Comparison of the PCR/ESI–MS technology with other commercially available assays

In **Table 20,** a categorized comparison of PCR/ES-MS with other commercially available assays can be found.

It has to be kept in mind that bloodstream infection may be caused by a wide range of pathogens. Given that designing a highly multiplexed diagnostic assay is challenging, one inherent limitation of these assays is that they may miss those microorganisms that are less commonly associated with sepsis [130 – 132]. Thus, in terms of **broadness of range of pathogens identified,** the PCR/ESI-MS would be comparable only to the SepsiTest (Molzym) [96], as both methods use broad-range primers. However, the PCR/EI-MS technology uses multiple pairs of primers targeting simultaneously universal genetic regions (such as 16S rRNA), structural genes (RNase P) and housekeeping genes which are highly conserved among the major groups of microorganisms. This redundant information avoids the lack of identification due to a mismatch in one of the primer pairs. Furthermore, it improves the detection of polymicrobial infections, as the competition for PCR resources is avoided (the microorganisms can be amplified by several primer pairs) [116]. In the SepsiTest, polymicrobial infections are difficult to detect by sequencing due to the presence of mixed peaks in the electropherogram. In our studies, around 11 – 14 % of the infections were polymicrobial by either, or both methods. Although some of the microorganisms were not detected molecularly, most of them were detected by both culture and PCR/ESI-MS, with IRIDICA performing better than the PLEX-ID version.

Most assays also include the detection of several antibiotic resistance markers. The most commonly included are *mec*A (resistance to methicillin), and *van*A and *van*B (resistance to vancomycin and teicoplanin).

The Vyoo assay also detects bla_{SHV} i bla_{CTX-M} (resistance to β-lactamases) whereas the PCR/ESI–MS includes the detection of the bla_{KPC} gene (resistance to carbapenems). Carbapenemase–resistant *Klebsiella pneumonia* (bla_{KPC}) was first described in the United States and since then this country suffers from a high prevalence of carbapenemase-producing *Enterobacteriaceae* [133]. However, a broader panel targeting the major groups of β–lactamases encoding genes in Gram–negative bacteria is currently under development by the manufacturer.

Table 20. Comparative table of the molecular methods commercially available for the diagnosis of bloodstream infection from whole blood.

	SeptiFAST (Roche)	SepsiTest (Molzym)	Vyoo (SIRS-Lab)	Magicplex (Seegene)	IRIDICA (Ibis Biosciences–Abbott Molecular)
Technology	Real-time PCR	Broadrange PCR + sequencing	Multiplex PCR + electrophoresis	3 multiplex PCR (1 conventional, 2 real time)	Broad-range PCR + ESI-MS
N. of pathogens in the panel[1]	25	> 300	34	27	27
Resistance markers	YES (*mec*A, separate assay)	NO	YES (*mec*A, *van*A, *van*B, bla_{SHV}, bla_{CTX-M})	YES (*mec*A, *van*A, *van*B)	YES (*mec*A, *van*A, *van*B, bla_{KPC})
Volume	1,5 mL	2 mL	5 mL	1 mL	5 mL
Detection limit (CFU/mL)	3 – 30	20 – 460	5 – 100	–	4 – 16
Microbial DNA enrichment	NO	YES (prior PCR step)	YES (prior PCR step)	YES (prior PCR step)	YES (after PCR step)

1. The list of pathogens included in each assay can be found in Appendix I.

Turnaround time	4,5 – 6 h	8 – 12[2] h	8 h	6 h	6 h
Population studied	ER, ICU, Onco-hematology, others	ICU	ICU	ER, ICU, Onco-hematology, others	ER ICU
Sensitivity[3]	68 – 75 %	37 – 87 %	38 – 60 %	37 – 65 %	83 %
Positive Agreement[3]	–	–	–	–	74.8 %
Specificity[3]	86 – 92 %	85.5 – 100 %	–	77 – 92 %	93.6 %
Negative Agreement[3]	–	–	–	–	78.6 %
References	[102, 103]	[96 – 99]	[97, 106, 107]	[99, 108]	[114, 128, 135]

