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Original Article

# Comparative Assessment of Rapid Antigen Test Kits for Forensic Detection of Narcotic Substances in Blood and Urine Samples

Md. Ali Mazrui Rahman[1], Dipa Roy [2], Md. Ariful Haque [3], Taznin Jahan [4]

[1]Resident Surgeon, Rajshahi Medical College Hospital, Rajshahi, Bangladesh
[2]Post Doctoral Fellow, Institute of Biological Sciences, University of Rajshahi, Bangladesh
[3]Associate Professor, Institute of Biological Sciences, University of Rajshahi, Bangladesh
[4]Assistant Surgeon, Upazila Health Complex, Durgapur, Rajshahi, Bangladesh

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## Corresponding author

Dr. Md. Ariful Haque,
Associate Professor, Institute of Biological Sciences,
University of Rajshahi, Rajshahi, Bangladesh
Email: haque@ru.ac.bd

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## Abstract

***Background:*** *Illicit narcotic substance use is an important public health and medico-legal problem, requiring reliable detection in biological samples during forensic investigations. Rapid antigen test kits are simple, quick, and practical for preliminary screening, particularly in resource-limited settings, but their performance may vary between blood and urine samples.*

***Objective:*** *This study aimed to compare the detection pattern and diagnostic performance of rapid antigen test kits for narcotic substances in blood and urine samples among suspected narcotic exposure cases.*

***Methods:*** *This comparative cross-sectional study was conducted at the Department of Forensic Medicine, Rajshahi Medical College Hospital, Bangladesh, from July 2019 to July 2020. A total of 36 suspected narcotic exposure cases were included. Blood and urine samples were collected, preserved, and tested using rapid antigen test kits for commonly abused narcotic substances. Results were compared with available confirmatory forensic methods, including GC-MS, LC-MS/MS, or HPLC. Diagnostic performance was assessed by sensitivity, specificity, predictive values, accuracy, agreement rate, and kappa statistics.*

***Results:*** *Among 36 forensic cases, urine samples were more common (20; 55.6%) than blood samples (16; 44.4%). Methamphetamine/Yaba was the most frequently detected substance (12; 33.3%), followed by cannabis/THC (7; 19.4%) and opiates/codeine-related substances (6; 16.7%). Overall, 30 (83.3%) of samples were positive by rapid antigen test, with slightly higher positivity in urine (85.0%) than in blood (81.3%). Among 35 valid samples, sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 93.1%, 50.0%, 90.0%, 60.0%, and 85.7%, respectively. Overall agreement with confirmatory methods was 85.7%, with moderately significant agreement (kappa = 0.46, p = 0.006).*

***Conclusion:*** *Rapid antigen test kits are useful for preliminary forensic narcotic screening, especially in urine samples, but all medico-legally important results should be confirmed by GC-MS, LC-MS/MS, or HPLC before final reporting.*

**Keywords:** Rapid antigen test, Narcotic substances, Forensic toxicology and Confirmatory testing.

## Introduction

Illicit narcotic substance use is a major public health, clinical, and medico-legal concern worldwide. Drug use disorders contribute substantially to morbidity, mortality, violence, accidental injury, psychiatric illness, impaired judgment, unsafe behavior, and criminal activity[1]. In forensic practice, accurate detection of narcotic substances in biological samples is essential for investigating suspected poisoning, intoxication, drug-facilitated crime, road traffic incidents, custodial cases, unnatural deaths, and other medico-legal conditions. Blood and urine are commonly used biological matrices because blood may reflect more recent exposure, while urine often provides a wider detection window for many drugs and metabolites[2,3].

