Methylene Blue in Septic Shock – A Systematic Review and Meta-Analysis

Shannon M. Fernando, MD, MSc; Alexandre Tran, MD, MSc; Karim Soliman, MD; Barbara Flynn, BScRN; Thomas Oommen, PharmD; Li Wenzhe, MD; Neill K. J. Adhikari, MDCM, MSc; Salmaan Kanji, PharmD; Andrew J. E. Seely, MD, PhD; Alison E. Fox-Robichaud, MD, MSc; Randy S. Wax, MD, MEd; Deborah J. Cook, MD, MSc; François Lamontagne, MD, MSc; Bram Rochwerg MD, MSc

ELECTRONIC APPENDIX

Supplemental Figure 1: Search Strategy

MEDLINE Search

1. Exp Methylene blue/
2. Methylene blue.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
3. 1 or 2
4. Sepsis or septic.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
5. Multiple organ failure/
6. Critical illness/
7. Respiratory distress syndrome/
8. ((severe or serious or critical or intensive) adj3 (illness or infection* or shock or care)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
9. Organ adj3 failure.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
10. 4 or 5 or 6 or 7 or 8 or 9
11. 3 and 10

Supplemental Table 1: Standardized Data Extraction Sheet.

Data to be Extracted	Notes to Reviewer
Basic Study Information	
Study Title	
Journal	
Year of Publication	
Language	
Author	List first author only
Correspondence Email	
Study Design	
Number of Sites	
Country/Countries of Study	
Eligibility Assessment	
Does the study include adult patients (i.e. ≥ 16 years of age)?	If "No" – Exclude
Does the study enroll or present a subgroup of patients with sepsis or septic shock?	If "No" – Exclude
Definition of "Sepsis/Septic Shock" (e.g., Sepsis-3 criteria; SIRS criteria, etc.).	
Does the study evaluate the use of intravenous methylene blue (at any dose, duration of therapy, and with any timing)?	If "No" – Exclude
Does the study compare patients receiving methylene blue with those not receiving MB, or those administered placebo?	If "No" – Exclude
Trial Characteristics	
Was population was included?	E.g. mixed, surgical, etc.
What was the mean duration of hospitalization?	
Were elderly patients included?	
Were pregnant patients included?	
Were patients with any other co-morbidity included/excluded?	
Methylene Blue Dosing	
Control (if applicable)	
Risk of Bias Assessment	
How were patients randomized?	
In either the intervention or the control group, were there any deviations from the protocol?	
Was there any missing outcome data?	
Were the outcome measures objective?	

Were all important pre-specified outcomes presented?	
Any other bias noted?	
Outcome #1	
Outcome being evaluated	e.g. Short-term mortality
Dichotomous or continuous outcome?	
Methylene Blue: N analyzed	
Methylene Blue: Number of Events/Mean	
Control: N analyzed	
Control: Number of Events/Mean	
Comments	
Outcome #2	
Outcome being evaluated	e.g. Duration of vasopressors
Dichotomous or continuous outcome?	
Methylene Blue: N analyzed	
Methylene Blue: Number of Events/Mean	
Control: N analyzed	
Control: Number of Events/Mean	
Comments	
Author Contact	
Contact author?	If more information needed, indicate here to contact author

Supplemental Table 2: Characteristics of the 6 randomized trials. *Abbreviations:* MB = methylene blue; SIRS = systemic inflammatory response syndrome; SSRI = selective serotonin reuptake inhibitor

