ElAgen

HCV Ab (v.4) Kit

REF 071067 Σ 96

REF 071064 Σ 192

REF 071068 Σ 480

2°C 8°C

IVD

CE 0459

Citiți cu atenție prezentul prospect, înainte de efectuarea testului și respectați cu strictețe instrucțiunile din cuprinsul acestuia.
Fiabilitatea rezultatelor este garantată numai cu condiția respectării stricte a acestor instrucțiuni.

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ro

SIMBOLURI UTILIZATE PE ETICHETE							
	IVD	REF	LOT				Σ
	Dispozitiv medical pentru diagnostic in vitro	Număr de catalog	Număr de lot	Atenție, citiți instrucțiunile de utilizare	Limite de temperatură	A se utiliza până la data de	Număr de test
				MICROPLATE	CONTROL +	CONTROL -	CAL
Română RO	Producător	A se feri de contactul direct cu razele soarelui	Data fabricației	Microplacă	Control pozitiv	Control negativ	Calibrator
	CONJ	DIL SPE	SUBS TMB	SOLN STOP	WASH BUF 20X	DIL AS	RCNS x mL
	Conjugat	Diluant eșantioane	Substrat TMB	Soluție de stopare (H_2SO_4 0.3M)	Soluție de spălare tampon concentrată 20x	Diluant pentru probe	Se reconstituie cu x mL
	Risc biologic	Pericol	Atenție				

Atenţie:
Soluție de stopare clasificată ca: Skin Corr. 1A

- ***Avertisment:***
  Pericol
- ***Componente periculoase ce necesită etichetare:***
  Acid sulfuric
- ***Indicații de pericol:***
  H314 – Provoacă arsuri grave ale pielii și leziuni oculare grave.
- ***Recomandări de siguranţă:***
  P260 Nu inspirați praful/fumul/gazul/abutiivaporii/spray-ul.
  P303+P361 +P353 ÎN CAZ DE CONTACT CU PIELEA (sau cu părul): scoateți imediat toată îmbrăcămintea contaminată. Clătiţi pielea cu apă/faceţi un duş.
  P305+P351+P338 ÎN CAZ DE CONTACT CU OCHII: Clătiți cu atenție cu apă, timp de mai multe minute. Scoateţi lentilele de contact, dacă este cazul şi dacă acest lucru se poate face cu uşurinţă. Continuaţi să clătiţi.
  P310 Adresați-vă imediat unui CENTRU DE INFORMARE TOXICOLOGICĂ sau unui medic.
  P405 A se depozita sub cheie.
  P501 Eliminați conținutul/recipientul în conformitate cu legislația în vigoare la nivel local/regional/național/internațional.

Atenţie:
Controlul negativ, controlul pozitiv, calibratorul, conjugatul, diluantul pentru eșantioane, diluantul pentru probe și soluție concentrată tampon pentru spălare 20X sunt clasificate ca: Skin Sens. 1

- ***Avertisment:***
  Atenție
- ***Componente periculoase ce necesită etichetare:***
  Amestec de: 5-cloro-2-metil-2H-izotiazolin-3-one [Nr. CE 247-500-7]; 2-metil-2H-izotiazolin-3-one [Nr. CE 220-239-6] (3:1)
- ***Indicații de pericol:***
  H317 Poate provoca o reacție alergică a pielii
- ***Recomandări de siguranţă:***
  P261 Evitați să inspirați praful/fumul/gazul/aburiivaporii/spray-ul.
  P280 Purtați mănuși de protecție/îmbrăcăminte de protecție/ echipament de protecție a ochilor/echipament de protecție a feței.
  P321 Trattament specific (consultați această etichetă).
  P333+P313 În caz de iritare a pielii sau de erupție cutanată: adresați-vă medicului.
  P302+P352 ÎN CAZ DE CONTACT CU PIELEA: Spălați cu apă din abundență.
  P501 Eliminați conținutul/recipientul în conformitate cu legislația în vigoare la nivel local/regional/național/internațional.

Pentru Fișele cu Date de Securitate, consultați site-ul www.adaltis.net.

RO *ROMÂNĂ*

A. UTILIZARE

Test imuno-enzimatic (ELISA) de generația a patra, pentru detectarea anticorpilor la virusul hepatitei C, în probe de ser sau plasmă umană (EDTA, Heparină și Citrat). Trusa poate fi utilizată pentru detectarea anticorpilor din unitățile de sânge recoltate de la pacienți infectați cu virusul HCV.
Numai pentru diagnosticarea in vitro.

B. INTRODUCERE

Organizația Mondială a Sănătății (OMS) definește infecția cu virusul hepatitic C ca fiind:

”Hepatita C este o infecție virală a ficatului, denumită, înainte de 1989, până la identificarea agentului cauzator, hepatită ”non A, non B”, cu mecanism de transmitere parenteral. Descoperirea și caracterizarea virusului hepatitic C (HCV) au permis înțelegerea rolului primordial al acestuia în hepatitele post-transfuzionale și a tendinței acestuia de a induce infecții persistente”.

Virusul HCV este una din cauzele majore ale hepatitei acute şi a bolilor hepatice cronice, printre care ciroză şi cancer la ficat. La nivel global, sunt infectate cu virusul HCV, estimativ, 170 de milioane de persoane, iar între 3 spre 4 milioane de persoane sunt nou infectate in fiecare an. Cele mai frecvente cauze de transmitere a virusului HCV la nivel mondial sunt transfuziile de unități de sânge necontrolate serologic, precum și folosirea, de la o persoană la alta, a acelor și seringilor care nu au fost corect sterilizate. În prezent nu există niciun vaccin disponibil pentru prevenirea hepatitei C, iar tratamentul hepatitelor C cronice este prea costisitor, pentru ca populațiile din țările în curs de dezvoltare să și-l poată permite. Așadar, dintr-o perspectivă globală, cel mai mare impact asupra bolnavilor de hepatită C constă în focalizarea eforturilor asupra reducerii riscurilor de transmitere a virusului HCV pe cale nozocomială (ex.: transfuzii de sânge, injecții nesigure) și asupra reducerii comportamentelor de risc (ex.: injectarea de droguri).

Virusul hepatitei C (HCV) este unul dintre virușii (A, B, C, D și E) ce sunt responsabili de cele mai multe dintre cazurile de hepatită virală. Este un virus ARN încapsulat monocatenar, din familia Flaviviridae, ce are un spectru restrâns de gazde. Oamenii și cimpanzeii reprezintă singurele specii cunoscute ca fiind susceptibile la infecția cu virusul HCV și ambele specii dezvoltă boli similare. O caracteristică importantă a virusului o reprezintă relativa mutabilitate a genomului, legată probabil de o tendință marcată (80%) de a induce infecții cronice. Virusul HCV este reunit în mai multe genotipuri diverse, ce pot fi importante în determinarea gravității bolii și a răspunsului la tratament.

Perioada de incubație a infecției cu virusul HCV, înainte de manifestarea simptomelor clinice, variază de la 15 la 150 de zile. În infecțiile acute, simptomele cele mai frecvente sunt oboseala și icterul; în orice caz, majoritatea cazurilor (un procent cuprins între 60% și 70%), inclusiv cele ce dezvoltă o infecție cronică, sunt asimptomatice. Aproximativ 80% dintre noii pacienți infectați dezvoltă o infecție cronică. Ciroza apare la un procent cuprins aproximativ între 10% și 20% dintre pacienții cu infecție cronică, în timp ce cancerul hepatic apare la un procent cuprins între 1% și 5% dintre persoanele care prezintă o infecție cronică, pe o perioadă de timp cuprinsă între 20 și 30 de ani. Majoritatea pacienților ce suferă de cancer hepatic fără a fi infectați cu virusul hepatitei B, prezintă infecție cu virusul HCV. Mecanismul prin care infecția cu virusul HCV cauzează cancerul hepatic nu este încă foarte clar. Hepatita C accentuează gravitatea bolilor ficatului, atunci când se manifestă concomitent cu alte probleme hepatice. Mai precis, bolile ficatului evoluează mai rapid, la persoanele cu boli hepatice cauzate de consumul de alcool și de infecția cu virusul HCV. Virusul HCV se transmite în principal prin contactul direct cu sângele infectat. Transmiterea virusului HCV prin transfuziile de sânge necontrolate serologic în vederea depistării prezenței virusului, folosirea, de la o persoană la alta, a acelor, seringilor și a altor echipamente medicale care nu au fost corespunzător sterilizate, sau schimbul de ace între consumatorii de droguri, este foarte documentată. Transmiterea se poate produce și pe cale sexuală sau perinatală, însă nu la fel de frecvent. Alte modalități de transmitere, ce țin de practici comportamentale, sociale, culturale (body piercing, circumcizii și tatuaje) sunt de asemenea posibile, dacă se utilizează instrumente care nu au fost corespunzător sterilizate. Virusul HCV nu se transmite pe calea strănutului, prin îmbrățișări, tuse, mâncare sau apă, dacă se folosesc aceleași tacâmuri sau pahare, sau prin contact întâmplător.
Atât în țările dezvoltate, cât și în cele în curs de dezvoltare, grupurile de risc includ consumatorii de droguri injectabile, primitorii de sânge necontrolat serologic, persoanele care suferă de hemofilie, pacienții dializați și persoanele cu numeroși parteneri sexuali, ce practică raporturi sexuale neprotejate. În țările dezvoltate, se estimează că 90% dintre persoanele infectate cu virusul hepatitei C cronice sunt în principal consumatori de droguri injectabile și persoane cărora li s-au administrat transfuzii de sânge necontrolat serologic, sau transfuzii de derivate din sânge. În majoritatea țărilor în curs de dezvoltare, unde se utilizează încă sânge și derivate din sânge neanalizate, principala cale de transmitere a infecției o reprezintă instrumentele pentru injecții nesterilizate și transfuziile de sânge necontrolate serologic. De asemenea, persoanele care practică ritualuri de sacrificare și circumcizii sunt persoane cu risc, dacă folosesc sau refolosesc instrumente metalice nesterilizate.

OMS estimează că aproximativ 170 de milioane de persoane, adică 3% din populația globului, sunt infectate cu virusul HCV și prezintă riscul de a se îmbolnăvi de ciroză și/sau cancer hepatic. Prevalența infecției cu virusul HCV în Africa, Orientul Mijlociu, Asia de Sud-Est și Pacificul de Nord este mult mai mare, față de America de Nord și de Europa.

