ЈАВНО ЗДРАВЈЕ

ВЕРИФИКАЦИЈА НА КВАЛИТАТИВНИ МЕТОДИ СО ТЕСТ ЛЕНТИ ЗА ИСПИТУВАЊЕ НА ДРОГИ КОИ СЕ ЗЛОУПОТРЕБУВААТ ВО УРИНА

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Извадок

Верификацијата на квалитативните методи со брзи тестови за детекција на осум дроги кои се злоупотребуваат во урината (амфетамини, метамфетамини, барбитурати, кокаин, марихуана, 3,4-метилендиокси-метамфетамин, метадон и опиати) беше направена со примена на резултатите од оценката од надворешната контрола на квалитетот, врз основа на протоколот за верификација во нашата лабораторија, кој опфаќа претходно дефинирани критериуми како што се: точноста, сензитивноста и специфичноста на методот. Нашите резултати покажаа дека квалитативниот метод за детекција на дрогите кои се злоупотребуваат во урина ги исполнува претходно дефинираните критериуми во поглед на сензитивноста, специфичноста и точноста за screening потреби. Постои добро совпаѓање на добиените резултати од наша страна со резултатите од надворешната контрола на квалитет. Веродостојноста на методот ги исполни претходно дефинираните критериуми со исклучок на амфетамините и метамфетаминот (слаба, односно никаква). Како заклучок можеме да кажеме дека брзите неинструментални тестови за детекција на дроги кои се злоупотребуваат во урината покажаа задоволителни резултати од верификацијата и ги исполнија критериумите за соодветната планирана намена во согласност со стандардот ISO 15189.

PUBLIC HEALTH

VERIFICATION OF THE QUALITATIVE METHODS WITH TEST DEVICES FOR DETECTION OF DRUGS OF ABUSE IN URINE

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Abstract

The verification of the qualitative method with test devices for detection of eight drugs of abuse in urine (amphetamines, methamphetamine, barbiturates, benzoylecgonine, marijuana, 3,4-methylendioxy-methamphetamine, methadone, and opiates) was done using the assessment results from External Quality Assessment Scheme according to verification protocol in our laboratory, which included predefined performance characteristics such as: accuracy, sensitivity and specificity of the method, as well as for method comparison analysis. Our results have shown that qualitative methods for detection of drugs of abuse in urine have fulfilled the predefined criteria in regard to sensitivity, specificity and accuracy for screening purposes. There was a good agreement between the observed results and assessment results. Reliability of the method has fulfilled the predefined criteria with the exception for amphetamine and methamphetamine (weak and none, respectively). As a conclusion we may say that the rapid non-instrumented test devices for detection of drugs of abuse in urine have shown satisfactory verification results and have fulfilled the criteria for intended purposes according to the ISO 15189 Standard.

Introduction

Laboratory medicine practice, as a part of the health care system, is regulated by the Law on Health Care of the Republic of Macedonia. The most recent changes of the Law, in regard to Laboratory Medicine, were made in order to provide a better diagnosis while maintaining standard quality credentials, where the standardisation of biochemical laboratories according to ISO 15189 Standard and their mandatory participation in EQAS (External Quality Assessment Scheme) or in External Quality Control (EQC) became obligatory[1]. Towards initial accreditation phase according to ISO 15189 Standard, the verification of the methods is required in order to decide whether or not the method is suitable for intended purposes of the Standard and to eliminate errors in the test results[2,3]. In addition to verification of quantitative tests, verification of the qualitative tests (such as the verification of non-instrumented qualitative method for drugs of abuse in urine) has to be performed before their implementation in the laboratory work[3]. Those methods have only two possible results - "positive" or "negative", and the obtained results have to be confirmed by gas chromatography-mass spectrometry. Performance characteristics and acceptance criteria of these methods are not always detailed and every laboratory has to choose the most suitable verification protocol. Therefore, this study was designed to verify the qualitative method for drugs of abuse in urine according to the requirements of the Standard, using alternative approaches - assessment results from the five-year participation in EQAS (Instand e.V.Gesellschaft zur Förderung der Qualitätssicherung in medizinischen Laboratorien e.V.) [3,4].

