Original Article *Ann Clin Biochem* 1997; **34**: 74–80

Evaluation of Bionike one-step tests for the detection of drugs of abuse in urine

E R S Brown, D R Jarvie and D Simpson
From the Department of Clinical Biochemistry, The Royal Infirmary, Edinburgh EH3 9YW, UK

SUMMARY. Bionike one-step tests were used to screen urine samples for amphetamines, methamphetamines, benzodiazepines, cannabinoids, methadone and opiates, and results were compared with those obtained using enzyme multiplied immunoassay technique d.a.u. assays. Taking into consideration different threshold levels and possible differences in cross-reactivities, there was good agreement between the methods. Results of Bionike tests correlated well with amphetamines, methadone and opiates detected in urine using gas chromatography–mass spectrometry. Bionike methods are rapid, simple to use, and relatively inexpensive for on-site testing of individual drugs or groups of drugs in urine.

Additional key phrases: amphetamines; opiates; methadone; cannabinoids

Screening for drugs of abuse in urine is now widely used in the management of drug misusers. Analyses are usually performed by immunoassays, which are reliable, sensitive, simple to use and cost-effective. There has, however, been increasing interest in on-site testing by methods that can be used by non-laboratory staff outside a traditional laboratory environment. This form of near-patient testing has a number of advantages over laboratory-based testing, including the almost immediate availability of results; turn-around times for requests sent to a recognized diagnostic laboratory for drug screening may be several days.

A number of methods for on-site screening for drugs of abuse in urine are now available commercially. EZ-SCREEN tests (Environmental Diagnostics Inc, Burlington, NC, USA) are based on QUIK-CARDS coated with antibody to which is added urine, enzyme and substrate to produce a coloured product. The Abuscreen ONTRAK system (Roche Diagnostics, Welwyn Garden City, UK) is a qualitative latex agglutination immunological slide test.[1] Roche Diagnostics have also developed ONTRAK TESTCUP which is a multi-analyte immunochromatographic screening method.[2] The Triage Screening Cassette (Merck, Darmstadt, Germany) is a device based on competitive immunoassay that simultaneously detects seven or eight groups of abused drugs at concentrations above the cut-off levels recommended by the USA National Institute of Drug Abuse (NIDA)/Substance Abuse and Mental Health Services Administration (SAMSHA).[3]

Evaluations of these tests have indicated that they are rapid, reliable and useful for on-site screening for suspected drug abuse and for checking for compliance.[1–6] However, George and Braithwaite[7] carried out a preliminary evaluation of five kits for on-site screening (manufacturers are not identified) and reported that all the kits were found to lack both sensitivity and specificity, and most kits gave an unacceptable proportion of false negative and false positive results.

Another method recently introduced into the UK is the Bionike A/Q One Step Tests (Bionike Inc, South San Francisco, CA, USA). These tests rely on competition for binding antibody between drugs coated on a membrane and drugs which may be present in the urine being tested. When a drug is present in the urine, it competes with membrane-bound drugs for the limited antibodies present as dye-antibody conjugates. When a sufficient amount of drug is present, it will prevent the binding of dye-antibody conjugate to the membrane-bound drug. Therefore, a positive urine sample will not generate a colour band in the test region while the presence of a

Correspondence: Dr D Simpson.

colour band in the test region indicates a negative result. Bionike tests are available commerically in the UK in the form of dip-sticks and test cards; one stick or card is required for each analyte.

We have carried out an evaluation of six Bionike tests (amphetamine, methamphetamine, benzodiazepines, cannabinoids, methadone and opiates); cocaine metabolite and barbiturates were not included because positive tests are rarely encountered in the Lothians area. Tests were performed 'blind' and results compared with Syva enzyme multiplied immunoassay technique (EMIT) d.a.u. assays (Behring Diagnostics UK Ltd, Milton Keynes, UK) and gas chromatography–mass spectrometry (GC-MS).

METHODS

Patients' specimens

Urine samples were obtained from suspected drug misusers and drug misusers on harm reduction therapy attending the Community Drug Problems Service, psychiatric clinics and general practice. Urines were also collected from drug users attending 'raves'. Rave samples were collected 6–12 h after ingestion of drugs. To minimize the risk of infection of staff by HIV and hepatitis B and C viruses, urines were heated to a temperature of 96°C for 30 min in a water bath prior to analysis.[8]

Bionike tests

The Bionike tests were carried out using dip-sticks which consist of a test pad, a test area and an absorbing pad (Fig. 1). For dip-stick tests, the end of the strip was placed into the sample until the urine was seen to move up through the test area. The strip was then placed in an upright position and coloured bands developed in the test area over a short period of time. Results were read between 3–8 min or 3–10 min depending on the test. A control band appears in all tests unless the test has failed. The presence of an additional coloured band in the test area indicates a negative result whilst the absence of this band indicates that the drug type for which the test is targeted is present in the urine at a concentration above the test threshold.

