Utility of PCR diagnosis of invasive fungal infections in neonates

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Disclosure of speaker‘s interests

No (potential) conflicts of interests

- ✓ Majority of fungal infections in neonates are caused by ***C. albicans*** **and** ***C. parapsilosis*** (*C. tropicalis* in Latin America).
- ✓ Neonatal infections continue to cause **morbidity and mortality in infants**.
- ✓ Infections by other fungal pathogens are very rare in neonates.
- ✓ Among approximately 400.000 infants followed nationally, the incidence rates of **early-onset sepsis infection within 3 days of life are 0.98 cases per 1000 live births**.
- ✓ Newborn infants are at increased risk for infections because they have relative **immunodeficiency**.

Kelly MS et al. Clin Perinatol 2015;42:105
Santos RP, Tristram D. Pediatr Clin North Am 2015;62:491
Joanne L. Calley, Adilia Warris J of Infection 2017; 74:108

- ✓ Invasive candidiasis is a leading infectious cause of morbidity and mortality in premature infants.
- ✓ Invasive candidiasis typically occurs in the **first 2 weeks of life** and presents with non-specific signs of sepsis.
- ✓ Neonates are particular susceptible to IC, **with a 3- to 5- fold higher incidence** compared with children and adults.
- ✓ Definitive diagnosis relies on the growth of *Candida* in **blood culture**, but this may identify fewer than **half of cases**.

Joanne L. Calley, Adilia Warris J of Infection 2017; 74:108
Kelly MS et al. Clin Perinatol 2015;42:105
Santos RP, Tristram D. Pediatr Clin North Am 2015;62:491

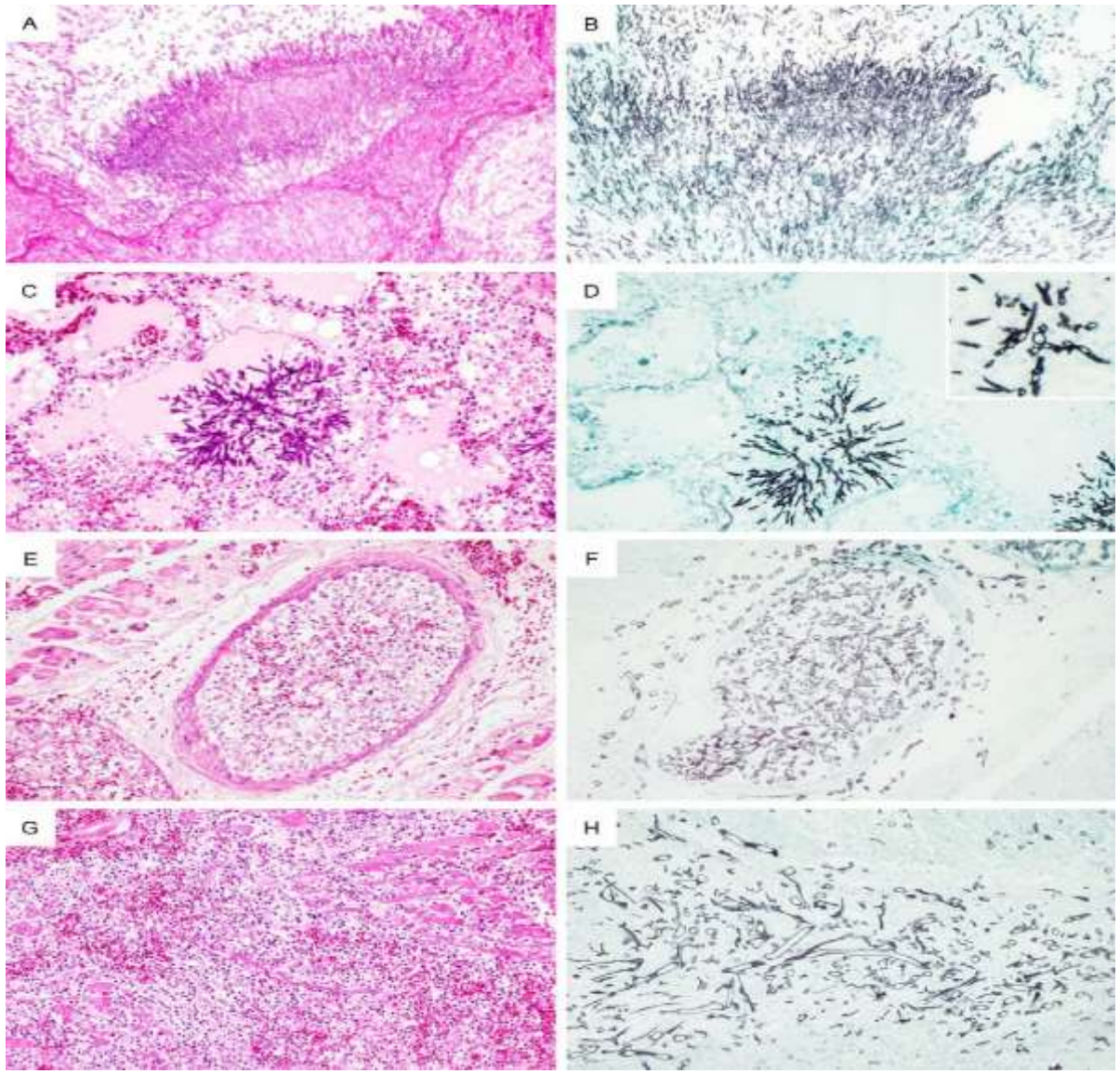
Overview of diagnostic tools available for the diagnosis of candidemia