Material and methods

The verification protocol of our laboratory, based on the Guidelines for the validation and verification of quantitative and qualitative test methods, comprised reproducibility testing of minimum 10 samples from each category (positive and negative)[5,6]. The reported and the obtained test results were used for calculation of accuracy, sensitivity and specificity of the method, as well as for the method comparison analysis (agreement between the methods and data-k-coefficient)[7,8] according to predefined performance characteristics. The predefined acceptable criteria for these tests for our laboratory were:

- The obtained data should meet the predefined performance characteristics and the claims of the manufacturer in regard to sensitivity and specificity and the combined sensitivity and specificity should be in total = 170;
- The test should have total accuracy of minimum 80%;
- The agreement between the methods (semi-qualitative method and gas chromatography-mass spectrometry) should be 80% and the value of kappa (κ) minimum of 0.6-0.798.

For verification of the method, non-instrumented test devices (cassettes or strips) (IVD products) manufactured by Nova, Dima, Human, IND Diagnostic Inc (IND) with CE conformity marking, were used. The devices were stored at room temperature, and used within the expiration date for detection of amphetamine,methamphetamine,barbiturates, benzoylecgonine, 11-nor-delta-9-THC-COOH(marijuana),3,4-methylendioxy-methamphetamine(MDMA) (ecstasy), methadone, and opiates in the assessment samples. These tests share the common immune-assay based test principle of competitive binding only providing preliminary results that must be confirmed by more specific quantitative methods (e.g. GC/MS)[9]. The external quality assessment specimens were transported and stored at $+40^{C}$ prior to testing, dissolved in the proper volume of double-distilled water and brought to a room temperature prior to testing. The urine samples were tested in triplicate within specified time limits and the test results were recorded separately as one of two options: negative or positive. The results were reported within the deadline dates, and obtained quantitative assessment results were used for method verification according to the predefined criteria. The sensitivity and specificity,

positive and negative predictive values, accuracy, and the ratio of agreement between the methods were calculated using assessment results as follows:

- Sensitivity (%): Number of true positives/ (Number of true positives + Number of false negatives multiplied with 100);
- Specificity (%): Number of true negatives/ (Number of true negatives + Number of false positives multiplied with 100);
- Positive predictive value (%) (PPV): Number of true positives/ (Number of true positives + Number of false positives multiplied with 100);

  according to the following formula:

- Negative predictive value (%) (NPV): Number of true negatives/ (Number of true negatives + Number of false negatives multiplied with 100);
- Accuracy (%): (Number of true positives + Number of true negatives) / (Number of true positives + Number of false positives + Number of true negatives + Number of false negatives) multiplied with 10010.
- Ratio of (agreement%): Number of agreement between the reported and assessment results/ number of samples multiplied with 100;
- Cohen's kappa coefficient (κ) (for reliability of the method) was calculated

$$k = \Pr(a) - \frac{\Pr(e)}{1} - \Pr(e)$$

Where Pr (a) is actual observed agreement and Pr (e) is expected agreement calculated according to the following formula:

$$Expected\,(Chance)\,Agreement = \frac{\frac{(cm^1 \times rm^1)}{n} + \frac{(cm^2 \times rm^2)}{n}}{n}$$

Where:

- cm^1 represents column 1 marginal;
- cm^2 represents column 2 marginal;
- rm^1 represents row 1 marginal;
- rm^2 represents row 2 marginal;
- n represents the number of observation[8].

Results

The performance characteristics of non-instrumented method for drugs of abuse in urine are presented in Table 1. The highest diagnostic sensitivity (100%) was obtained for detection of methamphetamine, benzoylecgonine and MDMA and the lowest for barbiturates (71%).

The obtained diagnostic sensitivity for barbiturates was lower than the predefined one, which might be due to the borderline assessment sample concentration. The highest diagnostic specificity was obtained for methadone and opiates (93%) and the lowest for marijuana (78%) (Table 1).

The highest positive predictive value (PPV) (%) was obtained for benzoylecgonine (91%) and the lowest for methamphetamine (67%). Positive predictive values for other tested drugs were between 80% (amphetamines) and 86% (for opiates). Negative predictive values were between 71% (for barbiturates) and 100% (for methamphetamine, benzoylecgonine and MDMA) (Table 1).

The highest overall accuracy was observed for benzoylecgonine (95%) and the lowest for barbiturates (77%) (Table 1).