Globally, narcotic use patterns are changing with the increasing availability of synthetic drugs, amphetamine-type stimulants, opioids, cannabis products, benzodiazepines, and new psychoactive substances[1,4]. These changes have created new challenges for forensic laboratories, especially in low- and middle-income countries where advanced confirmatory facilities may not always be readily available. In Bangladesh, substance abuse has become an increasing social and health problem, particularly among young adults. Studies from Bangladesh have reported the use of heroin, cannabis, yaba or methamphetamine, sedatives, and multiple substances among treatment-seeking and community-based populations[5,6]. Yaba, a tablet form commonly containing methamphetamine and caffeine, has received particular attention because of its increasing use among young people and its association with behavioral, social, and criminal consequences[6,7].

Rapid antigen or immunochromatographic test kits are widely used as preliminary screening tools because they are simple, inexpensive, quick, and suitable for settings with limited laboratory infrastructure[2,8]. These kits can provide immediate presumptive evidence of narcotic exposure and may help guide early forensic decisions. However, rapid screening tests are not confirmatory, and their performance may be affected by drug concentration, cut-off values, cross-reactivity, sample quality, timing of sample collection, adulteration, and the type of biological specimen tested[3,8,9]. Therefore, positive or doubtful screening results should ideally be verified by confirmatory analytical techniques such as gas chromatography, mass spectrometry, GC-MS, liquid chromatography, tandem mass spectrometry, LC-MS/MS, or high-performance liquid chromatography, HPLC[4,10].

Although advanced analytical methods provide higher sensitivity and specificity, they require costly instruments, skilled personnel, longer processing time, and well-equipped forensic laboratories[4,10]. In contrast, rapid test kits are more practical for initial screening in routine medico-legal settings. However, local evidence remains limited regarding their comparative performance in blood and urine samples in Bangladesh, underscoring the need for this comparison. There is also insufficient information on detection rates, invalid results, diagnostic agreement, and substance-wise performance of rapid kits in routine forensic practice.

Therefore, the present study was designed to assess the forensic usefulness of rapid antigen test kits for detecting narcotic substances in blood and urine samples. The study aimed to compare the detection pattern and diagnostic performance of rapid antigen test kits in blood and urine samples among suspected narcotic exposure cases at the Department of Forensic Medicine, Rajshahi Medical College Hospital, Bangladesh.

## Methods

This comparative cross-sectional study was conducted in the Department of Forensic Medicine, Rajshahi Medical College Hospital, Rajshahi, Bangladesh, from July 2019 to July 2020. A total of 36 suspected narcotic exposure cases were included in the study. Blood and

urine samples were collected from suspected cases referred for forensic examination, in accordance with standard medico-legal procedures. Cases with adequate sample volume, proper labeling, and complete medico-legal documentation were included. Decomposed, contaminated, improperly preserved, or insufficient samples were excluded.

Blood samples were collected in clean, sterile containers with appropriate preservative where required, while urine samples were collected in sterile containers. All samples were labeled, sealed, and preserved, maintaining chain-of-custody principles. Each sample was tested using commercially available rapid antigen test kits for commonly abused narcotic substances, including methamphetamine/Yaba, cannabis/THC, opiates/codeine-related substances, benzodiazepines, and cocaine. Test procedures were performed according to the manufacturer's instructions. Results were recorded as positive, negative, or invalid. Invalid tests were repeated where possible, and unresolved invalid results were excluded from diagnostic accuracy analysis.

Rapid antigen test results were compared with confirmatory forensic testing, such as GC-MS, LC-MS/MS, HPLC, or other standard laboratory-based chemical analyses, as available. Sensitivity, specificity, positive predictive value, negative predictive value, accuracy, and agreement rate were calculated. Data were checked, coded, and analyzed using standard statistical methods. Categorical variables were expressed as frequency and percentage. Agreement between rapid antigen test kits and confirmatory methods was assessed using the kappa statistic; p-values <0.05 were considered statistically significant. Ethical approval was obtained from the concerned authority, and confidentiality of all medico-legal records was maintained.

## Results

Table 1 shows the distribution of biological samples tested in the study. Among 36 forensic cases, urine samples were slightly more common (20; 55.6%) than blood samples (16; 44.4%).