	Study population	Patients, *n*	Sample volume	Sensitivity	Specificity
Blood culture	Adults	37		50%[a]	100%
Fungal antigens					
Mannan	Neonates	70	300 µL	92%	84%
Mannan	Chemotherapy	51		71%	77%
Mannan	Children and adults	92	300 µL	41%	100%
1->3 β-D glucan	Chemotherapy	51		76%	60%
1->3 β-D glucan	Children and adults	92	500 µL	47%	100%
Fungal antibodies					
Anti-mannan	Chemotherapy	51		43%	90%
Anti-mannan	Children and adults	92		47%	100%
PCR					
Semi-nested	**Children and adults**	**92**		**88%**	**100%**
Nested	**Hospitalized patients**	**110 (24 neonates)**	**200 µL**	**86%**	**54%**
Real-time	**Hospitalized patients**	**110 (24 neonates)**	**200 µL**	**81%**	**96%**
Real-time	**Inpatients**	**23**		**93%**	**66%**
Real-time	**Inpatients**	**23**		**77%**	**100%**
Real-time	**Immunocompromised**	**384**	**2500 µL**	**88%**	**94%**
NASBA	**Samples from positive blood cultures**				

[a]<50% sensitivity and 100% specificity in the clinical setting
NASBA, nucleic acid sequence-based amplification; PCR – polymerase chain reaction

Downey LC. et al. Curr Fungal Infect Rep 2010;4:17

Histopathology of IFD



A, B. ***Aspergillus*** *fumigates* forming fungal ball in lung parenchyma.

C, D. Intraalveolar hyphae of ***Fusarium*** *equiseti*. Inset shows high power appearance of intercalated **chlamydoconidia** (i.e. vesicular swellings), which are useful in **differential diagnostics between fusariomycosis and aspergillosis.**

E, F. **Cardiac mucormycosis with invasion and obstruction of blood vessel**.

G, H. Myocardial microabscess caused by *Rhizopus oryzae*.

A, C, E, G. **Hematoxylin & eosin**;

B, D, F, H. **Gomori's methenamine silver**; x 200, inset x 600.
All fungi species were classified by panfungal PCR.