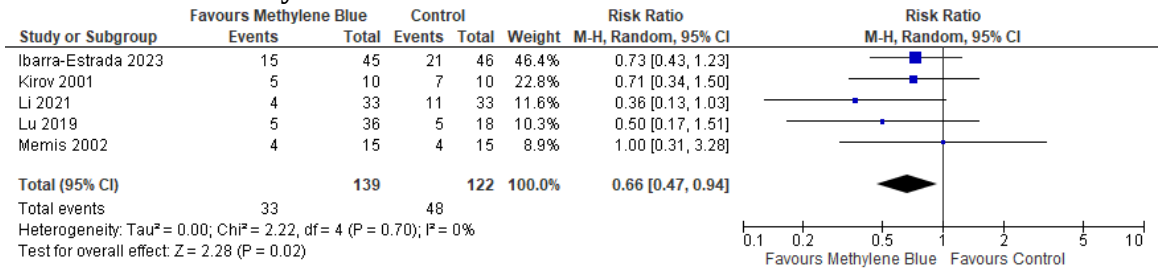
First Author, Year	Journal	Sites	Country	Population	Inclusion Criteria	Exclusion Criteria	Dose of Methylene Blue	Timing of Methylene Blue Administration	Total Sample Size	Mean Age (Years)	% Male	% Mortality (Short-term)
Ibarra-Estrada, 2023	*Crit Care*	1	Mexico	Intensive Care Unit	Age ≥ 18 years, with septic shock (Sepsis-3 criteria)	>24h of norepinephrine infusion; Pregnancy; High probability of death; Concurrent hemorrhage; Obstructive or hypovolemic shock; Pending surgery; Major burn injury; Personal or family history of G6PD deficiency; Allergy to MB, phenothiazides, or food dyes; Recent SSRI intake	Intravenous infusion of 100mg MB in 500mL of 0.9% normal saline over 6 hours daily; 3 days total	Within 24 hours of initiation of norepinephrine	92	46.5	60.9	39.6
Kirov, 2001	*Crit Care Med*	1	Norway	Intensive Care Unit	Age ≥ 18 years, with septic shock (≥ 2 SIRS criteria and a mean arterial pressure of <70mmHg at 30 mins despite fluid resuscitation or receiving vasopressors)	Pregnancy; Receiving corticosteroids; Immunosuppressants; Chemotherapy; Known irreversible underlying disease (such as end-stage neoplasms)	Intravenous bolus of 2mg/kg for 15 mins) followed by infusion of escalating doses of 0.25-2 mg/kg/hr for 1 each	Within 2 hours of randomization	20	57.4	55	40.0
Li, 2021	*Diet Health*	1	China	Intensive Care Unit	Septic shock (Unclear definition)	Abnormal liver and kidney function; Patients with incomplete data	Intravenous bolus of 2mg/kg for 15 mins	Unclear	66	52.7	59.1	22.7
Lu, 2019	*Nat Med J China*	1	China	Intensive Care Unit	Age ≥ 18 years, with septic shock (Sepsis-3 criteria)	History of previous myocardial infarction or stroke in the preceding 3 months; Pregnancy; Known anaphylaxis to MB; Nitrate use in previous 3 days	Intravenous bolus of 2mg/kg with or without 2mg/kg infusion over 24 hours	Unclear	54	64	53.7	18.5
Memis, 2002	*Anaesth Intensive Care*	1	Turkey	Operating Room and Intensive Care Unit	Age ≥ 18 years, with septic shock (≥ 2 SIRS criteria and a mean arterial pressure of <70mmHg at 30 mins despite fluid resuscitation or receiving vasopressors)	Pregnancy; Receiving corticosteroids; Immunosuppressants; Chemotherapy; Known irreversible underlying disease (such as end-stage neoplasms)	Intravenous infusion of 0.5 mg/kg/hr over 6 hours	Unclear	30	52.4	60	26.7
Xiong, 2010	*China J Anesthesiol*	1	China	Operating Room and Intensive Care Unit	Septic shock (Unclear definition) undergoing emergency surgery	Abnormal liver or kidney function; Previous methemoglobinemia; Previous carbon monoxide or cyanide poisoning	Intravenous infusion of 0.5-1.0 mg/kg/hr during surgery	Intraoperative	40	47.5	52.5	Not Recorded

Supplemental Table 3: Quality Assessment for Risk of Bias of the 6 studies. N.B. "Yes" = low risk of bias.

