REGIONE CAMPANIA
AZIENDA OSPEDALIERA DI CASERTA
SANT'ANNA E SAN SEBASTIANO
DI RILIEVO NAZIONALE E DI ALTA SPECIALIZZAZIONE

Determina Dirigenziale N. 492 del 06/08/2018

PROPONENTE: **UOC PROVVEDITORATO ED ECONOMATO**

OGGETTO: **PROCEDURA DI AFFIDAMENTO AI SENSI DELL'ART.36 CO. 2 LETT.A) DEL D.LGS. N.50/2016 E SS.MM.II. DELLA FORNITURA DI TEST PER ACCERTAMENTI TOSSICOLOGICI PER LA UOC PATOLOGIA CLINICA. CIG Z4124924DE**

REGIONE CAMPANIA

AZIENDA OSPEDALIERA DI CASERTA

SANT'ANNA E SAN SEBASTIANO

DI RILIEVO NAZIONALE E DI ALTA SPECIALIZZAZIONE

Oggetto: PROCEDURA DI AFFIDAMENTO AI SENSI DELL'ART.36 CO. 2 LETT.A) DEL D.LGS. N.50/2016 E SS.MM.II. DELLA FORNITURA DI TEST PER ACCERTAMENTI TOSSICOLOGICI PER LA UOC PATOLOGIA CLINICA. CIG Z4124924DE

Direttore UOC PROVVEDITORATO ED ECONOMATO

Premesso che

- questa AORN ha attivato un Protocollo d'intesa con la Procura della Repubblica per gli accertamenti dello stato di ebbrezza alcolica e alterazione psicofisica per effetto di assunzione di sostanze stupefacenti nei conducenti di vetture;
- il Direttore della UOC Patologia Clinica, con nota del 25/07/2018, assunta al protocollo n.0019966/i, ha avanzato la richiesta per l'acquisizione di Test per esami tossicologici non oggetto di convenzione So.Re.Sa, comunicandoci le ditte a cui inviare preventivo (all.1);
- vista l'urgenza rappresentata, in data 26.07.2018 è stata richiesta offerta alle ditte: Randox Laboratories Kimited, Werfen-Instrumentation Laboratory spa e Shimadzu;
- hanno fatto pervenire offerta, entro il termine di scadenza, le ditte: Randox Laboratories Kimited, Werfen-Instrumentation Laboratory spa (all.2);
- il Direttore della UOC Patologia Clinica ha attestato la conformità tecnica di entrambe le offerte;
- l'offerta conforme al prezzo più basso è risultata quella presentata dalla ditta Randox, Laboratories Kimited per una spesa complessiva pari ad €.19.950,00 + iva al 22% comprensiva del sistema Evidence Investigator (stazione caricamento dei carrier, termo agitatore per l'incubazione dei campioni, CCD camera integrata, pc, monitor, tastiera e stampante se necessaria) e dei costi di noleggio e assistenza full risk per un anno (all.3);

Considerato che

- il decreto n.58 del 18.07.11 a firma del Commissario ad acta per la prosecuzione del piano di rientro del settore sanitario della Regione Campania prevede che "*per gli acquisti di beni, attrezzature e servizi sanitari e non sanitari di importo pari o inferiore ad € 50.000,00, le Aziende Sanitarie possano porre in essere autonome procedure di acquisto….*";
- ravvisata l'urgenza di procedere all'acquisto di quanto richiesto, al fine di ottemperare al Protocollo d'intesa sottoscritto con la Procura della Repubblica;
- a seguito di apposita riunione, tenutasi in Direzione Generale in data 09/07/2018, è stato dato impulso per una rapidissima acquisizione del materiale sopra riportato e stante l'assoluta urgenza di adempiere alle prestazioni stabilite nel protocollo d'Intesa;

Attestata

la legittimità della presente proposta di determinazione, che è conforme alla vigente normativa in materia;

REGIONE CAMPANIA

AZIENDA OSPEDALIERA DI CASERTA

SANT'ANNA E SAN SEBASTIANO

DI RILIEVO NAZIONALE E DI ALTA SPECIALIZZAZIONE

DETERMINA

1. **PRENDERE ATTO** di quanto rappresentato nelle premesse e per l'effetto di procedere all'acquisto urgente, ai sensi dell'art.36 co.2 lett. a) del D.lgs. n.50/2016 e ss.mm.ii., di test per esami tossicologici compreso il sistema Evidence Investigator (stazione caricamento dei carrier, termo agitatore per l'incubazione dei campioni, CCD camera integrata, pc, monitor, tastiera e stampante se necessaria) e dei costi di noleggio e assistenza full risk per un anno, presso la Ditta Randox Laboratories Kimited con sede in Viale Vittoria,34 - Roma, per una spesa complessiva pari ad €. 24.339,00 IVA inclusa al 22% per effettuare le prestazioni di cui al Protocollo di Intesa di cui in premessa;
2. **IMPUTARE** la spesa complessiva di € 24.339,00 iva inclusa, sulla corrispondente autorizzazione n.7 sub 2 conto economico 501010501 del corrente bilancio;
3. **INSERIRE** nel contratto la clausola di recesso, ai sensi del combinato disposto degli artt.92 e 100 del D.lgs 159/2011, qualora vengano accertati elementi relativi a tentativi di infiltrazione mafiosa;
4. **PUBBLICARE** integralmente la presente determinazione;
5. **RENDERE** la stessa immediatamente eseguibile stante l'urgenza;
6. **TRASMETTERE** copia del presente atto al Collegio Sindacale, ai sensi di legge, nonché, oltre che al proponente, ai Direttori UOC Gestione Economico Finanziaria e Direttore UOC Patologia Clinica.

IL DIRETTORE UOC PROVVEDITORATO
ED ECONOMATO
dr.ssa Marisa Di Sano

Azienda Ospedaliera di Caserta
Sant'Anna e San Sebastiano
di rilievo nazionale e di alta specializzazione

Azienda Ospedaliera di Caserta
"Sant'Anna e San Sebastiano"
di rilievo nazionale e di alta specializzazione
Via Palasciano – 81100 Caserta (CE)
Direttore Generale: Dott. Mario Nicola Vittorio Ferrante

ALLEGATO N. 1

U.O.C. PATOLOGIA CLINICA
Direttore f.f. **Dott.ssa Maddalena Schioppa**
Tel/Fax 0823-232135 patologiaclinica@ospedale.caserta.it

Al Direttore UOC Provveditorato

Oggetto: protocollo d'intesa esami tossicologici

In riferimento all'oggetto si specifica ulteriormente che i prodotti richiesti per l'esecuzione dei test tossicologici di screening su matrice ematica non sono oggetto di convenzione SO.RE.SA. e che la fornitura, in base ad un'indagine di mercato può essere offerta dale seguenti Aziende:

1. Randox
2. Instrumentation Laboratory
3. Shimadzu

Distinti saluti

Direttore f.f. Dott.ssa Maddalena Schioppa

Da "instrumentationlaboratory.gare@legalmail.it" <instrumentationlaboratory.gare@legalmail.it>
A "provveditorato@ospedalecasertapec.it" <provveditorato@ospedalecasertapec.it>
Data martedì 31 luglio 2018 - 10:42

INVIO OFFERTA ESAMI TOSSICOLOGICI

ALLA C.A. DR.SSA MARISA DI SANO - UOC PROVVEDITORATO ED ECONOMATO

Si trasmette in allegato offerta inerente ai prodotti in oggetto.

Cordiali saluti

ALLEGATO N. 2

Tiziana Scalzo

Ufficio Gare e Offerte

Instrumentation Laboratory SpA
A Werfen Company

Allegato(i)

8100050309.pdf (318 Kb)

31-7-18 [illegible] per verifica conformità alle [illegible]

Werfen

Instrumentation Laboratory SpA
A Werfen Company

Spettabile
Azienda Ospedaliera di Caserta
"Sant'Anna e San Sebastiano"
di rilievo nazionale e di alta specializzazione
Via Palasciano – 81100 Caserta (CE)

Codice Cliente 197610

Unità Operativa Complessa Provveditorato ed Economato
Direttore: dr.ssa Marisa Di Sano
Pec: provveditorato@ospedalecasertapec.it

Ns.rif. Nr 8100050309/int del 30/07/18

OGGETTO: Fornitura urgente test tossicologici. Vs. rif. prot. N. 0020101/u del 26/07/2018

In riferimento all'oggetto ed alla Vs. gradita richiesta di fornitura, si offre quanto di seguito riportato:

- Fornitura di un sistema analitico ILAB TAURUS comprensivo di deionizzatore e gruppo di continuità al canone annuale di € 8.000,00.=(ottomila/00) comprensivo di assistenza tecnica full-risk a ns. carico per tutto il periodo della fornitura. Fatturazione trimestrale/posticipata
- Consumabili relativi allo strumento ed al deionizzatore in sconto merce.
- Reattivi, calibratori e controlli ai prezzi come da dettaglio riportato nella tabella

CONDIZIONI DI FORNITURA:
Consegna strumento: entro 60 gg ric. ordine – f.co Vs.indirizzo
Consegna materiale: entro 7 giorni ric. ordine - f.co Vs. indirizzo
Spedizione: a mezzo corriere
Imballo: standard, compreso
I.V.A. 22%: a Vs. carico, esclusa dai prezzi indicati in offerta
Pagamento: Rimessa Diretta 60 giorni d.f.
Validità: 5 anni dalla data della Vs. accettazione.

In attesa di un VS. cortese riscontro, è gradita l'occasione per porgere cordiali saluti

Instrumentation Laboratory SpA

Serenella Anna Natella
Procuratore

L'offerta è conforme
31/07/18

Testo breve materiale	Codice Reag	Caratt. Reag.	Qtà test richiesti	Qtà test offerti	tot qtà annua	Test x kit	Prezzo netto x Kit	Prezzo netto annuo
MultiCal Neg Serum Tox DRI	W150962	CALIB			2		136,50	273,00
Serum DoA Negative	00018162900	CALIB			12		175,00	2.100,00
Serum DoA OPI/COC Calibrators	00018163000	CALIB			10		350,00	3.500,00
Serum DoA THC Calibrators	00018163100	CALIB			10		350,00	3.500,00
Serum DoA METH Calibrators	00018163200	CALIB			10		350,00	3.500,00
Serum DoA AMP/XTC Calibrators	00018163300	CALIB			10		350,00	3.500,00
CQ Serum DoA AMP/XTC Controls	00018163400	CONTR			10		150,00	1.500,00
CQ Serum DoA OPI/COC/METH Control	00018163500	CONTR			10		150,00	1.500,00
CQ Serum DoA THC Controls	00018163600	CONTR			10		150,00	1.500,00
ionTANK bombola letto misto resine QI	W25Q100010	CONSU			3		-	-
Prefiltro uQ miniDIA QI	W25Q100025	CONSU			6		-	-
Cannabinoidi CEDIA	W15100091	REAGEN	500	1072	2	536	960,00	1.920,00
Alcool Etilico DRI	W150037	REAGEN	500	1774	2	887	478,84	957,68
Barbiturici Serum Tox DRI	W150911	REAGEN	100	212	2	106	575,00	1.150,00
Benzodiazepine Serum Tox DRI	W150920	REAGEN	500	636	6	106	575,00	3.450,00
Cocaina CEDIA	W15100086	REAGEN	500	1072	2	536	960,00	1.920,00
Metadone CEDIA	W15100088	REAGEN	500	1072	2	536	960,00	1.920,00
Oppiacei CEDIA	W15100089	REAGEN	500	1608	3	536	960,00	2.880,00
Amfetamine/Ecstasy CEDIA	W15100104	REAGEN	100	886	2	443	960,00	1.920,00
MultiCal 1 Serum Tox DRI	W150963	CALIB			1		136,50	136,50
MultiCal 2 Serum Tox DRI	W150965	CALIB			1		136,50	136,50
MultiCal 3 Serum Tox DRI	W150967	CALIB			1		136,50	136,50
MultiCal 4 Serum Tox DRI	W150976	CALIB			1		136,50	136,50
Cal Neg Alcool Etilico DRI	W151405	CALIB			2		-	-
Cal 100 Alcool Etilico DRI	W151406	CALIB			2		-	-
CQ 50 Alcool Etilico DRI	W150239	CONTR			2		-	-
CQ 300 Alcool Etilico DRI	W150243	CONTR			2		-	-
CQ TOX MP 3 Liv MAS	W1510011608	CONTR			4		159,00	636,00
Detergente Alcalino	00018253710	CONSU			4		-	-
Detergente Acido	00018253800	CONSU			4		-	-
Detergente Alcalino cuvette	00018469000	CONSU			4		-	-
Detergente Acido cuvette	00018469100	CONSU			4		-	-
Additivo per Bagno	00018469400	CONSU			2		-	-
Asciuga Cuvette	00018470810	CONSU			1		-	-
Cups Campione (3 ml)	00018262910	CONSU			1		-	-

Instrumentation Laboratory SpA
Viale Monza 338 - 20128 Milano - Italia
Serenella Anna Rosetta
Procuratore

Werfen

Werfen

Instrumentation Laboratory SpA
A Werfen Company

Spett.le
Azienda Ospedaliera di Caserta
"Sant'Anna e San Sebastiano"
Di rilievo nazionale e di alta specializzazione
Via Palasciano – 81100 Caserta (CE)

Milano, 30 Luglio 2018

Oggetto: **Dichiarazione infungibilità Serum DoA**

Con riferimento alla Vs. cortese richiesta in merito all'oggetto Instrumentation Laboratory S.p.A. dichiara che:

la linea di prodotti Serum DoA sono costituiti da calibratori e controlli su matrice ematica di origine umana con valori assegnati e tracciabili rispetto ad una tecnologia di riferimento.
Si dovranno utilizzare esclusivamente questi componenti forniti da Instrumentation Laboratory S.p.A. per l'esecuzione delle droghe d'abuso su matrice ematica sugli analizzatori ILab 650 e ILab Taurus.

L'occasione è gradita per porgere cordiali saluti.

Dr. Roberto Galli
Direttore Marketing

DC/mp

Werfen

Instrumentation Laboratory SpA
A Werfen Company

DICHIARAZIONE ESCLUSIVITA' TECNOLOGICA *

Il sottoscritto Roberto Galli nato a Milano il 8.10.1955 domiciliato per la carica a Milano in V.le Monza 338, in qualità di Procuratore della Società Instrumentation Laboratory S.p.A. Viale Monza, 338 - 20128 Milano consapevole delle sanzioni penali, nel caso di dichiarazioni non veritiere e falsità negli atti, richiamate dall'art. 76 D.P.R. 445 del 28/12/2000

DICHIARA
(ai sensi dell'art. 46 e 47 del D.P.R. 28.12.2000 N. 445)

l'Unicità Tecnologica ed esclusiva del Sistema così composto:

- Analizzatore ILab 650/ ILab Taurus
- Reagenti con tecnologia CEDIA®
- Materiali di calibrazione e controllo della Linea Serum DoA

Il sistema diagnostico proposto è l'unica soluzione automatizzata per lo screening delle droghe d'abuso su matrice ematica dichiarato conforme alla normativa CE (secondo la direttiva dell'Unione Europea 98/79/EC) e che utilizza un analizzatore random access, dotato di software che consente completa tracciabilità del dato analitico e completamente aperto all'inserimento di nuove metodiche tra cui, substrati, enzimi, proteine specifiche, farmaci ed analisi tossicologiche su altre matrici biologiche quali urinaria e cheratinica.

In base alle informazioni in nostro possesso gli elementi essenziali che qualificano detta unicità tecnologica, alla data odierna, non sono riscontrabili su alcun sistema per lo screening tossicologico su matrice ematica delle droghe d'abuso presente sul mercato.

In fede.

Milano, 30 Luglio 2017

Dott. Roberto Galli
Procuratore

*(riferimento a quanto regolato da D. LGS. n. 50/2016 art.63, comma 2°, lett. b).

Da "randox" <randox@pec.it>

A "provveditorato@ospedalecasertapec.it" <provveditorato@ospedalecasertapec.it>, "provveditorato@ospedale.caserta.it" <provveditorato@ospedale.caserta.it> **ALLEGATO N..2..**

Cc "LUCIANO" <luciano.colucci@randox.com>

Data lunedì 30 luglio 2018 - 17:57

RIF. FORNITURA URGENTE TEST TOSSICOLOGICI VS. PROT. N. 0020103/u DEL 26/07/2018

Spett.le UOC dell'Azienda Ospedaliera Sant'Anna e San Sebastiano,
in merito alla richiesta del 26/07/2018 protocollo numero 0020103/u per fornitura urgente di esami tossicologici, alleghiamo alla presente la Ns. migliore offerta e relativa documentazione e dichiarazioni.
Distinti saluti
Randox Laboratories Limited

Allegato(i)

Relazione_tecnica_Investigator 30072018.pdf (293 Kb)
dichiarazione esclusivita.pdf (176 Kb)
dichiarazione INFUNGIBILITA'.pdf (167 Kb)
David James Bowden Passaporto colori.pdf (559 Kb)
EV4204.pdf (1159 Kb)
EV4206 - batch 9569 (12556EV - 125557EV) v1.pdf (190 Kb)
OFFERTA SS Anna e Sebast -Caserta- 30072018-01.pdf (107 Kb)

31-7-18 [illegible]

RANDOX

Spett.le
AORN S. ANNA E S. SEBASTIANO DI CASERTA
VIA F. PALASCIANO snc
88100 CASERTA

Oggetto: **FORNITURA URGENTE TEST TOSSICOLOGICI – DICHIARAZIONE DI ESCLUSIVITA'**

DICHIARAZIONE SOSTITUTIVA DI ATTO DI NOTORIETA'
(art. 46 e 47 del D.P.R. 28 dicembre 2000, n. 445 e ss.mm.ii.)

Con la presente il Sottoscritto David James Bowden, nato a Larne (Irlanda del Nord), il 16/07/1966, residente in Ballyclare (prov.Antrim), Henryville Lodge n. 24 , c.f. BWDDDJ66L16Z114U, in qualità di Legale Rappresentante della Società **Randox Laboratories Limited,** con sede legale in Roma, Viale Della Vittoria n. 34 - 00122, codice fiscale n. 92015900589 e con partita I.V.A. n. 07197321008

Consapevole delle conseguenze penali cui puo'andare incontro in caso di dichiarazioni non veritiere e falso in atti previste dall'art. 76 del D.P.R. 28 dicembre 2000, n.445.

