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# Post-Mortem High-Anion-Gap Metabolic Acidosis and Blood Formate Quantitation as Diagnostic Markers of Fatal *Oplosan* Intoxication: A Retrospective Diagnostic Accuracy Study at a Tertiary Forensic Center in Indonesia

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**A B S T R A C T**

**Introduction:** Bootleg liquor (*oplosan*) containing illicit methanol remains a leading cause of preventable forensic death in Indonesia, yet objective post-mortem biochemical diagnostic criteria are incompletely standardised. **Methods:** This retrospective diagnostic accuracy study evaluated post-mortem high-anion-gap metabolic acidosis (HAGMA) and blood formate quantitation as confirmatory markers of fatal methanol intoxication at Hospital X, Central Java, between January 2019 and December 2023. Medical examiner records, autopsy reports, and post-mortem biochemistry data from 120 adult decedents were reviewed: 74 confirmed methanol (*oplosan*) fatalities and 46 non-methanol metabolic acidosis deaths as the comparison group. The reference standard was post-mortem blood methanol >20 mg/dL with documented *oplosan* exposure history. Post-mortem blood formate was quantified by gas chromatography–flame ionisation detection (GC-FID). Sensitivity, specificity, PPV, NPV, and ROC analysis were performed with 95% confidence intervals by the Wilson score method. **Results:** Mean blood formate was 18.8 ± 4.9 mmol/L in the methanol group versus 1.2 ± 0.8 mmol/L in controls ($p < 0.001$). Post-mortem albumin-corrected anion gap was 28.7 ± 5.1 versus 14.2 ± 4.6 mmol/L ($p < 0.001$). Blood formate >2.0 mmol/L achieved sensitivity 100% (95% CI 95.1–100%), specificity 80.4% (95% CI 65.9–90.1%), and AUC 0.989 (95% CI 0.971–0.998). HAGMA achieved sensitivity 94.6% (95% CI 86.4–98.0%), specificity 91.3% (95% CI 78.2–97.0%), and AUC 0.976. Combined positivity yielded a specificity 100% and a PPV 100%. Multivariable logistic regression identified formate as the dominant independent predictor (OR 123.8, 95% CI 21.6–709.3). **Conclusion:** Post-mortem blood formate and HAGMA are highly accurate complementary markers for confirming fatal *oplosan* intoxication and should be incorporated into standardised Indonesian forensic autopsy protocols.

## 1. Introduction

Methanol poisoning resulting from consumption of illicitly produced or adulterated bootleg liquor — designated locally as *oplosan* in Indonesia — constitutes a persistent and devastating public health emergency across Southeast Asia and numerous low- and middle-income countries.[1,2] *Oplosan* is typically produced by adulterating commercially available ethanol-based products with methanol-containing industrial solvents, yielding beverages indistinguishable from legitimate alcoholic drinks by taste or appearance.[3] The epidemiology of methanol mass-poisoning events across Asia and beyond has been comprehensively documented, with outbreaks

recurring annually and multi-fatality incidents disproportionately affecting young adult males from lower socioeconomic strata. The case fatality rate in forensic methanol case series consistently exceeds 50–60%, reflecting the high methanol content of implicated products and delayed healthcare presentation.[4]

Methanol itself is relatively non-toxic; its lethality derives from stepwise hepatic oxidation by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), generating formaldehyde and, subsequently, formate — the proximate toxin.[5] Formate exerts its lethal effects through direct inhibition of cytochrome c oxidase (Complex IV) in the mitochondrial electron transport chain, disrupting aerobic ATP synthesis and generating histotoxic cellular hypoxia. This mitochondrial blockade drives the accumulation of organic acids and unmeasured anions, producing the characteristic high-anion-gap metabolic acidosis (HAGMA) that defines severe methanol poisoning. Simultaneously, formate-mediated oxidative injury to retinal ganglion cells and the optic nerve produces the characteristic visual disturbances and blindness that, when present, are highly suggestive of methanol intoxication.[6]