**Table 1.** Distribution of biological samples tested

| Sample type | Number of samples tested (n) | Percentage (%) |
|---|---|---|
| Blood | 16 | 44.4 |
| Urine | 20 | 55.6 |
| Total | 36 | 100 |
| Sample type | Number of samples tested (n) | Percentage (%) |

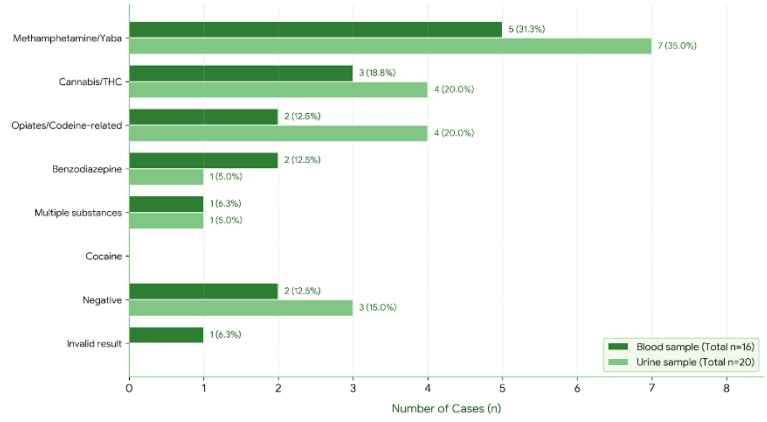



**Figure 1: Distribution of suspected narcotic substances detected by rapid antigen test kit**

Figure 1 presents the distribution of suspected narcotic substances detected by the rapid antigen test kit. Methamphetamine/Yaba was the most frequently detected substance (12; 33.3%), followed by cannabis/THC, 7 (19.4%), and opiates/codeine-related substances, 6 (16.7%). No cocaine-positive case was detected.

**Table 2.** Rapid antigen test kit results according to sample type

| Sample type | Positive n (%) | Negative n (%) | Invalid n (%) | Total |
|---|---|---|---|---|
| Blood | 13 (81.3) | 2 (12.5) | 1 (6.3) | 16 |
| Urine | 17 (85.0) | 3 (15.0) | 0 (0.0) | 20 |
| Total | 30 (83.3) | 5 (13.9) | 1 (2.8) | 36 |

Table 2 shows the results of rapid antigen test kits by sample type. Overall, 30 (83.3%) samples were positive, 5 (13.9%) were negative, and 1 (2.8%) was invalid. Positivity was slightly higher in urine samples (17; 85.0%) than in blood samples (13; 81.3%).

**Table 3.** Comparison of rapid antigen test kit with confirmatory forensic method

| Rapid antigen test result | Confirmatory positive (n) | Confirmatory negative (n) | Total |
|---|---|---|---|
| Positive | 27 | 3 | 30 |
| Negative | 2 | 3 | 5 |
| Total | 29 | 6 | 35 |

Table 3 compares rapid antigen test kit results with the confirmatory forensic method. Among 35 valid samples, 27 were true positives, and 3 were true negatives. False-positive results were found in 3 samples, while false-negative results were observed in 2 samples.

**Table 4.** Diagnostic performance of rapid antigen test kit by sample type

| Performance indicator | Blood samples (%) | Urine samples (%) | Overall (%) |
|---|---|---|---|
| Sensitivity | 92.3 | 93.8 | 93.1 |
| Specificity | 50 | 50 | 50 |
| Positive predictive value | 92.3 | 88.2 | 90 |
| Negative predictive value | 50 | 66.7 | 60 |

Table 4 shows the diagnostic performance of the rapid antigen test kit. The overall sensitivity was 93.1%, specificity was 50.0%, positive predictive value was 90.0%, negative predictive value was 60.0%, and overall accuracy was 85.7%. Sensitivity was slightly higher in urine samples (93.8%) than in blood samples (92.3%).