Dichiara sotto la propria responsabilita'

Che i materiali offerti sono **prodotti e distribuiti in esclusiva da Randox Laboratories Limited.**

Nessun'altra societa' o azienda terza ha titolo ad offrire direttamente o indirettamente i prodotti sopra citati per la Vs. Azienda Ospedaliera.

RANDOX LABORATORIES LIMITED
Viale della Vittoria, 34 - 00122 ROMA
P.IVA 07197321008 - C.F. 92015900589
Tel. 06/98968954 - Fax 06/60513810

D James Bowden

_____Roma____, lì 30/07/2018
luogo di sottoscrizione *data di sottoscrizione*

_____
(firma per esteso del dichiarante)

RANDOX

Spett.le
AORN S. ANNA E S. SEBASTIANO DI CASERTA
VIA F. PALASCIANO snc
88100 CASERTA

Oggetto: **FORNITURA URGENTE TEST TOSSICOLOGICI – DICHIARAZIONE DI INFUNGIBILITA'**

DICHIARAZIONE SOSTITUTIVA DI ATTO DI NOTORIETA'
(art. 46 e 47 del D.P.R. 28 dicembre 2000, n. 445 e ss.mm.ii.)

Con la presente il Sottoscritto David James Bowden, nato a Larne (Irlanda del Nord), il 16/07/1966, residente in Ballyclare (prov.Antrim), Henryville Lodge n. 24 , c.f. BWDDDJ66L16Z114U, in qualità di Legale Rappresentante della Società **Randox Laboratories Limited,** con sede legale in Roma, Viale Della Vittoria n. 34 - 00122, codice fiscale n. 92015900589 e con partita I.V.A. n. 07197321008

Consapevole delle conseguenze penali cui puo'andare incontro in caso di dichiarazioni non veritiere e falso in atti previste dall'art. 76 del D.P.R. 28 dicembre 2000, n.445.

Dichiara sotto la propria responsabilita'

Che la linea di prodotti EVIDENCE®, che include l'analizzatore EVIDENCE INVESTIGATOR ed i relativi kit, calibratori e controlli, presenta delle caratteristiche uniche al mondo che sono prodotte esclusivamente dalla Randox Laboratories Ltd, basate sull'esclusiva tecnologia BAT (Biochip Array Technology) mediante carrier con rilevazione in chemiluminescenza.

RANDOX LABORATORIES LIMITED
Viale della Vittoria, 34 - 00122 ROMA
P.IVA 07197321008 - C.F. 92015900589
Tel. 06/98968954 - Fax 06/60513810

D James Bowden

_____Roma____, li 30/07/2018
luogo di sottoscrizione *data di sottoscrizione*

_____
(firma per esteso del dichiarante)

RANDOX

CE

DRUGS OF ABUSE ARRAY I WHOLE BLOOD PLUS (DOA I WB P) (CE)

—— evidence ——
INVESTIGATOR

INTENDED USE

The Evidence Investigator™ Drugs of Abuse I Whole Blood Plus Assays are tests for the semi-quantitative determination of the parent molecule and metabolites of drugs in human blood. They are competitive enzyme immunoassays run on the biochip array analyser, Evidence Investigator™.

The Evidence Investigator™ Drugs of Abuse I Whole Blood Plus Assays provide only a preliminary analytical test result. A more specific alternative chemical method must be used to obtain a confirmed analytical result. Gas Chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method[1]. Other chemical confirmation methods are available. Clinical consideration and professional judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Cat. No. EV 4204

Containing the following components:

1. DOA WB ASSAY DILUENT	1 x 10 mL
2. DOA I WB P CONJ	1 x 10 mL
3. DOA I WB P BIOCHIP	54 biochips
4. DOA I WB P CAL	9 x 1 mL
5. LUM – EV841	1 x 10 mL
6. PX	1 x 10 mL
7. DIL SPE	1 x 10 mL
8. BUF WASH (Conc.)	1 x 32 mL
9. Calibrator Disc and Barcodes	1

LABELLING GUIDE

DOA I WB P	Drugs of Abuse I Whole Blood Plus
DOA WB DIL ASY	Assay Diluent
CONJ	Conjugate
BIOCHIP	Biochip
CAL	Calibrator
LUM-EV841	Luminol-EV841
PX	Peroxide
DIL SPE	Sample Diluent
BUF WASH (conc.)	Wash buffer Concentrate

CLINICAL SIGNIFICANCE

It is now accepted that drug misuse is a large and growing problem. Drug abuse has effects not only on the user but also on the whole of society. This can be in the form of crime; danger to other members of society and it can also cause an increased risk of the spread of transmittable diseases[2]. Blood drug testing can provide a tool for detecting users and for monitoring the compliance of subjects in recovery programs[3].

PRINCIPLE

The Evidence Investigator™ analyser performs simultaneous detection of multiple analytes from a single patient sample. The core technology is the Randox Biochip, a solid-state device containing an array of discrete test regions containing immobilised antibodies specific to different DOA compound classes. A competitive chemiluminescent immunoassay is employed for the DOA assays with the drug in the specimen and drug labelled with horseradish peroxidase (HRP) being in direct competition for the antibody binding sites. Increased levels of drug in a specimen will lead to reduced binding of drug labelled with HRP and thus a reduction in the chemiluminescent signal emitted. The light signal generated from each of the test regions on the biochip is detected using digital imaging technology and compared to that from a stored calibration curve. The concentration of analyte present in the sample is calculated from the calibration curve.

Several different immunoassay based multi-analyte arrays have been developed for use on Evidence Investigator™. The DOA I WB P array will semi-quantitatively test for Amphetamine class (AMPH/MAMP), Barbiturates (BARB), Benzodiazepine class (BENZ 1/BENZ 2), Cocaine metabolite (BZG), Methadone (MDONE), Opiates (OPIAT), Phencyclidine (PCP), Cannabinoids (THC), Methylenedioxy-Methamphetamine (MDMA), Buprenorphine (BUP) and Tricyclic Antidepressants (TCA) simultaneously.

LIMITATION

- The Evidence Investigator™ DOA I WB P Assays are designed for use only with human whole blood samples.
- There is a possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g. technical or procedural errors).
- These assays have been designed to reduce HAMA and other heterophilic antibodies interference. However, HAMA and other heterophilic antibodies can react with the immunoglobulins included in the assay components. Clinical consideration and professional judgment should be applied to any DOA I WB P result.

SPECIMEN COLLECTION AND PREPARATION

- The Evidence Investigator™ DOA I WB P array is designed for use with human whole blood.
- Sample preparation should be carried out in accordance with the collection tube manufacturers recommendations.
- **All whole blood samples should be centrifuged up to 13000 rpm (11000 G) for 10 minutes prior to the 4-fold dilution in sample diluent. Alternatively 4000 rpm (1000 G) for 20 minutes can be used.**
- Whole blood samples should be diluted 4-fold in Sample Diluent (DOA I WB P DIL SPE), e.g. 50 µL sample + 150 µL Sample Diluent prior to analysis. The diluted sample is now ready for application to the biochip.

SAMPLE STORAGE AND STABILITY

- If specimens are not to be analysed immediately, they should be frozen in small aliquots at -20°C. Repeat freeze/thaw cycles should be avoided.
- The premixed sample is stable for up to 24 hours at +2°C to +8°C or 7 days at -20°C.

SAFETY PRECAUTIONS & WARNINGS

Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.
Samples: Human blood samples should be handled and treated as if they were potentially infectious.
Signal reagent–EV841: Avoid contamination and exposure to direct sunlight.
Water: Use double deionised water.

Health and Safety Data Sheets are available on request.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

REAGENT COMPOSITION

1. **DOA WB ASSAY DILUENT**
   20 mM phosphate buffer, pH 7.2 containing protein, detergents and preservatives.
2. **DOA I WB P CONJ**
   20 mM Tris based buffer, pH 7.0 containing protein, preservatives and horseradish peroxidase - labelled drug derivatives.
3. **DOA I WB P BIOCHIP**
   Solid substrate containing immobilised antibody in discrete test regions.
4. **DOA I WB P CAL**
   Nine vials of lyophilised material containing analytes for the full DOA I WB P array.
5. **LUM –EV841 / PX**
   Luminol-EV841 (1 x 10 mL) and Peroxide (1 x 10 mL) are provided, and when mixed in a ratio of 1:1 gives the working signal reagent –EV841
6. **DIL SPE**
   Phosphate buffer, pH 7.2 containing detergents and preservatives.
7. **BUF WASH (conc.)**
   20 mM Tris buffered saline, pH 7.4, containing surfactant and preservatives. Do not use if buffer is gelatinous or crystallised. Contact Randox technical support for further advice.

STABILITY AND PREPARATION OF REAGENTS

1. **DOA WB ASSAY DILUENT**
   Buffer solution is ready for use and is stable up to the expiry date when stored at +2°C to +8°C.
2. **DOA I WB P CONJ**
   The conjugate solution is ready for use and is stable up to the expiry date when stored at +2°C to +8°C, protected from light.
3. **DOA I WB P BIOCHIP**
   Biochips are ready for use. 9 chips are contained in each carrier and 6 carriers are contained in each kit. The biochips are stable up to the expiry date when stored desiccated at +2°C to +8°C, protected from light.
   Open biochips are stable for 7 days when stored in closed Evidence Investigator™ ziplock bags with desiccant at room temperature. Biochip orientation should be marked on the base of the well prior to removal from the biochip carrier.
4. **DOA I WB P CAL**
   Open the vial very carefully, avoiding loss of material. Reconstitute in exactly 1 mL of deionised distilled water. Replace the rubber stopper and roll for 30 minutes. Avoid the formation of foam. Once reconstituted calibrators are stable up to 5 days when stored at +2°C to +8°C. After reconstitution, calibrators can be frozen and are stable up to 14 days when stored at -20°C. Only the required amount of product should be removed. After use, any residual product should NOT BE RETURNED to the original vial.
5. **LUM-EV841 / PX**
   Two components, Luminol-EV841 (1 x 10 mL) and Peroxide (1 x 10 mL) are provided, and when mixed in a 1:1 ratio give the working signal reagent. A carrier (9 wells + dead volume) requires approximately 3 mL of working strength signal reagent-EV841.
   Calculate the volume of working strength signal reagent – EV841 required based on 250 µL volume per well. E.g. 2 carriers require 6 mL of working strength signal reagent-EV841 – 3 mL of Luminol-EV841 and 3 mL of Peroxide. Measure required volumes of each component (1:1) and aliquot, using sterile disposable plastic pipettes, into separate opaque clean vials. Care should be taken to avoid contamination when handling these reagents.
   Mix components, rolling gently for 15 minutes prior to use. For consistency, it is recommended that the Luminol-EV841 be added to the Peroxide component when mixing. Protect Luminol–EV841 component and working strength reagent from light.
   Working signal reagent-EV841 has a stability of 4 hours.
6. **DIL SPE**
   Sample Diluent is ready to use and is stable up to the expiry date when stored at +2 to +8°C, protected from light.
7. **BUF WASH (conc.)**
   Wash buffer is provided as a concentrate, which requires dilution prior to use. The dilution factor is 31.25 i.e. 32 mL of concentrate should be added to 968 mL of water and mixed by inversion. Diluted wash buffer is stable for 30 days when stored at +2 to +8°C.

TEST PROCEDURE

MATERIALS PROVIDED

1.	DOA WB ASSAY DILUENT	1 x 10 mL
2.	DOA I WB P CONJ	1 x 10 mL
3.	DOA I WB P BIOCHIP	54 biochips
4.	DOA I WB P CAL	9 x 1 mL
5.	LUM-EV841	1 x 10 mL
6.	PX	1 x 10 mL
7.	DIL SPE	1 x 10 mL
8.	BUF WASH (Conc.)	1 x 32 mL
9.	Calibrator Disc and Barcodes	1

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipette and pipette tips
2. Wash bottle
3. Deionised distilled water
4. Evidence Investigator™ Ziplock bags (EV 3664)

CALIBRATION

A nine-point calibration should be performed using the Evidence Investigator™ DOA I WB P Calibrators, which cover the measuring range of all assays. A maximum of 6 biochip carriers may be assayed simultaneously, and it is recommended that a new calibration be constructed for each assay series.

QUALITY CONTROL

Evidence® DOA I WB P Controls (EV 4206) are recommended for quality control in the monitoring of accuracy and precision. Control results should fall within acceptable limits, otherwise corrective action should be taken as established by laboratory guidelines.

MATERIALS

All materials should be equilibrated to room temperature prior to use.

Remove the required number of biochip carriers from their packaging. Place the handling tray supplied with the investigator thermo shaker unit onto the working surface. Insert each carrier into the handling tray, ensuring they are flat and secure by clicking into position. All sample and reagent additions, washing and incubations are performed using the handling tray and carriers should only be removed from this for the final signal addition and imaging stage of the procedure.

It is recommended that the thermo shaker unit be equilibrated to +25°C for 30 minutes prior to use.

PROCEDURE

CDROM INSTALLATION

Insert disc into CDROM drive
The Updater program will auto run.
Click on the system to be updated to ensure correct array is updated on the software prior to concentration upload.
Close panel update and the Update Concentration window will be displayed.
Answer YES to question 'ARE YOU SURE YOU WANT TO UPDATE CONCENTRATIONS?'
The PDF can be viewed by opening My Computer and select explore on the CDROM.
Calibrator concentrations for this panel have now been updated. Please recalibrate associated array before further sample analysis.

SPECIAL INSTRUCTIONS

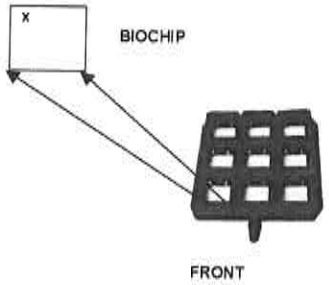
Array and Calibrator settings are an essential update for your Evidence® system and should be installed for use with the relevant Evidence® calibrators.

ASSAY PROTOCOL

For manual Evidence Investigator™ DOA I WB P assays, it is recommended that the reagent/sample loading time should not exceed 10 minutes.

The addition of reagents is carried out by pipetting towards the front edge of the biochip, taking care not to touch the biochip surface with pipette tips.

Fig. 1 Optimal position for addition of assay reagents and samples



Assay reagents and samples should be added with the tip of the pipette pointing towards the back to the well. 'X' marks the area optimal for sample addition.

1. Pipette 120 µL of assay diluent per well.
2. Pipette 60 µL of calibrator/control/diluted sample per well (refer to Specimen Collection and Preparation for details on how to dilute sample).
3. Pipette 120 µL of conjugate per well. Mix by tapping the edge of the handling tray.
4. Secure the handling tray to the base plate of the thermo shaker. Incubate for 30 minutes at +25°C and 330 rpm.
5. Following incubation, remove the handling tray containing the carriers from the thermo shaker. Discard reagents to waste using a sharp, flicking action of the handling tray.
6. Immediately carry out 6 quick wash cycles. Using wash bottle with diluted wash buffer (refer to kit insert for dilution), add approximately 350 µL wash buffer to each well, gently tap all edges of the handling tray to release any reagents trapped below the biochip, and flick to waste with a sharp action. Take care not to overfill wells during wash in order to reduce potential for well-to-well contamination. Carry out a further 6 wash cycles, for each cycle gently tap all edges of the handling tray for approximately 10 to 15 seconds then leave the biochips to soak in wash buffer for 2 minutes. After final wash tap onto lint free tissue to remove any residual wash buffer.
7. After the final wash, fill wells with wash buffer and leave to soak until directly prior to imaging. No carrier should be left to soak for longer than 30 minutes.

IMAGING

Please refer to the Operator's Manual for general operating procedure and creation of worklists.

- Process carriers individually. Those waiting imaging should be protected from light.
- Remove the first carrier to be imaged from the handling tray. Directly before addition of signal, remove wash buffer using a sharp, flicking action and tap the carrier onto lint free tissue to remove any residual wash buffer.
- Add 250 µL of working strength signal reagent-EV841 to each biochip well and cover to protect from light.
- Place the carrier into Evidence Investigator™ after exactly 2 minutes (+/-10 seconds). Use of a timer is recommended to ensure imaging occurs at the correct time.
- Capture of images will be automatically initiated as defined by dedicated software (please refer to Software Manual).

RESULTS PROCESSING

Results are processed automatically using the dedicated software.

REFERENCES

1. Hawks RL. Analytical Methodology from Urine Testing for Drugs of Abuse. National Institute on Drug Abuse (NIDA), *Research Monograph*, 1986; 73:30-41
2. Galloway JH and March ID, Detection of drug misuse – an addictive challenge, *J Clin Pathol*, 1999; 52: 713-718
3. Glass L R, Ingalls S T, Schilling C L and Hoppel C L, Atypical Urinary Opiate Excretion Pattern, *Journal of Analytical Toxicology*, October 1997; 21:509-514.

AMPHETAMINE CLASS ASSAY (AMPH/MAMP)

This insert is a supplement to the generic Drugs of Abuse Array I Whole Blood Plus package insert with information specific to the Amphetamine Class assays.

INTENDED USE

The Evidence Investigator™ amphetamine assays have been designed for use only on the Evidence Investigator™ analyser for semi-quantitative detection of the amphetamine class of compounds in blood.

This assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred method. Clinical consideration and professional judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

CLINICAL SIGNIFICANCE

Amphetamines are synthetic drugs, which cause powerful central nervous system (CNS) stimulation resulting in euphoric effects similar to that of cocaine. They can also cause increased alertness, self-confidence and the ability to concentrate[1,2]. They are potent sympathomimetic agents with a range of therapeutic applications for example; they can be used to treat mild depression, obesity, narcolepsy, and certain behavioural disorders in children[1,3]. Isomeric forms of amphetamine and methamphetamine exist and the D-isomer (dextroamphetamine) is four times as potent as the L-isomer[2].