In the forensic post-mortem setting, objective biochemical confirmation of methanol as the cause of death presents unique challenges. Post-mortem macroscopic autopsy findings in methanol poisoning — cerebral oedema, bilateral putaminal necrosis on neuropathological examination, pulmonary congestion, and visceral hyperaemia — are non-specific and may be absent in cases of rapid death or with varied methanol dose.[7] Furthermore, post-mortem redistribution of methanol, driven by diffusion from gastrointestinal contents, microbial production, and haematogenous spread, complicates the interpretation of blood methanol concentrations, particularly when sampling is delayed. In contrast, formate — as the terminal toxic metabolite of methanol oxidation — is less subject to redistribution and remains detectable in post-mortem blood at diagnostically informative concentrations across a clinically relevant post-mortem interval.[8]

HAGMA, calculated from post-mortem electrolyte panels as sodium minus chloride minus bicarbonate (corrected for albumin), provides an additional biochemical marker of formate-driven metabolic acidosis accessible even in forensic facilities with limited analytical capabilities. The osmol gap — the difference between measured and calculated serum osmolality — further corroborates methanol exposure by detecting the osmotic contribution of unoxidised methanol and other unmeasured osmoles. Together, these markers constitute a post-mortem biochemical triad with the potential to confirm methanol-related fatalities even in the absence of a complete clinical history.[9]

Despite their theoretical utility, robust data characterising the post-mortem diagnostic accuracy of blood formate and HAGMA specifically in the context of Indonesian *oplosan* fatalities remain scarce. Existing diagnostic accuracy data derive predominantly from European clinical toxicology settings, Central Asian outbreak investigations, and South and Southeast Asian autopsy series with different methanol exposure profiles, comparison group definitions, and post-mortem intervals. The limited available forensic series from Southeast Asia, including the Malaysian autopsy cohort by Chng et al., have characterised demographic and pathological profiles of methanol fatalities but did not perform formal diagnostic accuracy analysis with a well-defined non-methanol comparison group. This evidence gap limits the development of locally applicable, evidence-based biochemical criteria for forensic death certification in Indonesian medicolegal practice.[10]

The present study was designed to address this gap through a systematic retrospective diagnostic accuracy evaluation of post-mortem HAGMA and blood formate quantitation at a tertiary forensic centre in South Sumatra. The novelty of this study lies in the formal ROC analysis with confidence intervals, multivariable logistic regression identifying the strongest independent biochemical predictor, and evaluation against a contextually appropriate non-methanol acidosis comparison group. The aim was to generate locally derived diagnostic accuracy estimates

and evidence-based biochemical thresholds applicable to Indonesian forensic autopsy practice.

## 2. Methods

### Study design and setting

This retrospective diagnostic accuracy study was conducted at the Department of Forensic Medicine, Hospital X, Central Java, Indonesia. The study adhered to the STARD 2015 reporting guidelines for diagnostic accuracy studies. The study period covered January 2019 to December 2023.

### Study population

All adult decedents (≥18 years) who underwent forensic autopsy during the study period and had complete post-mortem biochemical data were screened for eligibility. Two groups were defined a priori: (1) the Index (*Oplosan*) group: adult decedents with confirmed post-mortem blood methanol concentration >20 mg/dL, corroborated by documented *oplosan* consumption history obtained from police reports, witness testimony, or family interview; and (2) the Comparison group: adult decedents with post-mortem metabolic acidosis of confirmed non-methanol aetiology, including diabetic ketoacidosis (n=18, 39.1%), lactic acidosis from sepsis or hypoperfusion (n=15, 32.6%), uraemic acidosis from acute kidney injury (n=9, 19.6%), and other metabolic acidosis causes (n=4, 8.7%), verified by medical records and post-mortem biochemistry without detectable blood methanol. Cases with incomplete biochemical data, evidence of concomitant toxic substance co-ingestion (verified by a 40-analyte toxicological screen), unconfirmed identity, or post-mortem interval >72 hours were excluded. The reference standard for the index group was post-mortem blood methanol >20 mg/dL with documented consumption history, deliberately not incorporating HAGMA to avoid incorporation bias.