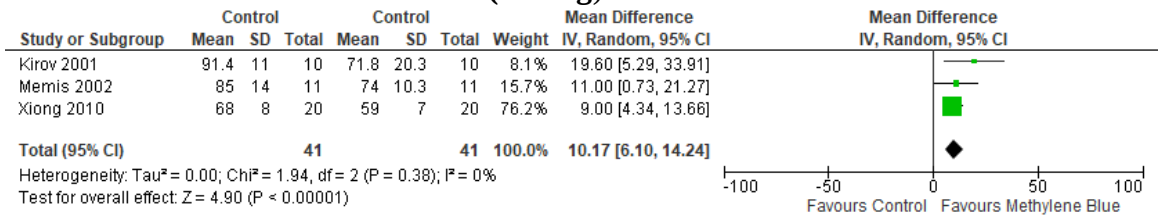
Author, Year	Allocation Sequencing?	Allocation Concealment?	Blinding?	Infrequent Loss to Follow-up?	Free of Selective Outcome Reporting?	No Other Risk of Bias?	Overall Risk of Bias
Ibarra-Estrada, 2023	Probably Yes	Definitely Yes	Probably Yes	Definitely Yes	Definitely Yes	Definitely Yes	Low
Kirov, 2001	Definitely Yes	Definitely Yes	***Probably No***	Definitely Yes	Probably Yes	Probably Yes	High
Li, 2021	***Definitely No***	***Definitely No***	***Probably No***	Definitely Yes	Probably Yes	Probably Yes	High
Lu, 2019	***Probably No***	***Probably No***	***Probably No***	Definitely Yes	Probably Yes	Probably Yes	High
Memis, 2002	Probably Yes	Definitely Yes	Probably Yes	Definitely Yes	Probably Yes	Probably Yes	Low
Xiong, 2010	Probably Yes	Probably Yes	***Probably No***	Definitely Yes	Probably Yes	Probably Yes	High

Supplemental Figure 2: Forest plots depicting efficacy and safety of methylene blue versus placebo or usual care.

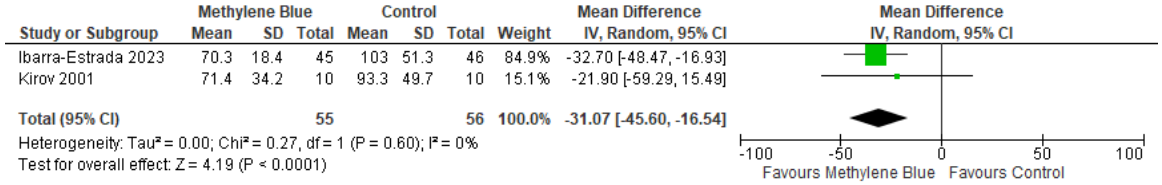
Short-term Mortality



Mean Arterial Pressure at 6 Hours (mmHg)



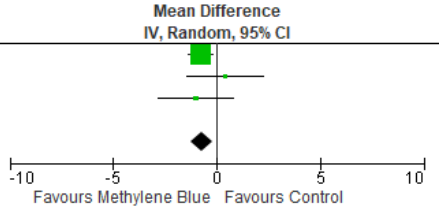
Duration of Vasopressors (Hours)



Duration of Invasive Mechanical Ventilation (Days)

Study or Subgroup	Methylene Blue Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Ibarra-Estrada 2023	4.2	1.4	45	5	1.5	46	82.7%	-0.80 [-1.40, -0.20]
Kirov 2001	3.5	1.8	10	3.1	2.4	10	8.5%	0.40 [-1.46, 2.26]
Memis 2002	7	2	15	8	3	15	8.8%	-1.00 [-2.82, 0.82]
Total (95% CI)			**70**			**71**	**100.0%**	**-0.72 [-1.26, -0.17]**

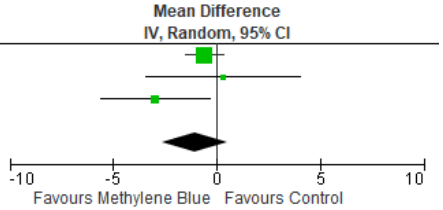
Heterogeneity: Tau² = 0.00; Chi² = 1.55, df = 2 (P = 0.46); I² = 0%
Test for overall effect: Z = 2.59 (P = 0.010)



Intensive Care Unit Length of Stay (Days)