Ruangritchankul K. et al. Int J Clin Exp Pathol 2015;8:14840

Correlation between fungi species identified by histopathology and panfungal PCR

Histopathological diagnosis	Samples (cases*)	Species by PCR	Samples (cases*)
***Aspergillus* spp.**	48 (30)	*Aspergillus flavus*	19
		Aspergillus fumigatus	13
		Aspergillus niger	2
		Aspergillus not specified	10
		Aspergillus spp.	44 (24)
		Fusarium equiseti	**3**
		***Fusarium* not specified**	**5**
		***Fusarium* spp.**	**8 (6)**
***Candida* spp.**	12 (10)	*Candida albicans*	4
		Candida tropicalis	8
		Candida spp.	12 (10)
Mucorales	11 (6)	*Lichtheimia corymbifera*	1
		Basidiobolus ranarum	2
		Mucor circinelloides	2
		Rhizopus oryzae	6
		Mucorales	11 (6)
Cryptococcus	1 (1)	*Cryptococcus neoformans*	1 (1)
Histoplasma	1 (1)	*Histoplasma capsulatum*	1 (1)
Pneumocystis	3 (3)	*Pneumocystis jirovecii*	3 (3)

*Total number of cases exceeds 48 because of **6 patients with mixed IFD.**

polymicrobial infections with ascomycetes indistinguishable with histology

Ruangritchankul K. et al. Int J Clin Exp Pathol 2015;8:14840

Organisms detected by blood culture and urine PCR in neonatal septicemia (*n* = 50)

	Blood culture	Urine PCR band	Chi-square test	*p*-value
	No (%)	No (%)		
Positive	30 (60.0)	38 (76)	34.64	<0.001
Klebsiella pneumoniae	11 (22.0)	12 (24.0)		
Escherichia coli	5 (4.5)	6 (16.0)		
Staphylococcus aureus	3 (6.0)	8 (10.0)		
Pseudomonas aeuroginosa	5 (10)	6 (12.0)		
Citrobacter freundii	2 (4)	2 (4.0)		
Acinetobacter baumannii	2 (4)	4 (4.0)		
***Non-albicans Candida* spp.**	**2 (4)**	–		
Negative	20 (40.0)	12 (24.0)		

Usually *Candida* species are among the top 5 causative agents.

Number and percentage of the etiologic agents causing sepsis

Causal agent	No.	%Total cases	% Positive cases
Bacteria	88	73.33	88.00
Viruses	20	16.67	-
Yeasts	**12**	**10.00**	**12.00**
Total	130	100	100

The isolated yeast species from blood to neonates

Yeast isolates	No = 12	%
Candida albicans	**11**	**91.7**
Debaryomyces hansenii	**1**	**8.3**
Total	12	100

Sensitivity comparison between the different methods used in the identification of yeast isolates

Method	No = 12	%
Blood culture	**12**	**100**
PCR	**12**	**100**
Buffy Coat	8	66.7

Detection of fungal DNA in lysis-centrifugation blood culture for the diagnosis of invasive candidiasis in neonatal patients

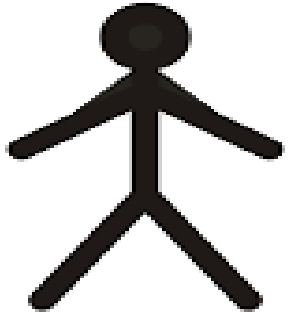
- ✓ Report of data concerning the **detection of fungal DNA directly from lysis-centrifugation blood culture** to assess its value in the detection of fungaemia in 86 of the 347 patients admitted to the neonatal intensive-care unit between January 2009 and December 2010.

- ✓ The **sensitivity and specificity of the PCR were 87.5% and 98.5% respectively**, with a **positive predictive value of 93.3% and a negative predictive value of 97.1%.**

- ✓ Detection of fungal DNA directly from blood culture isolator 1.5 microbial tubes, **without prior cultivation**, is a **promising approach for the rapid detection of *Candida* spp. in neonates with suspected candidemia.**

Trovato L. et al. CMI 2011;18:E63

A multiplex nested PCR for the detection and identification of *Candida* species in blood samples of critically ill paediatric patients