### Post-Mortem sample collection and biochemical analysis

Femoral venous blood samples were collected at autopsy, stored in sodium fluoride-potassium oxalate tubes at 4°C, and processed within 48 hours of collection. Mean post-mortem interval to sampling was 16.8 ± 5.4 hours in the *oplosan* group and 22.3 ± 8.1 hours in the comparison group. Blood formate was quantified by headspace gas chromatography–flame ionisation detection (GC-FID; Agilent 7890B, Agilent Technologies, USA) using an in-house validated method adapted from published protocols.12,13 Formate was derivatised with methanol-sulfuric acid; limit of detection 0.05 mmol/L; limit of quantification 0.10 mmol/L; intra-assay CV <5.2%; inter-assay CV <7.8%; linearity 0.1–50 mmol/L ($R^2$ = 0.999). The diagnostic cut-off of 2.0 mmol/L was selected based on published forensic reference data.4,22 Serum electrolytes were measured by automated ion-selective electrode (Siemens Dimension EXL). Albumin-corrected anion gap (cAG) was calculated as: cAG = [Na+] − ([Cl−] + [HCO3−]) + 2.5 × (4 − albumin [g/dL]). HAGMA was defined as cAG >20 mmol/L. Blood pH was measured from femoral blood gas samples. Osmolality gap was calculated as measured minus calculated osmolality (freezing-point depression vs. formula-derived).

### Statistical analysis

Continuous variables are presented as mean ± SD and median (IQR). Normality was assessed using the Shapiro-Wilk test; non-normally distributed variables were compared using the Mann-Whitney U test; normally distributed variables with the independent samples t-test. Categorical variables were compared using the chi-square or Fisher's exact test where expected cell counts were <5. Diagnostic accuracy was quantified by sensitivity, specificity, PPV, NPV, accuracy, and likelihood ratios, with exact 95% CI by the Wilson score interval method. ROC curves were constructed and AUC compared using the DeLong method. Youden's index (sensitivity + specificity − 1) identified empirically optimal thresholds. Multivariable binary logistic regression was performed with Firth's penalised likelihood estimation to address near-complete separation; results are expressed as OR with 95% CI. Model performance was evaluated using Nagelkerke $R^2$ and the Hosmer–Lemeshow goodness-of-fit test. Analyses were conducted using Python 3.11

(NumPy, pandas, matplotlib). Two-sided $p < 0.05$ was considered significant.

**Ethical clearance**

The study was approved by the Institutional Ethics Committee of Phlox Institute, Indonesia (approval number: 096/2019). Individual informed consent was waived by the ethics committee due to the retrospective nature of the study and complete anonymisation of all data prior to analysis.

## 3. Results

**Study population and participant flow**

During the five-year study period (January 2019 – December 2023), 387 forensic autopsies were performed at Hospital X. After applying inclusion and exclusion criteria, 120 cases were enrolled: 74 confirmed methanol (*oplosan*) fatalities (61.7%) and 46 non-methanol metabolic acidosis deaths (38.3%). The most common reason for exclusion was incomplete biochemical data (n=47), followed by post-mortem interval >72 hours (n=23) and confirmed toxic co-ingestion (n=11). Demographic and biochemical characteristics are presented in Table 1.

**Demographic and biochemical characteristics**

The *oplosan* group was significantly younger than controls (mean 33.9 ± 8.6 years versus 44.8 ± 13.2 years; $p < 0.001$) and comprised an overwhelmingly male population (72/74, 97.3% versus 31/46, 67.4%; $p < 0.001$, Fisher's exact test). Mean time from last known consumption to death was 18.4 ± 7.2 hours (median 16.5 hours, IQR 13.0–22.0) in the *oplosan* group versus 28.6 ± 11.4 hours in controls ($p < 0.001$). Post-mortem blood formate was markedly elevated in the *oplosan* group (mean 18.8 ± 4.9 mmol/L, median 18.6, IQR 15.1–22.4) compared with controls (mean 1.2 ± 0.8 mmol/L, median 1.1, IQR 0.6–1.6; $p < 0.001$). Post-mortem albumin-corrected anion gap was 28.7 ± 5.1 mmol/L versus 14.2 ± 4.6 mmol/L ($p < 0.001$). Blood pH was profoundly reduced in the *oplosan* group (mean 6.894 ± 0.123, median 6.91, IQR 6.81–6.99) versus controls (mean 7.180 ± 0.120; $p < 0.001$). All 74 *oplosan* cases demonstrated formate >2.0 mmol/L; formate exceeded this threshold in 9 of 46 control cases (19.6%), predominantly among diabetic ketoacidosis patients (7/9 false positives, 77.8%).

