Polymerase-chain reaction/electrospray ionization-mass spectrometry for the detection of bacteria and fungi in bronchoalveolar lavage fluids: a prospective observational study

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Abstract

PLEX-ID uses polymerase chain reaction-electrospray ionization/mass spectrometry for rapid identification of infectious agents in clinical samples. We evaluated its concordance with our centre's standard methods (SM) for bacterial and fungal detection in bronchoalveolar lavage (BAL) fluid in a prospective observational cohort study. The primary outcome was concordance (%) between SM and PLEX-ID. Secondary outcomes included concordance when excluding commensal oral flora, detection of resistance genes, and PLEX-ID's potential impact on clinical management, as determined by two independent reviewers. Included were 101 specimens from 94 patients. BALs were performed primarily for suspected pneumonia (76/101, 75%) and lung transplant work-ups (12/101, 12%). Most specimens yielded at least one organism by either method (92/101, 91%). Among all microorganisms detected ($n = 218$), 83% and 17% were bacterial and fungal, respectively. Overall concordance between SM and PLEX-ID was 45% (45/101). Concordance increased to 66% (67/101) when discordance for commensal flora was excluded. PLEX-ID failed to detect 21% of all 183 SM-identified organisms, while SM did not identify 28% of the 191 PLEX-ID-identified organisms ($p < 0.001$). There was low concordance for *mecA* detection. Two infectious-disease specialists' analyses concluded that in most of the 31 discordant, non-commensal cases, PLEX-ID results would have had little or no impact on patient management; in eight cases, however, PLEX-ID would have led to 'wrong decision-making'. The tested version of PLEX-ID concurred weakly with standard methods in the detection of bacteria and fungi in BAL specimens, and is not likely to be useful as a standalone tool for microbiological diagnosis in suspected respiratory infections.

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Introduction

Definitive identification of pathogens causing lower respiratory tract infections (LRTIs) requires time and specialized laboratory personnel. In the days preceding microbiological diagno-sis, patients often receive broad-spectrum antimicrobials that may nonetheless be ineffective. Molecular diagnostic tools such as polymerase chain reaction (PCR) have increased sensitivity and reduced turnaround time, but even multiplex PCR remains only partially broad-range, as it requires anticipation of specific pathogens for their detection [1].

Currently research-use-only (RUO), the Abbott PLEX-ID system is a platform for pathogen detection combining broad-range PCR with electrospray ionization-mass spectrometry (PCR/ESI-MS). Minute quantities of deoxyribonu-cleic acid (DNA) are extracted from clinical specimens and amplified via PCR [2]. Amplicons undergo ESI-MS, allowing

for the determination of their base composition. Computerized triangulation employs an internal database containing listings of base compositions with linking orders for known microbes to determine pathogens' genotypic identity.

PLEX-ID remains experimental and requires validation in the clinic. A recent retrospective study comparing PLEX-ID with standard blood-culturing techniques in blood-borne bacterial and yeast infections yielded concordances of 98.7% and 96.6% at genus and species levels, respectively [3]. For pure yeast detection, Simner *et al.* [4] reported a concordance of 91.8% between traditional culture and PLEX-ID's broad fungal assay in formalin-fixed, paraffin-embedded tissue samples, some of which were more than a decade old. The PLEX-ID/flu assay [5] was tested against nasopharyngeal specimens positive by PCR for the influenza virus and concurred at 91.3% and 95.3% for influenza A and B, respectively [6].

Prospective studies across a wider variety of clinical specimens are lacking, however. In this proof-of-concept study, our objectives were to quantify PLEX-ID's concordance with our centre's standard methods (SM) for bacterial and fungal detection in the bronchoalveolar lavage (BAL) fluid of patients undergoing bronchoscopy, and qualitatively evaluate the clinical consequences of PLEX-ID results in discordant cases.

Materials and Methods

Study design, patients and samples

This prospective, single-centre observational cohort study included all consecutive BAL specimens from any in- or outpatients undergoing clinically indicated bronchoscopy and with at least 5 mL of fluid remaining after extraction for the SM, collected between 1 January and 1 September 2013 at the Geneva University Hospitals, an 1800-bed tertiary-care medical centre. No more than two samples from the same patient were included. Immediately after sterile extraction of BAL fluid for SM processing, samples were stored at −80°C for later batch-testing via PLEX-ID.