Neonates n = 54
suspicious of
candidemia

Blood →
- Blood cultures: 14.8 %
- PCR: 24.0 %

Demographic, clinical, and laboratory data of the 13 patients with positive PCR

Patients	G	Age	Underlying disease	BSAT	CVC	AFT	Outcome	Blood cultures	Nested PCR multiplex
1	M	19 d	Ichthyosis, prematurity	Yes	Yes	Yes	Died	*C.parapsilosis*	*C. parapsilosis*
2	M	14 d	Prematurity	Yes	Yes	Yes	Survived	*C. albicans*	*C. albicans*
3	F	44 d	Esophageal atresia	Yes	Yes	Yes	Survived	*C. albicans*	*C. albicans*
4	F	20 m	Acute lymphoblastic leukemia	Yes	Yes	Yes	Died	*C. tropicalis*	*C. tropicalis*
5	M	20 m	Non-Hodgkin lymphoma	Yes	Yes	Yes	Survived	*C. albicans*	*C. albicans*
6	F	11 m	Hydrocephalus	Yes	Yes	Yes	Survived	*C. albicans*	*C. albicans*
7	M	5 y	Disseminated medullobastoma	Yes	Yes	Yes	Died	*C. krusei*	*C. krusei*
8	F	16 m	Hydrocephalus	Yes	Yes	Yes	Died	*C. albicans*	*C. albicans*
9	M	3 y	Bone marrow transplantation, neuroblastoma	Yes	Yes	Yes	Died	**Negative**	***C. tropicalis* and *C. parapsilosis*[a]**
10	F	15 y	Osteosarcoma, septic shock	Yes	Yes	Yes	Died	**Negative**	***C. parapsilosis*[a]**
11	M	35 d	Congenital diaphragmatic hernia	Yes	Yes	Yes	Survived	**Negative**	***C. tropicalis*[a]**
12	M	9 d	Congenital diaphragmatic hernia	Yes	Yes	Yes	Survived	**Negative**	***C. tropicalis* and *C. parapsilosis*[a]**
13	M	55 d	Necrotizing enterocholitis	Yes	Yes	Yes	Died	**Negative**	***C. tropicalis* and *C. parapsilosis*[a]**

G = gender; M = male; F = female; d = days; m = months; y = years; **BSAT** = broad spectrum antibiotic therapy; **CVC** = central venous catheter;
AFT = presumptive antifungal therapy after blood sample collections.
[a] = Sequencing analysis showed 99-100% identity with *C. parapsilosis stricto sensu* [GenBank: JN997459.1] and 99-100% identity with *C. tropicalis* [GenBank: EU266571.1].

Taira CL. et al. BMC Infect Dis 2014;14:406

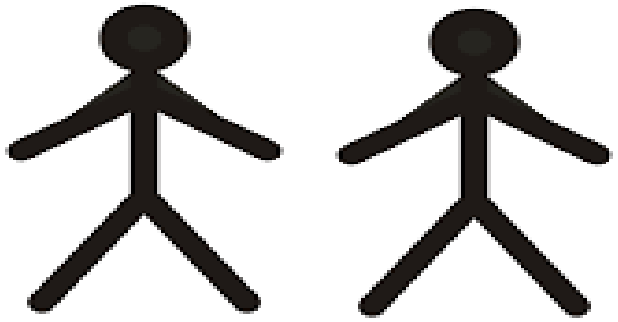
Characteristics of patients with positive PCR and negative blood culture for fungus

	Index of suspicion*	Clinical factors	Antifungal therapy	Relevant cultures
1	High	NEC	Completed amphotericin course	Blood: *C. albicans* 28 days prior to sample collection
2	High		On amphotericin	Blood: *C. albicans* 3, 5 and 8 days prior to sample collection.
3	High	NEC, intestinal perforation		Blood: *Enterococcus faecalis* Peritoneal fluid: *Klebsiella* Blood: *C. glabrata* 28 days prior to sample collection
4	High		On amphotericin	Blood: *C. albicans* 14 days prior to sample collection.
5	High			Urine: *C. albicans*
6	High			Urine: *C. albicans* 4 days after study sample collection
7	High	NEC, intestinal perforation		Peritoneal fluid: Heavy *C. albicans* 4 days after study sample collection
8	High			Blood: *Klebsiella* sp.
9	High	NEC		Blood: *Enterobacter cloacae*
10	High	ARDS, endocarditis		Blood: *Pseudomonas aeruginosa*
				Sputum: heavy *C. albicans*, many PMNs
11	Moderate	Wrist abscess		Abscess fluid: *Pseudomonas aeruginosa*
12	Moderate			Blood: *Staphylococcus epidermidis*
13	Low			None

* Index of suspicion for septicemia at the time of sample collection.
NEC=necrotizing enterocolitis.