Abuse of amphetamines is a significant problem, although abuse of amphetamine is not widespread, methamphetamine abuse is and there has been much concern over 'Ice', the solid form of methamphetamine[4]. Amphetamine abusers can develop a tolerance for the drug, resulting in a psychological dependence and leading to drug abuse[3]. Chronic abuse of amphetamine can lead to weight loss, hallucinations and paranoid psychosis, while acute overdose can cause agitation, hyperthermia, convulsions, coma and respiratory and/or cardiac failure[2]. Large quantities of illegally synthesised amphetamines are manufactured. Many amphetamine analogues from the illicit market of the 1960s have been reintroduced and new analogues have been synthesised and marketed for their increased potency, altered pharmacological effects and difficulty of detection[1,4].

MDMA (Methylenedioxy-methamphetamine), MDA (Methylenedioxyamphetamine) and MDEA (Methylenedioxyethylamphetamine) are synthetically modified amphetamines. MDMA is one of the most common amphetamine analogues on the illicit market. It was previously used as an adjunct to psychotherapy but it was placed on the schedule of controlled substances in 1988. Despite this, it still remains very popular as a recreational drug. MDMA is metabolised to MDA, another drug known for its central stimulant properties[2,4].

Amphetamines are usually taken orally, intravenously or by insufflation[3]. The half-life of amphetamines in humans is in the range 10 to 30 hours depending on the drug and the amount taken[5].

Evidence Investigator™ performs two amphetamine class assays, based on d-amphetamine and methamphetamine in the form of two discrete test regions (DTR) on the Drugs of Abuse (DoA) biochip. By performing both tests simultaneously the main amphetamines, metabolites and analogues can be detected giving a more accurate indication of recent drug use.

PRINCIPLE

The Evidence Investigator™ Amphetamine Class Assay is a competitive chemiluminescent immunoassay for the detection of amphetamines in blood.

REAGENT COMPOSITION

Refer to the DOA I WB P Assay Array package insert for reagent compositions. The biochip substrate contains immobilised monoclonal antibodies raised to amphetamines.

MATERIAL HANDLING

For further information on precautions, reagent composition, preparation and storage, specimen collection and handling, quality control materials and analyser procedure, please refer to the DOA I WB P Assay Array package insert for further details.

SEMI-QUANTITATIVE RESULTS

The Evidence Investigator™ DOA I WB P Calibrators are used in determining equivalent concentration of amphetamine class compounds present in a sample via comparison to the primary target analytes in those calibrators.

SPECIFICITY

Specificity/cross-reactivity of the Evidence® Amphetamine Class assays were assessed using a dose-response series diluted in drug screen negative blood. Percentage cross reactivities of amphetamines, as determined by the Evidence® Amphetamine Class Assays, are shown in Table 1.

Compounds that elicit a negative response by the Evidence® Amphetamine Class Assays are shown in Table 2.

Table 1. Cross reactivity of Amphetamine compounds

Compound	AMPH (%CR)	MAMP (%CR)
d-Amphetamine	100	0.6
(+) Methamphetamine	<0.1	100
dl-Amphetamine	29.5	0.5
MDA	426	0.6
MDMA	0.4	38
MDEA	1.4	2.1
BDB	177	0.4
MBDB	<0.1	96
Fenfluramine	<0.1	12.4
Phentermine	35.2	<0.4
Para-Methoxymeth-Amphetamine (PMMA)	<0.1	70.7
3-Trifluoromethylphenyl-Piperazine (TFMPP)	<0.1	23.5
4-Methoxyamphetamine (PMA)	138	<0.1
Mephedrone HCl	<1	1.8
Methylone HCl	<1	1.6
Methcathinone (Randox)	<1	2.4
Flephedrone HCl	<1	<1
R(+)-Methcathinone HCl	<1	3.4
3-Fluoromethcathinone HCl	<1	3.3
S(-) Methcathinone HCl	<1	2.1
Methylethcathinone	<1	1.7
N-Ethylcathinone HCl	<1	5.6
Ethylone HCl	<1	3.0
Buphedrone HCl	<1	7.0
MDPBP HCl	<1	0.4
MDPV HCl	<1	<1
Naphyrone HCl	<1	<1
4-Methyl-alpha-pyrrolidine butiophenone HCl	<1	<1
MDPPP HCl	<1	<1

Table 2. Concentrations of compounds failing to produce a positive response

Compound	AMPH (µg/mL)	MAMP (µg/mL)
Phenobarbital	10	10
Secobarbital	10	10
Pentobarbital	10	10
Oxazepam	10	10
Lorazepam	10	10
Methadone	10	10
Morphine	10	10
Codeine	10	10
Phencyclidine	10	10
Benzoylecgonine	10	10
11-nor-Δ^9-THC-COOH	10	10
(+) Ephedrine	10	10
(-) Pseudoephedrine	10	10
(+) Pseudoephedrine	10	10
Buprenorphine	0.15	0.15
Nortriptyline	0.15	0.15

INTERFERENCE

The effect of Triglycerides, Lipids, Haemoglobin and Bilirubin were assessed to establish the maximum concentration at which the interferent will not cause a significant increase or decrease in Amphetamine Class Assay performance and this is shown in Table 3.

Table 3. Interference in d-Amphetamine and Methamphetamine assays

d-Amphetamine assay

Substance	Concentration
Triglycerides	15 g/L
Lipids	20 g/L
Haemoglobin	10 g/L
Bilirubin	0.15 g/L

Methamphetamine assay

Substance	Concentration
Triglycerides	4 g/L
Lipids	20 g/L
Haemoglobin	5 g/L
Bilirubin	0.15 g/L

LIMITATIONS

- Other substances not listed may interfere with the assay.

CALIBRATION

The Amphetamine and Methamphetamine assays are standardised to d-Amphetamine and (+)Methamphetamine respectively and quantified by GC/MS.

TYPICAL ASSAY RANGE

ANALYTE	ASSAY RANGE
d-Amphetamine	0 - 550 ng/mL
Methamphetamine	0 – 1000 ng/mL

Note individual ranges of calibrator batches may vary slightly.

SPECIFIC PERFORMANCE DATA

Limit of Detection

The limit of detection of the Evidence Investigator™ Amphetamine Class test assay was established by analysing 20 negative human blood samples for d-amphetamine and methamphetamine. This was determined to be 1.42 ng/mL* and 7.08 ng/mL* respectively. *This is equivalent to 5.68 ng/mL and 28.30 ng/mL for amphetamine and methamphetamine respectively in a neat sample.

Intra Assay Precision

Intra assay precision was determined by assaying 20 replicates of each of three control levels.

Table 4. Analysis of Intra Assay Precision (n=20)

AMP	Sample 1	Sample 2	Sample 3
Conc ng/mL	18	43	73
% CV	5.9	5.4	5.3

MAMP	Sample 1	Sample 2	Sample 3
Conc ng/mL	49	158	308
% CV	15.9	8.2	10.6

Inter Assay Precision

Inter assay precision was determined by assaying 3 controls in replicates of 2 in 10 separate assays.

Table 5. Analysis of Inter Assay Precision (n=20)

AMP	Sample 1	Sample 2	Sample 3
Conc ng/mL	18	44	74
% CV	13.1	11.3	10.2

MAMP	Sample 1	Sample 2	Sample 3
Conc ng/mL	57	204	295
% CV	14.4	6.9	14.2

Recovery

Table 6. Analysis of Recovery

AMP	Recovery concentration (ng/mL)	Recovery (%)
Level 1	28	117
Level 2	44	96
Level 3	54	111

MAMP	Recovery concentration (ng/mL)	Recovery (%)
Level 1	156	117
Level 2	202	110
Level 3	238	109

REFERENCES

1. Lee M-R, Yu S-C, Lin C-L and Yeh Y-Cia, Solid-phase extraction in Amphetamine and methamphetamine analysis of Urine, *Journal of Analytical Toxicology*, July/August 1997; 21: 278-282
2. Wild D. (ed), *The Immunoassay Handbook*, second edition, Nature Publishing Group, London, Basingstoke, New York, 2001; 783-788.
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4. Cody J T, Detection of D, L-Amphetamine, D, L-Methamphetamine and illicit Amphetamine Analogs using Diagnostic Products Corporation's Amphetamine and Methamphetamine Radioimmunoassay, *Journal of Analytical Toxicology*, 1990; 14: 321-324
5. Albertson T E, Derlet R W, Van Hoozen B E, Methamphetamine and the expanding complications of amphetamines. *West J Med*. April 1999;**170(4)**:214-9.

BARBITURATE ASSAY (BARB)

This insert is a supplement to the generic Drugs of Abuse Array I Whole Blood Plus package insert with information specific to the Barbiturate assay.

INTENDED USE

The Evidence Investigator™ Barbiturate assay has been designed for use only on the Evidence Investigator™ analyser for semi-quantitative detection of barbiturates, in blood.

This assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred method. Clinical consideration and professional judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

CLINICAL SIGNIFICANCE

Barbiturates are a class of around 12 compounds derived from barbituric acid. They are central nervous system (CNS) depressants and they can be used as sedatives, hypnotics, anaesthetics and anti-epileptic drugs.

Barbiturates can be divided into three main groups according to their duration of action. The ultra-short-acting barbiturates are used clinically as anaesthetics whilst the long-acting barbiturates have anti-convulsing properties. The short-acting compounds are typically used as hypnotics[1,2].

It is the short-acting barbiturates that are the most favoured by drug abusers due to their ability to reduce tension and produce a feeling of tranquillity without too much drowsiness. These include secobarbital, pentobarbital and amobarbital. The duration of action of short acting barbiturates is 3-6 hours[1,2].

It is because of the barbiturate's sedative-hypnotic properties that they are frequently abused. Administration by abusers is nearly always by oral ingestion of tablets or capsules. An overdose of barbiturates can lead to a dramatic fall in blood pressure and body temperature, depressed respiration and coma. A continued use of barbiturates can lead to the development of tolerance and withdrawal can be dangerous for an addict[1,3].

The short-acting barbiturates are extensively metabolised by the liver to more pharmacologically inactive hydroxylated compounds[1]. Typical of short-acting barbiturates, secobarbital has a half-life of 19-34 hours. Whereas long-acting barbiturate is much longer, e.g. phenobarbital has a half-life of about 24-140 hours[5].

PRINCIPLE

The Evidence Investigator™ Barbiturate assay is a competitive chemiluminescent immunoassay for the detection of barbiturates in blood.

REAGENT COMPOSITION

Refer to the DOA I WB P Assay Array package insert for reagent compositions.

The biochip substrate contains immobilised polyclonal antibodies raised in sheep to barbiturates.

MATERIAL HANDLING

For further information on precautions, reagent composition, preparation and storage, specimen collection and handling, quality control materials and analyser procedure, please refer to the DOA I WB P Assay Array package insert for further details.

SEMI-QUANTITATIVE RESULTS

The Evidence Investigator™ DOA I WB P Calibrators are used in determining equivalent concentration of barbiturate compounds present in a sample via comparison to the primary target analyte in those calibrators.

SPECIFICITY

Specificity/cross-reactivity of the Evidence® Barbiturate assay was assessed using a dose-response series diluted in drug screen negative blood. Percentage cross reactivities of barbiturates, as determined by the Evidence® Barbiturate assay, are shown in Table 1. Compounds that elicit a negative response by the Evidence® Barbiturate assay are shown in Table 2.

Table 1. Cross reactivity of barbiturate compounds

Compound	% Cross reactivity
Phenobarbital	100
Secobarbital	371
Pentobarbital	151
Amobarbital	44
Barbital	33.3
Butalbital	51.1
Alphenal	117
Butabarbital	166
Cyclopentobarbital	70.1
p-Hydroxyphenobarbital	64

Table 2. Concentrations of compounds failing to produce a positive result

Compound	Concentration (µg/mL)
d-Amphetamine	10
(+)Methamphetamine	10
MDA	10
MDMA	10
Oxazepam	10
Lorazepam	10
Methadone	10
Morphine	10
Codeine	10
Phencyclidine	10
Benzoylecgonine	10
11-nor-Δ^9-THC-COOH	10
Buprenorphine	0.15
Notriptyline	0.15

INTERFERENCE

The effect of Triglycerides, Lipids, Haemoglobin and Bilirubin were assessed to establish the maximum concentration at which the interferent will not cause a significant increase or decrease in Barbiturate assay performance and this is shown in Table 3.

Table 3. Interference in Barbiturate assay

Substance	Concentration
Triglycerides	10 g/L
Lipids	15 g/L
Haemoglobin	10 g/L
Bilirubin	0.014 g/L

LIMITATIONS

- Other substances not listed may interfere with the assay.

CALIBRATION

The Barbiturate assay is standardised to Phenobarbital and quantified by GC/MS.

TYPICAL ASSAY RANGE

ANALYTE	ASSAY RANGE
Barbiturate	0 - 250 ng/mL

Note individual ranges of calibrator batches may vary slightly.

SPECIFIC PERFORMANCE DATA

Limit of Detection

The limit of detection of the Evidence Investigator™ Barbiturate Assay was established by analysing 20 negative human blood samples. This was determined to be 1.62 ng/mL*. *This is equivalent to 6.46 ng/mL of Barbiturate in a neat sample.

Intra Assay Precision

Intra assay precision was determined by assaying 20 replicates of each of three control levels.

Table 4. Analysis of Intra Assay Precision (n=20)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	13.1	29.5	94.2
% CV	13.6	10.9	9.1

Inter Assay Precision

Inter assay precision was determined by assaying 3 controls in replicates of 2 in 10 separate assays.

Table 5. Analysis of Inter Assay Precision (n=20)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	15.1	60.1	93.4
% CV	12.9	10.5	9.3

Recovery

Table 6. Analysis of Recovery

	Recovery concentration (ng/mL)	Recovery (%)
Level 1	36.3	114
Level 2	58.1	107
Level 3	72.0	114

REFERENCES

1. Widdop B. (2001) Chapter 37, Drugs of Abuse. In: Wild D. (ed), (2001) The Immunoassay Handbook, Second edition, Nature Publishing Group, London, Basingstoke, New York, 781-816.
2. Simpson D., Braithwaite R.A., Jarvie D.R., Stewart M.J., Walker S., Watson I.W. and Widdop B. (1997) Screening for drugs of abuse (II): cannabinoids, lysergic acid diethylamide, buprenorphine, methadone, barbiturates, benzodiazepines and other drugs. *Ann Clin Biochem*, 34(5):460-510.
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4. Shen D. (1989) [Saliva phenobarbital concentration in epileptics] *Zhonghua Shen Jing Jing Shen Ke Za Zhi.* 22(6):369-370, 382.
5. Tokugawa K., Ueda K., Fujito H. and Kurokawa, T. (1986) Correlation between the saliva and free serum concentration of phenobarbital in epileptic children. *Eur. J. Pediatr.* 145(5):401-402

BENZODIAZEPINE ASSAY (BENZ1/BENZ2)

This insert is a supplement to the generic Drugs of Abuse Array 1 Blood Plus package insert with information specific to the Benzodiazepine Class assay.

INTENDED USE

The Evidence Investigator™ Benzodiazepine assays have been designed for use on the Evidence Investigator™ analyser for semi-quantitative detection of benzodiazepine compounds, drugs with sedative and hypnotic effects, in blood. Evidence Investigator™ performs two Benzodiazepine assays based on oxazepam (Benzodiazepine Assay 1) and lorazepam (Benzodiazepine Assay 2).

This assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred method. Clinical consideration and professional judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

CLINICAL SIGNIFICANCE

The benzodiazepines are a group of over 20 structurally related central nervous system depressant drugs, each group varying considerably in their potency and clinical effects. The benzodiazepines are the most widely prescribed sedative/hypnotic drugs due to their wide range of uses[1,2,6]. They are prescribed to treat anxiety, stress and insomnia. They are also used both as pre-medication and for induction of general anaesthesia. Most specific uses include the management of alcohol withdrawal, control of epileptic fits and relief of muscle spasms[3].

Benzodiazepines were first developed as hypnotic sedatives in the late 1960s and replaced the more problematic barbiturates, then associated with numerous overdoses[4]. Benzodiazepines have the potential to elicit dependence and be abused, both in high doses and in low, therapeutic doses[5]. They are taken because of their mood-altering properties and are known as 'downers' due to their ability to lessen the effects of opiate withdrawal and after-effects of ecstasy, amphetamine and LSD. However, chronic abuse leads to blurred vision, confusion, slow reflexes, slurred speech and hypotension[2]. In spite of this, benzodiazepines are relatively safe drugs and have almost completely replaced the more toxic barbiturates.

Overdose and death are usually the result of the combination of benzodiazepine with other drugs or alcohol rather than taken alone[2,6].

Benzodiazepines are extensively metabolised by the liver by processes of N-dealkylation and hydroxylation followed by glucuronidation and only trace amounts of the parent compound are excreted. The most common metabolites are oxazepam and nordiazepam[2,5]. Benzodiazepines are classified according to elimination half-life. Long-acting, intermediate and short-acting, and ultra-short acting benzoziazepines show half-lives exceeding 24 hours, between 5 and 24 hours, and less than 5 hours, respectively[7].

The highly addictive nature of benzodiazepines together with their wide availability and low-cost on the illicit drug market has created a population of people dependent on one or more benzodiazepines. There is therefore a need to monitor the uses and abuse of these drugs[3,6].

By performing both Benzodiazepine Assays 1 and 2 simultaneously in the form of two discrete test regions (DTR) on the Drugs of Abuse biochip, the main benzodiazepines and their metabolites can be detected.

PRINCIPLE

The Evidence Investigator™ Benzodiazepine Assays are competitive chemiluminescent immunoassays for the detection of benzodiazepines in blood.

REAGENT COMPOSITION

Refer to the DOA 1 WB P Assay Array package insert for reagent compositions. The biochip substrate contains immobilised polyclonal antibodies raised in sheep to benzodiazepines.

MATERIAL HANDLING

For further information on precautions, reagent composition, preparation and storage, specimen collection and handling, quality control materials and analyser procedure, please refer to the DOA 1 WB P Assay Array package insert for further details.

SEMI-QUANTITATIVE RESULTS

The Evidence Investigator™ DOA 1 WB P Calibrators are used in determining equivalent concentration of benzodiazepine compounds present in a sample via comparison to the primary target analytes in those calibrators.