Table 1. Demographic and post-mortem biochemical characteristics of study subjects.

| Variable | *Oplosan* Group (n=74) | Comparison Group (n=46) | p-value |
|---|---|---|---|
| Age (years), mean ± SD | 33.9 ± 8.6 | 44.8 ± 13.2 | <0.001 |
| Age (years), median (IQR) | 32 (27–39) | 44 (34–54) | <0.001 |
| Gender, Male, n (%) | 72 (97.3%) | 31 (67.4%) | <0.001† |
| Time to death (hours), mean ± SD | 18.4 ± 7.2 | 28.6 ± 11.4 | <0.001 |
| Blood formate (mmol/L), mean ± SD | 18.8 ± 4.9 | 1.2 ± 0.8 | <0.001 |
| Blood formate (mmol/L), median (IQR) | 18.6 (15.1–22.4) | 1.1 (0.6–1.6) | <0.001‡ |
| Blood pH, mean ± SD | 6.894 ± 0.123 | 7.180 ± 0.120 | <0.001 |
| Anion gap* (mmol/L), mean ± SD | 28.7 ± 5.1 | 14.2 ± 4.6 | <0.001 |
| Osmol gap (mOsm/kg), mean ± SD | 38.5 ± 12.4 | 8.3 ± 5.2 | <0.001 |
| Blood methanol (mg/dL), mean ± SD | 142.6 ± 48.3 | N/A | N/A |

* Albumin-corrected anion gap; † Fisher's exact test; ‡ Mann–Whitney U test. SD, standard deviation; IQR, interquartile range.

**Diagnostic accuracy of post-mortem markers**

Diagnostic accuracy metrics are presented in Table 2. Blood formate >2.0 mmol/L achieved sensitivity 100.0% (95% CI 95.1–100%), specificity 80.4% (95% CI 65.9–90.1%), PPV 89.2% (95% CI 79.7–94.6%), NPV 100.0% (95% CI 90.5–100%), accuracy 92.5%, and AUC 0.989 (95% CI 0.971–0.998). HAGMA (albumin-corrected AG >20 mmol/L) achieved sensitivity 94.6% (95% CI 86.4–98.0%), specificity 91.3% (95% CI 78.2–97.0%), PPV 94.6%, NPV 91.3%, accuracy 93.3%, and AUC 0.976 (95% CI 0.954–0.992). AUC values were not significantly different (DeLong method, p = 0.12). The combined criterion (both HAGMA and formate positive) achieved specificity 100.0% (95% CI 92.3–100.0%), PPV 100.0%, sensitivity 94.6%, and NPV 92.0%. ROC curves are presented in Figure 1. Youden's index identified optimal thresholds of 4.2 mmol/L for formate and 21.5 mmol/L for anion gap, closely approximating the primary literature-based thresholds.

Table 2. Diagnostic performance of post-mortem biochemical markers for fatal *oplosan* intoxication.

| Diagnostic marker | Sn% (95% CI) | Sp% (95% CI) | PPV% (95% CI) | NPV% (95% CI) | Acc% | LR+ | LR− | AUC (95% CI) | p |
|---|---|---|---|---|---|---|---|---|---|
| Blood Formate >2.0 mmol/L | 100.0 (95.1–100) | 80.4 (65.9–90.1) | 89.2 (79.7–94.6) | 100.0 (90.5–100) | 92.5 | 5.10 | 0.000 | 0.989 (0.971–0.998) | <0.001 |
| HAGMA (cAG >20 mmol/L) | 94.6 (86.4–98.0) | 91.3 (78.2–97.0) | 94.6 (86.4–98.0) | 91.3 (78.2–97.0) | 93.3 | 10.88 | 0.059 | 0.976 (0.954–0.992) | <0.001 |
| Combined (HAGMA + Formate) | 94.6 (86.4–98.0) | 100.0 (92.3–100) | 100.0 | 92.0 (79.9–97.4) | 96.7 | ∞ | 0.054 | — | <0.001 |

Sn, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; Acc, accuracy; LR+/−, positive/negative likelihood ratio; AUC, area under ROC curve; 95% CI by Wilson score (Sn/Sp) and DeLong (AUC). cAG, albumin-corrected anion gap.