Tirodker U. et al. J Perinatol 2003;23:117

T2Candida provides rapid and accurate species identification in pediatric cases of candidemia



n = 9
controls

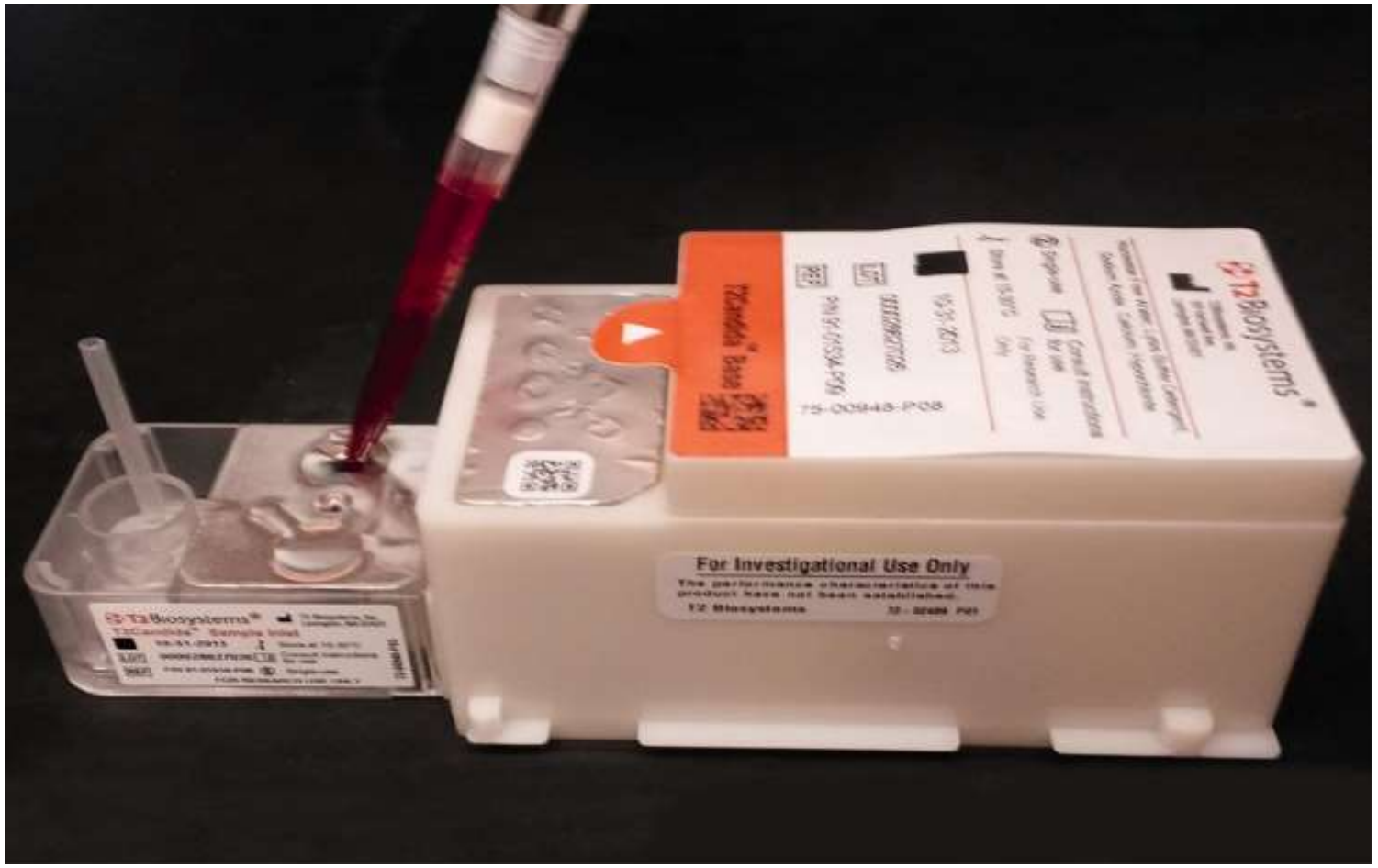
n = 15
with candidemia

5-12 years

U
whole blood
samples 2 ml

Blood cultures → 15 positive results
time ~