SPECIFICITY

Specificity/cross-reactivity of the Evidence® Benzodiazepines Class assays were assessed using a dose-response series diluted in drug screen negative blood. Percentage cross reactivities of benzodiazepines, as determined by the Evidence® Benzodiazepines Class Assays, are shown in Table 1. Compounds that elicit a negative response by the Evidence® Benzodiazepines Class assays are shown in Table 2.

Table 1. Cross reactivity of Benzodiazepine compounds

Compound	BENZ 1 Assay (%CR)	BENZ 2 Assay (%CR)
Oxazepam	100	5
Lorazepam	13	100
7-Aminoclonazepam	<0.1	0.3
7-Aminonitrazepam	2.4	<0.1
7-Aminoflunitrazepam	0.4	<0.1
α-OH-Alprazolam	310	<0.1
2-OH-Ethylflurazepam	188.1	<0.1
Alprazolam	258	<0.1
Bromazepam	21.6	0.5
Chlordiazepoxide	46.8	0.1
Clobazam	203.6	<0.1
Clonazepam	6.9	28.2
Desalkylflunitrazepam	54.4	8.3
Diazepam	256	<0.1
Estazolam	253	<0.1
Flunitrazepam	114.1	0.1
Lormetazepam	50.2	<0.1
Lorazepam glucuronide	<0.1	24.8
Midazolam	115.5	<0.1
Nitrazepam	194	0.8
Nordiazepam	317	1.9
Oxazepam glucuronide	2.0	3.5
Prazepam	172	<0.1
Temazepam	382	<0.1
Temazepam glucuronide	6.8	<0.1
Triazolam	29.6	<0.1
Phenazepam	61.2	72.8

Table 2. Concentrations of compounds failing to produce a positive result

Compound	BENZ 1 Assay (µg/mL)	BENZ 2 Assay (µg/mL)
d-Amphetamine	10	10
(+)Methamphetamine	10	10
MDA	10	10
MDMA	10	10
Phenobarbital	10	10
Secobarbital	10	10
Pentobarbital	10	10
Methadone	10	10
Morphine	10	10
Codeine	10	10
Phencyclidine	10	10
Benzoylecgonine	10	10
11-nor-Δ⁹-THC-COOH	10	10
Buprenorphine	0.15	0.15
Nortriptyline	0.15	0.15

INTERFERENCE

The effect of Triglycerides, Lipids, Haemoglobin and Bilirubin were assessed to establish the maximum concentration at which the interferent will not cause a significant increase or decrease in Benzodiazepines Class assays performance and this is shown in Table 3.

Table 3. Interference in Benzodiazepine Class assays

Benzodiazepine Assay 1

Substance	Concentration
Triglycerides	15 g/L
Lipids	15 g/L
Haemoglobin	10 g/L
Bilirubin	0.15 g/L

Benzodiazepine Assay 2

Substance	Concentration
Triglycerides	15 g/L
Lipids	20 g/L
Haemoglobin	1 g/L
Bilirubin	0.15 g/L

LIMITATIONS

- Other substances not listed may interfere with the assay.

CALIBRATION

The Benzodiazepine 1 and Benzodiazepine 2 assays are standardised to Oxazepam and Lorazepam respectively and quantified by GC/MS.

TYPICAL ASSAY RANGE

ANALYTE	ASSAY RANGE
Benzodiazepine 1	0 - 65 ng/mL
Benzodiazepine 2	0 – 150 ng/mL.

Note individual ranges of calibrator batches may vary slightly.

SPECIFIC PERFORMANCE DATA

Limit of Detection

The limit of detection of the Evidence Investigator™ Benzodiazepine Class assays, oxazepam (Benzodiazepine Assay 1) and lorazepam (Benzodiazepine Assay 2) was established by analysing 20 negative human blood samples for oxazepam and lorazepam. These were determined to be 0.30 and 0.33 ng/mL* respectively.

*This is equivalent to 1.22 and 1.30 ng/mL for oxazepam (Benzodiazepine Assay 1) and lorazepam (Benzodiazepine Assay 2) respectively in a neat sample.

Intra Assay Precision

Intra assay precision was determined by assaying 20 replicates of each of three control levels.

Table 4. Analysis of Intra Assay Precision (n=20)

Oxazepam (Benzodiazepine Assay 1)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	4.7	21.7	38.8
% CV	14.8	9.4	15.6

Lorazepam (Benzodiazepine Assay 2)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	8.1	30.4	61.3
% CV	16.2	5.3	8.9

Inter Assay Precision

Inter assay precision was determined by assaying 3 controls in replicates of 2 in 10 separate assays.

Table 5. Analysis of Inter Assay Precision (n=20)

Oxazepam (Benzodiazepine Assay 1)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	4.8	20.8	37.5
% CV	14.6	18.2	19.6

Lorazepam (Benzodiazepine Assay 2)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	7.3	28.8	58.6
% CV	15.2	12.6	13.4

Recovery

Table 6. Analysis of Recovery

Oxazepam (Benzodiazepine Assay 1)

	Recovery concentration (ng/mL)	Recovery (%)
Level 1	17.5	113
Level 2	23.4	117
Level 3	29.6	102

Lorazepam (Benzodiazepine Assay 2)

	Recovery concentration (ng/mL)	Recovery (%)
Level 1	25.9	115
Level 2	37.9	103
Level 3	46.8	104

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COCAINE METABOLITE ASSAY (BZG)

This insert is a supplement to the generic Drugs of Abuse Array I Whole Blood Plus package insert with information specific to the Cocaine Metabolite assay.

INTENDED USE

The Evidence Investigator™ Cocaine metabolite assay is intended for use on the Evidence Investigator™ analyser for semi-quantitative detection of cocaine in the form of the cocaine metabolite, benzoylecgonine, in blood.

This assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred method. Clinical consideration and professional judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

CLINICAL SIGNIFICANCE

Cocaine is a potent psychoactive substance, also known as 'coke' and 'snow' and it is extracted from the leaves of the South American shrub *Erythroxylon coca*[1,2]. The Coca leaves are traditionally chewed or sucked resulting in a slow absorption of the drug and hence a slow onset of action. Cocaine hydrochloride is a fine powder that may be insufflated (snorted), producing quick absorption and onset of effects. Intravenous use leads to a quick and powerful but brief effect. 'Crack' is a freebase form of cocaine which when smoked produces an immediate 'high', with a more intense euphoria and so has become the choice for many users[1,3,4]. Cocaine causes severe psychological dependence. The abuse of cocaine dramatically increased in the middle and late 1980s and it continues to receive much attention by federal and local law enforcement[4].

Cocaine is administered in small doses. It stimulates the central nervous system (CNS) and the resulting effects include; increased alertness, euphoria, sense of confidence and physical strength that may encourage risk-taking behaviour[2,3]. It can also have local anaesthetic properties and cause an increased blood pressure, heart rate and body temperature. The euphoric effects show rapid onset, however within an hour they wear off leaving anxiety, fatigue and depression[3]. Chronic abuse and overdose of cocaine can cause acute cocaine intoxication, which can lead to profound CNS stimulation, resulting in seizures and cardiac arrest[3,4]. Cocaine is also used in combination with other recreational drugs such as heroin, which contributes to many cocaine-associated deaths[5].

In the body, cocaine is rapidly converted to metabolites benzoylecgonine, by non-enzymatic loss of the methyl ester function, and ecgonine methyl ester, by enzymatic demethylation. Ecgonine can be formed by further hydrolysis of both these metabolites[8]. The apparent half-life for cocaine is short; approximately 0.8 ± 0.2 hours, while the half-life of benzoylecgonine is 6 hours[9].

PRINCIPLE

The Evidence Investigator™ Cocaine Metabolite Whole Blood Plus Assay is a competitive chemiluminescent immunoassay for the detection of Benzoylecgonine in blood.

REAGENT COMPOSITION

Refer to the DOA I WB P Assay Array package insert for reagent compositions.

The biochip substrate contains immobilised sheep polyclonal antibodies raised to Benzoylecgonine.

MATERIAL HANDLING

For further information on precautions, reagent composition, preparation and storage, specimen collection and handling, quality control materials and analyser procedure, please refer to the Drugs of Abuse I Whole Blood Plus Assay Array package insert for further details.

SEMI-QUANTITATIVE RESULTS

The Evidence Investigator™ DOA I WB P Calibrators are used in determining equivalent concentration of cocaine metabolites present in a sample via comparison to the primary target analyte in those calibrators.

SPECIFICITY

Specificity/cross-reactivity of the Evidence® Cocaine Metabolite assay was assessed using a dose-response series diluted in drug screen negative blood. Percentage cross reactivities of cocaine/cocaine metabolites, as determined by the Evidence® Cocaine Metabolite assay, are shown in Table 1.

Compounds that elicit a negative response by the Evidence® Cocaine Metabolite assay are shown in Table 2.

Table 1. Cross reactivity of cocaine metabolites

Compound	% Cross reactivity
Benzoylecgonine	100
Cocaine	84.8
Cocaethylene	56.8
Ecgonine methyl ester	<0.1
Norcocaine	0.28

Table 2. Concentrations of compounds failing to produce a positive result

Compound	Concentration (µg/mL)
d-Amphetamine	10
(+)Methamphetamine	10
MDA	10
MDMA	10
Phenobarbital	10
Secobarbital	10
Pentobarbital	10
Oxazepam	10
Lorazepam	10
Methadone	10
Morphine	10
Codeine	10
Phencyclidine	10
11-nor-Δ⁹-THC-COOH	10
Buprenorphine	0.15
Nortriptyline	0.15

INTERFERENCE

The effect of Triglycerides, Lipids, Haemoglobin and Bilirubin were assessed to establish the maximum concentration at which the interferent will not cause a significant increase or decrease in the Cocaine Metabolite assay performance and this is shown in Table 3.

Table 3. Interference in Cocaine metabolite assay

Substance	Concentration
Triglycerides	15 g/L
Lipids	20 g/L
Haemoglobin	10 g/L
Bilirubin	0.15 g/L

LIMITATIONS

- Other substances not listed may interfere with the assay.

CALIBRATION

The Cocaine Metabolite assay is standardised to Benzoylecgonine and quantified by GC/MS.

TYPICAL ASSAY RANGE

ANALYTE	ASSAY RANGE
Cocaine Metabolite (BZG)	0 - 225 ng/mL

Note individual ranges of calibrator batches may vary slightly.

SPECIFIC PERFORMANCE DATA

Limit of Detection

The limit of detection of the Evidence Investigator™ Cocaine Assay was established by analysing 20 negative human blood samples. This was determined to be 0.15 ng/mL*.
*This is equivalent to 0.61 ng/mL of Benzoylecgonine in a neat sample.

Intra Assay Precision

Intra assay precision was determined by assaying 20 replicates of each of three control levels.

Table 4. Analysis of Intra Assay Precision (n=20)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	24.1	41.5	52.5
% CV	7.7	12.9	10.1

Inter Assay Precision

Inter assay precision was determined by assaying 3 controls in replicates of 2 in 10 separate assays.

Table 5. Analysis of Inter Assay Precision (n=20)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	25.0	38.2	53.5
% CV	7.26	12.5	11.0

Recovery

Table 6. Analysis of Recovery

	Recovery concentration (ng/mL)	Recovery (%)
Level 1	10.1	101
Level 2	39.8	80
Level 3	85.3	85

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METHADONE ASSAY (MDONE)

This insert is a supplement to the generic Drugs of Abuse Array I Whole Blood Plus package insert with information specific to the Methadone assay.

INTENDED USE

The Evidence Investigator™ methadone assay has been designed for use only on the Evidence Investigator™ analyser for semi-quantitative detection of methadone, a narcotic pain-relieving drug, in blood.

This assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred method. Clinical consideration and professional judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

CLINICAL SIGNIFICANCE

Methadone is a synthetic opioid analgesic, structurally related to Propoxyphene, and used clinically to decrease pain. Methadone was first synthesised as a substitute for morphine in Germany during the Second World War and has similar analgesic properties[1,2].

Methadone is used in opioid withdrawal programs to replace the dependence of heroin, an illegal and short acting drug. Methadone being longer acting is taken to decrease the drug craving and the aim of the program is to help drug abusers to develop a lifestyle free of street drugs[4].

It is available in tablet and linctus form or as a solution for intramuscular injection. Methadone is prescribed on a wide scale and so there is danger of the drug finding its way into the street market of drug abusers[2,5].

The side effects associated with methadone use can include physiological dependence, sedation, respiratory depression, respiratory failure and hypoxia. In cases of overdose this can cause coma, seizures, hypotension, and death[2-4].

Methadone is rapidly absorbed from the gastrointestinal tract and undergoes extensive metabolism in the liver. It is metabolised by mono- and di-*N*-demethylation to unstable metabolites that cyclise to give 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP)[1,2,6]. These metabolites are excreted in the urine and bile. The half-life of (R,S)-methadone is 15-60 hours, and 10-40 hours for (R)-methadone[7]. Unchanged methadone is the primary compound for detection by the Evidence Investigator™ Methadone assay.

PRINCIPLE

The Evidence Investigator™ Methadone assay is a competitive chemiluminescent immunoassay for the detection of methadone in blood. For further information, refer to the DOA I WB P Assay Array package insert.

REAGENT COMPOSITION

Refer to the DOA I WB P Assay Array package insert for reagent compositions. The biochip substrate contains immobilised polyclonal antibodies raised in sheep to methadone.

MATERIAL HANDLING

For further information on precautions, reagent composition, preparation and storage, specimen collection and handling, quality control materials and analyser procedure, please refer to the Drugs of Abuse I Whole Blood Plus Assay Array package insert for further details.

SEMI-QUANTITATIVE RESULTS

The Evidence Investigator™ DOA I WB P Calibrators are used in determining equivalent concentration of methadone class compounds present in a sample via comparison to the primary target analyte in those calibrators.

SPECIFICITY

Specificity/cross-reactivity of the Evidence® Methadone assay was assessed using a dose-response series diluted in drug screen negative blood. Percentage cross reactivities of methadone and metabolites, as determined by the Evidence® Methadone assay, are shown in Table 1. Compounds that elicit a negative response by the Evidence® Methadone assay are shown in Table 2.

Table 1. Cross reactivity of methadone compounds

Compound	% Cross reactivity
Methadone.HCl	100
EDDP	<0.1
EMDP	<0.1
LAAM	0.7

Table 2. Concentrations of compounds failing to produce a positive result

Compound	Concentration (µg/mL)
d-Amphetamine	10
(+)Methamphetamine	10
MDA	10
MDMA	10
Phenobarbital	10
Secobarbital	10
Pentobarbital	10
Oxazepam	10
Lorazepam	10
Morphine	10
Codeine	10
Phencyclidine	10
Benzoylecgonine	10
11-nor-Δ^9-THC-COOH	10
Buprenorphine	0.15
Nortriptyline	0.15

INTERFERENCE

The effect of Triglycerides, Lipids, Haemoglobin and Bilirubin were assessed to establish the maximum concentration at which the interferent will not cause a significant increase or decrease in the Methadone assay performance and this is shown in Table 3.

Table 3. Interference in Methadone assay

Substance	Concentration
Triglycerides	15 g/L
Lipids	20 g/L
Haemoglobin	10 g/L
Bilirubin	0.15 g/L

LIMITATIONS

Other substances not listed may interfere with the assay.

CALIBRATION

The Methadone assay is standardised to methadone.HCl and quantified by GC/MS.

TYPICAL ASSAY RANGE

ANALYTE	ASSAY RANGE
Methadone	0 - 100 ng/mL

Note individual ranges of calibrator batches may vary slightly.

SPECIFIC PERFORMANCE DATA

Limit of Detection

The limit of detection of the Evidence Investigator™ Methadone Assay was established by analysing 20 negative human blood samples. This was determined to be 0.88 ng/mL*. *This is equivalent to 3.50 ng/mL of Methadone in a neat sample.

Intra Assay Precision

Intra assay precision was determined by assaying 20 replicates of each of three control levels.

Table 4. Analysis of Intra Assay Precision (n=20)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	4.0	19.7	35.5
% CV	12.9	10.9	13.8

Inter Assay Precision

Inter assay precision was determined by assaying 3 controls in replicates of 2 in 10 separate assays.

Table 5. Analysis of Inter Assay Precision (n=20)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	5.1	10.9	37.8
% CV	14.7	15.8	14.4

Recovery

Table 6. Analysis of Recovery

	Recovery concentration (ng/mL)	Recovery (%)
Level 1	16.1	116
Level 2	24.6	113
Level 3	31.1	113

REFERENCES

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OPIATES ASSAY (OPIAT)

This insert is a supplement to the generic Drugs of Abuse Array I Whole Blood Plus package insert with information specific to the Opiates assay.

INTENDED USE

The Evidence Investigator™ Opiates assay has been designed for use only on the Evidence Investigator™ analyser for the semi-quantitative detection of opiates in blood.

This assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred method. Clinical consideration and professional judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

CLINICAL SIGNIFICANCE

Morphine and codeine are opiates obtained from the opium poppy and are narcotic analgesic drugs. Morphine is one of the most effective and commonly used analgesics for the control of severe pain. Heroin and hydrocodone are semi-synthetic derivatives of morphine and codeine but are more correctly classified as opioids. These drugs have potent effects on the central nervous system including; analgesia, euphoria state, apathy, sedation and respiratory depression[1,2,3].

Illicit opiate use remains a major problem. Continued use can lead to tolerance and physical dependence. Heroin is the most commonly abused derivative and it is 2-3 times more potent as an analgesic than morphine. Being more lipid soluble, heroin crosses the blood-brain barrier more readily than morphine. Addicts can take heroin either by injection, nasal insufflation or smoking[4,5,6].

Opiate poisoning can result in individuals suffering respiratory depression, pinpoint pupils and often deep coma. Death from heroin overdose usually is a result of respiratory failure[3,6,7]. The testing of patient samples has become increasingly necessary to monitor known or identify suspected drug abusers for opiates[8].

The plasma concentration of morphine declines rapidly within the first 10 minutes of intravenous administration due to tissue distribution and metabolism. Only 7% of an intravenously administered dose remains in the general circulation after 6 minutes. The elimination half-life of morphine is 177 minutes. Morphine is a common metabolite for several of the abused drugs in this category. For example, heroin is metabolised in humans to 6-mono-acetylmorphine (6-MAM) by chemical and enzymatic processes and it is then further metabolised rapidly to morphine and morphine conjugates[1]. The Evidence Investigator™ Opiates assay is directed towards morphine.