Regarding the **volume of blood,** both Vyoo and IRIDICA [114] use 5 mL, the largest amount in comparison with the other molecular tests. Although many scientists advocate that a higher volume of blood is directly related to higher sensitivity of the molecular tests [111, 123 – 125], this fact is not so clear considering the SeptiFAST assay. B. Regueiro [101] found similar sensitivities when performing the automated DNA extraction using the MagnaPure (Roche) from 1.5 mL as when using the manual extraction method from 3 mL. Despite this difference in the volume analyzed, the sensitivity and specificity of SeptiFAST (using the automated method from 1.5 mL of blood) and IRIDICA are comparable. Being the first CE marked commercial assay for the molecular diagnosis of sepsis from whole blood, SeptiFAST has been largely evaluated in different patient types (severe sepsis, intensive care, general medicine and pediatrics) [134]. It should be noted that the results obtained with this assay are inconsistent across different studies, with sensitivity ranging between 15 % and 90.9 %, and specificity between 70 % and 100 %. Recently, two different systematic reviews

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2. The PCR result can be obtained after 4h. However, if a microorganism is detected, sequencing has to be subsequently performed

3. Sensitivity, specificity, positive and negative agreement in comparison with conventional methods.

have been published [102, 103] and the pooled sensitivity and specificity were, respectively, 75 % and 92 % (S.S Chang *et al.*), and 68 % and 86 % (P. Dark *et al.*). As reported above, the sensitivity and specificity obtained for the IRIDICA system was 74.8 – 83.0 % and 78.6 – 93.6 %, respectively. Although only three studies have been published so far using this system their results are comparable, demonstrating its robustness.

7. Implementation of the IRIDICA system in a clinical microbiology laboratory

As shown in **Figure 20,** IRIDICA workflow consists of four processes: DNA extraction, DNA amplification, purification of the PCR products and MS analysis. All processes are highly automated and the hands-on time is short, which is an essential factor for the implementation of any new technology in a clinical laboratory. For one specimen, the hands-on time and work–flow are shown in the **Figure 24.**
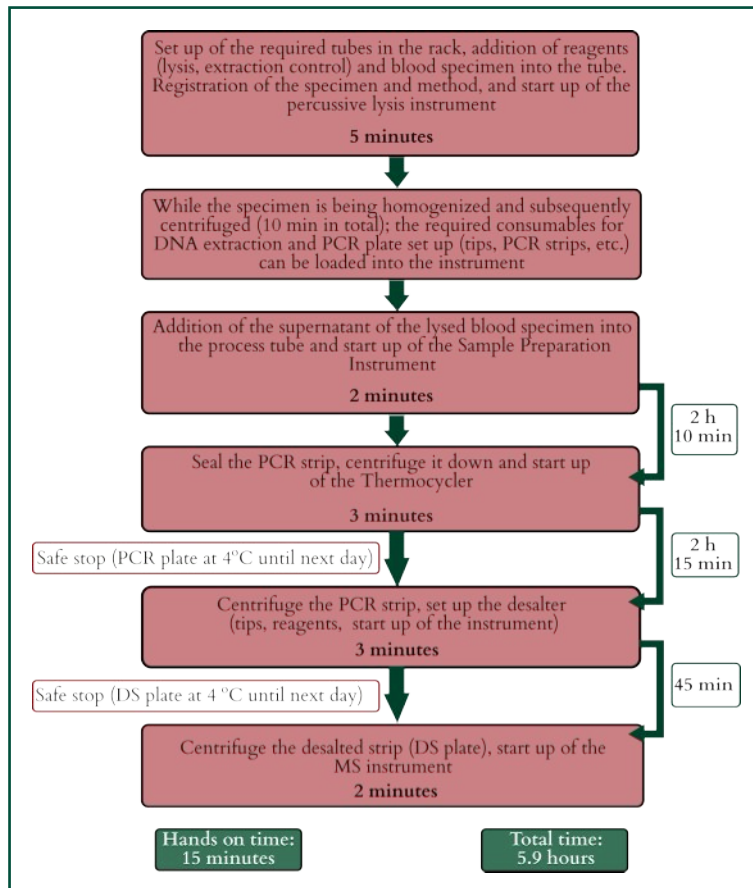

Figure 24. Workflow and hands-on time for the IRIDICA system.