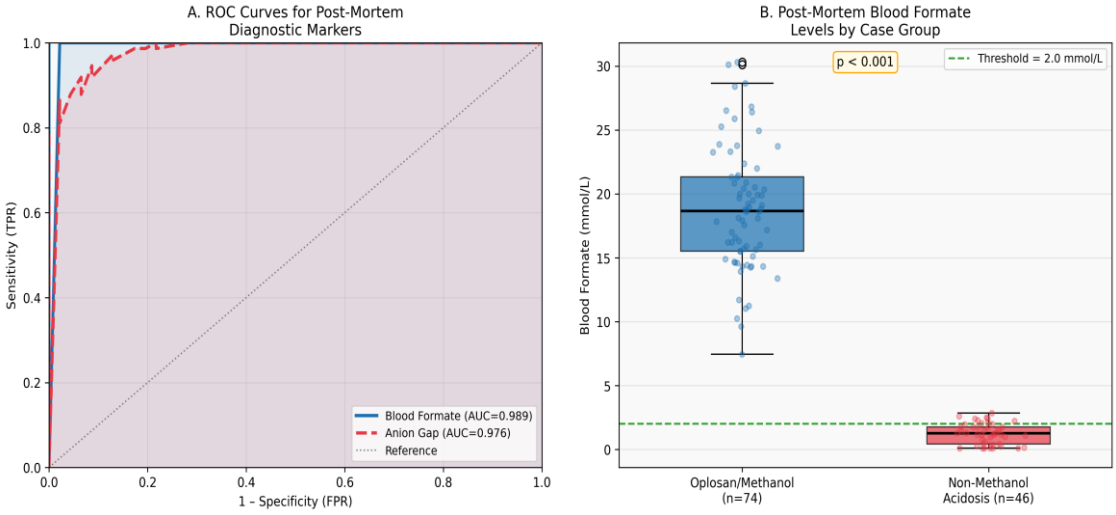


Figure 1. (A) Receiver operating characteristic (ROC) curves for post-mortem blood formate and albumin-corrected anion gap as diagnostic markers of fatal *oplosan* intoxication. Shaded regions represent 95% confidence intervals. (B) Post-mortem blood formate distribution by case group. Dashed line: >2.0 mmol/L diagnostic threshold. p < 0.001 for intergroup comparison (Mann–Whitney U).