Study or Subgroup	Methylene Blue Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Ibarra-Estrada 2023	6.3	2.1	45	6.9	2.5	46	61.3%	-0.60 [-1.55, 0.35]
Kirov 2001	6.4	4	10	6.1	4.5	10	14.4%	0.30 [-3.43, 4.03]
Memis 2002	13	2	11	16	4	11	24.3%	-3.00 [-5.64, -0.36]
Total (95% CI)			**66**			**67**	**100.0%**	**-1.05 [-2.62, 0.51]**

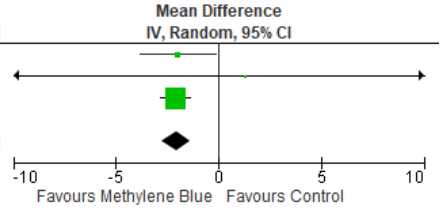
Heterogeneity: Tau² = 0.81; Chi² = 3.17, df = 2 (P = 0.21); I² = 37%
Test for overall effect: Z = 1.32 (P = 0.19)



Hospital Length of Stay (Days)

Study or Subgroup	Methylene Blue Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Ibarra-Estrada 2023	8.2	2.3	45	10.2	6	46	13.6%	-2.00 [-3.86, -0.14]
Kirov 2001	17.4	15.5	10	16.1	15.6	10	0.3%	1.30 [-12.33, 14.93]
Li 2021	6.6	1.2	33	8.7	1.8	33	86.2%	-2.10 [-2.84, -1.36]
Total (95% CI)			**88**			**89**	**100.0%**	**-2.08 [-2.76, -1.39]**

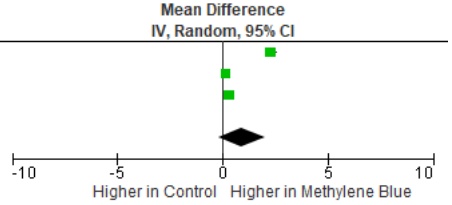
Heterogeneity: Tau² = 0.00; Chi² = 0.25, df = 2 (P = 0.88); I² = 0%
Test for overall effect: Z = 5.94 (P < 0.00001)



Methemoglobin Concentration (%)

Study or Subgroup	Methylene Blue Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Ibarra-Estrada 2023	2.8	0.84	45	0.53	0.23	46	33.1%	2.27 [2.02, 2.52]
Kirov 2001	1.06	0.22	10	0.9	0.05	10	33.5%	0.16 [0.02, 0.30]
Memis 2002	1.03	0.26	11	0.73	0.18	11	33.4%	0.30 [0.11, 0.49]
Total (95% CI)			**66**			**67**	**100.0%**	**0.91 [-0.21, 2.02]**

Heterogeneity: Tau² = 0.97; Chi² = 212.53, df = 2 (P < 0.00001); I² = 99%
Test for overall effect: Z = 1.59 (P = 0.11)



Supplemental Table 4: GRADE Evidence Profile.

Author(s):
Question: Methylene blue compared to usual care for distributive shock
Setting:
Bibliography:

Certainty assessment							№ of patients		Effect		Certainty	Narrative
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	methylene blue	usual care	Relative (95% CI)	Absolute (95% CI)		
Mortality												
5	randomised trials	serious[a]	not serious	not serious	serious[b]	none	33/139 (23.7%)	48/122 (39.3%)	**RR 0.66** (0.47 to 0.94)	**134 fewer per 1,000** (from 209 fewer to 24 fewer)	⨁⨁◯◯ Low	Methylene blue may decrease mortality.
MAP at 6 hours (assessed with: mmHg)												
3	randomised trials	serious[a]	not serious	not serious	serious[b]	none	41	41	-	MD **10.17 mmHg higher** (6.1 higher to 14.24 higher)	⨁⨁◯◯ Low	Methylene blue may increase MAP at 6 hours.
ICU Length of Stay (assessed with: days)												
3	randomised trials	serious[a]	not serious	not serious	serious[d]	none	66	67	-	MD **1.05 days fewer** (2.62 fewer to 0.51 more)	⨁⨁◯◯ Low	Methylene blue may reduce ICU length of stay.
Hospital Length of Stay (assessed with: days)												
3	randomised trials	serious[a]	not serious	not serious	serious[b]	none	88	89	-	MD **2.08 days fewer** (1.39 fewer to 2.76 fewer)	⨁⨁◯◯ Low	Methylene blue may decrease hospital length of stay.
Duration of IMV (assessed with: days)												
3	randomised trials	serious[a]	not serious	not serious	serious[e]	none	70	71	-	MD **0.72 days fewer** (1.26 fewer to 0.17 fewer)	⨁⨁◯◯ Low	Methylene blue may have no effect on duration of IMV.