Dip-stick tests for methadone were not available in the UK at the time of this study, and test cards in which strips are mounted on a card were used (Fig. 2). The card contains a sample well into which two to three drops of

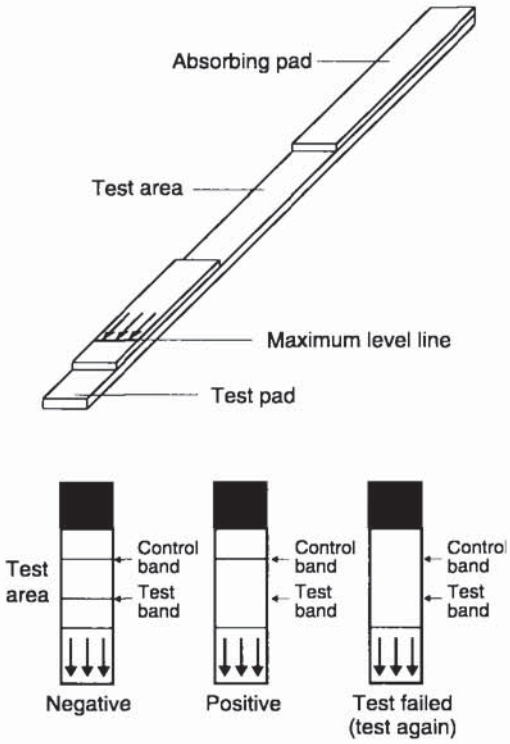

FIGURE 1. *Bionike A/Q™ Dip-stick Test (Reproduced with permission from Bionike Inc).*

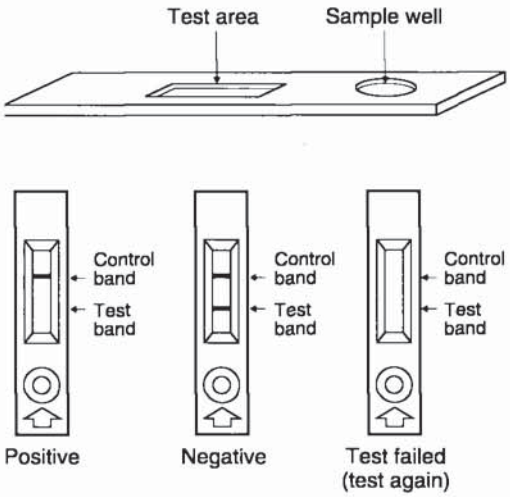

FIGURE 2. *Bionike A/Q™ Test Card Device (Reproduced with permission from Bionike Inc).*

urine were added; results are indicated as for dip-stick tests.

Each dip-stick and test card is supplied in a sealed packet under dry conditions and should be stored at 4°C to 30°C. The products are manufactured with a shelf life of 14–20 months.

Information about threshold levels and cross-reactivities of tests is given in the manufacturer's package inserts and cross-reaction information sheets and is summarized in Table 1.

EMIT assays

Urines were screened by EMIT d.a.u. assays adapted for use on the Cobas Bio analyser[8–10] (Roche Diagnostics, Welwyn Garden City, UK). The EMIT d.a.u. polyclonal assay is currently used for amphetamines because of cross-reaction with phenothiazine metabolites in the monoclonal assay[11,12] (in our laboratory about 10% of urines are from patients in psychiatric hospitals and clinics, many of whom are on phenothiazine therapy). The manufacturer's recommended threshold limits of 300 μg/L of d-amphetamine (for amphetamines), 300 μg/L for methadone, 300 μg/L of morphine (for opiates), 100 μg/L for cannabinoids, and 200 μg/L of oxazepam (for benzodiazepines) were used to classify results as positive or negative. Quantitative results were obtained by using multi-point calibration.[8–10]

GC-MS

Confirmation of positive tests for methadone and identification of individual amphetamines and opiates was carried out by GC-MS. Urines were extracted without prior hydrolysis by Bond Elut Certify™ solid-phase extraction columns using methods supplied by the manufacturer (Varian Sample Preparation Products, Harbor City, CA, USA). Extracts were applied to a DB-5MS column, 30 m long, 0·25 mm internal diameter and 0·25 μm film thickness (J & W Scientific, Folsom, CA, USA). The instrument was a Hewlett-Packard 5890 Series 2 GC with splitless injection and a 5971A mass-selective detector (Hewlett Packard, Winnersh, UK).