PRINCIPLE

The Evidence Investigator™ Opiates assay is a competitive chemiluminescent immunoassay for the detection of opiates in blood.

REAGENT COMPOSITION

Refer to the DOA I WB P Assay Array package insert for reagent compositions. The biochip substrate contains immobilised polyclonal antibodies raised in sheep to morphine.

MATERIAL HANDLING

For further information on precautions, reagent composition, preparation and storage, specimen collection and handling, quality control materials and analyser procedure, please refer to the DOA I WB P Assay Array package insert for further details.

SEMI-QUANTITATIVE RESULTS

The Evidence Investigator™ DOA I WB P Calibrators are used in determining equivalent concentration of opiate class compounds present in a sample via comparison to the primary target analyte in those calibrators.

SPECIFICITY

Specificity/cross-reactivity of the Evidence® Opiates assay was assessed using a dose-response series diluted in drug screen negative blood. Percentage cross reactivities of opiates, as determined by the Evidence® Opiates assay, are shown in Table 1.

Compounds that elicit a negative response by the Evidence® Opiates assay are shown in Table 2.

Table 1. Cross reactivity of opiate compounds

Compound	% Cross reactivity
Morphine	100
6-MAM	1214
Codeine	106.6
Morphine-3-glucuronide	16.2
Hydromorphone	27.2
Hydrocodone	6
Dihydrocodeine	5

Table 2. Concentrations of compounds failing to produce a positive result

Compound	Concentration (µg/mL)
d-Amphetamine	10
(+)Methamphetamine	10
MDA	10
MDMA	10
Phenobarbital	10
Secobarbital	10
Pentobarbital	10
Oxazepam	10
Lorazepam	10
Methadone	10
Phencyclidine	10
Benzoylecgonine	10
11-nor-Δ^9-THC-COOH	10
Buprenorphine	0.15
Nortriptyline	0.15

INTERFERENCE

The effect of Triglycerides, Lipids, Haemoglobin and Bilirubin were assessed to establish the maximum concentration at which the interferent will not cause a significant increase or decrease in the Opiates assay performance and this is shown in Table 3.

Table 3. Interference in Opiates assay

Substance	Concentration
Triglycerides	15 g/L
Lipids	15 g/L
Haemoglobin	1 g/L
Bilirubin	0.05 g/L

LIMITATIONS

- Other substances not listed may interfere with the assay.

CALIBRATION

The Opiate assay is standardised to Morphine and quantified by GC/MS.

TYPICAL ASSAY RANGE

ANALYTE	ASSAY RANGE
Opiate	0 - 300 ng/mL

Note individual ranges of calibrator batches may vary slightly.

SPECIFIC PERFORMANCE DATA

Limit of Detection

The limit of detection of the Evidence Investigator™ Opiate Assay was established by analysing 20 negative human blood samples. This was determined to be 0.45 ng/mL*.
*This is equivalent to 1.80 ng/mL of Opiate in a neat sample.

Intra Assay Precision

Intra assay precision was determined by assaying 20 replicates of each of three control levels.

Table 4. Analysis of Intra Assay Precision (n=20)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	17.4	20.3	44.7
% CV	12.7	13.9	13.7

Inter Assay Precision

Inter assay precision was determined by assaying 3 controls in replicates of 2 in 10 separate assays.

Table 5. Analysis of Inter Assay Precision (n=20)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	15.0	31.9	43.3
% CV	17.8	12.7	11.4

Recovery

Table 6. Analysis of Recovery

	Recovery concentration (ng/mL)	Recovery (%)
Level 1	19.1	111
Level 2	26.9	114
Level 3	33.7	108

REFERENCES

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PHENCYCLIDINE ASSAY (PCP)

This insert is a supplement to the generic Drugs of Abuse Array I Whole Blood Plus package insert with information specific to the Phencyclidine assay.

INTENDED USE

The Evidence Investigator™ Phencyclidine assay has been designed for use only on the Evidence Investigator™ analyser for semi-quantitative detection of phencyclidine in blood.

This assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred method. Clinical consideration and professional judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

CLINICAL SIGNIFICANCE

Phencyclidine, 1-phenylcyclohexylpiperidine, is also known as PCP and 'angel dust'[1,2]. It is a synthetic drug developed in the 1950s as an anaesthetic and analgesic but was removed from the market due to its hallucinogenic properties and the unpredictable behavioural reactions, which occurred following anaesthesia[3,4,5].

PCP still has a legitimate use as a veterinary tranquilliser, however, in the 1960s PCP became a popular recreational drug leading to widespread street drug use and often resulting in incidences of overdose intoxication and death[1,3]. The toxic effects include; hypertension, seizures, coma and respiratory depression[5].

PCP is used by smoking with tobacco or marijuana, nasal insufflation ('snorting'), intravenous injection and oral ingestion[3,5]. It is a drug of abuse due to its euphoric and hallucinogenic effects. However, these effects can get quite erratic, with violent or bizarre behaviour often occurring[4]. The effect of PCP may vary with dose, the user's settings and previous experiences with PCP[3].

PCP is hydroxylated and then glucuronidated and the metabolites are excreted renally. Up to 15% of PCP is eliminated unchanged in urine[6]. The half-life of PCP ranges from 7-46 hours with the average being 21 hours[11].

The Evidence Investigator™ Phencyclidine Assay, tests for the parent molecule, 1-phenylcyclohexylpiperidine. Few substances cross-react with PCP in immunoassays[7].

PRINCIPLE

The Evidence Investigator™ Phencyclidine Assay is a competitive chemiluminescent immunoassay for the detection of phencyclidine in blood.

REAGENT COMPOSITION

Refer to the Drugs of Abuse Blood I Plus Assay Array package insert for reagent compositions. The biochip substrate contains immobilised polyclonal antibodies raised to phencyclidine.

MATERIAL HANDLING

For further information on precautions, reagent composition, preparation and storage, specimen collection and handling, quality control materials and analyser procedure, please refer to the DOA I WB P Assay Array package insert for further details.

SEMI-QUANTITATIVE RESULTS

The Evidence Investigator™ DOA I WB P Calibrators are used in determining equivalent concentration of PCP present in a sample via comparison to the primary target analytes in those calibrators.

SPECIFICITY

Specificity/cross-reactivity of the Evidence® PCP assay were assessed using a dose-response series diluted in drug screen negative blood. Percentage cross reactivities of PCP class compounds, as determined by the Evidence® PCP assay, are shown in Table 1.

Compounds that elicit a negative response by the Evidence® PCP assay are shown in Table 2.

Table 1. Cross reactivity of PCP compounds

Compound	% Cross reactivity
Phencyclidine	100
Dextromethorphan	0.1

Table 2. Concentrations of compounds failing to produce a positive result

Compound	Concentration (µg/mL)
d-Amphetamine	10
(+)Methamphetamine	10
MDA	10
MDMA	10
Phenobarbital	10
Secobarbital	10
Pentobarbital	10
Oxazepam	10
Lorazepam	10
Methadone	10
Morphine	10
Codeine	10
Benzoylecgonine	10
11-nor-Δ^9-THC-COOH	10
Buprenorphine	0.15
Nortriptyline	0.15

INTERFERENCE

The effect of Triglycerides, Lipids, Haemoglobin and Bilirubin were assessed to establish the maximum concentration at which the interferent will not cause a significant increase or decrease in the PCP assay performance and this is shown in Table 3.

Table 3. Interference in Phencyclidine assay

Substance	Concentration
Triglycerides	15 g/L
Lipids	20 g/L
Haemoglobin	1 g/L
Bilirubin	0.05 g/L

LIMITATIONS

- Other substances not listed may interfere with the assay.

CALIBRATION

The PCP assay is standardised to phencyclidine and quantified by GC/MS.

TYPICAL ASSAY RANGE

ANALYTE	ASSAY RANGE
PCP	0 - 80 ng/mL

Note individual ranges of calibrator batches may vary slightly.

SPECIFIC PERFORMANCE DATA

Limit of Detection

The limit of detection of the Evidence Investigator™ Phencyclidine Assay was established by analysing 20 negative human blood samples. This was determined to be 0.63 ng/mL*. *This is equivalent to 2.52 ng/mL of Phencyclidine in a neat sample.

Intra Assay Precision

Intra assay precision was determined by assaying 20 replicates of each of three control levels.

Table 4. Analysis of Intra Assay Precision (n=20)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	3.8	13.6	22.2
% CV	11.5	10.0	14.8

Inter Assay Precision

Inter assay precision was determined by assaying 3 controls in replicates of 2 in 10 separate assays.

Table 5. Analysis of Inter Assay Precision (n=20)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	4.1	9.0	20.3
% CV	15.0	16.5	13.3

Recovery

Table 6. Analysis of Recovery

	Recovery concentration (ng/mL)	Recovery (%)
Level 1	7.6	123
Level 2	12.1	115
Level 3	16.4	118

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CANNABINOIDS ASSAY (THC)

This insert is a supplement to the generic Drugs of Abuse Array I Whole Blood Plus package insert with information specific to the Cannabinoids assay.

INTENDED USE

The Evidence Investigator™ Cannabinoids assay has been designed for use only on the Evidence Investigator™ analyser for semi-quantitative detection of cannabinoids in blood.

This assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred method. Clinical consideration and professional judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

CLINICAL SIGNIFICANCE

The Cannabinoids are a group of more than 60 C-21 compounds found in the plant, *Cannabis sativa*. The active ingredient in Cannabinoids is Δ-9-tetrahydrocannabinol (THC)[1]. THC produces effects of euphoria, sedation and an altered time sense[1,2,3]. Cannabinoids can be used in the treatment of various medical conditions, including glaucoma, asthma, multiple sclerosis and as an anti-emetic in patients undergoing cancer chemotherapy[3].

Marijuana is one of the most commonly used illegal substances in the United States and in many countries around the world[4]. As a 'street' drug it is sold primarily as marijuana (flowering tops of the female plant) or hashish (the plant resin). Pure THC is almost never available on the 'black market'[1]. The primary route of illicit consumption of cannabis is by smoking in cigarettes or pipes. It can also be ingested in cakes and confectionery and hashish oil (the most potent form) can be taken intravenously. An overdose of THC can cause hallucinations, coma and death[2,3].

The illicit use of marijuana as a recreational drug has led to the wide development of methods to detect if an individual has been using the drug[4]. After inhalation the plasma concentration of THC rapidly declines with a half-life of 3.1 to 4.5 minutes. This is due to a combination of tissue absorption of the highly lipophilic THC and its metabolism. Thereafter, there is a much slower decline in plasma THC concentration with half-life between 19 and 36 hours. THC is rapidly metabolised to the principle inactive metabolite 11-nor-Δ^9-THC-9-carboxylic acid (ΔCOOH-THC) and is conjugated with water-soluble glucuronic acid[1]. Most of these water-soluble metabolites are excreted in the faeces and to a lesser extent in the urine[1]. The large tissue store of THC and its gradual re-entry into the bloodstream results in THC metabolites being detected as much as 1-2 weeks after marijuana use depending on the route of administration, potency of THC and frequency of use[1]. The most widely used test procedures employ immunoassay techniques to detect the presence of ΔCOOH-THC[3]. The Evidence Investigator™ Cannabinoid Assay detects the two main THC metabolites: delta-9 and delta-8 analogues, 11-Nor-Δ^9-THC-9-carboxylic acid and 11-Nor-Δ^8-THC-9-carboxylic acid.

PRINCIPLE

The Evidence Investigator™ Cannabinoid Assay is a competitive chemiluminescent immunoassay for the detection of cannabinoids in blood.

REAGENT COMPOSITION

Refer to the DOA I WB P Assay Array package insert for reagent compositions. The biochip substrate contains immobilised monoclonal antibodies raised to cannabinoids.

MATERIAL HANDLING

For further information on precautions, reagent composition, preparation and storage, specimen collection and handling, quality control materials and analyser procedure, please refer to the DOA I WB P Assay Array package insert for further details.

SEMI-QUANTITATIVE RESULTS

The Evidence Investigator™ DOA I WB P Calibrators are used in determining equivalent concentration of cannabinoids present in a sample via comparison to the primary target analyte in those calibrators.

SPECIFICITY

Specificity/cross-reactivity of the Evidence® Cannabinoids assay were assessed using a dose-response series diluted in drug screen negative blood. Percentage cross reactivities of cannabinoids as determined by the Evidence® Cannabinoids assay, are shown in Table 1.

Compounds that elicit a negative response by the Evidence® Cannabinoids assay are shown in Table 2.

Table 1. Cross reactivity of Cannabinoid compounds

Compound	% Cross reactivity
11-nor-Δ^9-THC-9-carboxylic acid	100
11-hydroxy-Δ^9-THC	4.5
(-) Δ^9-THC	1
Cannabinol	0.3

Table 2. Concentrations of compounds failing to produce a positive result

Compound	Concentration (µg/mL)
d-Amphetamine	10
(+)Methamphetamine	10
MDA	10
MDMA	10
Phenobarbital	10
Secobarbital	10
Pentobarbital	10
Oxazepam	10
Lorazepam	10
Methadone	10
Morphine	10
Codeine	10
Phencyclidine	10
Benzoylecgonine	10
Buprenorphine	0.15
Nortriptyline	0.15

INTERFERENCE

The effect of Triglycerides, Lipids, Haemoglobin and Bilirubin were assessed to establish the maximum concentration at which the interferent will not cause a significant increase or decrease in the THC assay performance and this is shown in Table 3.

Table 3. Interference in Cannabinoid assay

Substance	Concentration
Triglycerides	10 g/L
Lipids	20 g/L
Haemoglobin	10 g/L
Bilirubin	0.15 g/L

LIMITATIONS

- Other substances not listed may interfere with the assay.

CALIBRATION

The THC assay is standardised to 11-nor-Δ^9-THC-9-carboxylic acid and quantified by GC/MS.

TYPICAL ASSAY RANGE

ANALYTE	ASSAY RANGE
THC	0 - 250 ng/mL

Note individual ranges of calibrator batches may vary slightly.

SPECIFIC PERFORMANCE DATA

Limit of Detection

The limit of detection of the Evidence Investigator™ Cannabinoids Assay was established by analysing 20 negative human blood samples. This was determined to be 1.90 ng/mL*.
*This is equivalent to 7.59 ng/mL of THC in a neat sample.

Intra Assay Precision

Intra assay precision was determined by assaying 20 replicates of each of three control levels.

Table 4. Analysis of Intra Assay Precision (n=20)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	10.6	37.1	87.1
% CV	11.2	9.6	16.6

Inter Assay Precision

Inter assay precision was determined by assaying 3 controls in replicates of 2 in 10 separate assays.

Table 5. Analysis of Inter Assay Precision (n=20)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	9.7	18.4	49.8
% CV	9.9	16.1	19.1

Recovery

Table 6. Analysis of Recovery

	Recovery concentration (ng/mL)	Recovery (%)
Level 1	26.6	108
Level 2	49.2	94
Level 3	66	102

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3,4 METHYLENEDIOXY METHAMPHETAMINE ASSAY (MDMA)

This insert is a supplement to the generic Drugs of Abuse Array I Whole Blood Plus package insert with information specific to the MDMA assay.

INTENDED USE

The Evidence Investigator™ MDMA assay has been designed for use only on the Evidence Investigator™ analyser for the semi-quantitative detection of MDMA in blood.

This assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used to obtain a confirmed analytical result. Gas Chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method. Other chemical confirmation methods are available. Clinical consideration and professional judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

CLINICAL SIGNIFICANCE

3,4 methylenedioxymethamphetamine (MDMA) is a ring substituted analogue of amphetamine commonly referred to as Ecstasy[1,2]. It was first synthesized in 1914 as an anorectic but was never marketed as such because of its side-effects. During the 1970s psychotherapists became interested in using MDMA as a therapeutic agent as it was reported to reduce the inhibition of the patient to speak openly in a therapy session[2,3]. Widespread abuse of the drug began in the early 1980s[2] with control action prepared in 1984 and emergency scheduling of MDMA into C1 of the CSA in 1985 with permanent scheduling in 1988[3]. Toxic effects include anxiety, depression, tachycardia, elevated blood pressure, cardiac arrhythmias, pupil dilation and sleep disorders[2]. It is now known that Ecstasy produces long term neurochemical changes after a single administration and selective and permanent brain damage during repetitive use[3]. Many deaths associated with Ecstasy use have been reported. Many of these deaths have resulted from hyperpyrexia brought on by high levels of exertion, high ambient temperatures and inadequate fluid intake. Death from Ecstasy use has also been attributed to sudden cardiac arrest, suicide, acute intoxication and accidents secondary to Ecstasy use[2]. The US Drug Enforcement Administration reported that in 2000 more than 6.4 million people aged 12 or over had reported that they had used Ecstasy at least once in their lives[4]. A 2004 review on drug abuse trends among youth in the US found an increased use of MDMA with this being evidenced by increased numbers of emergency department visits[5]. Psychotherapists have again become interested in using MDMA as a therapeutic agent and it was reported in 2002 that the FDA and the Spanish Ministry of Health have concluded that the risk/benefit ratio is favourable under certain circumstances for clinical studies investigating MDMA assisted psychotherapy. These studies are the only ones into therapeutic use of MDMA[6]. In the UK Ecstasy is a Class A drug under the Misuse of Drugs Act 1971[7].

