Rapid Blood Culture Identification Panel in Pediatric Patients in Guatemala

NCT05314816

Statistical Analysis Plan

v. 2/23/2023

Statistical Analysis Plan

Objective: to evaluate the clinical impact of the BioFire BCID2 panel in children with bacteremia in Guatemala.

Study Design: Pragmatic clinical trial comparing intervention to two different control groups. Intervention and concurrent control group allocated by time of blood culture positivity. Also comparing to historical controls.

Primary outcome: time to optimal antimicrobial therapy

Secondary outcomes:

- Time to effective antimicrobial therapy
- Time to pathogen identification
- Mortality
- Length of hospital stay
- Antibiotic days of therapy (DOT)
- Number of unique antibiotics
- Total ICU days

Definitions to consider:

Patient Group

Intervention= patient was enrolled in the intervention group and received the BCID2 intervention within 12h timeframe

Concurrent control= patient was enrolled in the study and did not receive the BCID2 intervention within 12h from blood culture positivity

- Weekday controls= Patient with positive blood culture from 1pm-11:59pm Mon-Thurs
- Weekend controls= Patient with positive blood culture from 1pm Fri- 11:59pm Sunday

Per protocol =patient received intervention based on original allocation.

- Per protocol intervention= Patient allocated to intervention based on timing of blood culture AND BCID2 was performed within 8h timeframe.
- Per protocol control= Patient allocated to control group based on timing of blood culture AND no BCID2 was performed.

Protocol deviation=Patient intended to remain in the allocated group, but there was an unintentional deviation from protocol.

- Protocol deviation intervention= BCID2 run between 8-12h in patient allocated to intervention.
- Protocol deviation control= BCID2 was sent clinically >12h from positive culture in patient allocated to control group OR patient originally allocated to intervention group but BCID2 was not performed.

Sub-groups

On-panel true pathogens= Pathogens on the BCID2 panel that are always considered real pathogens per protocol. These include, *E. faecalis, E. faecium, Listeria, Staph aureus, Strep pneumonia, Strep agalactiae, Strep pyogenes, Acinetobacter baumannii, B. fragilis, Enterobacterales, Enterobacter cloacae, E. coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus, Salmonella, Serratia matcescens, Haemophilus influenza, Neisseria meningitidis, Pseudomonas aeruginosa, Stenotrophomonas maltophilia, Cryptococcus, Candida species.*

Gram negative on-panel pathogen= On-panel true pathogens as above that are Gram negatives only. These include *Acinetobacter baumannii, B. fragilis, Enterobacterales, Enterobacter cloacae, E. coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus, Salmonella, Serratia matcescens, Haemophilus influenza, Neisseria meningitidis, Pseudomonas aeruginosa, Stenotrophomonas maltophilia.*

Gram negative pathogen= On or off-panel pathogens that are Gram negatives.

On-panel classic contaminant= Gram positive pathogens on the BCID2 panel that are often considered contaminants (note, may be considered real in the analysis). These include, *Staph epi, Staph lugdunensis, other Staph species, other Strep species.*

Off-panel non-Gram negatives (other)= typically contaminants.

True pathogens (on and off panel)= excluding common contaminants.

Polymicrobial= More than one organism was detected by BICD2 and/or Vitek.

Primary outcomes

Optimal antibiotic=typically narrowest spectrum agent based on predefined consensus guidelines

Effective antibiotic= any antibiotic susceptible based on susceptibilities even if overly broad.

Time to optimal antimicrobial therapy=time blood culture received in lab to time of first dose of "optimal" antibiotic. Censor at 14 days. Censor for death if "never reached" optimal. Stoppage of antibiotics if deemed a contaminant.

Time to effective antimicrobial therapy= time of blood culture received in lab to time of first dose of "effective" antibiotic. Censor at 14 days. Censor for death if "never reached" effective. Stoppage of antibiotics if deemed a contaminant.

Clinician discretion (Intention to treat analysis (ITT))=effective and optimal therapy determined based on whether **treating physicians** considered the pathogen real or a contaminant. Optimal therapy allowed additional antibiotics if the treating team was also treating for other syndromic diagnoses, such as culture-negative sepsis (if pathogen detected deemed to be a contaminant), nosocomial pneumonia, etc. if called out in diagnoses. Definitions created for optimal therapy for nosocomial pneumonia, culture-negative sepsis, etc.