Amphetamines

Samples were analysed as previously described.[12] The limit for positive identification of the compounds tested was 100 μg/L.

Methadone

Methadone was identified in the basic drugs fraction of the Certify general extraction procedure. GC-MS detection was as described for amphetamines except that derivatization was not used and the mass range was 50–450 amu. The limit for positive identification of methadone was 300 μg/L. The metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine was normally present in samples from patients taking methadone.

TABLE 1. *Bionike tests concentrations (μg/L) of substances which produce a similar result to that of the threshold calibrator (data from manufacturer)*

Substance (threshold μg/L)	Result
Amphetamine (threshold 1000 μg/L d-amphetamine)	
d,l-Amphetamine	2000
d,l-3,4-Methylenedioxyamphetamine	1000
Phentermine	100 000
Methamphetamine (threshold 1000 μg/L d-methamphetamine)	
d,l-Methamphetamine	1000
d,l-3,4-Methylenedioxymethamphetamine	1000
d,l-3,4-Methylenedioxyamphetamine	1000
d-Ephedrine	25 000
d-Pseudoephedrine	10 000
d,l-Amphetamine	10 000
d-Amphetamine	5000
Phentermine	20 000
Benzodiazepines (threshold 300 μg/L oxazepam)	
Diazepam	300
Desmethyldiazepam	300
Clorazepate-HCl	300
Estazolam	300
Flurazepam	300
Nitrazepam	300
Alprazolam	500
Medazepam	500
Temazepam	750
Cannabinoids (threshold 50 μg/L 11-nor-Δ-9-THC)	
11-nor-Δ-9-THC-9-carboxylic acid	100
Δ-9-tetrahydrocannabinol	500
Cannabinol	500
Methadone (threshold 300 μg/L methadone)	
Methadone Hydrochloride	300
Meperidine	10 000
d-Methamphetamine	10 000
Opiates (threshold 300 μg/L morphine)	
Morphine-3-B-d-glucuronide	300
Hydromorphone	300
Nalorphine	300
Norcodeine	300
Hydrocodone bitartrate	300
Codeine	500
Ethylmorphine	500
Normorphine	500
Oxycodone	1000
Heroin (diamorphine)	5000
Thebaine	5000

TABLE 2. *Comparison of results of Bionike tests and EMIT assays*

EMIT	Bionike	Amphet No. (%)	Methamp No. (%)	Benzos No. (%)	Opiates No. (%)	Methadone No. (%)	Cannabin No. (%)	Cannabin* No. (%)
Positive	Positive	16 (34)	21 (45)	18 (35)	17 (33)	13 (23)	18 (38)	26 (54)
Negative	Negative	17 (36)	15 (32)	29 (57)	30 (58)	37 (65)	19 (40)	19 (40)
Positive	Negative	13 (28)	8 (17)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Negative	Positive	0 (0)	1 (2)	0 (0)	2 (4)	5 (9)	8 (17)	2 (4)
Others†		1 (2)	2 (4)	4 (8)	3 (6)	2 (4)	3 (6)	1 (2)
No. of urines tested		47 (–)	47 (–)	51 (–)	52 (–)	57 (–)	48 (–)	48 (–)
Percentage agreement		– (70)	– (77)	– (92)	– (91)	– (88)	– (78)	– (94)

*Using EMIT threshold of 50 μg/L.
†Includes borderline results for EMIT and/or Bionike methods.
Amphet = amphetamine; Methamp = methamphetamine; Benzos = benzodiazepines; Cannabin = cannabinoids.

Opiates

Morphine, codeine, dihydrocodeine and pholcodine were extracted by the Certify opiates extraction procedure, using nalorphine as internal standard. Trimethylsilyl derivatives were made by reacting the dried residue with BSTFA containing 1% TMCS (Pierce Chemical Co, Rockford, IL, USA), and were injected without further evaporation or dilution. Initial oven temperature was 120°C, held for 1 min, rising at 30°/min to 270° then at 5°/min to 330°, held at 330° for 1 min. Opiate derivatives were detected by selected ion monitoring, and quantitated with reference to a standard curve prepared by dilution of the pure drugs in human urine. A cut-off concentration of 50 μg/L was used for classification of samples as positive or negative for each opiate.

RESULTS

A comparison of the results of Bionike tests versus EMIT assays is given in Table 2. A threshold level of 100 μg/L is currently used in this laboratory for EMIT cannabinoids assays, but, in addition, a cut-off level of 50 μg/L was used to allow a more meaningful comparison. A cut-off of 50 μg/L is also recommended by SAMSHA, and 65 μg/L is the current threshold for performance in the UK National External Quality Assurance Scheme (UKNEQAS) for drugs of abuse in urine. The percentage agreement between the Bionike and EMIT methods increased from 78% to 94% and the number of urines giving a positive result by Bionike and negative by EMIT was reduced from eight to two by using the 50 μg/L level. The two samples which tested positive by the Bionike method had values of 40 and 44 μg/L by the EMIT method.