T2 test → 15 positive results
time ~ 3-5 hours



Direct pipetting of whole blood into a T2Candida cartridge

Hamula CL. et al. Am J Clin Pathol 2016;145:858

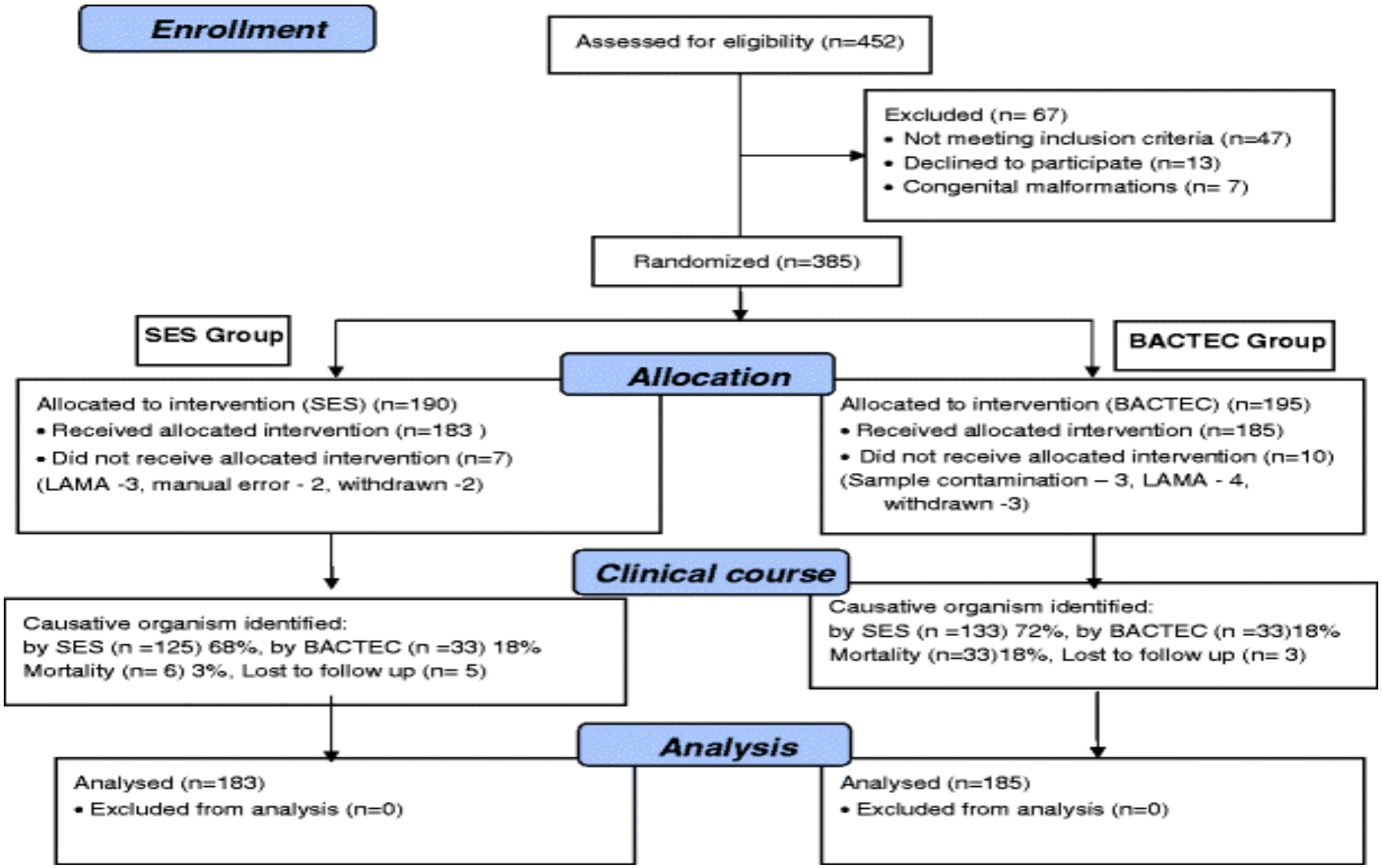
Agreement between T2Candida and blood culture results for 24 pediatric samples pipetted directly into a T2Candida cartridge