Ethics

The study protocol and related materials were approved by the University of Geneva's ethics committee (reference n° 12-265); the study was carried out in accordance with the Declaration of Helsinki, 6th revision. A waiver of informed consent was granted given the study's observational nature.

Outcomes

The primary outcome was concordance (%) between SM and PLEX-ID for bacteria and fungi at genus and species levels.

Secondary outcomes included concordance (i) beyond non-commensal oral flora, as SM do not typically identify all oral flora at the species and/or genus level (see below), (ii) for genus identification, and (iii) for detection of resistance genes *mecA*, *vanA*, *vanB* and *KPC* (although the latter three genes have rarely been detected at our institution). Finally, (iv) PLEX-ID's potential impact on therapeutic decision-making for discordant non-commensal specimens was qualitatively assessed via a clinical analysis undertaken independently by two infectious-disease specialists.

Definitions

Immunosuppression. In accordance with the Centers for Disease Control and Prevention's definition [7], patients were considered immunosuppressed if they had one or more of the following: neutropenia (absolute neutrophil count <500/mm^3), leukaemia, lymphoma, human immunodeficiency virus (HIV) with CD4 count <200/μL or early post-transplant state (<6 months), or were receiving cytotoxic chemotherapy or high-dose steroids.

Lower respiratory tract infection. Lower respiratory tract infection was defined, per European guidelines, as pneumonia and/or an acute illness present for 21 days or less, usually with cough as the main symptom, with at least one other lower respiratory-tract symptom (sputum production, dyspnoea, wheeze or chest discomfort/pain) and no alternative explanation (e.g. sinusitis or asthma) [8].

Standard methods

Our centre's standard diagnostic methods for BAL analysis include direct microscopic specimen examination with Gram, acridine orange and calcofluor white staining, and bacterial and fungal cultures, as well as *Mycoplasma* and *Chlamydia*-specific PCR. Bacterial and fungal cultures are performed by streaking a calibrated loop on various generic and selective media [9]; buffered charcoal yeast agar is routinely employed for the detection of *Legionella* spp. Colonies are quantitatively reported (e.g. >10^3 cfu/mL) and identified using a combination of manual (e.g. optochin, pneumo-agglutination) and molecular assays (matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF), Bruker MALDI Bio-Typer 2.0$^®$, Billerica, MA, USA). Fungi are identified by morphology when grown on specific media as well as by MALDI-TOF (after extraction, using the commercially available Bruker database), and by 18S and/or internal transcribed spacer (ITS) gene sequencing when discordant.

Upon request, specific PCRs are performed for the detection of *Pneumocystis jirovecii*, other fungi, mycobacteria (using GeneXpert$^®$; Cepheid, Sunnyvale, CA, USA), and a

panel of respiratory viruses (adenovirus, coronaviruses, human metapneumovirus, influenza A and B, parainfluenza viruses, picornaviruses and respiratory syncytial virus). PCR detection is considered positive for both *P. jirovecii* and viruses if the cycling threshold (CT) value is ≤39.

By SM, the identification of commensal oral flora in culture is often not performed at the species level. An optochin test is used to identify the presence of *Streptococcus pneumoniae* among oral streptococci. Other bacteria are identified by MALDI-TOF MS. When only commensal oral bacteria are identified (e.g. *Neisseria flavus* and *Micrococcus* spp.), the result returned by the laboratory is 'oral flora' with quantification.

PLEX-ID analysis

For both bacterial (including resistance genes coding for *mecA*, *vanA*, *vanB*, *vanC* and *KPC*) and fungal analyses, nucleic acids were extracted from 1 mL of BAL native fluid and recovered in 280 μL using a magnetic-bead-based method with the PLEX-ID SP instrument (extractor) and the PLEX-ID FH instrument (fluid handler) provided in PLEX-ID's Ultrapure DNA Prep Kit (from Abbott Molecular; not commercially available). For each sample, 15 μL of nucleic acids were distributed using the PLEX-ID FH into 16 reaction wells of 96-well assay plates. Amplification was performed with either the PLEX-ID Bac Spectrum SF Assay or the PLEX-ID Fungal Spectrum Assay amplification reagent kits (Abbott Molecular, Des Plaines, IL, USA), which were designed to detect a spectrum of more than 800 bacterial and fungal nucleic acids.

Electrospray ionization and MS identified the base compositions of the amplicons; their linking order was determined via bioinformatic triangulation using an internal database. Of note, the tested PLEX-ID assay was devised to detect pathogens in sterile fluids only; it was not designed for use in BAL pathogen detection.