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As mentioned above, IRIDICA allows analysis of 1 – 6 specimens. Any additional specimen would increase the hands-on-time by 3 min, and the total time-to-result by 25 min. Thus, for a complete run of 6 specimens, the total hands-on time would be 30 minutes and the complete analysis by the mass spectrometer would take 2 hours. Regarding negative controls, at least one per day is recommended, as long as no identification is found.

In the clinical laboratory, the molecular diagnosis of infectious diseases, for which obtaining a rapid result is not critical, is commonly organized in batches. However, in the case of the diagnosis of sepsis, this would reduce the impact of a rapid diagnostic, as some of the specimens would have to wait to be analyzed. Besides the increment in the volume of blood analyzed, another important improvement of IRIDICA over the PLEX-ID is the fact that specimens can be tested individually. This approximation is mandatory when analyzing urgent specimens, such as blood from potentially septic patients, as they may be submitted to the laboratory at any time. However, the DNA extraction process takes 2 hours, and urgent specimens might be received by the laboratory while the instrument is already in use. In order to minimize the impact of this waiting time, acquiring two Sample Preparation (SP) instruments should be considered . Furthermore, it has also to be kept in mind that IRIDICA offers a broad panel of assays covering severe syndromes (**Appendix 1, Table 3**). Given the initial investment needed for the acquisition of the technology, it would make sense to use the system for several diagnostic purposes at the same time. Then, having two extractors would be useful in its implementation into the routine of a clinical laboratory.

The second aspect that is usually considered when implementing a new molecular assay into the clinical laboratory is whether it requires an extensive knowledge in molecular methods or, on the contrary, it is user-friendly and easy enough to be implemented even in a 24 hours laboratory (including the shifts when not all   staff is available). The most critical step in the IRIDICA work-flow is the initial preparation of the

specimen, as the specimen can be easily contaminated. However, although a technician trained in molecular methods would be desirable, it can be considered that, with minimum training of the manipulation of this kind of specimen and the precautions needed, most laboratory technicians would be able to perform this step. Once the specimens are placed in the Sample Preparation instrument, the rest of the work-flow is certainly easy enough.

Another consideration in this regard is the separation of the process into different laboratory areas. Most laboratories using molecular assays have different areas for DNA extraction (Pre-PCR), and manipulation of PCR products (Post-PCR). Initially, IRIDICA was designed to be installed in a single room, although it is recommended to separate the pre-amplification process from the rest of the processes (PCR, desalting and MS analysis). In our experience, no cross-contamination was detected when having these two parts of the IRIDICA workflow in different rooms. However, although this design is safer in terms of contamination, it would also be necessary to have at least two technicians that were familiar with the technology: one dedicated to the DNA extraction process and the other one dedicated to the desalting and MS analysis, in order to avoid circulation from the Post-PCR to the Pre-PCR area.

The ideal situation would be installing the IRIDICA in a 24-hour laboratory, as in this case the IRIDICA system would show all its potential impact in patient management. For laboratories with only a day-shift, some blood specimens arriving at the laboratory at the end of the shift would suffer a delay in the time-to-result, as they could not be completely analyzed until the next morning (the workflow can be safely stopped at two points and be continued on the following day as detailed in **Figure 24**). Thus, it should be considered whether or not those specimens arriving in the laboratory later than a stated time should be analyzed by the IRIDICA. Either way, those decisions should be made depending on individual laboratory organization and taking into consideration the available human resources.