MDMA is a ring substituted analogue of amphetamine and has a chiral center with a pair of enantiomers with the racemic mixture being consumed. The enantiomers show different pharmacologic activities, stereoselective metabolism and body disposition. MDMA is metabolised by two main metabolic pathways. One of the pathways is by *O*-demethylenation followed by catechol-O-methyltransferase (COMT)-catalysed methylation and/or glucuronide/sulphate conjugation. The other pathway involves N-dealkylation, deamination and oxidation to the corresponding benzoic acid derivatives conjugated to glycine. The first pathway gives 3,4-methylenedioxyamphetamine (MDA) by N-demethylation with the MDA and also MDMA both being further *O*-demethylenated to 3,4-dihydroxyamphetamine (HHA) and 3,4-dihydroxymethamphetamine (HHMA), respectively. HHMA and HHA are *O*-methylated to 4-hydroxy-3-methoxymethamphetamine (HMMA) and 4-hydroxy-3-methoxyamphetamine (HMA), respectively. In plasma and urine these metabolites are mainly as their glucuronide or sulphate conjugates. In the other pathway successive degradation of the side chains gives rise to N-dealkyl and deaminooxo metabolites. MDMA is subsequently metabolised to glycine conjugates of the corresponding 3,4-disubstituted benzoic acids. The main metabolites in urine and plasma are HHMA and HMMA with MDA a minor metabolite. The HHMA and HMMA are conjugated to glucuronic acid or sulphate and are not usually found in free form[8]. In a study where MDMA, MDA and HMMA were assayed in oral fluid samples after MDMA administration MDMA was the principle analyte detected with only very small quantities of MDA and trace amounts of HMMA detected[9].

PRINCIPLE

The Evidence Investigator™ MDMA Assay is a competitive chemiluminescent immunoassay for the detection of MDMA in blood.

REAGENT COMPOSITION

Refer to the DOA I WB P Assay Array package insert for reagent compositions. The biochip substrate contains immobilised monoclonal antibodies raised to MDMA.

MATERIAL HANDLING

For further information on precautions, reagent composition, preparation and storage, specimen collection and handling, quality control materials and analyser procedure, please refer to the DOA I WB P Assay Array package insert for further details.

SEMI-QUANTITATIVE RESULTS

The Evidence Investigator™ DOA I WB P Calibrators are used in determining equivalent concentration of MDMA present in a sample via comparison to the primary target analyte in those calibrators.

SPECIFICITY

Specificity/cross-reactivity of the Evidence® MDMA test kit was assessed using a dose-response series. Each compound was diluted in certified drug free human blood to the ranges specified. Compounds listed were tested in duplicate to a maximum of 0.5 mg/mL. Concentrations of the cross-reactants, which produce a response equal to that of the target compound at the cut-off, were calculated.

Percentage cross-reactivity with MDMA and related compounds, as determined by the Evidence® MDMA Assay, are shown in Table 1.

Compounds that elicit a negative response by the Evidence® MDMA Assay are shown in Table 2.

Table 1. Cross reactivity of MDMA compounds

Compound	% Cross reactivity
MDMA	100
MDEA	384
PMMA	30.5
MDA	5.5
MBDB	123
D,L BDB	4.8
Mephedrone HCl	0.3
Methylone HCl	3.9
Methcathinone (Randox)	0.01
Flephedrone HCl	<1
R(+)-Methcathinone HCl	0.02
3-Fluoromethcathinone HCl	0.1
S(-) Methcathinone HCl	0.1
Methylethcathinone	0.4
N-Ethylcathinone HCl	0.0
Ethylone HCl	4.6
Buphedrone HCl	0.05
MDPBP HCl	0.7
MDPV HCl	<1
Naphyrone HCl	<1
4-Methyl-alpha-pyrrolidine butiophenone HCl	<1
MDPPP HCl	2.4

Table 2. Concentrations of compounds failing to produce a positive result

Compound	Concentration (µg/mL)
HMMA	500
PMA	500
d-Amphetamine	500
l-Amphetamine	500
d,l-Amphetamine	500
l-Ephedrine	500
d-Methamphetamine	500
l-Methamphetamine	500
d,l-Methamphetamine	500
Phentermine	500
d,l phenylpropanolamine	500
Pseudoephedrine	500
Phenobarbital	0.32
Oxazepam	0.1
Lorazepam	0.16
Methadone	0.12
Phencyclidine	0.1
Morphine	0.16
11-nor-Δ^9-THC-COOH	0.2
Benzoylecgonine	0.6
Buprenorphine	0.15
Nortriptyline	0.15

INTERFERENCE

The effect of Triglycerides, Lipids, Haemoglobin and Bilirubin were assessed to establish the maximum concentration at which the interferent will not cause a significant increase or decrease in the MDMA assay performance and this is shown in Table 3.

Table 3. Interference in MDMA assay

Substance	Concentration
Triglycerides	10 g/L
Lipids	15 g/L
Haemoglobin	5 g/L
Bilirubin	0.1 g/L

LIMITATIONS

- Other substances not listed may interfere with the assay.

CALIBRATION

The MDMA assay is standardised to MDMA and quantified by GC/MS.

TYPICAL ASSAY RANGE

ANALYTE	ASSAY RANGE
MDMA	0 - 1000 ng/mL

Note individual ranges of calibrator batches may vary slightly.

SPECIFIC PERFORMANCE DATA

Limit of Detection

The limit of detection of the Evidence Investigator™ MDMA Assay was established by analysing 20 negative human blood samples. This was determined to be 1.76 ng/mL*.

*This is equivalent to 7.03 ng/mL of MDMA in a neat sample.

Intra Assay Precision
Intra assay precision was determined by assaying 20 replicates of each of three control levels.

Table 4. Analysis of Intra Assay Precision (n=20)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	44.3	108.5	274.7
% CV	10.3	8.7	6.8

Inter Assay Precision
Inter assay precision was determined by assaying 3 controls in replicates of 2 in 10 separate assays.

Table 5. Analysis of Inter Assay Precision (n=20)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	45.4	101.3	274.0
% CV	10.3	10.5	12.0

Recovery

Table 6. Analysis of Recovery

	Recovery concentration (ng/mL)	**Recovery (%)**
Level 1	134	112
Level 2	191	101
Level 3	230	111

REFERENCES

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BUPRENORPHINE ASSAY (BUP)

This insert is a supplement to the generic Drugs of Abuse Array I Whole Blood Plus package insert with information specific to the Buprenorphine assay.

INTENDED USE

The Evidence Investigator™ Buprenorphine assay has been designed for use only on the Evidence Investigator™ analyser for the semi-quantitative detection of buprenorphines in blood.

This assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used to obtain a confirmed analytical result. Gas Chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method. Other chemical confirmation methods are available. Clinical consideration and professional judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

CLINICAL SIGNIFICANCE

Buprenorphine is a highly lipophylic derivative of thebaine[1] and is a powerful partial agonist analgesic[1,2]. It is effective in treating pain and is 25 to 40 times more potent than morphine. Since the 1980s it has been widely prescribed for the treatment of moderate to severe pain and in anaesthesiology for pre-medication and/or anaesthetic induction[2]. Soon after buprenorphine became clinically available there were reports of abuse as it was frequently used as the drug of choice when there was a shortage of heroin supplies[1]. Buprenorphine hydrochloride in a sublingual tablet called Subutex® was developed and registered for use in France in 1995, and subsequently other countries for opiate dependence treatment as it was found that withdrawal syndrome was milder than with methadone and that fewer symptoms emerge during detoxification with buprenorphine[3]. There is potential for misuse of the higher dose tablets by intravenous injection and it has been reported that in France this has happened regularly in 10-15% of the cases with irregular use in as many as 20-30% of patients[4].

Buprenorphine undergoes extensive first pass metabolism, has a large volume of distribution and is highly protein bound[5]. It is metabolized by *N*-dealkylation to norbuprenorphine (*N*-desalkylbuprenorphine) with both the buprenorphine and the norbuprenorphine then conjugated to glucuronic acid[6,7]. Most of the dose of buprenorphine is eliminated in the faeces with approximately 10-30% excreted in urine[5]. It has been reported that no unconjugated buprenorphine was found in urine with the buprenorphine and the norbuprenorphine excreted as glucuronide conjugates. However, in a different study a small amount of unconjugated buprenorphine was found in urine[8]. Until recently these were the only reported metabolites of buprenorphine in human subjects. However, a number of other minor metabolites have been identified[9].

PRINCIPLE

The Evidence Investigator™ Buprenorphine Assay is a competitive chemiluminescent immunoassay for the detection of buprenorphine in blood.

REAGENT COMPOSITION

Refer to the DOA I WB P Assay Array package insert for reagent compositions. The biochip substrate contains immobilised monoclonal antibodies raised to buprenorphine.

MATERIAL HANDLING

For further information on precautions, reagent composition, preparation and storage, specimen collection and handling, quality control materials and analyser procedure, please refer to the DOA I WB P Assay Array package insert for further details.

SEMI-QUANTITATIVE RESULTS

The Evidence Investigator™ DOA I WB P Calibrators are used in determining equivalent concentration of buprenorphine present in a sample via comparison to the primary target analyte in those calibrators.

SPECIFICITY

Specificity/cross-reactivity of the Evidence® Buprenorphine test kit was assessed using a dose-response series. Each compound was diluted in certified drug free human whole blood to the ranges specified. Compounds listed were tested in duplicate to a maximum of 0.5 mg/mL. Concentrations of the cross-reactants, which produce a response equal to that of the target compound at the cut-off, were calculated.

Percentage cross-reactivity with Buprenorphine is shown in Table 1. Compounds that elicit a negative response are shown in Table 2.

Table 1. Cross reactivity of Buprenorphine compounds

Compound	% Cross reactivity
Buprenorphine	100
Buprenorphine Glucuronide	51.7

Table 2. Concentrations of compounds failing to produce a positive result

Compound	Concentration (µg/mL)
Methamphetamine	0.18
d-Amphetamine	0.5
Phenobarbital	0.32
Oxazepam	0.1
Lorazepam	0.16
Methadone	0.12
Morphine	0.16
Phencyclidine	0.1
Benzoylecgonine	0.06
11-nor-Δ^9-THC-9-carboxylic acid	0.2
MDMA	1.0
Nortriptyline	0.15

INTERFERENCE

The effect of Triglycerides, Lipids, Haemoglobin and Bilirubin were assessed to establish the maximum concentration at which the interferent will not cause a significant increase or decrease in the Buprenorphine assay performance and this is shown in Table 3.

Table 3. Interference in Buprenorphine assay

Substance	Concentration
Triglycerides	2 g/L
Lipids	2 g/L
Haemoglobin	10 g/L
Bilirubin	0.15 g/L

LIMITATIONS

- Other substances not listed may interfere with the assay.

CALIBRATION

The Buprenorphine assay is standardised to Buprenorphine and quantified by LC/MS.

TYPICAL ASSAY RANGE

ANALYTE	ASSAY RANGE
BUP	0 - 90 ng/mL

Note individual ranges of calibrator batches may vary slightly.

SPECIFIC PERFORMANCE DATA

Limit of Detection

The limit of detection of the Evidence Investigator™ Buprenorphine Assay was established by analysing 20 negative human blood samples. This was determined to be 0.18 ng/mL*. *This is equivalent to 0.70 ng/mL of Buprenorphine in a neat sample

Intra Assay Precision

Intra assay precision was determined by assaying 20 replicates of each of three control levels.

Table 4. Analysis of Intra Assay Precision (n=20)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	3.9	13.9	21.6
% CV	18.0	16.1	15.8

Inter Assay Precision

Inter assay precision was determined by assaying 3 controls in replicates of 2 in 10 separate assays.

Table 5. Analysis of Inter Assay Precision (n=20)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	3.8	7.7	14.5
% CV	22.4	19.7	23.6

Recovery

Table 6. Analysis of Recovery

	Recovery concentration (ng/mL)	Recovery (%)
Level 1	7.3	111
Level 2	12.3	100
Level 3	15.8	115

REFERENCES

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TRICYCLIC ANTIDEPRESSANTS ASSAY (TCA)

This insert is a supplement to the generic Drugs of Abuse Array I Whole Blood Plus package insert with information specific to the TCA assay.

INTENDED USE

The Evidence Investigator™ TCA assay has been designed for use only on the Evidence Investigator™ analyser for the semi-quantitative detection of TCA in blood.

This assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography / mass spectrometry (GC/MS) is the preferred method. Clinical consideration and professional judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

CLINICAL SIGNIFICANCE

Tricyclic antidepressants (TCAs) are a group of drugs that contain a characteristic three ringed nucleus structure. They have been used in the treatment of various forms of depression, anxiety disorders, attention deficit hyperactivity disorder and enuresis in children. They can also be used at much lower doses for the treatment of certain types of pain. Members of the TCA group include amitriptyline, imipramine, nortriptyline, desipramine, clomipramine, and doxepin[1] with amitriptyline, imipramine, nortriptyline and desipramine widely prescribed[2]. There is a wide interindividual variation in pharmacokinetic factors of TCAs so that the relationship between TCA dose and antidepressant response is poorly delineated. Optimal plasma concentrations are achieved in less than 40-50% of patients treated with standard doses of TCAs. Therapeutic levels have been determined for some of the TCAs which has allowed the antidepressant response to therapy with TCAs to be improved by therapeutic drug monitoring[1]. Antidepressant drugs are among the most commonly encountered causes of self-poisioning with the TCAs generally more toxic in overdose than other types of antidepressants[3]. The therapeutic range for amitriptyline is 120 to 250 ng/mL, for desipramine it is 115 to 250 ng/mL, for imipramine it is 180 to 350 ng/mL and for nortriptyline it is 50 to 150 ng/mL. The toxic concentration for amitriptyline, nortriptyline, imipramine and desipramine is >500 ng/mL and lethal cardiotoxicity has been associated with plasma concentrations >1000 ng/mL[1]. The TCAs are almost entirely metabolised in the liver with the absorption varying markedly depending on the route of administration and the drug formulation. They are highly tissue and protein bound resulting in a large apparent volume of distribution. The metabolic pathways of TCAs are demethylation, hydroxylation followed by glucuronide coupling, N-oxidation and dealkylation[4]. Amitriptyline and imipramine are tertiary amines and are demethylated to the secondary amines nortriptyline and desipramine respectively. The secondary amines are then hydroxylated[1] with the plasma concentrations of hydroxylated metabolites of nortriptyline reported to be as much as threefold that of the parent drug. Only a very small percentage of the total TCA dose is eliminated in the urine unchanged[4].

PRINCIPLE

The Evidence Investigator™ TCA Assay is a competitive chemiluminescent immunoassay for the detection of cannabinoids in blood.

REAGENT COMPOSITION

Refer to the DOA I WB P Assay Array package insert for reagent compositions. The biochip substrate contains immobilised monoclonal antibodies raised to TCA.

MATERIAL HANDLING

For further information on precautions, reagent composition, preparation and storage, specimen collection and handling, quality control materials and analyser procedure, please refer to the DOA I WB P Assay Array package insert for further details.

SEMI-QUANTITATIVE RESULTS

The Evidence Investigator™ DOA I WB P Calibrators are used in determining equivalent concentration of TCA present in a sample via comparison to the primary target analyte in those calibrators.

SPECIFICITY

Specificity/cross-reactivity of the Evidence® TCA test kit was assessed using a dose-response series. Each compound was diluted in certified drug free human whole blood to the ranges specified. Compounds listed were tested in duplicate to a maximum of 0.5 mg/mL. Concentrations of the cross-reactants, which produce a response equal to that of the target compound at the cut-off, were calculated.

Percentage cross-reactivity with Nortriptyline is shown in Table 1. Compounds that elicit a negative response are shown in Table 2.

Table 1. Cross reactivity of TCA compounds

Compound	% Cross reactivity
Nortriptyline	100
Imipramine N Oxide	1127
Imipramine	294
Trimipramine	238
Desipramine	206
Cyclobenzaprine	201
Opipramol	167
Promazine	117
Maprotiline	96
Doxepin	95
Clomipramine	76
Protryptyline	67
Cyproheptadine	61
Lofepramine	58
Dothiepin	50
Chlorpromazine	24.3
2 Hydroxyl Imipramine	19.5
Nordoxepin	19.4
Perphenazine	17.3
Prochlorperazine	9.3
Amitriptyline	190

Table 2. Concentrations of compounds failing to produce a positive result

Compound	Concentration (µg/mL)
Methamphetamine	0.18
d-Amphetamine	0.5
Phenobarbital	0.32
Oxazepam	0.1
Lorazepam	0.16
Methadone	0.12
Morphine	0.16
Phencyclidine	0.1
Benzoylecgonine	0.06
11-nor-Δ^9-THC-9-carboxylic acid	0.2
Buprenorphine	0.15
MDMA	1.0
Amoxapine	0.8
Carbamazepine	0.8
Carbamazepine 10,11 Epoxide	0.8
Cis-10-OH-Nortriptyline	0.15
Diphenhydramine	1000
Iminostilbene	250
Mansein	0.8
Thioridazine	1000

INTERFERENCE

The effect of Triglycerides, Lipids, Haemoglobin, Bilirubin and Buflomedil were assessed to establish the maximum concentration at which the interferent will not cause a significant increase or decrease in the TCA assay performance and this is shown in Table 3.

Table 3. Interference in TCA assay

Substance	Concentration
Triglycerides	10 g/L
Lipids	6 g/L
Haemoglobin	5 g/L
Bilirubin	0.1 g/L
Buflomedil	4 g/L

LIMITATIONS

- Other substances not listed may interfere with the assay.

CALIBRATION

The TCA assay is standardised to Nortriptyline and quantified by LC/MS.

TYPICAL ASSAY RANGE

ANALYTE	ASSAY RANGE
TCA	0 - 450 ng/mL

Note individual ranges of calibrator batches may vary slightly.

SPECIFIC PERFORMANCE DATA

Limit of Detection

The Limit of detection of the Evidence Investigator™ TCA assay was determined to be 1.69 ng/mL*. This represents the lowest concentration of TCA that can be distinguished from zero.

*This is equivalent to 6.74 ng/mL for TCA in a neat sample.

Intra Assay Precision

Intra assay precision was determined by assaying 20 replicates of each of three control levels.

Table 4. Analysis of Intra Assay Precision (n=20)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	28.9	97.9	165.4
% CV	8.2	7.1	7.2

Inter Assay Precision

Inter assay precision was determined by assaying 3 controls in replicates of 2 in 10 separate assays.