Determining concordance for oral flora. As described above, SM allow for identification of commensal oral flora on a broad taxonomic level; PLEX-ID is by definition a genotypic diagnostic tool. We attempted to mitigate this inequality by prospectively deeming PLEX-ID results concordant with the laboratory designation 'oral flora' whenever PLEX-ID identified any organism on our centre's list of commensal oral flora (Appendix S1).

Analysis of PLEX-ID's potential impact on clinical decision-making

Two infectious-disease specialists not previously involved in the study (SE and SH) were asked to independently review PLEX-ID's potential clinical impact in cases of discordant, non-commensal specimens. The physicians first reviewed

patients' clinical charts, which included all data on the clinical course, including the results of BAL analysis by SM. PLEX-ID results were then revealed. The physicians were asked four questions, to be answered via Likert items (Fig. 1).

Statistical analysis

Descriptive analyses were performed within Stata, Release 12 (StataCorp, College Station, TX, USA). All tests were two-sided at the 0.05 significance level. Rates of SM and PLEX-ID positivity and concordance were compared using chi-squared tests. Logistic regression models were constructed to evaluate potential associations between specimen and patient characteristics and concordance between PLEX-ID and SM. The clinical analysis was assessed for inter-rater reliability by means of inter-rater agreement percentages and Cohen's kappa index.

Results

Patient characteristics

There were 101 consecutive BAL specimens from 94 patients included during the study period (Table 1). Patients' median age was 54 years (range, 1–89 years); 49 (52%) were male and 36 (38%) were immunosuppressed. Among the latter, 14/36 (38%) were lung-transplant recipients (LTR). Twenty-three patients (24%) were intubated at the time of their bronchoscopy. Patients underwent bronchoscopy primarily for presumed pneumonia (76/101, 75%), routine transplant work-ups (12/101, 12%) and malignancy work-ups (5/101, 5%). Most (59/101, 58%) post-bronchoscopy diagnoses were not infection related and included interstitial pneumopathies and transplant rejection. Pneumonia was diagnosed in 41/101 (41%); among these, 71%, 7% and 7% were considered to be bacterial, viral and tuberculous, respectively, while 10% were attributed to *P. jirovecii*; only two cases (5%) of non-*Pneumocystis* fungal pneumonia were diagnosed.

Sample characteristics

Positivity rate and microorganism taxonomy. As shown in Table 1, most BAL specimens yielded evidence of at least one organism by either SM or PLEX-ID (92/101, 91%); the median number of microorganisms per specimen was two (range, 0–7). Among all microorganisms detected (*n* = 218), 83% and 17% were bacterial and fungal, respectively. Among fungi, 26/36 (72%) were yeasts. In all, 56 distinct bacterial and fungal species were identified, 52 by SM and 46 by PLEX-ID. Viral panels were performed on 56/101 (55%) samples; of these, seven were positive (two yielding influenza B, two a picornavirus, one an adenovirus, one a parainfluenza virus and one a metapneumo-

1. **Would PLEX-ID results have had an impact on the choice of empiric antimicrobial therapy for this patient within 24 h after bronchoscopy?**

    1 = no impact at all
    2 = likely no impact
    3 = possible impact
    4 = probably an impact
    5 = definite impact

    If your answer was 1 or 2, please skip question 2 and move to question 3.

2. **Would PLEX-ID results have changed the current antimicrobial therapy by adding/stopping a drug or by broadening/narrowing the current spectrum?**
    (Several answers are possible.)

    1 = adding an antimicrobial (e.g. antifungal agent)
    2 = discontinuing an antimicrobial agent
    3 = broadening the current spectrum
    4 = narrowing the current spectrum

    (Please include explanatory comments in the corresponding column as needed.)

3. **Would PLEX-ID results have changed *overall* patient care (antimicrobial therapy aside)?**

    1 = not at all
    2 = probably not
    3 = possibly
    4 = probably
    5 = definitely

    If you answered 1 or 2, you may stop here.

4. **Please specify how PLEX-ID might have changed overall patient care:**
    (Several answers are possible.)