Testele diagnostice pentru HCV se utilizează pentru a preveni infecțiile prin screening-ul donatorilor de sânge și plasmă, pentru stabilirea diagnosticului clinic și pentru o mai bună luare a deciziilor privind tratamentul administrat unui pacient. Testele diagnostice disponibile în prezent se bazează pe dozări imuno-enzimatice (EIA) pentru detectarea unor anticorpi specifici HCV. Sistemul EIA poate depista peste 95% dintre pacienții cu infecții cronice, dar numai între 50% și 70% dintre infecțiile acute. Analiza RIBA (metoda recombinată de imunoblot) de identificare a anticorpilor care reacționează cu antigene individuale HCV se utilizează adesea ca test suplimentar pentru confirmarea unui rezultat pozitiv obținut prin teste EIA. Teste pentru HCV bazate pe amplificarea acizilor ribonucleici (de ex. PCR, probă cu ADN legat) au fost utilizate atât pentru confirmarea rezultatului serologic, cât și pentru stabilirea eficacităţii tratamentului antiviral folosit. Un rezultat pozitiv indică prezența infecției active și posibilitatea de extindere a

infecției și/sau de dezvoltare a unor boli cronice ale ficatului.
Medicamentele antivirale, cum ar fi interferonul, administrat ca atare sau împreună cu ribavirina, se pot utiliza pentru tratamentul pacienților cu hepatită C cronică, însă acest tratament este foarte costisitor. Tratamentul ce constă doar în administrarea interferonului este eficient la aproximativ 10-20% dintre pacienți. Interferonul administrat împreună cu ribavirina este eficient la 30-50% dintre pacienți. În schimb, se pare că ribavirina, administrată ca atare, nu este eficientă.

Nu există niciun vaccin eficient împotriva virusului HCV. Cercetările continuă, însă mutabilitatea accentuată a genomului virusului HCV îngreunează descoperirea unui vaccin eficient. De asemenea, și lipsa cunoștințelor privind un eventual răspuns imuno-protector, ulterior infecției cu virusul HCV, încetinește descoperirea vaccinului. Nici măcar nu se știe dacă sistemul imunitar este în stare să elimine virusul.

În orice caz, câteva studii au demonstrat prezența unor anticorpi ce neutralizează virusul, la pacienții infectați cu virusul HCV. În lipsa unui vaccin, trebuie adoptate toate măsurile de precauție pentru a preveni infecția, inclusiv (a) teste screening, testele de sânge și a organelor donate; (b) dezactivarea virusului în plasme și produse derivate; (c) dezvoltarea și consolidarea practicilor de control al infecției în protocoalele de acțiune sanitară, precum și o corectă sterilizare a instrumentelor medicale și stomatologice; (d) promovarea unor schimbări în relațiile dintre oamenii de rând și operatorii sanitari, pentru a reduce utilizarea excesivă a injecțiilor și pentru practicarea unor injecții sigure; (e) reducerea riscului pentru persoanele consumatoare de droguri și cele ce utilizează practici sexuale de mare risc".

Genomul codifică pentru componentele structurale, o proteină nucleocapsidică și două glicoproteine de suprafață și componentele funcționale implicate în replicarea virusului și în procesarea proteinelor acestuia. Regiunea de codificare nucleocapsidică pare a fi cea mai conservatoare, dintre probele izolate obținute în întreaga lume.

C. PRINCIPIUL TESTULUI

Microplăcile sunt tapetate cu antigene HCV specifice derivate din regiunile "core" și "ns" de codificare pentru antigenele conservatoare și determinanții antigenici imunodominanți (peptida de bază, NS3 recombinant, peptide NS4 și NS5).
Faza solidă este tratată mai întâi cu eșantionul diluat și, anticorpii HCV, dacă sunt prezenți, se vor lega la antigenele fixate. După etapa de îndepărtare prin spălare a tuturor celorlalte componente ale eșantionului, în cea de-a doua fază de incubare, după legarea anticorpilor HCV, anticorpii IgG și IgM sunt detectați prin adăugarea conjugatului cu anticorpi specifici policlonali anti-IgG&M, marcați cu peroxidază (HRP).
Enzima captată pe faza solidă, reacționând cu amestecul substrat TMB, generează un semnal optic care este proporțional cu cantitatea de anticorpi anti-HCV prezenţi în eșantion. O valoare cut-off permite să se interpreteze densitățile optice în rezultate pozitive și negative de anticorpi HCV.

D. COMPONENTE

Trusa conține reactivi pentru 96 de teste (cod 071067), 192 de teste (cod 071064), sau 480 de teste (cod 071068).

Microplacă	1
Control negativ	1x4 mL/fiolă
Control pozitiv	1x2 mL/fiolă
Calibrator	2 fiole
Soluție de spălare tampon concentrată 20x	1x50 mL/fiolă
Conjugat	1x16 mL/fiolă
Diluant pentru eșantioane	1x50 mL/fiolă
Substrat TMB	1x16 mL/fiolă
Soluție de stopare	1x15 mL/fiolă
Diluant pentru probe	1x8 mL/fiolă
Hârtie de sigilare placă	2
Număr de teste	96
Cod	071067

Microplacă	2
Control negativ	2x4 mL/fiole
Control pozitiv	1x4 mL/fiolă
Calibrator	3 fiole
Soluție de spălare tampon concentrată 20x	2x50 mL/fiole
Conjugat	2x16 mL/fiole
Diluant pentru eșantioane	2x50 mL/fiole
Substrat TMB	2x16 mL/fiole
Soluție de stopare	2x15 mL/fiole
Diluant pentru probe	2x8 mL/fiole
Hârtie de sigilare placă	4
Număr de teste	192
Cod	071064

Microplacă	5
Control negativ	1x20 mL/fiolă
Control pozitiv	1x10 mL/fiolă
Calibrator	7 fiole
Soluție de spălare tampon concentrată 20x	5x50 mL/fiole
Conjugat	2x40 mL/fiole
Diluant pentru eșantioane	5x50 mL/fiole
Substrat TMB	2x40 mL/fiole
Soluție de stopare	2x40 mL/fiole
Diluant pentru probe	1x40 mL/fiolă
Hârtie de sigilare placă	10
Număr de teste	480
Cod	071068

1. Microplacă

12 strip-uri de 8 minigodeuri tapetate cu peptidă Core, antigen recombinant NS3, peptide NS4 și NS5. Plăcile sunt sigilate în folie din aluminiu cu absorbant de umezeală.
Așteptați ca microplaca să ajungă la temperatura mediului ambiant (18…24°C) înainte de a deschide folia. Strip-urile nefolosite trebuie reintroduse la loc în folia cu absorbant de umezeală și trebuie păstrate la o temperatură de 2...8°C.

2. Control negativ

Control gata de utilizare. Conține 10 mM soluție tampon citrat de Na cu pH 6.0 ± 0.1, proteină de bază 2% cazeină și conservant 0.1% Proclin 150. Controlul negativ este colorat în culoarea verde măsliniu.

3. Control pozitiv

Control gata de utilizare. Conține proteine din ser de capră în procent de 1%, anticorpi umani pozitivi la virusul

HCV, 10 mM tampon citrat de Na cu ph 6.0±0.1, 0.5% Tween 20, 0.09% azidă de sodiu și conservant 0.1% Proclin 150. Controlul pozitiv este colorat în culoarea verde închis.

Notă importantă: *Lipsa agenților patogeni vitali în soluția de control pozitiv nu poate fi garantată în totalitate, prin urmare reactivul trebuie tratat ca fiind potențial infectat, în conformitate cu principiile de bună practică de laborator.*

4. Calibrator
Calibrator liofilizat. Se va dizolva în cantitatea de apă distilată cu aviz EIA indicată pe etichetă. Conține proteine din ser de fetus de vițel, anticorpi umani pozitivi la virusul HCV, cu conținut calibrat după codul NIBSC Working Standard 06/188-006, 10mM de tampon citrat de Na cu pH 6.0±0.1, 0.3 mg/mL sulfat de gentamicină și conservant 0.1% Proclin 150.
Notă importantă: *Lipsa agenților patogeni vitali în calibrator nu poate fi garantată în totalitate, prin urmare reactivul trebuie tratat ca fiind potențial infectat, în conformitate cu principiile de bună practică de laborator.*

Note: volumul necesar pentru dizolvarea conținutului fiolei poate varia de la un lot la altul. Vă rugăm să utilizați volumul corespunzător, indicat pe etichetă.

5. Soluție de spălare tamponconcentrată 20x
Soluție concentrată 20X. După diluare, soluția de spălare (tampon de spălare diluat) conține 10 mM tampon fosfat cu pH 7.0 ± 0.2, 0.05% Tween 20 și 0.05% Proclin 150.
După diluare, soluția de spălare rămâne stabilă timp de 1 săptămână, la temperaturi între 2...8°C.

6. Conjugat
Reactiv gata de utilizare, colorat în culoarea roșie. Conține peroxidază de hrean conjugată cu anticorpi policlonali de capră cu IgG și IgM umani, 5% BSA, 10 mM tampon Citrat cu pH 6.4 ± 0.1 și conservanți 0.1% Proclin și 0.05% Tween 20.

7. Substrat TMB
Componentă gata de utilizare. Conține 50 mM soluție tampon citrat-fosfat cu pH 3.5-3.8, 4% dimetil sulfoxid, 0.03% tetrametilbenzidină (TMB) și 0.02% peroxid de hidrogen (H_2O_2). Amestecați ușor, înainte de utilizare.
Notă: A se păstra ferit de lumina directă, deoarece este sensibil la surse de lumină puternice.

8. Diluant pentru probe
Componentă gata de utilizare. Conține ser de capră, 10 mM soluție tris tampon cu pH 8.0±0.1 ce conține 0.1% Proclin 150 și 0.09% azidă de sodiu pentru pre-tratarea eșantioanelor și a soluțiilor de control de pe placă, cu stoparea interferențelor.

9. Soluție de stopare
Componentă gata de utilizare.
Conține o soluție 0.3 M de H_2SO_4. Amestecați ușor, înainte de utilizare.

10. Diluant eșantion
Componentă gata de utilizare, colorată în culoarea verde închis. Conține 1% cazeină, 10 mM tampon citrat de Na cu pH 6.0 ± 0.1 și conservant 0.1% Proclin 150.
A se utiliza pentru diluarea eșantionului.