- Time to effective therapy in ITT:
    - Time from blood culture received in lab (time 0) to effective therapy
    - Time from blood culture positivity (time 0) to effective therapy
- Time to optimal therapy in ITT:
    - Time from blood culture received in lab (time 0) to effective therapy
    - Time from blood culture positivity (time 0) to effective therapy

Pathogen directed (Per protocol analysis) =effective and optimal therapy based on **strict definitions** for real pathogen or contaminant created by study team. Strict optimal antibiotic definitions without allowing for additional antibiotics for culture-negative sepsis or nosocomial pneumonia, etc. Does allow for additional antibiotics for clear other indications (pyelonephritis, CAP, dacrocystitis, intra-abdominal abscess, etc.) or another organism isolated (excluding urine and trach cultures). Not allowing for more controversial diagnoses such as sepsis or nosocomial pneumonia.

- Time to effective therapy in per protocol:
    - Time from blood culture received in lab (time 0) to effective therapy
    - Time from blood culture positivity (time 0) to effective therapy
- Time to optimal therapy in per protocol:
    - Time from blood culture received in lab (time 0) to effective therapy
    - Time from blood culture positivity (time 0) to effective therapy

Secondary outcomes

Time to pathogen identification= Time from time of blood culture receipt in lab (time 0) to identification of a pathogen. For control patients, this is the time to Vitek. For intervention patients, this is the time to BCID2 if the BCID2 was positive for an on-panel pathogen or time to vitek if the BCID2 was negative.

- Control: time to Vitek
- Intervention:
    - If BCID2 positive → calculate time to BCID2 result
    - If BCID2 negative → calculate time to Vitek

Total antibiotic days=all days that patient was on antibiotics (any dose of antibiotic) from date that blood culture was drawn. Capped at 14 days or date of death.

Antibiotic days of therapy =Sum of antibiotics each day over 14-day period. For example if 2 antibiotics were each given over 5 days, this would count as 10 DOT.

Number of different antibiotics=Number of unique antibiotics given over the 14-day period.

Length of stay=date admitted to date patient left the study (date of death/discharge or lapse of 30 days). Censor at 30 days.

Total ICU days=date admitted in ICU to date transferred out or date of death/discharge. Censor at 30 days.

Time to death= Time from blood culture obtained to time of death.

Number of deaths within 24h of blood culture draw= Use time of death calculation above to determine the number of patients who died <24h

Overview of Analyses to perform:

- All interventions vs. all concurrent controls (including protocol deviations) vs. historical controls
    - Demographics
    - All outcomes (primary and all secondary endpoints)
    - Sensitivity analysis comparing weekday vs. weekend controls
- Per protocol controls vs. per protocol intervention (excluding protocol deviations)
    - Demographics
    - All outcomes (primary and all secondary endpoints)
- Clinician discretion (ITT) analysis (controls vs. interventions)

    - Number of contaminants vs. true pathogens
    - Time to optimal therapy
    - Time to effective therapy
- Pathogen directed (Per protocol) analysis (controls vs. interventions)
    - Number of contaminants vs. true pathogens
    - Time to optimal therapy
    - Time to effective therapy
- Gram negatives only (on- and off- panel)
    - Demographics
    - Time to optimal therapy
    - Time to effective therapy
    - Mortality
- On-panel true pathogens only
    - Demographics
    - Time to optimal therapy
    - Time to effective therapy
    - Mortality
- On-panel Gram negatives only
    - Demographics
    - Time to optimal therapy
    - Time to effective therapy
    - Mortality
- True pathogens (on and off panel)
    - Demographics
    - Time to optimal therapy
    - Time to effective therapy
    - Mortality

Figure 1: CONSORT Diagram

Table 1: Patient Characteristics

	All Intervention N = 135	All Concurrent Control N = 219	p-value (interv. vs. concurrent control)	Weekend controls N=XX	p-value (interv. Vs. weekend)	Weekday controls N=XX	p-value (interv. Vs. weekday)	Historical controls N=99	p-value (intervention vs. historical control)
Age in months (median, IQR)*									
Categorical age groups (0-30 days, 1-5.99mo, 6-11.99mo, 12month-23.99month, 24 month-4.99y, >=5y)**									
Female (n, %)**									
No antecedent medical conditions (N, %)**									
Immunocompromised (N, %)**									
Central line (N, %)**									
Required ICU stay (N, %)**									

**Wilcoxon test of medians*

***chi-square test*

Table 1A: Supplemental table with demographics from sub-groups.