    1 = by leading to additional microbiologic testing
    2 = by leading to other diagnostic analyses (e.g. biomarkers, blood chemistry, etc.)
    3 = by leading to further radiologic imaging (e.g. ultrasonography, CT, etc.)
    4 = by leading to additional specific intervention(s) (e.g., consultations, surgeries, etc.)
    5 = other (please describe in the final column provided)

FIG. 1. Hypothetical questions asked for clinical management analysis.

virus). The picornaviruses and adenovirus were not considered causative; in all other cases the post-bronchoscopy diagnosis was viral pneumonia. PLEX-ID did not analyse viral pathogens.

Concordance

Overall concordance between SM and PLEX-ID was 45% (Table 2). Compared with all SM as a reference standard, sensitivity and specificity of PLEX-ID were, respectively, 59% (95% CI, 48–70%) and 21% (95% CI, 15–28%); positive and negative predictive values were, respectively, 30% (95% CI, 23–37%) and 48% (95% CI, 35–61%). In a subgroup of specimens (n = 41) from 40 patients with a post-bronchoscopy diagnosis of pneumonia, overall concordance between SM and PLEX-ID

was 30% (12/41). Table 3 lists the organisms identified by either or both methods in this subgroup.

Among all specimens, concordance increased to 66% (67/101) when discordance for commensal oral flora was excluded. In the subgroup of specimens from patients with a post-bronchoscopy diagnosis of pneumonia, concordance increased to 54% (22/41) when discordance for commensal flora was excluded.

Among discordant non-commensal specimens, the rule was complete discordance: only two samples concurred on genus but not species identification. Among specimens with no commensal organisms, PLEX-ID sensitivity increased to 65% (95% CI, 52–76%), while specificity declined slightly to 18% (95% CI, 7–35%); positive predictive value increased to 62%

TABLE 1. Baseline characteristics of patients and their BAL specimens

Variable	Data
Indications and diagnoses	
Indication for BAL	
LRTI suspected (%)	76/101 (75)
Routine pre- or post-transplant BAL (%)	12/101 (12)
Malignancy work-up (%)	5/101 (5)
Transplant rejection work-up (%)	4/101 (4)
Non-malignancy, non-infectious disease work-up (%)	4/101 (4)
Diagnosis post-BAL	
No evidence of pulmonary infection (total, %)	59/101 (58)
Rejection (%)	4/59 (7)
Interstitial pneumopathy (%)	6/59 (10)
Cryptogenic organizing pneumonia (%)	2/59 (3)
ARDS (%)	1/59 (2)
Sarcoidosis (%)	1/59 (2)
Other (non-infectious diagnosis not assigned)	45/59 (76)
Pneumonia (total, %)	41/101 (41)
Bacterial	29/41 (71)
Pneumocystis jirovecii	4/41 (10)
Viral	3/41 (7)
Tuberculous	3/41 (7)
Fungal (non-pneumocystis)	2/41 (5)
Pulmonary abscess (total, %)	1/101 (1)
Quantitative specimen findings	
Absolute number of organisms detected	
By standard methods	172
By PLEX-ID	175
Combined	218
Distribution of all microorganisms detected	
Bacterial (%)	182/218 (83)
Commensal oral flora	124/182 (68)
Fungal (%)	36/218 (17)
Moulds	10/36 (28)
Yeasts	26/36 (72)
Candida spp.	21/26 (81)
Number of distinct species identified	
By standard methods	52
By PLEX-ID	46
Combined	56
Median number of organisms per specimen (range; IQR)	2 (0–7; 1–3)
Number of specimens yielding no organism (%)	9/101 (9)
Number of specimens yielding commensal oral flora (%)	69/92 (75)
Number of specimens yielding a fungus (mould or yeast) (%)	31/92 (34)
Median number of white blood cells/mL (IQR)	
Positive specimens with evaluable data (*n* = 75)	200 (120–345)
Negative specimens with evaluable data (*n* = 8)	145 (68–300)

ARDS, acute respiratory distress syndrome; BAL, bronchoalveolar lavage; IQR, interquartile range; LRTI, lower respiratory tract infection.