Notă: Diluantul își schimbă culoarea din verde măsliniu, în verde închis-albastru, în prezența eșantionului.

E. MATERIALE NECESARE, DAR NEINCLUSE ÎN TRUSĂ
1. Micropipete calibrate (200 µL și 10 µL) și vârfuri de unică folosință.
2. Apă distilată cu aviz EIA (bidistilată sau deionizată, tratată cu cărbune activ pentru îndepărtarea oxidanților chimici folosiți ca dezinfectanți).
3. Cronometru cu interval de timp de 60 minute sau mai mult.
4. foi de hârtie absorbantă.
5. Incubator termostatic calibrat pentru microplăci ELISA, ce poate asigura o temperatură de +37°C.
6. Cititor calibrat de microplăci ELISA, cu capacitate de citire la 450 nm, prevăzut dacă este posibil cu filtre de 620-630 nm pentru detectarea blank-ului și filtre de 405 nm.
7. Spălător calibrat pentru microplăci ELISA.
8. Mixer vortex sau alte dispozitive asemănătoare pentru centrifugare.

F. AVERTISMENTE ȘI PRECAUȚII
1. Această trusă poate fi utilizată doar de personal tehnic specializat și corespunzător calificat, sub supravegherea medicului șef de laborator. Citiți cu atenție prezentul prospect, înainte de dozare și respectați cu strictețe instrucțiunile din cuprinsul acestuia.
2. Citiți cu atenție Fișa cu Date de Securitate (SDS), inainte de a efectua dozarea.
3. În cazul utilizării trusei pentru screening-ul unor unități de sânge și componente ale sângelui, acesta va trebui să fie utilizată într-un laborator certificat și autorizat de autoritatea națională responsabilă în domeniu (Ministerul Sănătății sau un organism similar), în vederea efectuării acestui tip de analize.
4. Întreg personalul implicat în executarea probei trebuie să fie echipat cu îmbrăcăminte de protecție de laborator, mănuși din latex fără talc și ochelari de protecție. Utilizarea oricăror dispozitive ascuțite (ace) sau tăioase (lame) este interzisă. Întreg personalul implicat trebuie să fie instruit cu privire la procedurile de siguranță personală, conform recomandărilor Centrului pentru Controlul Bolilor Atlanta, SUA, indicate în publicația Autorității Naționale pentru Sănătate: ”Siguranță personală în Laboratoarele de Microbiologie și Biomedicină”, ediția 1984.
5. Întreg personalul implicat în manipularea eșantioanelor trebuie să fie vaccinat împotriva virusurilor HBV și HAV, pentru care există vaccinuri sigure și eficiente.
6. Încăperea laboratorului trebuie să aibă un mediu controlat, pentru a se evita contaminarea cu praf sau cu agenți microbiologici din aer, în momentul deschiderii fiolelor și al microplăcii din trusă și în momentul efectuării testului. Substrat (TMB) trebuie ferit de lumina puternică. După începerea testului, evitați vibrațiile mesei de lucru.
7. După recepționarea trusei, aceasta trebuie păstrată la o temperatură de 2…8°C, într-un frigider sau într-o cameră rece, cu temperatură controlată.
8. Nu folosiți componente din truse aparținând unor loturi diferite. Nu se recomandă folosirea componentelor din două truse aparținând aceluiași lot.
9. Asigurați-vă că reactivii sunt limpezi și că nu conțin microorganisme sau particule de mari dimensiuni.

Dacă reactivii nu îndeplinesc aceste condiții, anunțați imediat responsabilul de laborator, pentru a demara procedurile necesare în vederea schimbării trusei.

10. Evitați contaminările încrucișate între eșantioane de ser/plasmă, folosind vârfuri de unică folosință, pe care să le înlocuiți la fiecare eșantion.
11. Evitați contaminările încrucișate între reactivii din trusă, folosind vârfuri de unică folosință pe care să le înlocuiți la fiecare componentă în parte.
12. Nu folosiți trusa după expirarea termenului de valabilitate a acesteia, tipărit pe cutie și pe eticheta aplicată pe fiecare fiolă în parte.
13. Toate eșantioanele trebuie considerate ca fiind potențial infectate. Toate serurile umane trebuie manipulate conform prevederilor Nivelului 2 de Bio-Siguranță, urmând recomandările Centrului pentru Controlul Bolilor Atlanta, SUA, precum și cele din cuprinsul publicației Autorității Naționale pentru Sănătate: "Bio-Siguranță în Laboratoarele de Microbiologie și Biomedicină", ediția 1984.
14. Pentru prepararea componentelor lichide, sau pentru componentele mutate pe stațiile de testare automatizate, se recomandă folosirea de recipiente din plastic de unică folosință, în felul acesta evitându-se contaminările încrucișate.
15. Deșeurile rezultate în urma folosirii trusei se vor elimina conform prevederilor legislației în vigoare la nivel național și ale legislației în materie de deșeuri rezultate din substanțe chimice și biologice de laborator. Mai precis, scurgerile de lichide, rezultate în urma procedurii de spălare, resturile de soluții de control și resturile de soluții eșantion trebuie considerate ca fiind potențial infectate și trebuie supuse procedurii de inactivare, înainte de a fi eliminate. Se recomandă procedura de inactivare prin tratarea cu o soluție de hipoclorit de sodiu cu concentrație de 10% timp de 16-18 ore sau dezactivarea la cald, în autoclavă, la 121°C timp de 20 minute.
16. Scurgerile accidentale de soluții eșantion, în timpul efectuării testelor, trebuie absorbite cu foi de hârtie înmuiate în hipoclorit de sodiu, iar apoi trebuie clătite cu apă. Ulterior, respectivele foi de hârtie se vor arunca într-un recipient special pentru deșeuri provenite din materiale biologice.
17. Soluția de stopare conține 0,3 M acid sulfuric. Evitați contactul acesteia cu pielea și ochii. În caz de contact, clătiți imediat și abundent cu apă.
18. Eliminarea soluțiilor reactive ce conțin azidă de sodiu sau thimerosal, drept conservanți, trebuie tratate conform prevederilor și legislației în vigoare în materie, în țara în care se utilizează testul. Eliminarea soluțiilor ce conțin azidă de sodiu prevede utilizarea unor mari cantități de apă de la robinet. Rețineți faptul că azida de sodiu poate forma compuși explozivi, în urma contactului prelungit cu plumbul sau cuprul.
19. Nu fumați, nu mâncați și nu aplicați produse cosmetice în zonele în care sunt manipulate eșantioanele și reactivii.
20. Celelalte deșeuri produse în urma utilizării trusei (de exemplu: vârfurile folosite pentru controale și eșantioane, microplăcile folosite) trebuie să fie manipulate ca și cum ar fi potențial infectate și trebuie colectate conform prevederilor legislației în vigoare la nivel național și ale legislației privind eliminarea deșeurilor de laborator.
21. Nu pipetați substanțele cu gura.

G. EȘANTIOANE: PREPARARE ȘI RECOMANDĂRI

1. Sângele se recoltează din venă prin metode aseptice, iar plasmele și serurile se prepară prin folosirea tehnicilor standard de preparare a eșantioanelor pentru analize clinice de laborator. Nu s-a depistat nicio influență, în cazul preparării eșantionului cu citrat, EDTA sau heparină.
2. Nu adăugați niciun fel de conservanți în eșantioane; evitați mai ales azida de sodiu, deoarece aceasta poate influența activitatea enzimatică a conjugatului, determinând obținerea unor rezultate fals negative.
3. Eșantioanele trebuie să fie clar identificate cu coduri sau nume, pentru a se evita confuziile în interpretarea rezultatelor. În cazul în care trusa se utilizează pentru teste screening ale unor unități de sânge, recomandăm insistent etichetarea acestora cu coduri de bare ce se vor citi cu un cititor electronic.
4. Eșantioanele intens hemolizate (roșii) sau lipemice (lăptoase) trebuie aruncate, deoarece pot duce la obținerea unor rezultate false. Eșantioanele ce conțin resturi de fibrină sau cheaguri și corpuri microbiologice trebuie aruncate, deoarece pot duce la obținerea unor rezultate false.
5. Serurile și plasmele se vor păstra la temperaturi de +2…8°C cel mult cinci zile după recoltare. Pentru conservarea acestora pe perioade mai îndelungate de timp, eșantioanele pot fi congelate la –20°C timp de câteva luni. Niciun eșantion congelat nu poate fi congelat și decongelat decât o singură dată, deoarece acest proces generează particule ce pot compromite rezultatul testului.
6. În cazul în care eșantionul conține particule, centrifugați la o viteză de 2.000 rpm timp de 20 minute, sau filtrați cu filtre de 0.2-0.8um pentru a curăța eșantionul ce trebuie testat.

H. PREPARAREA COMPONENTELOR ȘI AVERTISMENTE

Studiile efectuate pe o trusă deschisă nu au demonstrat nicio pierdere semnificativă de activitate la cel mult 1 reutilizare a aceluiași material, în termen de 6 luni.

1. Microplăci:
Așteptați până când microplaca ajunge la temperatura mediului ambiant (cel puțin 1 oră) înainte de a deschide folia. Verificați dacă absorbantul de umezeală nu și-a schimbat culoarea în verde închis, ceea ce ar indica o conservare deficitară a trusei. În astfel de situații, adresați-vă serviciului clienți din cadrul firmei Adaltis.
Strip-urile nefolosite trebuie introduse la loc în folie, cu tot cu absorbantul de umezeală. Folia se va sigila perfect și se va păstra la temperaturi de 2…8°C. După prima deschidere, strip-urile rămase vor fi stabile până când indicatorul de umezeală din interiorul foliei cu absorbant de umeazeală își va schimba culoarea din galben în verde.

2. Control negativ:
Gata de utilizare. Centrifugați în vortex, înainte de utilizare.

3. Control pozitiv:
Gata de utilizare. Centrifugați în vortex, înainte de utilizare. Această componentă trebuie tratată ca și cum ar fi potențial infectată.