8. Limitations of the studies

The studies had several limitations **(Articles I and II).** Firstly, the specimens were analyzed retrospectively. Thus, we were not able to perform any further testing when discrepancies were found, especially in those involving resistance markers. Secondly, in both studies, the specimens were stored for varying periods of time at –20 °C until tested at IBIS Biosciences. However, long-term stability of whole blood samples under these storage conditions had previously been demonstrated by the manufacturer on spiked samples (unpublished data), and statistical analysis ruled out any significant association between the storage time and the PCR/ESI-MS technology positivity rate. Finally, in some cases blood samples were drawn when the patients were already under antibiotic treatment, which could have led to the detection of clinically relevant microorganisms by the molecular method in patients with a negative BC. In the absence of a highly sensitive gold standard, reviewing clinical facts as well as other positive cultures was and is necessary in these cases. This data supports the fact that molecular technologies may be useful in those cases where the value of a traditional culture is limited; the identification of the etiological pathogen in treated patients could have a clinical impact in patient outcome through the adjustment of the initially administered antimicrobial therapy. It also has to be taken into account that molecular methods are able to detect the DNA either from living or dead bacteria, as well as DNA released into the bloodstream by translocation, while blood culture only detects viable microorganisms. Some of the PCR/ESI-MS findings could not be supported by evident clinical facts and, consequently, the results obtained should always be reviewed and interpreted by a clinical microbiologist considering all the available clinical data.

For the PLEX-ID study **(Article I),** 42 patients were sampled at several time-points during the same sepsis episode; serial blood cultures are often obtained in the clinical practice of patients that are not evolving favorably. Given that the PCR/ESI-MS results are quantitative, future

studies should explore the value of this technique for monitoring anti-biotic efficacy and predict clinical outcome of patients with sepsis.

For the IRIDICA study, only the specimens from patients admitted to the ICU were consecutively included; while we obtained a dedicated blood specimen from most of the patients admitted to the ER, only those with a paired positive blood culture were included, in order to make the study feasible. Thus, the specimen set did not reflect the usual blood culture positivity rate in the clinical setting (around 10 % in our hospital). Nevertheless, the ICU subanalysis does reflect the positivity rate in this department (7.5 – 13.7 %), and no other studies have tested as many patients with a positive blood culture using this technology.

9. Concluding remarks

Sepsis is a severe syndrome where time is crucial for optimal patient management. The administration of an empiric antibiotic therapy within the first hour of recognition of clinical symptoms of bloodstream infections is strongly recommended [64, 67]. The rapid identification of the causal agent of sepsis is of paramount importance for the best possible outcome of the patient, since it allows for the administration of an appropriate empiric treatment on the basis of clinical protocols regarding bacterial antimicrobial susceptibility in that area. Once antimicrobial susceptibility testing is made available, the initial therapy can be changed, if necessary, to assure adequate antibiotic activity against the etiologic agent, or its spectrum reduced to prevent antimicrobial resistance development. Thus, being able to detect and identify the causal pathogen directly from blood would speed the diagnosis and, therefore, improve the management of septic patients.

It should be also noticed that molecular methods are more expensive than conventional ones. This fact may imply that their use should be restricted to selected patients at high-risk for infection. In this regard, specific biomarkers of infection, such as C-reactive protein or procalcitonin, could be helpful for patient stratification [5, 49, 99]. Despite the cost of the diagnostic assays, the overall benefits for the patient have to be considered. A rapid identification of the pathogen may lead to the optimization of the administered therapy and, thus, to a prompter recovery of the patient and a shorter stay in the ICU department. Cost/benefit studies regarding the use of molecular assays in combination with conventional methods have been performed using the Septi-Fast assay and significant economic savings were reported due to the shortening of the ICU stay and a more rational use of antibiotics [136 – 138]. Although prospective studies are needed to assess the real impact and of the IRIDICA technology in the management of septic patients, a significant impact can be anticipated.

CONCLUSIONS

Determination of the system capability for the detection and identification of a wide-range of pathogens:

1. Using the BAC BSI Assay, the PCR/ESI-MS technology detected and identified a wide range of bacteria and *Candida* spp., including 20 different species from positive blood culture (PLEX-ID version) and 29 different species from uncultured blood (PLEX-ID and IRIDICA versions).

2. The PCR/ESI-MS (PLEX-ID and IRIDICA) detected a total of 7 species of microorganisms ($N = 11$ sepsis cases) not included in any of the other molecular tests commercialized for the diagnosis of sepsis either from positive blood cultures or whole blood.