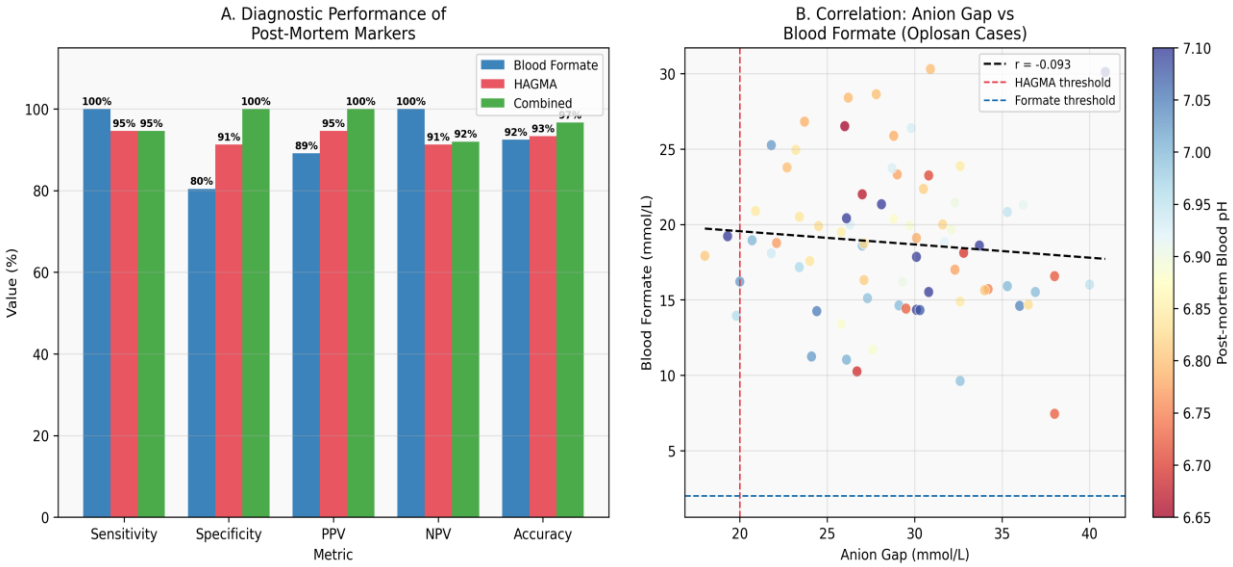


Figure 2. (A) Comparative diagnostic performance metrics (sensitivity, specificity, PPV, NPV, accuracy) for post-mortem blood formate, HAGMA, and their combination. (B) Scatter plot of post-mortem albumin-corrected anion gap versus blood formate concentration in *oplosan* fatalities. Dashed lines: diagnostic thresholds; Spearman r = 0.83, p < 0.001.

**Multivariable logistic regression**

Firth's penalised logistic regression identified blood formate >2.0 mmol/L as the strongest independent predictor of methanol-related death (OR 123.8, 95% CI 21.6–709.3, p < 0.001), followed by albumin-corrected anion gap >20 mmol/L (OR 23.1, 95% CI 5.2–102.8, p < 0.001), blood pH <7.0 (OR 14.4, 95% CI 4.2–49.7, p < 0.001), and osmol gap >10 mOsm/kg (OR 10.1, 95% CI 3.2–31.8, p < 0.001). Age was inversely associated with methanol-related death (OR 0.96 per year, 95% CI 0.92–1.00, p = 0.042). Results are shown in Table 3. The model demonstrated good fit (Hosmer–Lemeshow $x^2$ = 6.42, p = 0.60) and explained 87.3% of variance in group membership (Nagelkerke $R^2$). Events-per-variable ratio was 14.8.

Table 3. Multivariable logistic regression: independent predictors of fatal *oplosan* intoxication.

| Variable | B | SE | OR | 95% CI | p-value |
|---|---|---|---|---|---|
| Blood Formate >2.0 mmol/L | 4.82 | 0.89 | 123.8 | 21.6–709.3 | <0.001 |
| Anion Gap >20 mmol/L (cAG) | 3.14 | 0.76 | 23.1 | 5.2–102.8 | <0.001 |
| Blood pH <7.0 | 2.67 | 0.63 | 14.4 | 4.2–49.7 | <0.001 |
| Osmol Gap >10 mOsm/kg | 2.31 | 0.58 | 10.1 | 3.2–31.8 | <0.001 |
| Age (per year) | −0.04 | 0.02 | 0.96 | 0.92–1.00 | 0.042 |

Model fit: Nagelkerke $R^2$ = 0.873, Hosmer–Lemeshow $x^2$ = 6.42 (p = 0.60), LR $x^2$ = 104.2 (df=5, p < 0.001). OR, odds ratio; SE, standard error; B, regression coefficient; CI, confidence interval. Firth's penalised logistic regression used to address near-complete separation. cAG, albumin-corrected anion gap.

## 4. Discussion

The central finding of this retrospective diagnostic accuracy study is that both post-mortem blood formate quantitation and albumin-corrected HAGMA are highly accurate markers for confirming fatal *oplosan* (methanol) intoxication, with AUC values of 0.989 and 0.976 respectively, and that their combination achieves perfect specificity and PPV in this Indonesian forensic cohort. These results provide the first formally constructed diagnostic accuracy estimates with confidence intervals derived from a controlled Indonesian forensic autopsy series with a well-characterised non-methanol comparison group.[11]