4. Calibrator:
Dizolvați cu atenție conținutul liofilizat al fiolei cu cantitatea de apă distilată cu aviz EIA, indicată pe etichetă. Centrifugați în vortex, înainte de utilizare.
Această componentă trebuie tratată ca și cum ar fi potențial infectată.
***Notă**: După dizolvare, calibratorul nu este stabil. Păstrați în părți egale, la temperaturi de –20°C.*

5. Soluție de spălare concentrată 20x (flacon de 50 mL):
Întreaga cantitate de soluție concentrată 20x se va dizolva cu apă biodistilată, până la 1000 ml (volumul este indicat pe etichetă) și se va amesteca ușor, înainte de utilizare. Deoarece soluția poate să conțină formațiuni cristaline, aveți grijă să dizolvați întreaga cantitate. În timpul preparării, evitați producerea spumei, deoarece prezența bulelor poate compromite eficiența fazei de spălare.
Notă: După diluare, soluția de spălare rămâne stabilă timp de 1 săptămână, la +2..8°C.

6. Conjugat:
Gata de utilizare. Centrifugați în vortex, înainte de utilizare. Aveți grijă să nu contaminați lichidul cu oxidanți chimici, pulberi sau microbi prezenți în aer. Dacă este necesară mutarea acestei componente, folosiți exclusiv recipiente din plastic, pe cât posibil sterilizate.

7. Substrat TMB:
Gata de utilizare. Centrifugați în vortex, înainte de utilizare. Aveți grijă să nu contaminați lichidul cu oxidanți chimici, pulberi sau microbi prezenți în aer. A se feri de lumină puternică, agenți oxidanți și suprafețe metalice. Dacă este necesară mutarea acestei componente, folosiți exclusiv recipiente din plastic, pe cât posibil sterilizate.

8. Diluant pentru probă:
Gata de utilizare. Centrifugați în vortex, înainte de utilizare.

9. Soluție de stopare:
Gata de utilizare. Centrifugați în vortex, înainte de utilizare.

10. Diluant pentru eșantion:
Gata de utilizare. Centrifugați în vortex, înainte de utilizare.

I. INSTRUMENTAR UTILIZAT ÎMPREUNĂ CU TRUSA

1. Micropipetele trebuie să fie gradate, pentru a picura cantitatea corectă necesară pentru probă și este obligatorie o decontaminare regulată (cu spirt medicinal, înălbitor 10%, soluție dezinfectantă de uz spitalicesc) a acelor componente care pot intra accidental în contact cu eșantionul. Acestea trebuie să fie în permanență controlate, pentru a se asigura o precizie de 1% și o corectitudine de ±2%. La intervale regulate de timp, este obligatorie o dezinfectare a stropilor sau reziduurilor de componente din set.
2. Incubatorul ELISA trebuie să fie setat la 37°C (cu o toleranță de ± 0.5°C) și trebuie să fie verificat cu regularitate, pentru a se asigura menținerea unei temperaturi corecte. Pentru incubare se pot utilizat atât incubatoarele pe uscat, cât și băile de apă, dacă dispozitivele sunt omologate pentru incubarea testelor ELISA.
3. Spălătorul ELISA este extrem de important pentru efectuarea cu succes a probei. Spălătorul trebuie să fie omologat și trebuie să fie corect optimizat. De regulă, sunt suficiente 4-5 cicluri de spălare (aspirare + distribuire a unei cantități de 350 µL de soluție de spălare = 1 ciclu) pentru ca proba să permită obținerea unui rezultat corect. Se recomandă un interval de timp de înmuiere de 20-30 secunde, între cicluri. Pentru a stabili corect numărul acestora, se recomandă să se efectueze un test de probă cu soluțiile de control din trusă și cu eșantioane de referință clar stabilite ca fiind pozitive sau negative și să se verifice conformitatea cu valorile indicate mai jos, în secțiunea O ”Control de calitate intern”. Operațiunile de gradare corectă a volumului distribuit și de întreținere a spălătorului (decontaminare și curățare a acelor) se vor efectua conform instrucțiunilor producătorului.
4. Timpii de incubare au o toleranță de ±5%.
   - ✓ Metodă de incubare de scurtă durată (pentru prima/a 2-a incubare, toleranța este cuprinsă între 43 min. și 47 min.; pentru cea de-a 3-a incubare, toleranța este cuprinsă între 14 și 16 min.).
   - ✓ Metodă de incubare standard (pentru prima incubare, toleranța este cuprinsă între 57 min. și 63 min.; pentru cea de-a 2-a și cea de-a 3-a incubare, toleranța este cuprinsă între 29 și 31 min.).
5. Cititorul de microplăci ELISA trebuie să fie prevăzut cu un filtru de citire la 450nm și, pe cât posibil, și cu un al doilea filtru (620-630nm) pentru operațiunile de blank. Densitatea optică la 450 nm mai mare decât liniaritatea cititorului, poate fi citită la 405 nm și înmulțită cu factorul de conversie.
   Performanțele sale standard trebuie să fie (a) amplitudine de bandă ≤ 10nm; (b) interval de absorbție de la 0 la ≥ 2.0; (c) liniaritate ≥ 2.0; (d) repetabilitate ≥ 1%. Blank-ul este determinat în godeul descris în secțiunea ”Procedură de efectuare a probei”. Sistemul optic al cititorului trebuie să fie corect etalonat, pentru a asigura o măsurare corectă a densității optice. Efectuați cu regularitate operațiunile de întreținere, conform instrucțiunilor producătorului.
6. Atunci când se utilizează o stație automată pentru truse ELISA, toate etapele critice (distribuire, incubare, spălare, citire, manipulare a datelor) trebuie să fie atent verificate, etalonate și corect desfășurate, în vederea menținerii conformității cu valorile indicate în secțiunile O ”Control de calitate intern”. Protocolul probei trebuie să fie instalat în sistemul de operare al unității și trebuie să fie validat și pentru spălător și cititor. De asemenea, partea stației ce realizează manipularea componentelor lichide (distribuire și spălare) trebuie să fie validată și corect setată. O atenție deosebită se va acorda evitării transferului prin intermediul acelor utilizate pentru distribuire și spălare. Acesta trebuie să fie studiat și controlat, în vederea minimizării pericolului de contaminare a godeurilor alăturate. Utilizarea unor stații automate ELISA este recomandată pentru testele screening de sânge, atunci când numărul de eșantioane ce trebuie testate este de peste 20-30 unități pe tură.

L. CONTROALE ȘI OPERAȚIUNI PREMERGĂTOARE PROBEI

1. Verificați termenul de valabilitate al trusei, tipărit pe eticheta aplicată pe cutie. Nu folosiți trusa, dacă termenul de valabilitate este expirat.
2. Verificați componentele lichide, acestea nu trebuie să fie contaminate cu particule sau microorganisme vizibile cu ochiul liber. Asigurați-vă că substratul TMB este incolor sau de culoarea bleu pal, aspirând o cantitate mică din acesta cu o pipetă sterilă din plastic transparent. Verificați dacă ambalajul nu s-a rupt în timpul transportului și dacă nu s-au produs scurgeri de lichide în interiorul cutiei. Verificați ca folia de aluminiu, în care se află microplaca, să nu fie găurită sau deteriorată.
3. Diluați întreg conținutul soluției concentrate de spălare 20x, conform instrucțiunilor de mai sus.
4. Dizolvați calibratorul, conform instrucțiunilor de mai sus.
5. Așteptați până când toate componentele trusei ajung la temperatura camerei (aproximativ 1 oră) și apoi amestecați conform instrucțiunilor.
6. Setați incubatorul ELISA la +37°C și pregătiți spălătorul ELISA amorsându-l cu soluția de spălare diluată, conform instrucțiunilor producătorului. Setați numărul corect de cicluri de spălare, conform instrucțiunilor din secțiunea I.3.
7. Asigurați-vă că cititorul ELISA este pornit de cel puțin 20 de minute, înainte de a efectua citirea.
8. Dacă se utilizează o stație automată, porniți stația, verificați setările acesteia și asigurați-vă că utilizați protocolul corect.
9. Controlați dacă micropipetele au fost setate la volumul prevăzut.
10. Asigurați-vă că aveți la îndemână toate instrumentele necesare, gata de a fi utilizate.
11. În cazul sesizării unor probleme, nu continuați derularea testului, ci informați persoana responsabilă.

M. PROCEDURĂ DE EFECTUARE A PROBEI

Proba trebuie efectuată conform instrucțiunilor de de mai jos, având grijă să se păstreze aceeași incubare, pentru toate eșantioanele ce trebuie testate.
Proba se poate efectua prin două proceduri de incubare. Alegeți-o pe cea mai potrivită, conform regulamentelor în vigoare:

1. Incubare standard (prima incubare 60 minute, a 2-a și a 3-a incubare 30 minute)
2. Incubare de scurtă durată (prima și a 2-a incubare 45 minute, a 3-a incubare 15 minute)

1. Incubare standard - Probă manuală:

1. Introduceți numărul corect de godeuri în suportul respectiv. Lăsați primul godeu gol, pentru blank.
2. Adăugați 200 µL de control negativ în trei godeuri, 200 µL de calibrator în două și 200 µL de control pozitiv într-un singur godeu. Nu diluați controalele și calibratorul, deoarece sunt deja diluate și gata de utilizare!
3. Adăugați 200 µL de diluant pentru eșantioane în toate godeurile eșantioanelor; adăugați apoi 10 µL de eșantion în fiecare godeu corespunzător identificat. Agitați ușor placa, având grijă să evitați revărsarea și contaminarea godeurilor alăturate, pentru a dizolva complet eșantionul în diluantul acestuia.

Notă importantă: *Asigurați-vă că diluantul pentru eșantion, după adăugarea eșantionului, își schimbă culoarea din verde deschis în verde-albastru închis, pentru a semnala adăugarea eșantionului.*

4. Adăugați 50 µL de diluant pentru probă în toate godeurile cu soluții de control/calibrator și cu eșantioane. Verificați dacă eșantioanele își schimbă culoarea în albastru închis.
5. Incubați microplaca timp de **60 min la +37°C.**
   Notă importantă: Strip-urile trebuie să fie sigilate cu hârtia adezivă specială din dotare, numai atunci când testul este efectuat manual. Nu acoperiți strip-urile, dacă se folosește un dispozitiv ELISA automat.
6. Spălați microplaca într-un spălător automat, distribuind și aspirând 350 µL/godeu de soluție de spălare diluată conform instrucțiunilor din secțiunea I.3.
7. Pipetați 100 µL de conjugat enzimatic în toate godeurile, cu excepția celui pentru blank și apoi sigilați cu hârtie adezivă. Asigurați-vă că ați adăugat această componentă de culoare roșie în toate godeurile, cu excepția A1.