Certainty assessment							№ of patients		Effect		Certainty	Narrative
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	methylene blue	usual care	Relative (95% CI)	Absolute (95% CI)		
Duration of vasopressors (assessed with: days)												
2	randomised trials	serious[a]	not serious	not serious	serious[b]	none	55	56	-	MD **31.07 hours fewer** (16.54 fewer to 45.6 fewer)	⨁⨁◯◯ Low	Methylene blue may decrease the duration of vasopressors.
MetHgb Concentration (assessed with: percentage)												
3	randomised trials	serious[a]	serious[c]	not serious	serious[d]	none	66	67	-	MD **0.91 percentage higher** (0.21 lower to 2.02 higher)	⨁◯◯◯ Very low	Methylene blue has an uncertain effect on MetHgB concentration.

CI: confidence interval; **MD:** mean difference; **RR:** risk ratio

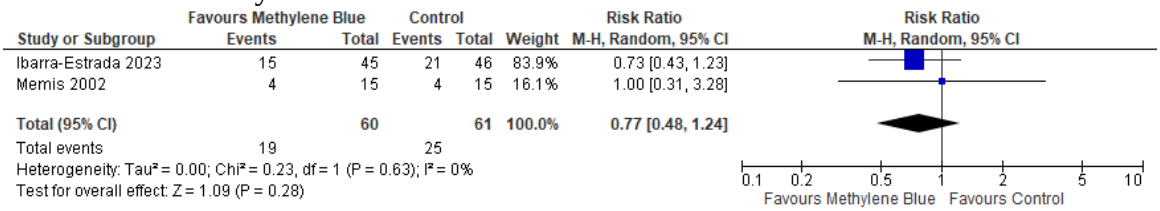
Explanations

a. Majority of evidence comes from studies at high risk of bias
b. small number of patients and events contributes to imprecision
c. Visual inspection of the forest plot suggests important variability amongst included studies
d. Wide confidence intervals that don't exclude harm or benefit
e. Small numbers, and confidence interval doesn't exclude benefit

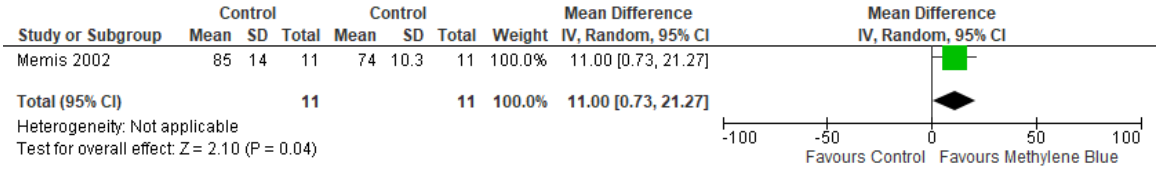
Supplemental Figure 3: Sensitivity Analyses – Forest Plots.