There was a 92% agreement between Bionike and EMIT methods for benzodiazepines, with no 'false positives' or 'false negatives'. The Bionike assay uses a threshold level of 300 μg/L compared to 200 μg/L of oxazepam for EMIT but none of the samples tested had concentrations in the 200–300 μg/L range.

When results for the EMIT amphetamines polyclonal assay (cut-off 300 μg/L for d- and dl-amphetamine, 650 μg/L for l-methamphetamine and 1000 μg/L for d-methamphetamine) were compared with those for the Bionike test for amphetamine and methamphetamine (cut-off levels both 1000 μg/L), the percentage agreement was 70% and 77%, respectively. The corresponding number of urines testing positive by EMIT and negative by Bionike for these tests were 13 (28%) and eight (17%), respectively. Table 3 gives a list of the amphetamines and related compounds detected in 29 urines by GC-MS compared with results for the Bionike and EMIT tests. For most samples there was good agreement between GC-MS and the other assays. As expected from cross-reactivity data, a urine containing only amphetamine tested positive by the Bionike amphetamine method but negative by the methamphetamine method. A 'false negative' result for 3,4-methylendioxymethamphetamine (MDMA) by the EMIT amphetamines assay could be explained by poor cross-reactivity of the assay for MDMA.[13]

A comparison of results for methadone by the Bionike and EMIT tests indicated 88% agreement. Of the five urines which tested

TABLE 3. *Comparison of compounds detected by the GC-MS amphetamines procedure with results of Bionike amphetamine and methamphetamine and EMIT amphetamines methods (No. of urines tested = 29)*

Compounds detected (GC-MS)	No. of samples	No. of positive results		
		Bionike		EMIT
		Amphet	Methamp	Amphet
MDMA	1	0	1	0
MDMA, MDA	3	3	3	3
MDMA, MDEA	3	3	3	3
MDMA, MDEA, MDA	5	5	5	5
MDEA, P/EPH, PPA	4	3	4	4
MDEA, P/EPH, PPA, methamphetamine	1	0	1	1
Amphetamine	1	1	0	1
P/EPH, PPA, U/M	4	1	4	4
PPA	6	0	0	4
PPA, U/M	1	0	0	0

GC-MS = Gas chromatography–mass spectrometry; MDMA = 3,4-methylenedioxymethamphetamine; MDEA = 3,4-methylenedioxyethamphetamine; MDA = 3,4-methylenedioxyamphetamine; P/EPH = pseudoephedrine/ephedrine; PPA = phenylpropanolamine; U/M = unidentified material.

TABLE 4. *Comparison of compounds detected by GC-MS screening procedure with results of Bionike and EMIT methadone methods (No. of urines tested = 49)*

Compounds detected (GC-MS)	No. of Samples	No. of positive results	
		Bionike	EMIT
Methadone and metabolite	12	12	11
Methadone (trace) and metabolite	2	2	0
Methadone *not* detected	35	1	0

GC-MS = Gas chromatography–mass spectrometry.

positive by Bionike but negative by EMIT, only one had a result close to the EMIT threshold, the others were in the concentration range 48–138 μg/L. Results of GC-MS analysis for methadone and metabolites compared well with results for the other tests (Table 4) but one false positive result was obtained by the Bionike test. Three samples tested negative by EMIT but positive by GC-MS and Bionike methods.

There was 91% agreement between the Bionike and EMIT tests for opiates but two samples tested positive by the Bionike method and negative by EMIT (*see* Table 2). The concentrations obtained for these samples by EMIT were 21 and 48 μg/L and GC-MS analysis suggested the presence of dihydrocodeine at a concentration less than 50 μg/L. There was one false positive result by the EMIT method (Table 5); opiates were not detected by GC-MS and the urine tested negative by Bionike.

DISCUSSION

In our experience the Bionike tests for the detection of drugs of abuse in urine are rapid, simple to use and reliable. The instructions provided with kits were clear and easy to follow.

Urines were collected in Universal containers and we found that dip-stick tests could be performed without the need to transfer the urine to the sample cup supplied by the manufacturer. Interpretation is subjective, but in most cases it was unambiguous although some results were classified as borderline. The manufacturer recommends that results should be read between 3–10 min but we found that interpretation of results was facilitated by leaving strips for 15–30 min; coloured bands appeared to be stable for at least several days. Appearance of a coloured band for negative tests and absence of a band for positive tests can be confusing

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.