Notă importantă: *Aveți grijă să nu loviți peretele intern din plastic al godeului, cu vârful plin cu conjugat. Se pot produce contaminări.*

8. Incubați microplaca timp de **30 min la +37°C.**
9. Spălați godeurile, urmând instrucțiunile din secțiunea I.3.
10. Pipetați 100 µL de amestec substrat TMB în fiecare godeu, inclusiv în cel pentru Blank. Incubați microplaca **la temperatura mediului ambiant (18-24°C) timp de 30 minute.**

Notă importantă: *Amestecul trebuie ferit de lumina directă puternică. Lumina puternică poate genera fundaluri accentuate.*

11. Pipetați 100 µL de soluție de stopare în fiecare godeu, folosind aceeași ordine de pipetare descrisă la punctul 10, pentru a bloca reacția enzimatică. La adăugarea soluției de stopare, controlul pozitiv și eșantioanele pozitive își vor schimba culoarea, din albastru în galben.
12. Măsurați intensitatea culorii soluției din fiecare godeu, conform instrucțiunilor din secțiunea 1.5, cu un filtru de citire optică la 450 nm și dacă este posibil cu un filtru de citire optică la 620-630 nm pentru blank-ul din godeul A1 de pe microplacă.

Note importante:

1. *Dacă nu aveți la dispoziție cel de-al doilea filtru de citire optică, asigurați-vă că nu există amprente digitale pe fundalul microplăcii, înainte de citirea optică la 450 nm. Astfel de amprente pot determina obținerea unor rezultate fals pozitive.*
2. *Citirea optică trebuie efectuată imediat după adăugarea soluției de stopare și, în orice caz, în maxim 20 de minute de la adăugarea acesteia. Este posibil să apară o ușoară auto-oxidare a substratului, cu generarea unui rezultat cu fundal accentuat.*
3. *Centrifugarea la 350 ± 150 rpm în timpul incubării determină o creștere a sensibilității de dozare, cu circa 20%.*

2. Incubare de scurtă durată - Probă manuală:

1. Introduceți numărul corect de godeuri în suportul respectiv. Lăsați primul godeu gol, pentru blank.
2. Adăugați 200 µL de control negativ în trei godeuri, 200 µL de calibrator în două și 200 µL de control pozitiv într-un singur godeu. Nu diluați controalele și calibratorul, deoarece sunt deja diluate și gata de utilizare!
3. Adăugați 200 µL de diluant pentru eșantioane în toate godeurile eșantioanelor; adăugați apoi 10 µL de eșantion în fiecare godeu corespunzător identificat. Agitați ușor placa, având grijă să evitați revărsarea și contaminarea godeurilor alăturate, pentru a dizolva complet eșantionul în diluantul acestuia.

Notă importantă: *Asigurați-vă că diluantul pentru eșantion, după adăugarea eșantionului, își schimbă culoarea din verde deschis în verde-albastru închis, pentru a semnala adăugarea eșantionului.*

4. Adăugați 50 µL de diluant pentru probă în toate godeurile cu soluții de control/calibrator și cu eșantioane. Verificați dacă eșantioanele își schimbă culoarea în albastru închis.
5. Incubați microplaca timp de **45 min la +37°C.**
   Notă importantă: Strip-urile trebuie să fie sigilate cu hârtia adezivă specială din dotare, numai atunci când testul este efectuat manual. Nu acoperiți strip-urile, dacă se folosește un dispozitiv ELISA automat.
6. Spălați microplaca într-un spălător automat, distribuind și aspirând 350 µL/godeu de soluție de spălare diluată conform instrucțiunilor din secțiunea I.3.
7. Pipetați 100 µL de conjugat enzimatic în toate godeurile, cu excepția celui pentru blank și apoi sigilați cu hârtie adezivă. Asigurați-vă că ați adăugat această componentă de culoare roșie în toate godeurile, cu excepția A1.

Notă importantă: *Aveți grijă să nu loviți peretele intern din plastic al godeului, cu vârful plin cu conjugat. Se pot produce contaminări.*

8. Incubați microplaca timp de **45 min la +37°C.**
9. Spălați godeurile, urmând instrucțiunile din secțiunea I.3.
10. Pipetați 100 µL de amestec substrat TMB în fiecare godeu, inclusiv în cel pentru Blank. Incubați microplaca la temperatura mediului ambiant (18-24°C) timp de **15 minute.**

Notă importantă: *Amestecul trebuie ferit de lumina directă puternică. Lumina puternică poate genera fundaluri accentuate.*

11. Pipetați 100 µL de soluție de stopare în fiecare godeu, folosind aceeași ordine de pipetare descrisă la punctul 10, pentru a bloca reacția enzimatică. La adăugarea soluției de stopare, controlul pozitiv și eșantioanele pozitive își vor schimba culoarea, din albastru în galben.
12. Măsurați intensitatea culorii soluției din fiecare godeu, conform instrucțiunilor din secțiunea 1.5, cu un filtru de citire optică la 450 nm și dacă este posibil cu un filtru de citire optică la 620-630 nm pentru blank-ul din godeul A1 de pe microplacă.

Note importante:

1. *Dacă nu aveți la dispoziție cel de-al doilea filtru de citire optică, asigurați-vă că nu există amprente digitale pe fundalul microplăcii, înainte de citirea optică la 450 nm. Astfel de amprente pot determina obținerea unor rezultate fals pozitive.*
2. *Citirea optică trebuie efectuată imediat după adăugarea soluției de stopare și, în orice caz, în maxim 20 de minute de la adăugarea acesteia. Este posibil să apară o ușoară auto-oxidare a substratului, cu generarea unui rezultat cu fundal accentuat.*
3. *Centrifugarea la 350 ± 150 rpm în timpul incubării determină o creștere a sensibilității de dozare, cu circa 20%.*

N. SCHEMĂ PROBĂ

Metodă	**Operațiuni (Incubare Standard)**	**Operațiuni (Incubare de scurtă durată)**
Soluții de control & Calibrator	200 µL	200 µL
Diluant pentru eșantioane și Eșantion	200 µL diluant+ 10 µL eșantion	200 µL diluant+ 10 µL eșantion
Diluant pentru probe	50 µL	50 µL
Prima incubare	**60 min (± 3)**	**45 min (± 2)**
Temperatură	+37°C	+37°C
Spălare	4-5 cicluri	4-5 cicluri
Conjugat enzimatic	100 µL	100 µL
A 2-a incubare	**30 min (± 1)**	**45 min (± 2)**
Temperatură	+37°C	+37°C
Spălare	4-5 cicluri	4-5 cicluri
Substrat TMB	100 µL	100 µL
A 3-a incubare	**30 min (± 1)**	**15 min (± 1)**
Temperatură	Temperatură mediu ambiant (18…24°C)	Temperatură mediu ambiant (18…24°C)
Soluție de stopare	100 µL	100 µL
Citire DO	450/620nm	450/620nm

Mai jos vă prezentăm un exemplu de schemă de distribuire (valabil pentru ambele proceduri de incubare):

Microplacă

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	E2										
B	CN	E3										
C	CN	E4										
D	CN	E5										
E	CAL	E6										
F	CAL	E7										
G	CP	E8										
H	E1	S9										

Legendă: BLK = Blank CN = Control Negativ
CAL = Calibrator CP = Control Pozitiv E = Eșantion

O. CONTROL DE CALITATE INTERN

Se va efectua un control de validare asupra soluțiilor de control și calibratorului, ori de câte ori se utilizează trusa, pentru a se verifica dacă performanțele probei sunt în conformitate atât cu valorile de DO 450/620nm, cât și cu valorile așteptate, indicate în tabelul de mai jos:

Verificați	**Cerințe**
Godeu blank	< 0.100 DO 450/620nm valoare
Control negativ (CN)	< 0.050 valoare medie DO450/620nm după extragerea blank-ului
Calibrator	S/Co >1.1
Control pozitiv	>1.000 DO450/620nm valoare

Dacă rezultatele testului corespund cerințelor de mai sus, treceți la secțiunea următoare.
În caz contrar, nu treceți mai departe și efectuați următoarele verificări:

Probleme	Verificați
Godeu blank > 0.100 DO450nm	1. dacă soluția substrat nu s-a contaminat în timpul probei
Control Negativ (CN) > 0.050 DO450nm după extragerea blank-ului	1. dacă procedura de spălare și setările spălătorului au fost setate conform studiilor de precalificare; 2. dacă s-a utilizat soluția corectă de spălare și dacă spălătorul a fost amorsat înainte de utilizare; 3. dacă nu s-a comis vreo eroare în procedura de efectuare a probei (adăugarea de soluție de control pozitiv, în locul celei de control negativ); 4. dacă nu s-a produs vreo contaminare a soluției de control negativ sau a godeurilor acestuia, din cauza unor stropi de soluție de control pozitiv sau de conjugat enzimatic; 5. dacă micropipetele nu s-au contaminat cu eșantioane pozitive sau cu conjugat enzimatic; 6. dacă acele spălătorului nu sunt blocate sau parțial înfundate.
Calibrator S/Co < 1.1	1. dacă procedurile au fost executate corect; 2. dacă nu a apărut nicio eroare în timpul adăugării acestuia (de ex. adăugarea de soluție de control negativ în locul calibratorului); 3. dacă procedura de spălare și setările spălătorului au fost setate conform studiilor de precalificare; 4. dacă nu s-a produs nicio contaminare externă a calibratorului.
Control pozitiv <1.000 DO450nm	1. dacă procedurile au fost executate corect; 2. dacă nu s-a comis nicio eroare în timpul adăugării controlului (adăugare de soluție de control negativ, în locul controlului pozitiv). În astfel de situații, controlul negativ va indica o DO 450nm > 0.150 3. dacă procedura de spălare și setările spălătorului au fost setate conform studiilor de precalificare; 4. dacă nu s-a produs nicio contaminare externă a controlului pozitiv

În cazul în care a apărut una dintre problemele de mai sus, anunțati responsabilul, pentru a se decide modul de acțiune.

P. REZULTATE
Rezultatele testului sunt calculate pe baza unei valori medii de cut-off stabilită cu ajutorul formulei de mai jos:

Cut-Off (Co) = valoare absorbantă medie CN (control negativ) + 0.350

Valoarea determinată pentru test se va utiliza pentru interpretarea rezultatelor, conform instrucțiunilor din paragraful următor.