Excluding "High Risk of Bias" Studies

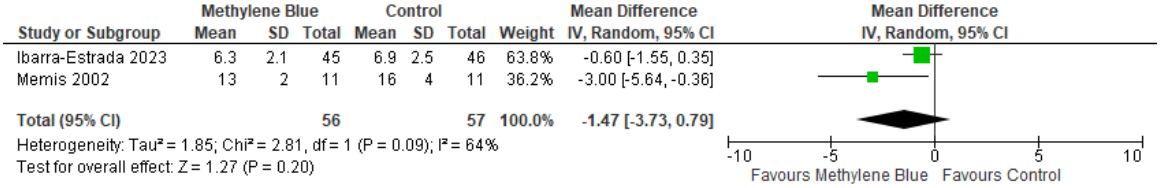
Short-term Mortality



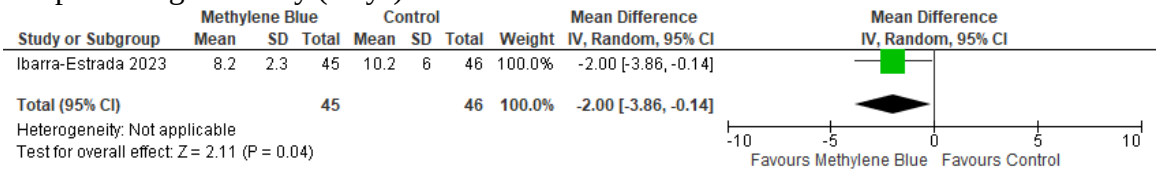
Mean Arterial Pressure at 6 hours (mmHg)



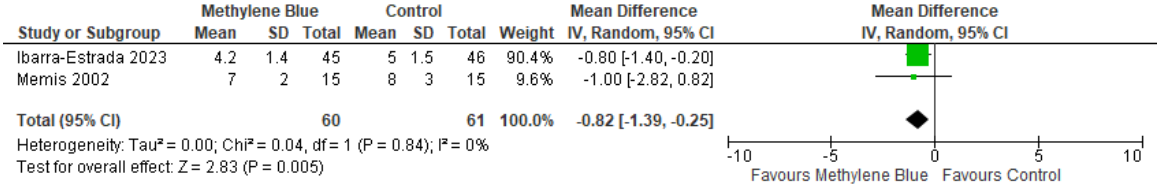
Intensive Care Unit Length of Stay (Days)



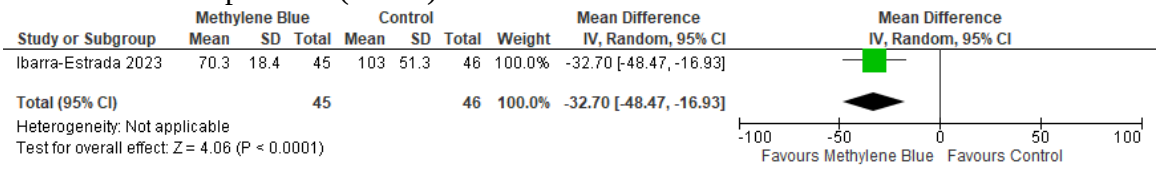
Hospital Length of Stay (Days)



Duration of Invasive Mechanical Ventilation (Days)



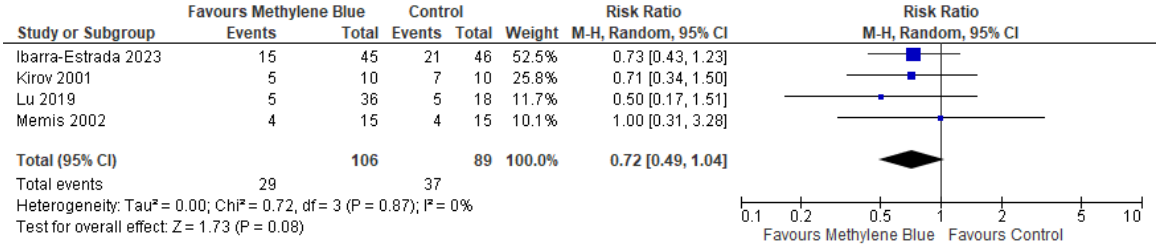
Duration of Vasopressors (Hours)