	Per protocol Intervention	Per protocol Control	p-value	True on-panel pathogen Intervention	True on-panel Pathogen controls	p-value	All Gram negative (GN) Interv.	All GN control	p-value	All GN weekDAY controls	p-value (compare to GN interv.)	On-panel GN Interv.	On-panel GN control	p-value

Age in months (median, IQR)*														
Categorical age groups (0-30 days, 1-5.99mo, 6-11.99mo, 12month-23.99month, 24 month-4.99y, >=5y)**														
Female (n, %)**														
No antecedent medical conditions (N, %)**														
Immunocompromised (N, %)**														
Central line (N, %)**														
Required ICU stay (N, %)**														

Table 2: Organisms isolated in culture (Vitek)

ORGANISM	**INTERVENTION N=135 (%)***	**CONCURRENT CONTROL N=219 (%)***	**STUDY TOTAL N=354 (%)***	**HISTORICAL CONTROLS N=99 (%)***	**Weekend controls N=XX (%)**	**Weekday controls N=XX (%)**
BCID2 Panel organisms						
Acinetobacter calcoacetius-baumannii complex (n, %)						
Bacteroides fragilis						
Haemophilus influenzae						
Neisseria meningitidis						
Pseudomonas aeruginosa						
Stenotrophomonas maltophilia						

Enterobacter cloacae complex						
Escherichia coli						
Klebsiella aerogenes						
Klebsiella oxytoca						
Klebsiella pneumoniae group						
Proteus spp.						
Salmonella spp.						
Serratia marcescens						
Enterococcus faecalis						
Enterococcus faecium						
Listeria monocytogenes						
Staphylococcus aureus						
Staphylococcus epidermidis						
Staphylococcus lugdunensis						
Streptococcus agalactiae (Group B)						
Streptococcus pneumoniae						
Streptococcus pyogenes (Group A)						
Candida albicans						
Candida auris						
Candida glabrata						
Candida krusei						
Candida parapsilosis						
Candida tropicalis						
Cryptococcus neoformans/gattii						

Other coagulase-negative Staphylococcus species						
Other Streptococcus species						
Total on-panel targets						
Non-BCID2 organisms						
Bacillus species						
Achromobacter species						
Burkholderia species						
Shewanella species						
Sphingomonas species						
Other Acinetobacter species						
Pantoea species						
Other Pseudomonas species						
Alloiococcus species						
Aeromonas species						
Leuconostoc species						
Kocuria species						
Citrobacter freundii						
Gram positive Bacillus (no ID)						
Moraxella species						
Pandoraea species						
Kluyvera species						
Total non-BCID2 targets						

*n= number of cultures. Some cultures grew more than one isolate. Percentage of each organism was calculated from the total number of cultures.

Table 2A: Supplemental table of organisms for sub-groups. (Will likely add historical controls to supplemental table)

ORGANISM	Per protocol Intervention N=112 (%)*	Per protocol controls N=192 (%)*	Protocol deviation interventions N=23 (%)*	Protocol deviation controls N=27(%)*
BCID2 Panel organisms				
Acinetobacter calcoacetius-baumannii complex				
Bacteroides fragilis				
Haemophilus influenzae				
Neisseria meningitidis				
Pseudomonas aeruginosa				
Stenotrophomonas maltophilia				
Enterobacter cloacae complex				
Escherichia coli				
Klebsiella aerogenes				
Klebsiella oxytoca				
Klebsiella pneumoniae group				
Proteus spp.				
Salmonella spp.				
Serratia marcescens				
Enterococcus faecalis				
Enterococcus faecium				
Listeria monocytogenes				
Staphylococcus aureus				
Staphylococcus epidermidis				
Staphylococcus lugdunensis				

Streptococcus agalactiae (Group B)				
Streptococcus pneumoniae				
Streptococcus pyogenes (Group A)				
Candida albicans				
Candida auris				
Candida glabrata				
Candida krusei				
Candida parapsilosis				
Candida tropicalis				
Cryptococcus neoformans/gattii				
Other coagulase-negative Staphylococcus species				
Other Streptococcus species				
Total on-panel targets				
Non-BCID2 organisms				
Bacillus species				
Achromobacter species				
Burkholderia species				
Shewanella species				
Sphingomonas species				
Other Acinetobacter species				
Pantoea species				
Other Pseudomonas species				
Alloiococcus species				
Aeromonas species				
Leuconostoc species				

Kocuria species				
Citrobacter freundii				
Gram positive Bacillus (no ID)				
Moraxella species				
Pandoraea species				
Kluyvera species				
Total non-BCID2 targets				