Q. INTERPRETAREA REZULTATELOR
Rezultatele testului se vor interpreta ca raport dintre valoarea DO 450 nm a eșantionului și valoarea Cutt-off (sau S/Co), pe baza următorului tabel:

S/Co	Interpretare
< 0.9	Negativ
0.9 - 1.1	Invalid
> 1.1	Pozitiv

Un rezultat negativ indică faptul că pacientul nu este infectat cu virusul HCV, sau că unitatea de sânge poate fi utilizată pentru transfuzie.
Pentru pacienții în cazul cărora rezultatul testului este invalid, va fi necesară repetarea testului, cu un eșantion prelevat după 1-2 săptămâni. Unitatea de sânge nu va putea fi utilizată pentru transfuzie.
Un rezultat pozitiv indică prezența infecției cu virusul HCV, prin urmare pacientul trebuie să fie supus tratamentului aferent, iar unitatea de sânge trebuie distrusă.

Note importante:
1. *Interpretarea rezultatelor se va face exclusiv sub supravegherea șefului de laborator, pentru a se reduce riscul unor erori de analiză.*
2. *Orice rezultat pozitiv trebuie să fie confirmat printr-o metodă alternativă, în măsură să detecteze anticorpii IgG și IgM (teste de confirmare), înainte de pronunțarea unui diagnostic de hepatită virală.*
3. *După cum am indicat în evaluarea performanțelor produsului, această analiză este în măsură să detecteze seroconversia la anticorpi anti-HCV core, înaintea unor alte truse din comerț. Așadar, un rezultat pozitiv, neconfirmat, obținut cu aceste truse din comerț, nu trebuie să fie exclus, ca rezultat fals pozitiv! În orice caz, eșantionul trebuie supus și unui test de confirmare.*
4. *Din moment ce proba este în măsură să determine și anticorpii de clasă IgM, este posibil să apară neconcordanțe cu alte produse din comerț, pentru detectarea anticorpilor anti-HCV, ce nu conțin conjugat anti IgM. Pozitivitatea reală a eșantionului pentru anticorpii HCV trebuie să fie confirmată ulterior, examinându-se și reactivitatea IgM, importantă pentru diagnosticarea infecției cu virusul HCV.*
5. *Atunci când rezultatele sunt transmise de la laborator către un sistem electronic, aveți grijă să nu transmiteți date greșite.*
6. *Diagnosticul de hepatită virală trebuie să fie stabilit și comunicat pacientului doar de personal medical calificat.*

În continuare vă prezentăm un exemplu de calcul:
Datele de mai jos nu trebuie folosite în locul datelor reale, obținute de către utilizator.
Control negativ: 0.019 – 0.020 – 0.021 DO450nm
Valoare medie: 0.020 DO450nm Mai mică de 0.050 – Acceptat

Control pozitiv: 2.189 DO450nm
Peste 1.000 – Acceptat

Cut-Off = 0.020+0.350 = 0.370
Calibrator: 0.550 - 0.530 DO450nm
Valoare medie: 0.540 DO450nm S/Co = 1.4
S/Co peste 1.1 – Acceptat

Eșantion 1: 0.070 DO450nm
Eșantion 2: 1.690 DO450nm
Eșantion 1 S/Co < 0.9 = negativ
Eșantion 2 S/Co > 1.1 = pozitiv

R. PERFORMANȚELE TESTULUI
Evaluarea performanțelor testului s-a realizat conform prevederilor Specificațiilor Tehnice Comune (CTS) (art. 5, Capitolul 3 din Directiva 98/79/CE) și s-a efectuat pentru ambele proceduri de incubare (standard și de scurtă durată).

1. LIMITE DE DETECTARE
Limita de detectare a probei a fost calculată folosindu-se procedura de incubare de scurtă durată, conform prevederilor Standardului de Lucru Britanic pentru detectarea anticorpilor anti-HCV, NIBSC cod 06/188-006. Tabelul de mai jos cuprinde valorile medii de DO450nm prevăzute de acest standard, diluat în plasmă negativă și apoi analizat.

Diluare	Lot#1	Lot#2	Lot#3
Factor	S/Co	S/Co	S/Co
1 X	3,50	4,00	4,30
2 X	2,10	2,60	2,60
4 X	1,3	1,40	1,30
Plasmă Negativă	0,25	0,20	0,20

De asemenea, s-a analizat ”in toto” eșantionul codificat Accurun 1 - seria 3000 – pus la dispoziție de Boston Biomedica Inc., USA și s-au obținut următoarele rezultate:

Accurun 1 series	Lot#1	Lot#2	Lot#3
Factor	S/Co	S/Co	S/Co
1 X	2,90	3,04	3,40

2. SPECIFICITATE ȘI SENSIBILITATE DE DIAGNOSTIC
Analiza performanței trusei s-a realizat printr-o testare externă, efectuată pe un număr de peste 5000 de eșantioane.
2.1 Specificitatea diagnosticului
Reprezintă probabilitatea ca proba să dea un rezultat negativ, în lipsa unei probe de analizat specifice. Au fost examinați peste 5000 de donatori aleatori, inclusiv donatori pentru prima dată.
Specificitatea diagnosticului a fost verificată cu un test omologat US FDA.
Au fost testați 5043 donatori și s-a obținut o specificitate de 99.5%.
210 pacienți spitalizați au fost testați pentru HCV; s-a obținut o specificitate a diagnosticului de 99.5%. De asemenea, specificitatea diagnosticului a fost verificată și prin testarea unui număr de 162 eșantioane potențial interferente (cu alte boli infecțioase, anticorpi pozitivi E.coli, pacienți cu boli hepatice nevirale, pacienți supuși dializei, femei însărcinate, eșantioane intens hemolizate, lipemice etc.). S-a obținut o valoare a specificității de 100%.
Nu s-a observat nicio falsă reactivitate determinată de metoda de preparare a eșantioanelor. Atât plasmele, derivate prin diferite tehnici standard de preparare (citrat, EDTA și heparină), cât și serurile au fost utilizate pentru stabilirea valorilor de specificitate. Au fost testate eșantioane congelate, pentru a se verifica eventualele interferențe determinate de recoltare și conservare. Nu s-a depistat nicio interferență.

2.2 Sensibilitate de diagnostic
Reprezintă probabilitatea ca proba să dea un rezultat pozitiv, în lipsa unei probe de analizat specifice. Sensibilitatea de diagnostic a fost verificată extern, pe un număr total de 348 eșantioane; s-a obținut o sensibilitate de diagnostic de 100%. Intern, au fost testate peste 50 de eșantioane pozitive, obținându-se și de această dată o sensibilitate de diagnostic de 100%.
Au fost testate eșantioane pozitive la infecții provocate de alte genotipuri decât HCV.

Mai mult de atât, s-au studiat majoritatea panelurilor de sero-conversie puse la dispoziție de Boston Biomedica Inc., USA, (PHV) și Zeptometrix, USA, (HCV).

Mai jos vă prezentăm rezultatele pentru unele dintre acestea.

Panel	Nr. eșantioane	Adaltis [1]	Ortho [1, 2]
PHV 901	11	9	9
PHV 904	7	2	4
PHV 905	9	3	4
PHV 906	7	7	7
PHV 907	7	3	2
PHV 908	13	10	8
PHV 909	3	2	2
PHV 910	5	3	3
PHV 911	5	3	3
PHV 912	3	1	1
PHV 913	4	2	2
PHV 914	9	5	5
PHV 915	4	3	0
PHV 916	8	4	3
PHV 917	10	6	6
PHV 918	8	2	0
PHV 919	7	3	3
PHV 920	10	6	6
HCV 10039	5	2	0
HCV 6212	9	6	7
HCV 10165	9	5	4

Notă:
1. Eșantioane pozitive
2. HCV v.3.0

De asemenea, produsul a fost testat pe panelul EFS Ac HCV, lot nr. 06.140817, produs de Etablissement Francais Du Sang (EFS), Franța, obținându-se următoarele rezultate:

Panel EFS Ac HCV

Eșantion	Lot#1 S/Co	Lot#2 S/Co	Lot#3 S/Co	Rezultate așteptate
HCV 1	0,53	0,52	0,55	**Negativ**
HCV 2	3,28	5,91	3,04	**Pozitiv**
HCV 3	2,17	3,18	2,56	**Pozitiv**
HCV 4	2,26	2,23	2,35	**Pozitiv**
HCV 5	6,10	7,06	6,90	**Pozitiv**
HCV 6	1,66	1,77	1,67	**Pozitiv**

3. PRECIZIE

A fost calculată pe cinci eșantioane, unul negativ și patru pozitive, examinate prin 4 replicări, fiecare în șase runde separate.

S-au obținut următoarele rezultate:

Rezultate în cadrul aceluiași lot: Trusă ElAgen HCV Ab (v.4) -

Primul lot (procedură incubare de scurtă durată)

		Precizie - %CV		
Eșantion	**S/Co Medie**	**În interiorul Probei**	**Între Probe**	**Total**
Negativ	0.03	6.66	10.56	12.48
Pozitive	1.20	8.52	8.49	12.03
	1.51	7.69	12.22	14.44
	3.57	7.43	11.82	13.97
	11.87	3.42	9.32	9.92

Rezultate în cadrul aceluiași lot: Trusă ElAgen HCV Ab (v.4) -

Primul lot (procedură incubare de lungă durată)

		Precizie - %CV		
Eșantion	**S/Co Medie**	**În interiorul Probei**	**Între probe**	**Total**
Negativ	0.04	4.67	12.34	13.19
Pozitive	1.47	9.62	11.40	14.92
	1.82	8.92	12.77	15.58
	4.31	4.59	12.88	13.67
	13.78	2.42	8.96	9.26

Rezultate între loturi: Trusă ElAgen HCV Ab (v.4) -
Primul, al 2-lea și al 3-lea lot (procedură incubare de scurtă durată)

	Precizie - %CV		
Eșantion	**Lot 1**	**Lot 2**	**Lot 3**
Negativ	8,65	8,29	6,13
Calibrator	4,98	4,44	5,38
Pozitiv	4,11	3,11	1,37

Variabilitatea indicată în tabele nu s-a soldat cu erori de clasificare a eșantioanelor.