TABLE 2. Concordance data between PLEX-ID and standard methods

Variable	Data (%)
Concordance	
Overall concordance	45/101 (45)
Concordance when excluding discordance for commensal oral flora	67/101 (66)
Overall concordance among specimens from patients diagnosed with pneumonia post-bronchoscopy	12/41 (30)
Concordance among specimens from patients diagnosed with pneumonia post-bronchoscopy, excluding commensal flora	22/41 (54)
Discordance only at species level (genus identified by both methods)	2/101 (2)
Undetected organisms and resistance	
Among all organisms detected, number not detected by PLEX-ID	43/218 (20)
Among all organisms detected, number not detected by SM	46/218 (21)
Among all organisms detected by SM, the number not detected by PLEX-ID	43/183 (24)
Among all organisms detected by PLEX-ID, the number not detected by SM	51/191 (26.7), p <0.001
Among non-commensals detected by SM, number not detected by PLEX-ID	24/68 (35)
Among non-commensals detected by PLEX-ID, number not detected by SM	26/70 (37), p <0.001
Among fungi detected by SM, number not detected by PLEX-ID	6/21 (28.6)
Among fungi detected by PLEX-ID, number not detected by SM	15/30 (50), p 0.030
Organisms most often 'missed' by PLEX-ID, *n* (%)	
Pneumocystis jirovecii	5/7 (71)
Actinomyces odontolyticus	4/18 (22)
Haemophilus influenzae	2/7 (29)
Escherichia coli	2/5 (40)
Organisms most often 'missed' by standard methods, *n* (%)	
Candida albicans	8/16 (50)
Streptococcus spp.	5/15 (33)
Streptococcus pneumoniae	5/6 (83)
Streptococcus group *mitis* (excluding *S. pneumoniae*)	3/22 (14)
'Missed' organisms, fungi, *n* (%)	
PLEX-ID	6/36 (17)
SM	15/36 (42), p 0.030
mecA identification (%)[a]	
PLEX-ID	7/7 (100)
SM	2/7 (29)

SM, standard methods.
[a]No other resistance genes were identified throughout the study.

(95% CI, 50–73%) and negative predictive value decreased to 20% (95% CI, 8–39%).

PLEX-ID failed to detect 21% of the 183 SM-identified organisms, while SM did not recover 28% of the 191 PLEX-ID-identified organisms (p <0.001); this pattern held when excluding commensal bacteria (24/68 (35%) vs. 26/70 (37%), respectively). Organisms most often missed by PLEX-ID were *P. jirovecii* (missed in 5/7 (71%)), *Actinomyces odontolyticus* (4/18, 22%) and *Haemophilus influenzae* (2/7, 29%). Conversely, SM most often missed *Candida albicans* (8/16, 50%), *Streptococcus* spp. (5/15, 33%) and *S. pneumoniae* (5/6, 83%) when these were detected by PLEX-ID. PLEX-ID detected fungal organisms significantly more frequently than SM (42% vs. 17%, p 0.030). The only resistance gene detected by either method was *mecA*. PLEX-ID identified *mecA* in seven specimens; SM concurred in only two of these, despite the use of a *mecA*-specific PCR performed on every suspect colony.

Possible predictors for discordance. Univariable logistic regression models failed to establish associations between specimen discordance and increased white blood cells on lavage, immunosuppression and patients' antimicrobial status. Patients who were mechanically ventilated during bronchoscopy appeared to have an increased risk of discordance (odds ratio (OR), 2.12; 95% CI, 1.06–4.15), but upon adjusting for increased number of microorganisms (≥3) per specimen in multivariable analysis, the association was no longer observed.

PLEX-ID's potential clinical impact in cases of discordant, non-commensal specimens

Inter-rater reliability for the questions depicted in Fig. 1 was slight to fair, with inter-rater agreement and kappa values ranging from 29 to 50% and 0.036 to 0.308, respectively (Table 4). Both reviewers found that in the majority of the 31 discordant, non-commensal cases, PLEX-ID results would have

TABLE 3. Species, genera or resistance genes identified by standard methods and/or PLEX-ID in 41 specimens from patients diagnosed with pneumonia after bronchoalveolar lavage. Organisms in bold are considered commensal oral flora