3. IRIDICA was able to identify pathogens such as *Mycoplasma hominis* and opportunistic pathogens such as *Mycobacterium simiae,* which are not usually isolated by culture.

Determination of the capability of the PLEX-ID version for the detection and identification of pathogens from blood culture:

4. The PCR/ESI-MS technology, in its PLEX-ID version, is highly sensitive in the detection of pathogens from positive blood cultures (91.8 % by microorganism and 96.7 % by specimen) in comparison with conventional methods.

5. The PLEX-ID detected clinically significant microorganisms that were not isolated by culture. Thus, the sensitivity when considering the clinical infection criterion was 92.2 % by microorganisms and 96.8 % by specimen.

6. The sensitivity and specificity of the PLEX-ID were similar to other commercially available methods. However, the time-to-result is longer and the costs are higher than for the MALDI-TOF, the preferred diagnostic platform for this specimen type.

Determination of the capability of the PCR/ESI–MS (PLEX–ID and IRIDICA versions) for the detection and identification of pathogens from whole blood

7. In patients from the ICU and ER departments, when comparing the PCR/ESI–MS with the conventional methods, the positive agreement was 43.5 % (moderate) for the PLEX–ID version and 73 % (good) for the IRIDICA version.

8. The efforts made by the manufacturer in order to improve the PCR/ESI–MS technology have been translated into a better sensitivity of the IRIDICA version in comparison with the PLEX–ID.

9. Both versions of the technology detected microorganisms that were not isolated by culture and half of them were clinically relevant. Thus, when comparing the PCR/ESI–MS results with the clinical infection criterion, the positive agreement with the blood culture was better (50 % for the PLEX–ID and 77.2 % for the IRIDICA), evidencing the diagnostic limitations of blood culture.

10. The results obtained with molecular methods should also be interpreted by the clinician in light of clinical signs and symptoms in order to differentiate contaminants from etiologic agents.

11. When used in combination with conventional methods, IRIDICA could lead to an increase of the number of microbiologically confirmed sepsis cases, including patients under antimicrobial treatment.

Evaluation of the usefulness of the IRIDICA version for the diagnosis of sepsis in the critically ill patient

12. When analyzing the subgroup of ICU patients, IRIDICA showed 83 % sensitivity in comparison with blood culture and 90 % in comparison with the clinical infection criterion.

13. IRIDICA showed a negative predictive value of 95 %, evidencing its usefulness for ruling out infections when the suspicion of sepsis is low.

14. A high proportion of ICU patients could beneficiate from early pathogen identification by IRIDICA, leading to a more appropriate antimicrobial treatment and a better patient management.

15. The current version of the technology, IRIDICA, is a rapid and reliable tool for the molecular diagnosis of sepsis, especially in ICU patients.

Theoretical implementation of the IRIDICA system in the routine of a clinical laboratory for the molecular diagnosis of sepsis

16. IRIDICA does not require extensive knowledge on molecular methods, facilitating the training of the laboratory staff.

17. More evidence is needed showing that the whole IRIDICA process can be performed in a single room, which could facilitate its implementation in clinical laboratories.

18. In order to maintain the time-to-diagnosis of 6 h, a 24/7 shift schedule would be required.

CONCLUSIONS

Determinació de la capacitat de la tècnica PCR/ESI-MS per a identificar un ampli ventall de patògens:

1. Mitjançant la utilització de l'assaig BAC BSI, la tecnologia PCR/ESI-MS va detectar i identificar un ampli rang de bacteris i *Candida* spp., incloent-hi 20 espècies a partir d'hemocultiu positiu (versió PLEX-ID) i 29 espècies a partir de mostra de sang directa (versions PLEX-ID i IRIDICA).

2. La tècnica PCR/ESI-MS (PLEX-ID i IRIDICA) va detectar un total de 7 espècies de microorganismes ($N = 11$ casos de sèpsia) que no estan inclosos en cap dels altres assajos comercialitzats per al diagnòstic molecular de la sèpsia, tant a partir d'hemocultiu positiu com de mostra de sang directa.