S. SUGESTII PENTRU SOLUȚIONAREA PROBLEMELOR

Respectarea strictă a procedurii și a specificațiilor, precum și o corectă utilizare a reactivilor și o distribuire corectă permit e

vitarea următoarelor tipuri de erori:

EROARE	CAUZE POSIBILE / SUGESTII
DO foarte diferite (± 50%) față de cele indicate în CC	-reactivi aplicați în cantități eronate (sugestie: verificați conformitatea dintre cantitatea setată în pipete și cea prevăzută de test, etalonați din nou) -temperatură eronată sau timp de incubare eronat (sugestie: o întreținere mai atentă a incubatorului, notați ora de începere a incubării) -eroare în executarea fazelor de spălare și de citire fotometrică (sugestie: verificați corecta funcționare sau setările respectivelor dispozitive) -contaminarea substratului sau a conjugatului (sugestie: folosiți numai recipiente curate din plastic de unică folosință)
Repetabilitate redusă a rezultatelor	-reactivii și eșantioanele au fost aplicați în cantități care nu sunt constante (sugestie: verificați precizia pipetelor și conformitatea dintre cantitatea aplicată și cea prevăzută de test; etalonați din nou) -eroare în executarea fazelor de spălare sau de citire (sugestie: verificați corecta funcționare sau setările respectivelor dispozitive) -contaminarea substratului (sugestie: folosiți numai recipiente curate din plastic de unică folosință) -murdărirea sau degradarea reactivilor (sugestie: folosiți vârfuri adecvate, recipiente curate din plastic de unică folosință și apă distilată sau un produs echivalent)
Nicio reacție colorimetrică, după adăugarea substratului	-unii reactivi nu au fost adăugați -contaminare accentuată a conjugatului sau a substratului -executare greșită a procedurii de testare (de ex. aplicare accidentală a reactivilor într-o ordine greșită, sau din recipientul greșit etc.)
Reacție prea puțin intensă (DO prea mici)	-timp de incubare prea scurt, temperatură de incubare prea joasă -diluare eronată a conjugatului
Reacție prea intensă (DO prea mari)	-diluare eronată a conjugatului -timp de incubare prea lung, temperatură de incubare prea ridicată -calitate proastă a apei folosite pentru soluția de spălare (grad redus de deionizare) -spălare insuficientă (conjugatele nu au fost corect îndepărtate)
Rezultate inexplicabile	-contaminarea pipetelor, a vârfurilor sau a recipientelor -spălarea nu este constantă sau nu este suficientă (conjugatele nu au fost corect îndepărtate)
%CV în interiorul probei prea ridicat	-reactivii și/sau strip-urile nu au ajuns la temperatura camerei, înainte de utilizare - spălătorul pentru microplăci nu spală corect (sugestie: curățați capul spălătorului)
%CV între probe prea ridicat	-condițiile de incubare nu sunt constante (durată, temperatură) -controalele și eșantioanele nu au fost adăugate în același timp (cu aceleași pauze) (verificați ordinea de adăugare) -modificări cauzate de personalul operator

T. AUTOMATIZARE

Procedura descrisă în prezentul prospect cu instrucțiuni de utilizare se referă exclusiv la testul efectuat prin metoda manuală. În cazul utilizării unor sisteme de analiză automate, se vor urma instrucțiunile din cuprinsul manualelor de utilizare ale respectivelor dispozitive. Fiecare laborator trebuie să respecte propriile proceduri de validare internă, pentru a atesta conformitatea cu sistemele automatizate.

U. RESTRICȚII

Procentul de repetabilitate a unor rezultate fals pozitive, neconfirmate de analiza RIBA de confirmare, sau de alte metode similare, a fost stabilit ca fiind de sub 0,1% din populația normală.

Eșantioanele congelate ce conțin particule de fibrină sau cheaguri după congelare au dus la obținerea unor rezultate fals pozitive.

BIBLIOGRAFIE

1. CDC. Public Health Service inter-agency guidelines for screening donors of blood, plasma, organs, tissues, and semen for evidence of hepatitis B and hepatitis C. MMWR 1991;40(No. RR-4):1-17.
2. Alter MJ. Epidemiology of hepatitis C. Hepatology 1997;26:62S-5S.
3. McQuillan GM, Alter MJ, Moyer LA, Lambert SB, Margolis HS. A population based serologic study of hepatitis C virus infection in the United States. In Rizzetto M, Purcell RH, Gerin JL, Verme G, eds. Viral Hepatitis and Liver Disease, Edizioni Minerva Medica, Turin, 1997, 267-70.
4. Dufour MC. Chronic liver disease and cirrhosis. In Everhart JE, ed. Digestive diseases in the United States: epidemiology and impact. US Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. Washington, DC: US Government Printing Office, 1994; NIH publication no. 94-1447, 615-45.
5. Alter MJ, Hadler SC, Judson FN, et al. Risk factors for acute non-A, non-B hepatitis in the United States and association with hepatitis C virus infection. JAMA 1990;264:2231-35.
6. Alter HJ, Holland PV, Purcell RH, et al. Posttransfusion hepatitis after exclusion of commercial and hepatitis-B antigen-positive donors. Ann Intern Med 1972;77:691-9.
7. Alter HJ, Purcell RH, Holland PV, Feinstone SM, Morrow AG, Moritsugu Y. Clinical and serological analysis of transfusionassociated hepatitis. Lancet 1975;2:838-41.
8. Seeff LB, Wright EC, Zimmerman HJ, McCollum RW, VA Cooperative Studies Group. VA cooperative study of post-transfusion hepatitis and responsible risk factors. Am J Med Sci 1975;270:355-62.
9. Feinstone SM, Kapikian AZ, Purcell RH, Alter HJ, Holland PV. Transfusion-associated hepatitis not due to viral hepatitis type A or B. N Engl J Med 1975;292:767-70.
10. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989;244:359-62.
11. Kuo G, Choo QL, Alter HJ, et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science 1989;244:362-4.
12. Alter HJ, Purcell RH, Shih JW, et al. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. N Engl J Med 1989;321:1494-1500.
13. Aach RD, Stevens CE, Hollinger FB, et al. Hepatitis C virus infection in post-transfusion hepatitis. An analysis with first- and secondgeneration assays. N Engl J Med 1991;325:1325-9.
14. Alter MJ, Margolis HS, Krawczynski K, Judson, FN, Mares A, Alexander WJ, et al. The natural history of community-acquired hepatitis C in the United States. N Engl J Med 1992;327:1899-1905.
15. Alter, MJ. Epidemiology of hepatitis C in the west. Semin Liver Dis 1995;15:5-14.
16. Donahue JG, Nelson KE, Muåoz A, et al. Antibody to hepatitis C virus among cardiac surgery patients, homosexual men, and intravenous drug users in Baltimore, Maryland. Am J Epidemiol 1991;134:1206-11.
17. Zeldis JB, Jain S, Kuramoto IK, et al. Seroepidemiology of viral infections among intravenous drug users in northern California. West J Med 1992;156:30-5.
18. Fingerhood MI, Jasinski DR, Sullivan JT. Prevalence of hepatitis C in a chemically dependent population. Arch Intern Med 1993;153:2025-30.
19. Garfein RS, Vlahov D, Galai N, Doherty, MC, Nelson, KE. Viral infections in short-term injection drug users: the prevalence of the hepatitis C, hepatitis B, human immunodeficiency, and human Tlymphotropic viruses. Am J Pub Health 1996;86:655-61.
20. Brettler DB, Alter HJ, Deinstag JL, Forsberg AD, Levine PH. Prevalence of hepatitis C virus antibody in a cohort of hemophilia patients. Blood 1990;76:254-6.
21. Troisi CL, Hollinger FB, Hoots WK, et al. A multicenter study of viral hepatitis in a United States hemophilic population. Blood 1993;81:412-8.
22. Kumar A, Kulkarni R, Murray DL, et al. Serologic markers of viral hepatitis A, B, C, and D in patients with hemophilia. J Med Virology 1993;41:205-9.
23. Tokars JI, Miller ER, Alter MJ, Arduino MJ. National surveillance of dialysis associated diseases in the United States, 1995. ASAIO Journal 1998;44:98-107.
24. Osmond DH, Charlebois E, Sheppard HW, et al. Comparison of risk factors for hepatitis C and hepatitis B virus infection in homosexual men. J Infect Dis 1993;167:66-71.
25. Weinstock HS, Bolan G, Reingold AL, Polish LB: Hepatitis C virus infection among patients attending a clinic for sexually transmitted diseases. JAMA 1993;269:392-4.
26. Thomas DL, Cannon RO, Shapiro CN, Hook EW III, Alter MJ. Hepatitis C, hepatitis B, and human immunodeficiency virus infections among non-intravenous drug-using patients attending clinics for sexually transmitted diseases. J Infect Dis 1994;169:990-5.
27. Buchbinder SP, Katz MH, Hessol NA, Liu J, O'Malley PM, Alter, MJ. Hepatitis C virus infection in sexually active homosexual men. J Infect 1994;29:263-9.
28. Thomas DL, Zenilman JM, Alter HJ, et al. Sexual transmission of hepatitis C virus among patients attending sexually transmitted diseases clinics in Baltimore--an analysis of 309 sex partnerships. J Infect Dis 1995;171:768-75.
29. Thomas DL, Factor SH, Kelen GD, Washington AS, Taylor E Jr, Quinn TC. Viral hepatitis in health care personnel at The Johns Hopkins Hospital. Arch Intern Med 1993;153:1705-12.
30. Cooper BW, Krusell A, Tilton RC, Goodwin R, Levitz RE. Seroprevalence of antibodies to hepatitis C virus in high-risk hospital personnel. Infect Control Hosp Epidemiol 1992;13:82-5.
31. Abdel-Hamid, M., M. El-Day, S. El-Kafrawy, N. Mikhail,G.T. Strickland, and A.D. Fix. 2002. Comparison ofsecond- and third-generation enzyme immunoassays fordetecting antibodies to hepatitis C virus. J. Clin.Microbiol. 40:1656-1659.
32. Dusheiko, G., H. Schlimovitz-Weiss, D. Brown, F. McOmish, P.-L. Yap, S. Sherlock, N. McIntyre, and P. Simmonds. 1994. Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. Hepatology 19:13-18.
33. Gretch, D. Diagnostic tests for hepatitis C. The article can be found at: http://www.hepnet.com/nih/gretch.html. It was written as part of a National Institute of Health Conference on Hepatitis C, held from March 24-26, 1997 in Bethesda Maryland
34. Mondelli, M.U., A. Cerino, F. Bono, A. Cividini, A. Maccabruni, M. Aricò, A. Malfitano, G. Barbarini, V. Piaza, L. Minoli, and E. Silini. 1994. Hepatitis C virus (HCV) core serotype in chronic HCV infection. J. Clin. Microbiol. 32:2523-2527.
35. Ohno, T., M. Mizokami, R.-R. Wu, M.G. Saleh, K.-I. Ohba, E. Orito, M. Mukaide, R. Williams, and J.Y.N. Lau. 1997. New hepatitis C virus (HCV) genotyping system that allows for the identification of HCV genotype 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. J. Clin. Microbiol. 35:201-207.
36. Takada, N., S. Takase, N. Enomoto, A. Takada, and T. Date. 1992. Clinical backgrounds of the patients having different types of hepatitis C virus genome. J. Hepatol. 14:35-40.
37. Yoshioka, K., S. Kakumu, T. Wakita, T. Ishikawa, Y. Itoh, M. Takayanagi, Y. Higashi, M. Shibata, and T. Morishima. 1992. Detection of hepatitis C virus by polymerase chain reaction and response to interferon-α therapy: relationships to genotypes of hepatitis C virus. Hepatology 16:293-299.