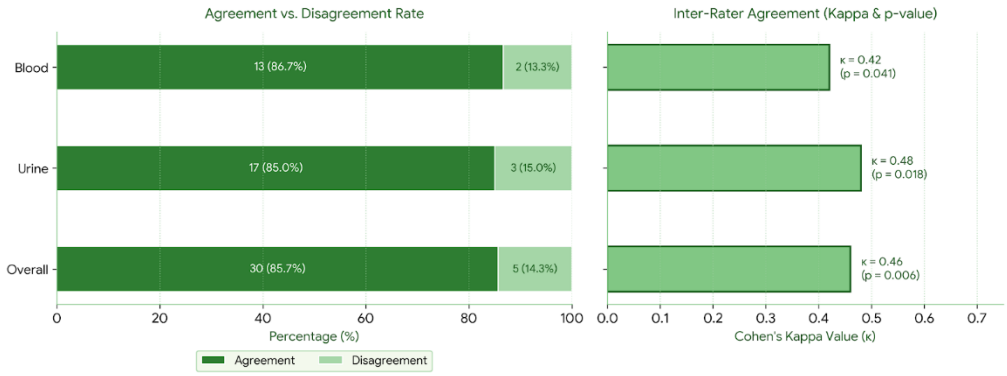



**Figure 2: Agreement between rapid antigen test kit and confirmatory forensic method**

Figure 2 shows the agreement between the rapid antigen test kit and the confirmatory forensic method. Overall agreement was 30 (85.7%), while disagreement was 5 (14.3%). The overall kappa value was 0.46 (p-value = 0.006), indicating moderate, statistically significant agreement.

## Discussion

The present study found that rapid antigen test kits detected narcotic substances in a high proportion of suspected forensic cases, with an overall positivity of 83.3%, slightly higher in urine (85.0%) than in blood (81.3%). Methamphetamine/Yaba was the commonest detected substance (33.3%), followed by cannabis/THC (19.4%), and opiates/codeine-related substances (16.7%). This pattern is consistent with Akbaba and Baransel, who reported methamphetamine and amphetamine as the most prevalent narcotics in paired forensic urine and blood samples, with urine positivity of 91.0% by biochip screening and 95.7% by LC-MS/MS[11]. The slightly lower positivity in the present study may reflect the smaller sample size, local case-mix, different rapid kit format, and inclusion of blood samples, for which detection windows are generally shorter than those for urine.

The overall sensitivity of the rapid test kit in this study was 93.1%, with comparable performance in blood (92.3%) and urine (93.8%). This supports the usefulness of rapid kits as a preliminary forensic screening tool. Similar high screening performance was reported by Bang et al., who found that Triage TOX showed 92.4–100% agreement with UPLC-MS/MS in 105 urine specimens[12]. Walsh et al. also reported opioid immunoassay sensitivity of 96–100% compared with LC-MS/MS, although specificity varied from 84–99%[13]. Avcioglu et al. reported immunochemical sensitivities of 97–100% and specificities of 84–100% for most urine drug parameters, except benzodiazepines[14]. Therefore, the sensitivity observed in the present study is broadly compatible with recent evidence, particularly for screening purposes.

However, the specificity in this study was only 50.0%, lower than that reported in many published studies. Rossi et al. reported specificity >0.99 for two benzodiazepine immunoassays compared with LC-MS/MS in 501 urine samples[15], whereas Bang et al. reported high overall agreement, with only a few discordant cases[12]. The lower specificity in the present study may be explained by the small number of confirmatory-negative samples, cross-reactivity, matrix interference, mixed substance exposure, or imperfect kit cut-off performance. Hughey and Colby showed that false-positive rates in urine drug screening immunoassays may vary widely, from 0% to 43%, especially for amphetamine-related assays[9]. Mina also emphasized that immunoassay performance differs across platforms and drug groups when assessed against GC-MS or LC-MS/MS [16]. These findings support the present observation that rapid tests should not be treated as definitive evidence.