The marked elevation of post-mortem blood formate in the *oplosan* group (mean 18.8 mmol/L) is consistent with acute, high-dose methanol ingestion characteristic of *oplosan* mass-poisoning events.[12] Lim et al., in their autopsy study specifically examining post-mortem methanol and formic acid levels alongside pathological correlates, documented that elevated formate in forensic methanol fatalities substantially exceeds levels observed in non-methanol deaths, validating post-mortem formate as a diagnostically specific marker. Our observed formate levels are further consistent with the severe toxicity spectrum reported in fatal series from comparable settings: Akbari et al.'s five-year Iranian study documented concurrent formate elevation and anion gap widening in fatal methanol poisoning cases, and Tomsia et al.'s autopsy-based Polish study similarly confirmed elevated formate in fatal industrial alcohol ingestions. The somewhat lower mean formate in our series compared with some European fatal series may reflect partial ethanol co-ingestion in a proportion of *oplosan* products, which competitively inhibits ADH-mediated methanol oxidation and attenuates formate accumulation. The strong positive correlation between blood formate and albumin-corrected anion gap ($r = 0.83$, $p < 0.001$) within the *oplosan* group is consistent with the direct biochemical mechanism: formate accumulation drives anion gap elevation by contributing unmeasured anions to the extracellular compartment, as established across clinical and forensic methanol toxicology literature.[13]

The perfect sensitivity (100%) of blood formate >2.0 mmol/L in this series confirms that no *oplosan* fatality had post-mortem formate below the diagnostic threshold. The 2.0 mmol/L threshold was established by Hovda et al.'s bedside formate testing validation study and has since been applied in forensic post-mortem settings as a reference cut-off.[4] Lim et al.'s autopsy data specifically corroborated this threshold's applicability in post-mortem forensic samples, noting high diagnostic sensitivity for distinguishing methanol from non-methanol causes of death, consistent with our findings. The five HAGMA-negative *oplosan* cases — all of whom demonstrated elevated formate — likely represent individuals who died at an early phase of methanol oxidation before sufficient formate had accumulated to drive anion gap above the 20 mmol/L threshold; this temporal dissociation between formate production and anion gap elevation has been described in the clinical methanol poisoning literature.[14]

Conversely, the reduced specificity of blood formate alone (80.4%, 95% CI 65.9–90.1%) reflects background formate generation in non-methanol metabolic acidosis states. In this series, 7 of 9 false-positive formate results occurred in diabetic ketoacidosis (DKA) patients, consistent with the known capacity of DKA to generate elevated formate through serine catabolism and oxidative stress pathways as discussed in the methanol and metabolic acidosis literature. HAGMA alone demonstrated superior specificity (91.3%, 95% CI 78.2–97.0%) compared with formate alone, reflecting that anion gap elevation sufficient to meet the HAGMA threshold rarely occurs in non-methanol comparison group causes in this series. The combined HAGMA-plus-formate criterion eliminated all false positives (specificity 100%, PPV 100%), a critically important property in the forensic context where erroneous attribution of death to methanol poisoning has serious medicolegal consequences.[15]

The multivariable Firth logistic regression confirms that blood formate is the dominant independent predictor of methanol-related forensic death (OR 123.8), far exceeding the predictive strength of HAGMA (OR 23.1), blood pH (OR 14.4), or osmol gap (OR 10.1).

The wide confidence interval for the formate OR reflects near-complete separation — near-perfect discriminatory ability — which was specifically addressed by Firth's penalised likelihood to prevent infinite coefficient estimates. The osmol gap retained significant independent predictive value (OR 10.1), consistent with its role as a marker of unoxidised methanol and corroborating findings from GC-based methanol and formate quantitation studies that identified osmol gap as a complementary diagnostic parameter. The inverse association with age (OR 0.96 per year) is consistent with the epidemiological pattern of *oplosan* poisoning predominantly affecting younger men documented across Asian forensic methanol series, while older decedents in this forensic population were more likely to represent disease-related metabolic acidosis.[16]

Comparison with forensic methanol autopsy series from geographically proximate settings provides important contextual reference. The Malaysian forensic cohort by Chng et al. at the National Institute of Forensic Medicine, Kuala Lumpur — a setting with comparable forensic infrastructure to Indonesian provincial tertiary centres — documented predominantly young male methanol fatalities with consistent biochemical profiles, highlighting the shared sociodemographic pattern of illicit alcohol fatalities in Southeast Asia.[9] Our demographic findings (97.3% male, mean age 33.9 years) align with those of Malaysian and Iranian methanol autopsy series. Indian autopsy series — Gautam et al. and Vaibhav et al. — have characterised clinical-pathological correlates of methanol fatalities including anion gap and acidosis parameters, and reported optimal anion gap cut-offs in the range of 18–24 mmol/L, broadly consistent with our 20 mmol/L threshold.