Table 5. Analysis of Inter Assay Precision (n=20)

	Sample 1	Sample 2	Sample 3
Conc ng/mL	26.6	95.9	158.8
% CV	14.0	12.1	12.2

Recovery

Table 6. Analysis of Recovery

	Recovery concentration (ng/mL)	Recovery (%)
Level 1	56.4	110
Level 2	91.6	104
Level 3	124.3	105

REFERENCES

1. Linder MW, Keck PE Jr. (1998) Standards of laboratory practice: antidepressant drug monitoring. National Academy of Clinical Biochemistry. *Clin Chem.* 44(5):1073-84.
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4. Scoggins BA, Maguire KP, Norman TR, Burrows GD. (1980) Measurement of tricyclic antidepressants. Part II. Applications of methodology. *Clin Chem.* 26(7): 805-15

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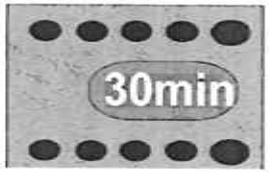
INVESTIGATOR

Assay Procedure for DOA I WB P Array

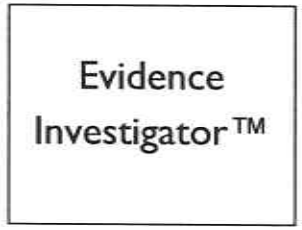
1. Centrifuge whole blood samples at 13000 rpm for 10 minutes or 4000 rpm for 20 minutes. Dilute whole blood samples 1 in 4 with sample diluent. Add 120 µL of assay buffer, 60 µL of diluted sample (or standard) and 120 µL of conjugate to each well.



2. Incubate the carrier at +25°C for 30 minutes @ 330 rpm on the thermoshaker.

3. Decant the liquid and wash each well Perform 6 quick washes and 6 two-minute soaks. Decant the liquid and tap the carrier onto lint free tissue paper.

4. Mix Luminol-EV841 & Peroxide (1:1). Add 250 µL of signal reagent-EV841 to each reaction well. Incubate for 2 minutes and protect from light.



5. Image each carrier on the Investigator™ System.

RANDOX CE

DRUGS OF ABUSE ARRAY I WHOLE BLOOD PLUS CONTROLS (DOA I WB P CONTROL) (CE)

evidence

CATALOGUE NO.	EV 4206
LOT NO.	9569 (12556EV-12557EV) v1
EXPIRY:	2018-12
PRESENTATION:	Lyophilised
SIZE:	4 x 2 x 1 mL

LABELLING GUIDE

DOA I WB P CONTROL	Drugs of Abuse Array I Whole Blood Plus Control

INTENDED USE

Randox Evidence® DOA I WB P controls are intended for use as an assayed quality control in the routine monitoring of accuracy and precision for the analytes listed in this insert. They are buffer based containing a variety of analytes at 2 different levels.

REAGENT COMPOSITION

Lyophilised 20 mmol/L phosphate buffer, pH 7.2 containing stabilisers, preservatives and drug concentrations as outlined.

STORAGE AND STABILITY

Unopened:	Store refrigerated at +2 to +8°C up to the expiration date.
Opened:	Reconstituted controls are stable for up to 5 days for all assays when stored at +2 to +8°C or up to 14 days when stored at -20°C in the original vial. Only the required amount of product should be removed. After use, any residual product should NOT BE RETURNED to the original vial.

SAFETY PRECAUTIONS AND WARNINGS

Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

The controls must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions. Avoid contact with eyes, skin and clothing. Avoid ingestion.

Health and Safety Data Sheets are available on request.

MATERIALS PROVIDED

Batch-specific Control Settings and Instructions for Use CD
Batch-specific Control barcodes

DOA I WB P CONTROL 1	4 x 1 mL
DOA I WB P CONTROL 2	4 x 1 mL

MATERIALS REQUIRED BUT NOT PROVIDED

Evidence® Drugs of Abuse Array I Whole Blood Plus Kit	EV 4202/4203
Evidence® Drugs of Abuse Array I Whole Blood Plus Calibrators	EV 4205
Evidence® Drugs of Abuse Array I Whole Blood Plus Investigator kit	EV 4204
2 mL Sample cups	EV 3574
16 mm Sample tubes	EV 3633

PROCEDURAL NOTES

CD ROM INSTALLATION

Insert disc into CDROM drive
Double click on **MY COMPUTER** icon on Windows desktop
Double click on **CD drive** icon
Double click on **UPDATECONTROLS.EXE**
Select the box next to either Evidence® or Evidence Investigator™ controls for the relevant panel.
Click on **UPDATE**
A progress bar will appear and once complete will say 'Update Complete'
Click on **EXIT**
Control information (lot, target, SD, barcode, display name) for this lot of controls has now been updated.

PREPARATION FOR USE
1. Open the vial very carefully, avoiding any loss of material.
2. Reconstitute in 1 mL of accurately measured distilled water.
3. Replace the rubber stopper, close vial and leave to roll for 30 minutes out of bright light before use.
4. Ensure that contents are completely dissolved by swirling gently.

FOR EVIDENCE® ANALYSER ONLY
1. Label appropriate sample tubes with batch-specific control barcodes provided.
2. Using a volumetric pipette, transfer the required volume of control to the appropriate barcode labelled tube or sample cup to be used on the Evidence® analyser.

LIMITATIONS
1. Do not use the product past expiry date.
2. Control settings are lot specific. Do not mix lots of reagent.
3. Do not use product if there is evidence of contamination.

VALUE ASSIGNMENT
Controls are assigned using a minimum of 24 replicates over Multiple Analysers. For each control level the mean is assigned as the target with the range being assigned as the mean +/- the Standard Deviation.

EVIDENCE® ANALYSER

Level 1

Component	Units	Target	Low Range	High Range	SD	Method
Methamphetamine	ng/mL	107.44	57.28	157.6	25.08	Evidence®
Barbiturates	ng/mL	29.08	18.9	39.26	5.09	Evidence®
Benzodiazepine1	ng/mL	11.25	7.31	15.19	1.97	Evidence®
Benzodiazepine2	ng/mL	18.51	12.03	24.99	3.24	Evidence®
Methadone	ng/mL	10.12	6.58	13.66	1.77	Evidence®
Opiates	ng/mL	15.76	10.24	21.28	2.76	Evidence®
PCP	ng/mL	4.25	2.77	5.73	0.74	Evidence®
BZG	ng/mL	22.01	14.31	29.71	3.85	Evidence®
MDMA	ng/mL	92.61	60.19	125.03	16.21	Evidence®
THC	ng/mL	7.34	4.76	9.92	1.29	Evidence®
TCA	ng/mL	27.16	17.66	36.66	4.75	Evidence®
Amphetamine	ng/mL	15.68	8.56	22.8	3.56	Evidence®
Buprenorphine	ng/mL	4.36	2.84	5.88	0.76	Evidence®

Level 2

Component	Units	Target	Low Range	High Range	SD	Method
Methamphetamine	ng/mL	252.81	164.33	341.29	44.24	Evidence®
Barbiturates	ng/mL	81.85	53.21	110.49	14.32	Evidence®
Benzodiazepine1	ng/mL	33.56	17.6	49.52	7.98	Evidence®
Benzodiazepine2	ng/mL	50.59	28.71	72.47	10.94	Evidence®
Methadone	ng/mL	36.02	23.42	48.62	6.3	Evidence®
Opiates	ng/mL	39.38	25.6	53.16	6.89	Evidence®
PCP	ng/mL	16.78	10.9	22.66	2.94	Evidence®
BZG	ng/mL	47	30.54	63.46	8.23	Evidence®
MDMA	ng/mL	206.59	134.29	278.89	36.15	Evidence®
THC	ng/mL	78.37	50.95	105.79	13.71	Evidence®
TCA	ng/mL	157.63	102.47	212.79	27.58	Evidence®
Amphetamine	ng/mL	53.42	34.72	72.12	9.35	Evidence®
Buprenorphine	ng/mL	20.51	13.19	27.83	3.66	Evidence®

EVIDENCE INVESTIGATOR™

Level 1

Component	Units	Target	Low Range	High Range	SD	Method
Methamphetamine	ng/mL	91.55	55.37	127.73	18.09	Investigator™
Barbiturates	ng/mL	30.76	20	41.52	5.38	Investigator™
Benzodiazepine1	ng/mL	9.33	5.13	13.53	2.1	Investigator™
Benzodiazepine2	ng/mL	18.38	11.94	24.82	3.22	Investigator™
Methadone	ng/mL	9.89	6.43	13.35	1.73	Investigator™
Opiates	ng/mL	15.57	10.13	21.01	2.72	Investigator™
PCP	ng/mL	3.66	1.94	5.38	0.86	Investigator™
BZG	ng/mL	19.96	10.86	29.06	4.55	Investigator™
MDMA	ng/mL	96.15	62.49	129.81	16.83	Investigator™
THC	ng/mL	7.02	4.46	9.58	1.28	Investigator™
TCA	ng/mL	24.91	16.19	33.63	4.36	Investigator™
Amphetamine	ng/mL	16.17	10.51	21.83	2.83	Investigator™
Buprenorphine	ng/mL	3.65	2.23	5.07	0.71	Investigator™

Level 2

Component	Units	Target	Low Range	High Range	SD	Method
Methamphetamine	ng/mL	242.5	157.62	327.38	42.44	Investigator™
Barbiturates	ng/mL	87.45	56.85	118.05	15.3	Investigator™
Benzodiazepine1	ng/mL	31.84	20.7	42.98	5.57	Investigator™
Benzodiazepine2	ng/mL	50.23	29.03	71.43	10.6	Investigator™
Methadone	ng/mL	33.27	21.63	44.91	5.82	Investigator™
Opiates	ng/mL	38.52	24.3	52.74	7.11	Investigator™
PCP	ng/mL	15.53	10.09	20.97	2.72	Investigator™
BZG	ng/mL	45.61	24.55	66.67	10.53	Investigator™
MDMA	ng/mL	205.85	133.81	277.89	36.02	Investigator™
THC	ng/mL	71.21	36.35	106.07	17.43	Investigator™
TCA	ng/mL	144.67	94.03	195.31	25.32	Investigator™
Amphetamine	ng/mL	51.56	33.52	69.6	9.02	Investigator™
Buprenorphine	ng/mL	18.26	9.18	27.34	4.54	Investigator™

28 Jun 17 bm

RELAZIONE TECNICA

1.DOTAZIONI STRUMENTALI

Per la fornitura di 500 test per i seguenti analiti: **benzodiazepine, cocaina, metadone, oppiacei, cannabinoidi, Anfetamine, barbiturici** per il laboratorio di tossicologia descritti nella Vostra richiesta, Randox Laboratories mettera a disposizione N. 1 strumento di ultima generazione denominato Evidence Investigator per l'effettuazione delle attività di screening delle droghe d'abuso nel sangue, urine, saliva.
La strumentazione e' di ultima generazione ed il software in italiano e' in via di implementazione. Il software e' tuttavia intuitivo e Windows-based, rendendolo fruibile pur in assenza di una conoscenza approfondita della lingua inglese.
La strumentazione Evidence Investigator possiede la marcatura CE. I kit per la ricerca delle droghe d'abuso su sangue e urina sono marcati CE.
Lo strumento sara' corredato di tutto il necessario al suo funzionamento (stampante, gruppo di continuita', consumabili).Randox Laboratories offre completa disponibilità ad effettuare aggiornamenti tecnologici software/hardware gratuiti a proprio carico.
Viene assicurata un'assistenza tecnica full-risk con interventi di assistenza effettuabili entro 48 ore dalla chiamata telefonica. Grazie alla nuova tecnologia utilizzata, la manutenzione ordinaria da effettuare sulla strumento e' minima ed effettuabile da parte dell'operatore. L'azienda fornira' l'addestramento necessario agli operatori per l'utilizzo in piena autonomia della strumentazione e sara' a disposizione per eventuali aggiornamenti e attivita' di supporto scientifico che potranno scaturire nel corso della fornitura.

La strumentazione Randox Evidence Investigator è uno strumento da banco, automatizzato, che permette l'analisi tossicologica di campioni per un'ampia varietà di test utilizzando le principali matrici quali sangue, urina, saliva, sangue post-mortem, tessuto, meconio, capello, umor vitreo.
Lo strumento Evidence Investigator e' da banco ed e' perfettamente in grado di soddisfare le esigenze del laboratorio, portando ad una piu' immediata locazione dello stesso e a risparmi in termini di spazio da dedicare alla strumentazione per il laboratorio. Evidence Investigator risulta essere facilmente posizionabile in laboratori di ogni dimensione. Non essendo necessari collegamenti dedicati a deionizzatore o a scarichi di reflui, il bancone da utilizzare per la sua installazione deve prevedere solo il collegamento delle prese elettriche per il funzionamento della strumentazione.

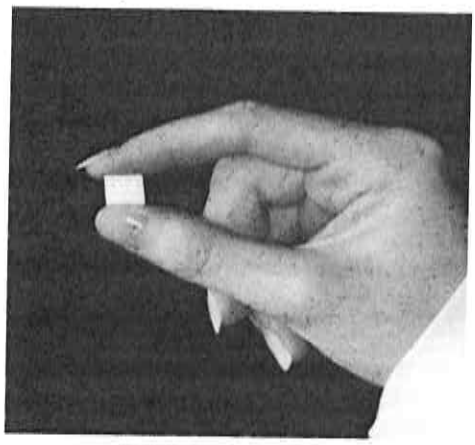
per conformità
31/07/18

La produttivita' oraria dello strumento Evidence Investigator risponde alle esigenze del laboratorio, con la possibilita' di ottenere il risultato per un pannello di droghe d'abuso in uno stesso campione nei tempi adeguati ai carichi di lavoro indicati.
Evidence Investigator utilizza la tecnologia del Biochip Array, basata sull'utilizzo di un biochip come piattaforma per reazioni immunologiche basate sul principio della chemiluminescenza. Tecniche immunologiche convenzionali sono usate per la misurazione della concentrazione degli analiti sulla superficie del biochip, con la conseguente creazione di un profilo di marcatori biologici specifici e simultanei.

IL BIOCHIP

Il fondamento della tecnologia Evidence Investigator e' il biochip. Un biochip ha una superficie di 9mm^2 ed e' la piattaforma su cui avvengono interazioni chimiche e molecolari. I biochip sono forniti come pre-fabbricati con regioni discrete di test (DTR) situate in precise posizioni con coordinate ben definite, ottenute mediante tecnologie di nanodispensazione sulla superficie del biochip. Ogni DTR corrisponde ad anticorpi diversi o ad altre specie reattive per ogni dosaggio.

Il biochip Randox



Ogni biochip e' dedicato al campione di ogni paziente ed e' eliminato dopo l'uso.

METODOLOGIA DI DOSAGGIO UTILIZZANDO IL BIOCHIP

La metodologia di dosaggio usando il biochip e' basata su tecniche immunologiche standardizzate. Nei dosaggi sono utilizzati metodi competitivi e sandwich. La metodologia adottata e' pannello-specifica e dipende dal peso molecolare dell'analita

di interesse. Il pannello Droghe d'Abuso utilizza, ad esempio, una reazione competitiva.

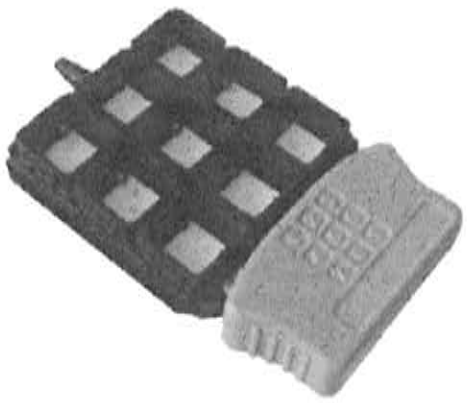
Il dosaggio competitivo utilizza analiti marcati con enzima per la produzione del segnale, mentre il dosaggio sandwich utilizza un anticorpo secondario marcato con enzima.

IL CARRIER

Un carrier e' un supporto quadrato con 9 pozzetti di reazione separati, posizionati in un formato 3x3. Ogni pozzetto contiene un biochip che e' bloccato alla base del pozzetto mediante appositi blocchi. Ogni pozzetto contenente un biochip e' usato come camera di reazione per il dosaggio del campione del paziente.

Dopo l'analisi dei campioni, i biochip contenuti nel carrier sono inseriti nella stazione di analisi dell'immagine per la lettura del segnale ed infine, il carrier con i biochip sono eliminati in un contenitore per rifiuti biologici. I biochip e i carrier non sono riutilizzabili.

I carrier sono forniti confezionati singolarmente. E' possibile utilizzare i biochip contenuti al loro interno 3 alla volta rispondendo in maniera adeguata ai carichi di lavoro indicati nella vostra richiesta di 500 test per anno in quanto i tre biochip potranno essere utilizzati per due campioni pazienti ed un controllo per seduta. I carrier sono pannello-specifici. La calibrazione viene effettuata su un carrier completo di 9 biochip ed e' stabile per tutti i biochip utilizzati dello stesso lotto fino a loro scadenza.



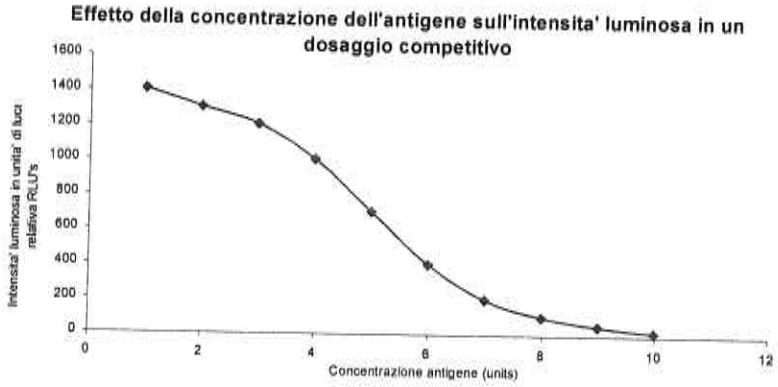
Un carrier

DOSAGGI COMPETITIVI

I dosaggi competitivi sono di solito utilizzati per la ricerca di molecole a basso peso molecolare con pochi siti di legame per il legame dell'anticorpo.