Table 3: Outcomes

	Interventions N=135	**Concurrent Controls N=219**	**p-value**	**Weekend control**	**p-value (interv. vs. weekend)**	**Weekday control**	**p-value (interv. vs. weekday)**	**Historical controls N=99**	**p-value (interven. vs. historic)**
Median time to optimal therapy per protocol (XX hours)									
Median time to effective therapy per protocol (XX hours)									
Median time to pathogen identification (XX hours)									
All-cause mortality (n, %)									
Median time to death from									

blood culture obtained (XX days)									
Deaths within 24h of blood culture drawn (N, %)**									
Deaths from sepsis (N, %)									
Deaths from other causes (N, %)									
Cause of death unknown (N, %)									
Median LOS (XX days)									
Median ICU days (XX days)									
Median total antibiotic days (XX days)									
Median antibiotics DOT									
Number of unique antibiotics in 14-day period									

Table 3A: For NICU kids only

	Interventions N=135	**Concurrent Controls N=219**	**p-value**	**Weekend control**	**p-value**	**Weekday control**	**p-value**	**Historical controls N=99**	**p-value**

Median time to optimal therapy (XX hours)									
Median time to effective therapy (XX hours)									
Median time to pathogen identification (XX hours)									
All-cause mortality (n, %)									
Median time to death from blood culture obtained (XX days)									
Deaths within 24h of blood culture drawn (N, %)**									
Deaths from sepsis (N, %)									
Deaths from other causes (N, %)									
Cause of death unknown (N, %)									
Median LOS (XX days)									
Median ICU days (XX days)									

Median total antibiotic days (XX days)									
Median antibiotics DOT									
Number of unique antibiotics in 14-day period									

Table 3B: For non-NICU patients only

	Interventions N=135	**Concurrent Controls N=219**	**p-value**	**Weekend control**	**p-value**	**Weekday control**	**p-value**	**Historical controls N=99**	**p-value**
Median time to optimal therapy (XX hours)									
Median time to effective therapy (XX hours)									
Median time to pathogen identification (XX hours)									
All-cause mortality (n, %)									
Median time to death from blood culture obtained (XX days)									

Deaths within 24h of blood culture drawn (N, %)**									
Deaths from sepsis (N, %)									
Deaths from other causes (N, %)									
Cause of death unknown (N, %)									
Median LOS (XX days)									
Median ICU days (XX days)									
Median total antibiotic days (XX days)									
Median antibiotics DOT									
Number of unique antibiotics in 14-day period									

Table 3C: Table of outcomes with sub-groups for all units

	Per protocol interventions	**Per protocol Controls**	**p-value**	**All Gram negative interventions**	**All Gram negative controls**	**p-value**	**All GN weekDAY controls**	**p-value (compare to GN interv.)**	**True on-panel pathogen interventions**	**True on-panel pathogen controls**	**p-value**	**On-panel Gram neg interv**	**On-panel Gram neg control**	**p-valu**

Median time to optimal therapy (XX hours)														
Median time to effective therapy (XX hours)														
Median time to pathogen identification (XX hours)														
All-cause mortality (n, %)														
Median time to death from blood culture obtained (XX days)														
Deaths within 24h of blood culture drawn (N, %)**														
Deaths from sepsis (N, %)														
Deaths from other causes (N, %)														
Cause of death unknown (N, %)														
Median LOS (XX days)														
Median ICU days (XX days)														

Median total antibiotic days (XX days)														
Median antibiotics DOT														
Number of unique antibiotics in 14-day period														

Table 4: BCID2/Vitek discrepancies in intervention patients

	Number of patients (N, %)
BCID2/Vitek agreed	
BCID2/Vitek disagreed	
BCID2 positive, Vitek negative	
BCID2 negative, Vitek positive	
Species misidentification	
Resistance gene did not match susceptibility	
Organism not on panel	

Other parameters:

- Look at distribution (median with IQR) of time between BCID2 result and team notification (only for intervention patients).
- Look at distribution (median with IQR) of time between BCID2 execution and team notification (only for intervention patients).
- Age distribution and gender between those excluded via initial screening and those included (initially enrolled).

Figures: Kaplan Meier curves for time to optimal and time to effective therapy (using both intention to treat AND per protocol analyses)

1st way- time of blood culture draw as time 0

2nd way- time of blood culture positivity as time 0

Figure 1: Compare interventions vs. concurrent controls vs. Historical controls (all pathogens)

Figure 1A: time to effective therapy ITT

Figure 1B: time to effective therapy per protocol

Figure 1C: time to optimal therapy ITT

Figure 1D: time to optimal therapy per protocol

Figure 1E: time to death (deaths only)

Figure 2: Compare interventions vs. concurrent controls vs. Historical controls (Gram negative rods on-panel and off-panel)

Figure 3: Compare interventions vs. concurrent controls vs. Historical controls (True on-panel pathogens only)

Figure 4: Compare interventions vs. concurrent controls vs. Historical controls (on-panel Gram negative pathogens only)