Organism or resistance gene identified	Total number of specimens in which identified	Number of specimens in which identified by SM	Number of specimens in which identified by PLEX-ID
Actinomyces odontolyticus	6	4	2
Aspergillus fumigatus	1	1	1
Bacillus cereus	1	1[a]	1
Candida albicans	7	2	7
Candida glabrata	1	1	1
Candida tropicalis	2	2	1
Chlamydophila pneumoniae	1	1	0
Corynebacterium propinquum	2	2	1
Corynebacterium pseudodiphtheriticum	1	0	1
Cryptococcus spp.	1	0	1
Eikenella corrodens	1	0	1
Escherischia coli	3	3	2
Fusobacterium nucleatum	1	1[a]	1
Gardnerella vaginalis	1	0	1
Gemella haemolysans	1	0	1
Gemella morbillorum	1	1[a]	1
Gemella sanguinis	2	2[a]	2
Granulicatella adiacens	2	0	2
Haemophilus influenzae	3	3	3
Klebsiella pneumoniae	1	1	1
Lactobacillus gasseri	2	2[a]	2
Morganella morganii	1	1	0
Mycobacterium tuberculosis	1	1	0
Mycoplasma pneumoniae	1	1	1
Neisseria meningitidis	1	1	1
Neisseria subflava	2	1[a]	2
Penicillium spp.	1	0	1
Pneumocystis jirovecii	5	5	2
Pseudomonas aeruginosa	7	5	6
Rhizomucor pusillus	3	3	3[b]
Rothia dentocariosa	1	1[a]	1
Rothia mucilaginosa	3	3[a]	3
Saccharomyces cerevisiae	2	1	2[b]
Staphylococcus aureus	2	2	2
S. aureus, methicillin-sensitive	1	1	1
Staphylococcus epidermidis	2	1	2
Streptococcus spp.	7	4	7
Streptococcus anginosus group	1	1	1
Streptococcus mitis group (excluding Streptococcus pneumoniae)	4	2[a]	4
S. pneumoniae	3	1	2
Streptococcus pseudopneumoniae	1	1[a]	1
Streptococcus salivarius	1	1[a]	1
Streptococcus suis	1	1[a]	1
Streptococcus thermophilus	1	0	1
Streptococcus viridans	1	0	1
mecA	4	1	4

[a]SM identified the organism at genus level only.
[b]In one of the cases, PLEX-ID identified the organism at genus level only.

had no impact at all, or probably no impact, on choice of antimicrobial therapy within the 24 h following bronchoscopy, as most patients were receiving broad-spectrum antimicrobials empirically. In the majority of cases where reviewers did find that PLEX-ID would probably or definitely have had an impact

on therapy, its impact would have been to allow for a narrowing of the current antimicrobial spectrum (13/25, 52%).

Similarly, when asked whether PLEX-ID results would have changed overall patient management, Reviewers 1 and 2 responded either not at all or probably not in 83% and 100% of cases, respectively. In the few cases where PLEX-ID results would have changed non-pharmacological management, Reviewer 1 believed they would have led either to additional microbiological testing or to other, non-interventional, diagnostic analyses such as biomarker testing.

Notably, PLEX-ID missed several clinically important pathogens on at least one occasion, among them *Mycobacterium tuberculosis*, *S. pneumoniae* and Gram-negative rods such as *Chlamydophila pneumoniae*, *Escherichia coli*, *H. influenzae* and *Morganella morganii*.

Discussion

This prospective study documented a concordance of 45% between PLEX-ID and standard diagnostic methods for bacterial and fungal detection in BAL specimens. Among patients with confirmed pneumonia post-bronchoscopy, overall specimen concordance was only 30%.

These results stand in contrast to those of earlier studies, in which PLEX-ID was retrospectively compared with clearly positive specimens other than BAL fluid. The version tested seems to perform best in the detection of yeasts and, possibly, resistance genes, though there were not enough samples positive for either to confirm such a hypothesis here.

It may be argued that our study's design hampered PLEX-ID's potential from the outset. PLEX-ID was tested as a standalone diagnostic tool; as such it was not compared with one alternative method but with a battery of techniques, many of them already state-of-the-art.

Both the specimen type and the study population, which included immunosuppressed patients, increased the probability of discordance given their higher likelihood of polymicrobial yields. Indeed, our specimens' overall positivity rate was high (91%), with up to seven microbes found per specimen. In earlier studies, specimens issuing from more sterile body compartments such as blood were tested; when positive, such samples are only rarely polymicrobial [3]. When non-blood tissue specimens were tested, these were controlled settings in which PLEX-ID was tested retrospectively for the detection of a single pathogen or pathogen type [5]. As noted above, the tested version was designed to detect pathogens in sterile fluids only. Appropriately, a PLEX-ID system including quantitative assays designed specifically for BAL and other non-sterile fluids is currently under development.