Blood Culture Result	T2Candida Result, No.			
	Candida albicans/ Candida tropicalis	*Candida parapsilosis*	*Candida krusei/ Candida glabrata*	Negative
C. parapsilosis	0	7	0	0
C. albicans	4	0	0	0
C. glabrata	0	0	3	0
C. tropicalis	1	0	0	0
Negative	0	0	0	9

Hamula CL. et al. Am J Clin Pathol 2016;145:858

Syndrome evaluation system (multiplex PCR) *versus* blood culture (BACTEC) in the diagnosis and management of neonatal sepsis – A randomized controlled trial

CONSORT flow chart



Bhat BV. Et al. Indian J Pediatr 2016;83:370-379

Microbiological data of SES and control arm

	SES arm (*N* = 185)		Control arm (*N* = 183)	
	Culture	SES	Culture	SES
Positive	33 (17.84 %)	125 (67.57 %)	33 (18.03 %)	133 (72.68 %)
Negative	152 (82.16 %)	60 (32.43 %)	150 (81.97 %)	50 (27.32 %)

Bhat BV. Et al. Indian J Pediatr 2016;83:370-379

Rank order of organisms detected in SES and culture

Organism	Detected in culture N (%)	Detected in SES N (%)
Klebsiella pneumoniae	39 (59.0)	66 (49.3)
Acinetobacter baumannii	16 (24.2)	28 (20.9)
Candida species	**5 (7.6)**	**11 (8.2)**
Escherichia coli	3 (4.5)	3 (2.2)
Pseudomonas aeruginosa	1 (1.5)	12 (9)
Enterobacter aerogenes	1 (1.5)	6 (4.5)
Staphylococcus aureus	Nil	2 (1.5)
Group B *streptococcus*	Nil	4 (3)
Enterococcus	1 (1.5)	Nil
Streptococcus pyogenes	Nil	1 (0.7)
Streptococcus pneumoniae	Nil	1 (0.7)

Bhat BV. Et al. Indian J Pediatr 2016;83:370-379

- ✓ **SES was better than BACTEC in identifying** the causative organism in both the groups (68 % *vs.* 18 % in SES group and 72 % *vs.* 18 % in control group).
- ✓ SES had 100 % concordance with blood culture by BACTEC.
- ✓ **Detection of bacteria and fungi were four and ten-fold higher** respectively with SES when compared to BACTEC culture.
- ✓ Microbiological diagnosis **was rapid** with SES compared to BACTEC (7 h *vs.* 72 h).
- ✓ Treatment based on SES resulted in significantly **less mortality (3 % *vs.* 18 %).**
- ✓ Readmission rate, duration of hospital stay and change in antibiotics were also significantly less in SES group.

The new molecular based diagnostic system (SES) helps in rapid and accurate diagnosis of neonatal sepsis and reduces mortality and morbidity in affected neonates.

- ✓ **Advantage fast time to result (24 h versus 48h-72h compared to blood cultures).**
- ✓ **Relative small samples volume of (1-2 mL).**
- ✓ **The efficiency of these in-house assays has not been widely studied, lacks thoroughly clinical evaluation and therefore can't be recommended as stand-alone, single approach in clinical routine diagnostics.**
- ✓ **Molecular-based diagnostic assays can be recommended as valuable add on tools that complement conventional diagnostic procedures.**