Table 1. Verification of the qualitative method for detection of drugs of abuse in urine

Narcotic drug in urine	Cut-off ` ng/ml	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Amphetamines	1000	80	91	80	91	87
Methamphetamine	1000	100	89	67	100	91
Barbiturates	300	71	83	83	71	77
Benzoylecgonine	300	100	91	91	100	95
Marijuana (THC)	50	92	78	78	90	83
MDMA	500	100	91	83	100	94
Methadone	300	85	93	85	93	91
Opiates	2000	85	93	86	93	91

Abbreviations: PPV, positive predictive value: NPV, negative predictive value

In order to measure the reliability of the method, the observed agreement and Cohen's kappa (κ) values were calculated (Table 2). The observed agreement was from 70% (methamphetamine) to 95% (benzoylecgonine and methadone respectively) meaning that 5-30% of the reported data were incorrect. Regarding our predefined criteria the percent of observed agreement was acceptable for screening purposes with the exception for methamphetamine and barbiturates. Nevertheless, this includes the expected agreement, which is the agreement by chance alone (Pe) and the agreement beyond chance. Due to the limitation of the simple proportion of agreement, the Cohen's kappa as a measure of agreement between two methods was calculated and the data are presented in Table 2.

Table 2. Statistical results of the reliability of qualitative method(s) for drugs of abuse in urine

Narcotic drug in urine	Cut-off ng/ml	% of agreement	κ for positive samples*	Level of agreement for positive samples	% of data that are reliable	κ for negative samples	Level of agreement for negative samples	% of data that are reliable
Amphetamines	1000	85	0.46	Weak	15-35	1	Almost Perfect	82-100
Methamphetamine	1000	70	0	None	0-4	0	None	0-4
Barbiturates	300	75	0.7	Moderate	35-63	0.5	Weak	15-35
Benzoylecgonine	300	95	0.8	Strong	64-81	0.9	Strong	64-81
Marijuana (THC)	50	80	0.6	Moderate	35-63	0.7	Moderate	35-63
MDMA	500	93	0.9	Strong	64-81	0.7	Moderate	35-63
Methadone	300	95	> 0.9	Almost Perfect	82-100	0.9	Strong	64-81
Opiates	2000	90	0.8	Strong	64-81	0.8	Strong	64-81

*κ– Cohen's kappa coefficient (κ) (for reliability of the method)

Cohen's kappa for benzoylecgonine, methadone and opiates has shown strong to almost perfect level of agreement for both, positive and negative samples. Although the observed agreement for marijuana was at the acceptable level, Cohen's kappa showed moderate agreement between the methods, similar to barbiturates. There was no agreement for methamphetamine and only a week one for amphetamines.

Discussion

The results obtained for the performance characteristics of the test devices were in agreement with the predefined acceptable criteria for intended purposes (screening) in our laboratory, as well as with those declared by the manufacturers.

The intended use of the tests was for screening purposes meaning that the performance goal for the screening procedure can be a very high sensitivity, which should be confirmed by a gold standard method(gas chromatography-mass spectrometry).. Both, percent agreement and kappa have strengths and limitations10,11. The percent agreement statistics is easily calculated and directly interpretable. Its key limitation is that it does not take into account the possibility that raters guessed on scores, and may overestimate the true agreement among raters. The kappa takes into account the possibility of guessing, but the assumptions it makes about rater independence and other factors are not well supported, and thus it may lower the estimate of agreement excessively. Low levels of interrater reliability are not acceptable in health care or in clinical research, especially when the results might imply legal procedures. We think that the best advice for laboratories is to calculate both percent agreement and kappa. It will be of a great importance for the beneficiaries to be aware of the limitations of the qualitative methods for drugs of abuse in urine.

Conclusions

In conclusion, the parameters tested for the verification of the qualitative method for drugs of abuse in urine have fulfilled the predefined acceptance criteria for diagnostic sensitivity and specificity and for accuracy. In regard to reliability, the qualitative methods for drugs of abuse have shown satisfactory results for the screening purposes of the tests with exclusion of methamphetamine. We may conclude that the verification results have fulfilled the predefined criteria and the Standard requirements and can be introduced as screening methods in our laboratory.

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