TABLE 4. Independent analysis of PLEX-ID's potential clinical impact in cases of discordant, non-commensal specimens

Question	Inter-rater agreement[a] Absolute	Grouped	Kappa[a] Absolute	Grouped	Likert items	Reviewer 1 response	Reviewer 2 response	Total score (%)	Comments
Would PLEX-ID results have had an impact on the choice of empirical antimicrobial therapy for this patient within 24 h after bronchoscopy?	29%	54.8	0.133	0.188	1 = no impact at all	6/31 (19)	20/31 (65)	26/62 (42)	In over half the cases, both reviewers noted that PLEX-ID results could have led to 'wrong decision-making.'
					2 = likely no impact	10/31 (32)	1/31 (3)	11/62 (18)	
					3 = possible impact	5/31 (16)	2/31 (6)	7/62 (11)	
					4 = probably an impact	8/31 (26)	3/31 (10)	11/62 (18)	
					5 = definite impact	2/31 (6)	5/31 (16)	7/62 (11)	
Would PLEX-ID results have changed the current antimicrobial therapy by adding/stopping a drug or by broadening/narrowing the current spectrum?	50%	NA	0.308	NA	1 = adding an antimicrobial	5/15 (33)	3/10 (30)	8/26 (31)	More than one response was allowed.
					2 = discontinuing an antimicrobial	1/15 (7)	0/10 (0)	1/26 (4)	
					3 = broadening the current spectrum	1/15 (7)	1/10 (30)	4/26 (15)	
					4 = narrowing the current spectrum	8/15 (53)	5/10 (50)	13/26 (50)	
Would PLEX-ID results have changed overall patient care (antimicrobial therapy aside)?	32%	81%	0.036	<0.001	1 = not at all	9/31 (29)	30/31 (97)	39/62 (62)	In most cases, reviewers noted that patients were already being treated for the organisms detected by PLEX-ID. In three cases, it was noted that PLEX-ID results could have harmed patient safety. More than one response was allowed.
					2 = probably not	16/31 (52)	1/31 (3)	17/62 (27)	
					3 = possibly	2/31 (6)	0/31 (0)	2/62 (3)	
					4 = probably	4/31 (13)	0/31 (0)	4/62 (6)	
					5 = definitely	0/31 (0)	0/31 (0)	0/62 (0)	
Please specify how PLEX-ID might have changed overall patient care.	NA[b]	NA	NA	NA	1 = by leading to additional microbiological testing	5/6 (83)	NA	5/6 (83)	More than one response was allowed.
					2 = by leading to other diagnostic analyses	5/6 (83)		5/6 (83)	
					3 = by leading to other radiological imaging	1/6 (17)		1/6 (17)	
					4 = by leading to additional specific intervention(s)	0/6 (0)		0/6 (0)	
					5 = other	0/6 (0)		0/6 (0)	

[a]Both absolute and 'grouped' agreement rates and kappa values are shown. In the grouped analysis, Likert items for the first and third questions were reduced to three groups: 'no impact or likely no impact,' 'possible impact' and 'probably or definitely an impact.'
[b]Reviewer 2 did not find that PLEX-ID would have changed overall patient management.

The PLEX-ID platform was further at a disadvantage in that fluid was first extracted from samples for SM analysis, and often only a small—sometimes diluted—amount remained for later PLEX-ID batch analysis. Indeed, the five instances of missed *P. jirovecii* occurred in the setting of *a prior* supernatant extraction for SM; in these cases, PLEX-ID did not actually analyse the same sample volume that SM had.

Nonetheless, in light of these findings, PLEX-ID cannot currently be recommended as a standalone diagnostic tool for the detection of bacteria and fungi in BAL specimens. The results of the clinical analysis would confirm as much. While in the majority of cases, PLEX-ID results would not have had an impact on antimicrobial therapy or other patient management, reviewers worryingly commented in eight cases that PLEX-ID would have led to 'wrong decision-making' with potential harm to patients. Indeed, some of the microbes missed by PLEX-ID rank among the most important and potentially lethal pathogens producing human respiratory infections (*M. tuberculosis*, *S. pneumoniae*, etc.). Of note, in most of the cases in which PLEX-ID was considered likely to have an impact, that impact would have been to enable clinicians to narrow the antimicrobial spectrum more quickly.

Conclusions

PLEX-ID analysis of BAL specimens concurred weakly with that of SM in the detection of bacteria and fungi. In its current state, PLEX-ID may be useful as an adjunctive diagnostic tool in situations where either slow cultivation would be required (e.g. certain fungal pneumonias), or combined SM yield no findings yet clinical suspicion remains high.

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Transparency Declaration

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. PCR/ESI-MS in BAL specimens.

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