The overall accuracy of 85.7% and agreement of 85.7% in this study are comparable to those reported in several forensic and emergency toxicology studies. Akbaba and Baransel reported accuracy of 88.4% for methamphetamine and 86.7% for amphetamine between biochip screening and LC-MS/MS, with kappa values of 0.661 and 0.663, respectively[11]. In the present study, kappa was 0.46, indicating moderate but statistically significant agreement. The lower kappa may be due to broader drug categories, fewer negative cases, and smaller sample size. Tanaka et al. reported benzodiazepine sensitivity of only 0.56 for Triage and 0.53 for DRIVEN-FLOW M7-II, although amphetamine accuracy was high, 0.96–1.00[17]. In another postmortem urine study, Tanaka et al. found benzodiazepine sensitivity of 0.52–0.59 across three on-site kits[18]. These studies indicate that agreement depends strongly on the target drug class.

False-negative results were also clinically and legally important in this study, where 2 confirmatory-positive cases were missed by the rapid kit. Fujikawa et al. reported variable kit sensitivity, including 78.6% and 90.5% for benzodiazepines and only 25.0% and 45.8% for tricyclic antidepressants[19]. Rosano et al. showed high false-negative rates for immunoassays, including benzodiazepines 40%, amphetamines 38%, opiates 25%, and cocaine 16%[20]. Similarly, Kahl et al. found ELISA missed 26% of benzoylecgonine, 33% of lorazepam, and 60% of oxymorphone positives compared with LC-MS/MS [10]. Swanson et al. showed that LC-MS/MS detected additional drugs outside the ELISA scope in 22% of postmortem blood cases, while ELISA had 8% false-positive and 5% false-negative results[21].

These findings have important forensic implications. Rapid antigen kits are valuable for early screening because they are simple, quick, inexpensive, and practical in resource-limited medico-legal settings. Nevertheless, because this study found 3 false positives, 2 false negatives, and a specificity of only 50.0%, positive results should be interpreted as presumptive. Confirmatory testing by GC-MS, LC-MS/MS, HPLC, or equivalent validated methods remains essential before legal reporting, court submission, or final medico-legal opinion. Tominaga et al. similarly recommended caution and instrumental confirmation after on-site immunoassay screening in forensic autopsy[22]. Yagihashi et al. also supported LC/MS as an effective method when exposure history is unclear[23]. Therefore, the present findings support a two-step forensic workflow: rapid testing for preliminary triage, followed by confirmatory analytical testing for legally defensible conclusions.

## Limitations of the study

This study had a small sample size and was conducted at a single forensic center, limiting generalizability. Only selected narcotic substances were tested, so substance-wise diagnostic accuracy could not be assessed in detail. Confirmatory testing was performed when available, and the timing of drug exposure and sample collection could not be fully controlled.

## Conclusion

Rapid antigen test kits serve as efficient preliminary screening tools for detecting narcotic substances in forensic blood and urine samples, demonstrating higher positivity rates in urine samples, with Methamphetamine/Yaba identified as the most frequently detected substance. While these kits offer high sensitivity and solid overall accuracy for early medico-legal decision-making, their relatively low specificity and moderate agreement with confirmatory methods mean that positive, negative, or indeterminate results cannot stand alone.

Consequently, all preliminary findings must be strictly confirmed using standard laboratory analytical methods such as GC-MS, LC-MS/MS, or HPLC prior to issuing final forensic reports. To ensure overall diagnostic reliability, strict protocols for sample collection, preservation, labeling, sealing, and chain of custody must be maintained throughout the process. Finally, larger multicenter studies are recommended to further validate the forensic performance and utility of these screening kits within Bangladesh.

**Ethical approval:** The study was approved by the Institutional Ethics Committee
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**Competing interests:** None declared.

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