3. IRIDICA fou capaç d'identificar patògens com *Mycoplasma hominis* i patògens oportunistes com *Mycobacterium simiae,* els quals no s'aïllen normalment per cultiu.

Determinació de la capacitat de la versió PLEX-ID per a detectar i identificar diferents patògens a partir d'hemocultiu positiu:

4. La tècnica PCR/ESI-MS, en la seva versió PLEX-ID, és molt sensible en la detecció de patògens a partir d'hemocultiu positiu (91,8 % per microorganisme i 96,7 % per mostra) en comparació amb els mètodes convencionals.

5. El PLEX-ID va detectar microorganismes clínicament rellevants que no van ser aïllats pel cultiu. Per tant, la sensibilitat quan es va considerar el criteri clínic d'infecció va ser de 92,2 % per microorganisme i 96,8 % per mostra.

6. La sensibilitat i especificitat del PLEX-ID van ser similars a les d'altres mètodes disponibles comercialment. No obstant això, el temps fins a l'obtenció del resultat és més llarg i els costos són més elevats que per al MALDI-TOF, la plataforma de diagnòstic preferida per a aquest tipus de mostres.

Determinació de la capacitat de la tècnica PCR/ESI-MS per a detectar i identificar diferents patògens a partir de sang total

7. En pacients dels departaments d'Urgències i Medecina Intensiva, quan es va comparar la tècnica PCR/ESI-MS amb els mètodes convencionals, la concordança positiva pa ser de 43,5 % (moderada) per a la versió PLEX-ID i 73 % (bona) per a la versió IRIDICA.

8. Els esforços realitzats pel fabricant per a millorar la tecnologia PCR/ESI-MS s'han traduït en un increment de la sensibilitat de l'IRIDICA en comparació amb el PLEX-ID.

9. Ambdues versions de la tecnologia van detectar microorganismes que no van ser aïllats per cultiu i la meitat d'aquests van ser clínicament rellevants. Per tant, la concordança positiva quan es van comparar els resultats de la tècnica PCR/ESI-MS amb el criteri clínic d'infecció va ser millor (50 % per al PLEX-ID i 77,2 % per a l'IRIDICA), evidenciant les limitacions diagnòstiques de l'hemocultiu.

10. Els resultats obtinguts amb mètodes moleculars també han de ser interpretats pel metge considerant els signes i símptomes clínics per tal de diferenciar els contaminants dels agents etiològics.

11. Utilitzant-se en combinació amb els mètodes convencionals, IRIDICA podria conduir a un augment del nombre de casos de sèpsia confirmats microbiològicament, inclosos els pacients sota tractament antimicrobià.

Avaluació de la versió IRIDICA per al diagnòstic de la sèpsia en malalts crítics

12. En analitzar el subgrup de pacients de la UCI, IRIDICA va mostrar un 83 % de sensibilitat en comparació amb l'hemocultiu i un 90 % en comparació amb el criteri d'infecció clínica.

13. IRIDICA va mostrar un valor predictiu negatiu del 95 %, el que evidencia la seva utilitat per descartar infeccions quan la sospita de sèpsia és baixa.

14. Una alta proporció de pacients de la UCI es podria beneficiar de la identificació precoç de patògens per IRIDICA, la qual cosa permetria administrar un tractament antimicrobià més adequat i un millor maneig dels pacients.

15. La versió actual de la tecnologia, IRIDICA, és una eina ràpida i fiable per al diagnòstic molecular de sèpsia, especialment en pacients d'UCI.

Implementació teòrica del sistema IRIDICA per al diagnòstic rutinari de la sèpsia

16. IRIDICA no requereix un ampli coneixement en mètodes moleculars, cosa que facilita la formació del personal de laboratori.

17. Es necessiten més proves que demostren que tot el procés IRIDICA es pot realitzar en una habitació individual, la qual cosa podria facilitar la seva aplicació en els laboratoris clínics.

18. Per tal de mantenir el temps d'obtenció de resultats de 6 h, seria necessari un horari de treball 24/7.

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