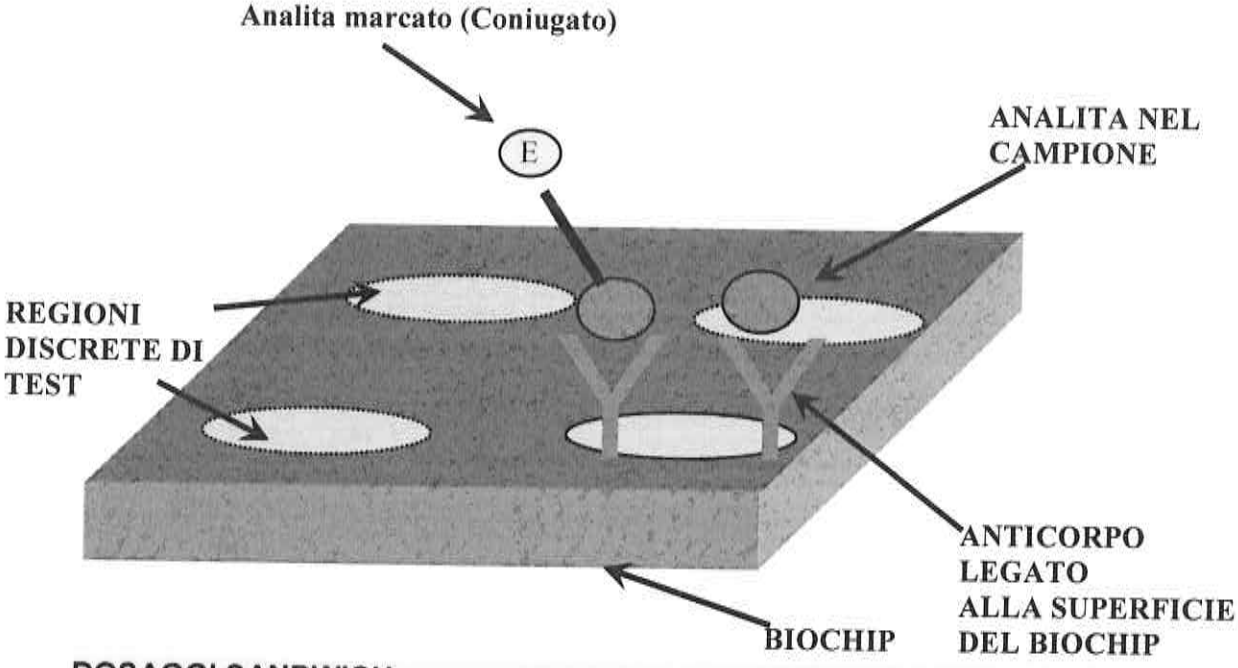
Gli anticorpi sono legati a regioni discrete di test (DTRs) sulla superficie del biochip e viene aggiunto l'analita marcato con enzima. In assenza di campione, l'analita marcato si lega all'anticorpo sulla superficie del biochip. Viene aggiunto il reattivo del segnale e l'intensità' del segnale prodotto e' proporzionale alla concentrazione dell'analita marcato che si e' legato agli anticorpi.

Quando e' presente il campione, contenente gli analiti di interesse, gli analiti del campione in esame competeranno con gli analiti marcati per legarsi agli anticorpi. Questo ridurrà gli analiti marcati legati agli anticorpi e quindi ridurrà la produzione di segnale luminoso.
La produzione di segnale luminoso in un dosaggio competitivo e' *inversamente proporzionale alla concentrazione di analita presente nel campione.*



Il reagente contenente gli analiti marcati contiene analiti specifici per ogni DTR sulla superficie del biochip. L'enzima utilizzato per marcare gli analiti e' la perossidasi di rafano. Il reattivo del segnale e' un substrato universale utilizzato per tutte le DTR sulla superficie del biochip.

DOSAGGIO COMPETITIVO

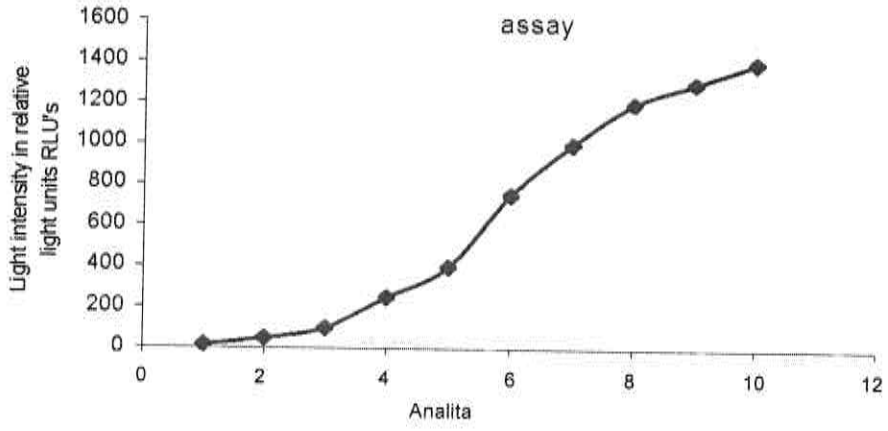


DOSAGGI SANDWICH

I dosaggi sandwich sono di solito utilizzati per la ricerca di molecole ad alto peso molecolare con diversi siti di legame per il legame dell'anticorpo.

Gli anticorpi sono legati a regioni discrete di test (DTRs) sulla superficie del biochip. Quando viene aggiunto il campione di un paziente, contenente gli analiti di interesse, questi si legano agli anticorpi presenti sulla superficie del biochip. Quindi viene aggiunto un reagente contenente un anticorpo marcato con enzima (coniugato), che si lega a un secondo gruppo funzionale sull'analita catturato. Viene aggiunto il reattivo del segnale e l'intensità' del segnale prodotto e' proporzionale alla concentrazione dell'analita presente nel campione del paziente.

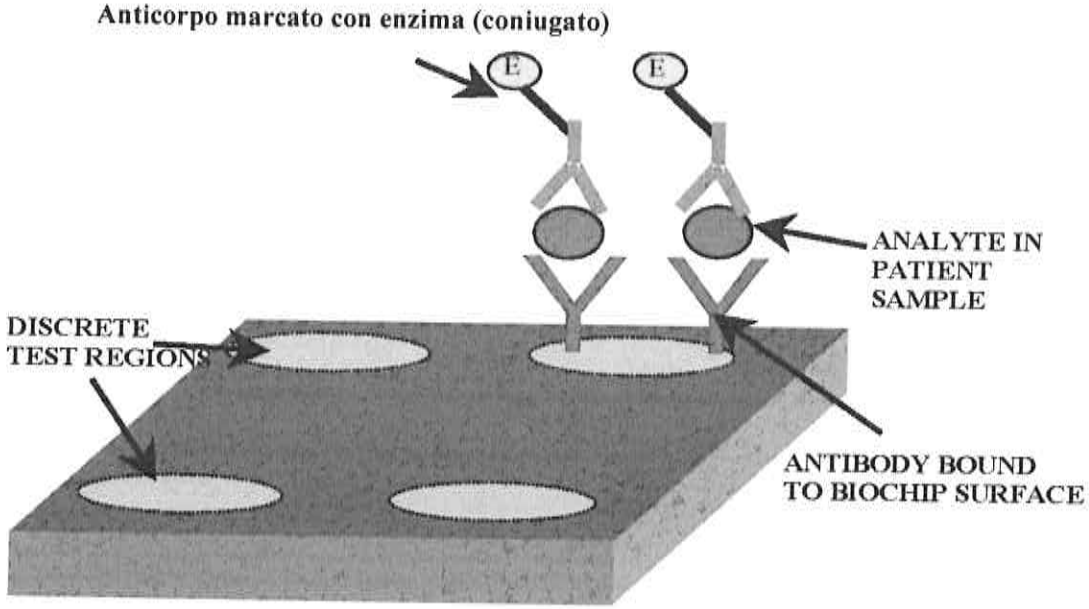
Effetto della concentrazione di analita sulla produzione di luce in un test sandwich



In assenza del campione, non ci sono analiti disponibili per il legame alla superficie del biochip, e quindi non viene prodotto alcun segnale.

Il reagente che contiene gli anticorpi marcati con enzima possiede al suo interno anticorpi specifici per tutte le DTR presenti sulla superficie del biochip. Per marcare gli anticorpi viene utilizzata la perossidasi di rafano. Il reattivo del segnale e' un substrato universale utilizzato per tutte le DTR sulla superficie del biochip.

DOSAGGIO SANDWICH



TECNOLOGIA CHEMILUMINESCENTE

La chemiluminescenza e' la produzione di luce mediante una reazione chimica. E' usata negli immunodosaggi per determinare il livello di analita presente nei campioni. Evidence Investigator utilizza un substrato chemiluminescente marcato con perossidasi di rafano (HRP) per l'identificazione di anticorpi o analiti legati alla superficie del biochip. La produzione intensa di segnale rende possibile l'identificazione di picogrammi di antigene o anticorpo.

Il reagente del segnale utilizzato da Evidence Investigator e' formato dalla miscela 1:1 di una soluzione di Luminolo e una di Perossido. Le soluzioni sono automaticamente miscelate prima del dosaggio.

SISTEMA DI RILEVAMENTO DI EVIDENCE INVESTIGATOR

L'emissione di luce dalle reazioni chemiluminescenti che avvengono sulle DTR sulla superficie del biochip e' rilevata e quantificata utilizzando una camera CCD (Charge Coupled Device). Questo sistema permette di rilevare basse intensità luminose grazie alla sua elevata sensibilità, rende possibile una risposta lineare a fotoni incidenti, la rilevazione di un ampio intervallo di concentrazioni e una buona risoluzione, garantendo una buona cadenza analitica del campione. La camera CCD registra l'emissione di luce da tutte le DTR poste su ciascun biochip contenuto in un carrier. Un software di gestione dell'immagine la processa e quantifica il segnale.

BREVE DESCRIZIONE DI EVIDENCE INVESTIGATOR

Lo strumento Evidence Investigator è formato dalle seguenti componenti:

- Stazione di caricamento dei carrier
- Termoagitatore per l'incubazione dei campioni
- CCD Camera integrata
- PC, monitor e tastiera
- Stampante se necessaria

Tutti i dosaggi presenti su un biochip sono effettuati simultaneamente dopo l'aggiunta di reagenti e campioni. Evidence Investigator utilizza la rilevazione mediante

chemiluminescenza, e la quantificazione del risultato non avverrà a meno che il test sia stato selezionato mediante il software al momento della creazione della lista di lavoro. Quindi non tutti i dosaggi presenti sul biochip devono essere richiesti se non necessario.
Grazie all'unicità' del software, i risultati non richiesti inizialmente possono essere ottenuti in un secondo momento; questa caratteristica e' definita analisi retrospettiva.
L'immagine prodotta dalla reazione chemiluminescente di ogni specifico dosaggio e' salvata nel software ed i risultati corrispondenti possono essere richiamati senza dover ricaricare i campioni. La capacità di eseguire l'analisi retrospettiva e' uno dei principali vantaggi di questa nuova tecnologia.
I risultati possono essere riportati come qualitativi, o semi-quantitativi. L'operatore non deve accedere all'immagine della reazione, poiché il software la processa in automatico.

I reagenti sono multiparametrici e contenuti nel kit che viene conservato in frigorifero quando non in uso. Non si rende necessaria la presenza di un vano reagenti refrigerato all'interno dello strumento.
Ogni campione ed ogni lotto di reagenti utilizzato puo' essere inserito nella lista di lavoro mediante codice a barre permettendo la gestione delle informazioni riguardanti campioni e reagenti tramite il software (es. pannello, lotto, scadenza,operatore, ecc.).
Pipettando i campioni utilizzando pipette con puntali monouso si garantisce l'assenza di carry-over.
Il processamento automatizzato dei biochip assicurano la massima performance. L'errore e la variabilità causata dall'operatore sono ridotti al minimo; i passaggi critici sono stati ottimizzati per ogni applicazione.
La manutenzione da parte dell'operatore e' minima e poco dispendiosa a livello di ore-uomo, non essendoci fluidica o parti mobili come nei tradizionali strumenti di chimica clinica.
I biochip sono incubati in un termoagitatore dedicato ad una temperatura ottimale e pannello-specifica.
L'analisi di immagine del biochip è effettuata mediante una CCD camera ed un software di analisi di immagine all'avanguardia.
Il software interpreta i dati e genera risultati qualitativi o semi-quantitativi a seconda del pannello selezionato.

E' previsto un programma di gestione per il controllo qualita' che permette di monitorare la validita' dei lotti in uso, delle calibrazioni e delle sessioni di lavoro mediante l'utilizzo di grafici Levey-Jennings.

Attualmente i test che verranno forniti comprendendo gli analiti da Voi richiesti sono 13 ed elencati di seguito:

DoA I+

Amphetamine
Barbiturates
Benzodiazepines I (Oxazepam)
Benzodiazepines II (Lorazepam)
Buprenorphine
Benzoylecgonine (Cocaine Metabolite)
Cannabinoids (THC)
Creatinine (Urine only)
Methamphetamine
Methadone
MDMA
Opiate
Phencyclidine (PCP)
Tricyclic Antidepressants (TCA)

Data

30/07/2018

In fede

RANDOX LABORATORIES LIMITED
Viale della Vittoria, 34 - 00122 ROMA
P.IVA 07197321008 - C.F. 92015900589
Tel. 06/98968954 - Fax 06/60513810

RANDOX
QUALITY CONTROL

Randox Laboratories Ltd
Viale della Vittoria, 34
00122 Roma
Italy
Tel.: +39 06 9696 8954
Fax: +39 06 6051 3810

ALLEGATO N.. 3

Offerta

Destinatario:	Azienda Ospedaliera di Caserta "Sant'Anna e San Sebastiano"	Offerta n.:	1
Indirizzo:	Via Palasciano, sn 81100 CASERTA (CE)	Data:	30/07/2018
		Referente Randox:	LUCIANO COLUCCI
		Tel :	3207404291
Rif. Richiesta del:	26/07/2018	Email :	luciano.colucci@randox.com
Validità offerta:	28/02/2019		

Gentile Provveditorato,

La ringraziamo per l'interesse mostrato verso i prodotti Randox. Con la presente invio alla Sua attenzione l'offerta relativa ai prodotti di Vostro interesse:

Codice	Descrizione	Metodo	Formato	Quantità	Prezzo Unitario	Prezzo Totale
EV4204	DRUG OF ABUSE ARRAY I+ (Sangue intero) *	Evidence Investigator	biochip	500	€ 39.90	19,950
EV4204	DRUG OF ABUSE ARRAY I+ (Sangue intero) Necessari per calibrazione e controllo interno	Evidence Investigator	biochip	256	0	0
EV4206	Drug of Abuse Array I (Sangue intero) Controllo interno	Evidence Investigator	kit	3	0	0
	* ogni biochip permette il dosaggio simultaneo dei seguenti analiti richiesti : benzodiazepine, cocaina, metadone, oppiacei, cannabinoidi, Anfetamine, barbiturici. Lo stesso biochip permette di analizzare senza costi aggiuntivi i seguenti analiti: Buprenorfine, Metamfetamine, MDMA, PCP, TCA.					
					totale Netto (senza IVA)	€ 19,950.00
					Iva	22%

L'OFFERTA E' COMPRENSIVA DEL SISTEMA EVIDENCE INVESTIGATOR CORREDATO DALLE SEGUENTI UNITA':

Stazione di caricamento dei carrier
Termoagitatore per l'incubazione dei campioni
CCD Camera integrata
PC, monitor e tastiera
Stampante se necessaria

ED E' COMPRENSIVA DEI COSTI DI NOLEGGIO E ASSITENZA FULL RISK PER 1 ANNO

Termini di pagamento: 60 gg. Ricezione fatturazione elettronica

Spese di spedizione : Porto Franco.

Cordiali Saluti,

RANDOX LABORATORIES LIMITED
Viale della Vittoria, 34 - 00122 ROMA
P.IVA 07197321008 - C.F. 92015900589
Tel. 06/96968954 - Fax 06/60513810

REGIONE CAMPANIA
AZIENDA OSPEDALIERA DI CASERTA
SANT'ANNA E SAN SEBASTIANO
DI RILIEVO NAZIONALE E DI ALTA SPECIALIZZAZIONE

DETERMINA DIRIGENZIALE

PARERE CONTABILE

Registro Autorizzazioni n°:	UFFAUT	del	
Budget Economico:	2018		
Codice Conto:	501010501		
Descrizione:	DISPOSITIVI MEDICO-DIAGNOSTICI IN VITRO (IVD) (MAT. DIAGNOST.)		
Presente Autorizzazione:	€24.339,00	n° 7	SUB 2
Registro Autorizzazioni n°:		del	
Budget Economico:			
Codice Conto:			
Descrizione:			
Presente Autorizzazione:	€0,00	n°	SUB
Registro Autorizzazioni n°:		del	
Budget Economico:			
Codice Conto:			
Descrizione:			
Presente Autorizzazione:	€0,00	n°	SUB

Caserta, li 03/08/2018

UOC GESTIONE ECONOMICO FINANZIARIA E DELLA

CHIANESE EDUARDO

REGIONE CAMPANIA
AZIENDA OSPEDALIERA DI CASERTA
SANT'ANNA E SAN SEBASTIANO
DI RILIEVO NAZIONALE E DI ALTA SPECIALIZZAZIONE

Determina Dirigenziale N. 492 del 06/08/2018

PROPONENTE: **UOC PROVVEDITORATO ED ECONOMATO**

OGGETTO: **PROCEDURA DI AFFIDAMENTO AI SENSI DELL'ART.36 CO. 2 LETT.A) DEL D.LGS. N.50/2016 E SS.MM.II. DELLA FORNITURA DI TEST PER ACCERTAMENTI TOSSICOLOGICI PER LA UOC PATOLOGIA CLINICA. CIG Z4124924DE**

In pubblicazione dal 06/08/2018 e per il periodo prescritto dalla vigente normativa in materia (art.8 D.Lgs 14/2013, n.33 e smi)

Atto immediatamente esecutivo

UOC AFFARI GENERALI

Direttore Eduardo Chianese

Elenco firmatari

In Sostituzione Del Dir Uoc Provveditorato Tiziana Simone - UOC PROVVEDITORATO ED ECONOMATO

Eduardo Chianese - UOC GESTIONE ECONOMICO FINANZIARIA E DELLA PROGETTUALITA' EUROPEA

Per delega del Direttore della UOC AFFARI GENERALI E LEGALI, il funzionario Gabriella Perrotta