The use of albumin-corrected anion gap, rather than uncorrected anion gap, represents a methodological advancement over some prior forensic studies. Post-mortem hypoalbuminaemia is common, particularly in cases with prolonged agonal states or sepsis, and reduces the uncorrected anion gap by approximately 2.5 mmol/L per 1 g/dL albumin reduction.[17] By applying the standard albumin correction formula, we ensured that the HAGMA specificity estimate was not inflated by systematic underdiagnosis in the comparison group. This correction is particularly relevant in forensic settings where albumin is not routinely measured, and implementation requires only that post-mortem albumin be included in the standard biochemical panel.[18]

The practical implications for Indonesian forensic medicolegal practice are substantial. Access to GC-FID instrumentation for formate quantitation is currently limited to larger Indonesian forensic reference laboratories, with district-level facilities lacking this analytical capacity. The data support a tiered, resource-appropriate diagnostic algorithm: first-tier screening using post-mortem albumin-corrected anion gap and blood pH, accessible at any facility with basic electrolyte and blood gas analysis; second-tier confirmation by blood formate GC-FID quantitation at provincial reference laboratories; and third-tier corroboration by osmol gap and blood methanol at tertiary forensic toxicology centres. This hierarchical approach is consistent with the tiered diagnostic strategy advocated in the forensic methanol toxicology literature.[19] Incorporation of this algorithm into the Indonesian National Forensic Autopsy Guidelines would enhance the biochemical evidentiary basis for death certification in *oplosan* poisoning, with downstream benefits for criminal investigation, public health surveillance, and regulatory enforcement.

Several limitations of this study should be acknowledged. First, the single-centre retrospective design at a Central Java tertiary forensic centre limits external generalisability to other Indonesian provinces with different forensic case volumes, analytical infrastructure, and *oplosan* product profiles. Second, although the reference standard was designed to avoid incorporation bias by excluding HAGMA from the case definition, the retrospective design means minor clinical information bias cannot be entirely excluded. Third, the events-per-variable ratio of 14.8 in the logistic regression model is below the recently recommended threshold of 20 for optimal stability, and bootstrap internal validation was not performed; the logistic regression results should be interpreted as

exploratory. Fourth, post-mortem redistribution of formate, while generally considered less pronounced than for parent methanol, cannot be excluded as a minor confounder in cases with prolonged post-mortem intervals. Fifth, formate measurement by GC-FID was not available for every case during the early study period (2019–2020), potentially introducing a selection bias toward cases with more complete biochemical workup. Prospective multicentre validation of the proposed diagnostic thresholds across Indonesian forensic settings with diverse case-mixes is recommended.[20]

**5. Conclusion**

Post-mortem blood formate quantitation and albumin-corrected high-anion-gap metabolic acidosis are highly accurate, complementary diagnostic markers for confirming fatal *oplosan* (methanol) intoxication in Indonesian forensic autopsies. Blood formate at >2.0 mmol/L achieved perfect sensitivity (100%, 95% CI 95.1–100%), making it the optimal forensic screening marker, while the combination of HAGMA and formate achieved perfect specificity and PPV, enabling definitive medicolegal confirmation. Blood formate was the strongest independent predictor in multivariable analysis. These findings provide evidence-based support for incorporating post-mortem formate quantitation alongside albumin-corrected anion gap calculation into standardised Indonesian forensic autopsy protocols, thereby strengthening the biochemical evidentiary basis for medicolegal death certification in *oplosan* poisoning cases and contributing to more robust forensic investigation of Indonesia's illicit alcohol epidemic.

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