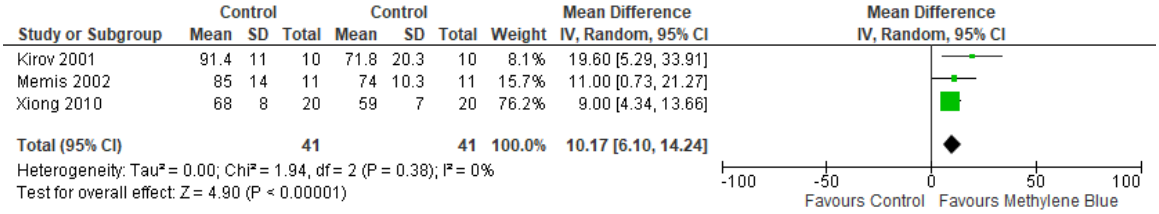
Methylene Blue in Septic Shock – Supplement

Excluding Studies with MB Bolus Only (i.e. without infusion)

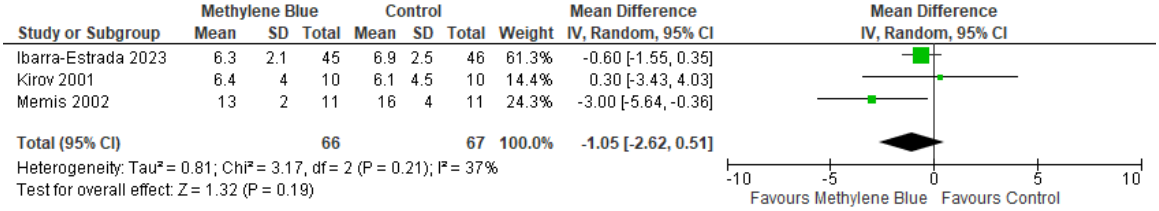
Short-term Mortality



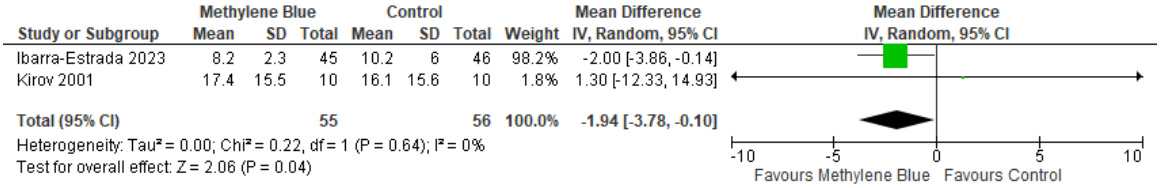
Mean Arterial Pressure (6 Hours)



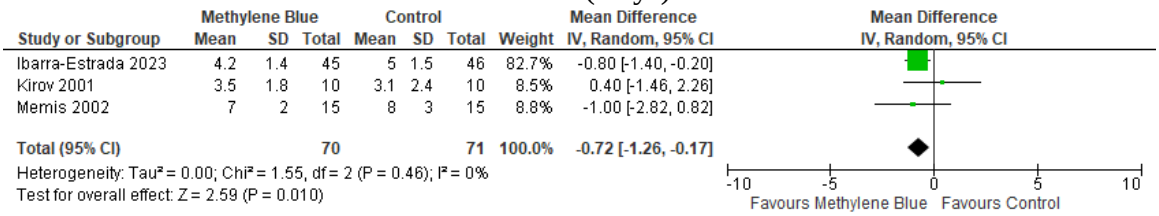
Intensive Care Unit Length of Stay (Days)



Hospital Length of Stay (Days)

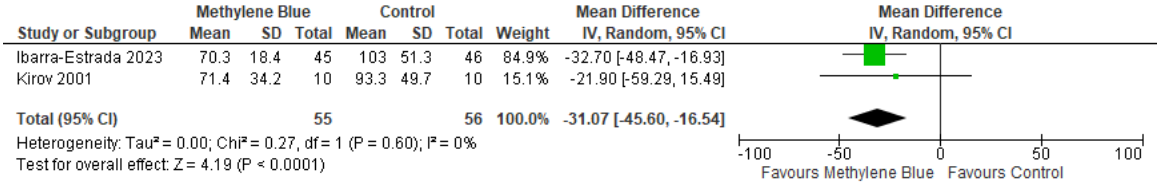


Duration of Invasive Mechanical Ventilation (Days)



Methylene Blue in Septic Shock – Supplement

Duration of Vasopressors (Hours)



Methemoglobin Concentration (%)