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Blood Grouping Reagents:
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SLIDE AND TUBE TESTS

IVD **For *In-Vitro* and professional use only**

2°C 8°C **Store at 2- 8°C**

INTENDED USE

The blood grouping reagents are used to detect the presence or absence of A, B or Rhesus Antigens on the surface of human red blood cells based on hemaglutination using slide or tube test techniques in whole blood samples or anticoagulant blood samples collected in EDTA , citrate or heparin tubes.

INTRODUCTION & PRINCIPLES

Blood grouping reagents are prepared from In-Vitro culture supernatants of hybridized immunoglobulin-secreting mouse cell lines. The reagents are diluted with phosphate buffer containing sodium chloride, EDTA and bovine albumin to give reagents that are optimized for use in tube and slide procedures. **Anti-A monoclonal reagent is colored with acid blue (patent blue) dye, Anti-B monoclonal reagent is colored with acid yellow (tartrazine) dye, and Anti-AB monoclonal reagent is not colored.** The test procedure is based on hemaglutination principle, where red cells possessing the antigen agglutinate in the presence of the corresponding antibody indicating that the result is positive. The test is considered negative when no agglutination appears.

Anti-D IgG/IgM blend reagent is prepared from carefully blended human monoclonal IgM and IgG. Anti-D IgG/IgM blend reagent is suitable for slide and tube test procedures. The reagent will directly agglutinate Rh D positive cells, including majority of variants (but not D^{VI}) and a high proportion of weak D (Du) phenotypes. The reagent will agglutinate category D^{VI} and low grade weak D (D^{u}) phenotypes by the indirect anti-globulin techniques.

Anti-D IgG/IgM blend reagent is diluted with a sodium chloride solution, sodium phosphate solution and bovine albumin (sodium caprylate free). Anti-D IgG/IgM blend reagent is not colored. The procedure is based on hemaglutination principle, where red cells' possessing the antigen agglutinates in the presence of the corresponding antibody in the reagent indicating that the result is positive. The test is considered negative when no agglutination appears.

MATERIALS

MATERIALS PROVIDED

Blood Grouping Reagents:

- Anti-A monoclonal reagent (10 ml/vial), Clone: (9113D10).
- Anti-B monoclonal reagent (10 ml/vial), Clone: (9621A8).
- Anti-AB monoclonal reagent (10ml/vial), Clone: (152D12+9113D10).
- Anti-D IgG/IgM Blend reagent (10 ml/vial), Clone: (P3X61 + P3X21223B10 + P3X290 + P3X35).

MATERIALS NEEDED BUT NOT PROVIDED

- Plastic test tube or glass.
- Isotonic saline solution (% 0.9) NaCl).
- Applicator sticks.
- Centrifuge (100-1200 (g) for tube test).
- Timer.
- Incubator
- Anti-Human Globulin Reagent (can be ordered from Atlas Medical).
- White or transparent glass slide.

PRECAUTIONS

- The reagents are intended for in vitro diagnostic use only.
- The test is for well trained professional healthy user not for lay user.
- These reagents are derived from animal and human sources, thus, appropriate care must be taken in the use and disposal of these reagents, as there are no known test methods that can guarantee absence of infectious agents.
- Do not use reagents if it is turbid or contain particles as this may indicate reagent deterioration or contamination.
- Protective clothing should be worn when handling the reagents.
- **The reagents contain (0.1-0.2%) Sodium Azide and 0.02% sodium arseniate which is toxic and can be absorbed through the skin. When drained, the drains should be thoroughly flushed with water.**
- The reagents should be used as supplied and in accordance to the procedure mentioned below. Don't use beyond expiration date.
- Avoid cross contamination of reagents or specimens.
- Visible signs of microbial growth in any reagent may indicate degradation and the use of such reagent should be discontinued.
- Don't use these reagents if the label is not available or damaged.
- Do not use dark glass slide.
- Don't use the kit if damaged or the glass vials are broken or leaking and discard the contents immediately.
- Test materials and samples should be discarded properly in a biohazard container.
- Wash hands and the test table top with water and soap once the testing is done.
- Heamolysed blood sample should not be used for testing.
- The test should be performed at room temperature in a well let area with very good visibility.
- Failure to follow the procedure in this package insert may give false results or safety hazard.
- Close the vial tightly after each test.
- The reagent is considered toxic, so don't drink or eat beside it.
- If spillage of reagent occurs clean with disinfectant (disinfectant used could be irritable so handle with care).

STORAGE CONDITIONS

- The reagents should be stored refrigerated between 2 - 8°C.
- Never Freeze or expose to elevated temperature.
- The reagent is stable until the expiry date stated on the product label. Do not use the reagents past the expiry date.

REAGENT PREPRATION

- The reagents are intended for use as supplied, no prior preparation or dilution of the reagent is required.
- All reagents should be brought to room temperature before use.

SPECIMEN COLLECTION AND PREPARATION

- Blood collected with or without anticoagulant (EDTA, Heparin or Citrate) can be used for Antigen typing.

Note: Blood collected without anticoagulant should be tested immediately.

- The specimens should be tested as soon as possible after collection. If testing is delayed, the specimens should be stored at 2- 8 ºC, Sample must be retained to room temperature prior to analysis. (Testing should be carried out within five days of collections).
- Insure that there is no sign of hemolysis.
- At the time of the test, centrifuge the blood sample at 1200 RCF for 3 minutes.
- Blood collection is to be done with great care.

PROCEDURES

A. DIRECT TUBE METHOD AT ROOM TEMPERATURE

1. Prepare a 5% suspension of red blood cells in isotonic solution.
2. Using the vial dropper, transfer a drop (40±10µl) of each reagent into a separate and appropriately marked tube.
3. Add 50 µl of red blood cell suspension prepared in step 1.
4. Shake to homogenize the mixture, then centrifuge at 500g for **1 minute.**
5. Gently shake the tube in such a way to detach the cell pellet and macroscopically observe for any possible agglutination.
6. Read the reaction immediately.
7. For Anti-D tube, if the reaction is weak or negative, shake the tubes and incubate at 37°C for **15 minutes.**
8. Wash the red blood cells twice with isotonic saline solution (NaCl 0.9%) and discard the last washing liquid.
9. Add one drop (50µl) of the AHG reagent into the tube. Mix and centrifuge at 120g for **1 minute.**
10. Gently shake the tube in such a way to detach the cell pellet and macroscopically observe for any possible agglutination.
11. Read the reaction immediately.

B. ANTIGLOBULIN INDIRECT METHOD for ANTI-D

1. After immediately centrifuging and reading as above, if the reaction is weak or negative, shake the tubes and incubate at 37°C for 15 minutes.
2. Wash the red blood cells twice with isotonic saline solution (NaCl 0.9%) and discard the last washing liquid.
3. Add one drop (40 µl ± 10 µl) of ANTI-HUMAN GLOBULIN to the tube. Mix and centrifuge at 120 (g) for **1 minute**.
4. Gently shake the tube in such a way to detach the cell pellet and macroscopically observe for any possible agglutination.
5. Read the reaction immediately.

C. DIRECT SLIDE METHOD AT ROOM TEMPERATURE

1. Bring reagents and samples to room temperature (18-25°C).
2. Using the wax pen divide the slide into appropriate numbers of divisions.
3. Using the provided dropper, place one drop (40 µl ± 10 µl) of each reagent onto its correspondent division on the slide.
4. Add 25µl of the precipitated cells next to each drop of reagents.
5. Mix the reagent and the cells using a clean stirring stick over an area with a diameter of approximately 20-40mm.
6. Incubate the slide at room temperature (18-25°C) without stirring for **30 seconds**.
7. Hold the slide and gently rock the slide for **3 minutes** and observe macroscopically for any agglutination.
8. Read the reaction immediately.

READING THE RESULT

POSITIVE: If Agglutination appears.

NEGATIVE: If no agglutination is observed.

Use the below table to determine the blood group:

Result of each reaction				ABO Group
Anti-A monoclonal reagent	Anti-B monoclonal reagent	Anti-AB monoclonal reagent	Anti-D IgG/IgM blend reagent	
+	-	+	+	**A+**
+	-	+	-	**A-**
-	+	+	+	**B+**
-	+	+	-	**B-**
+	+	+	+	**AB+**
+	+	+	-	**AB-**
-	-	-	+	**O+**
-	-	-	-	**O-**

STABILITY OF THE REACTIONS

- ABO Blood Grouping Tube tests should be read immediately following centrifugation.
- Slide tests should be interpreted within three minutes to avoid the possibility that a negative result may be incorrectly interpreted as positive due to drying of reagents.
- Delay in reading and interpreting results may result in weekly positive or falsely negative reactions. Slide tests should be interpreted at the end of the three minutes.

PROCEDURE LIMITATION

1. False positive/ negative results may occur due to:
   - Contamination from test materials.
   - Improper storage, cells concentration, incubation time or temperature.
   - Improper or excessive centrifugation.
   - Deviation from the recommended technique.
   - Blood samples of weak A or B subgroups may give rise to false negative results or weak reactions when tested using slide test method. It is advisable to re-test weak subgroups using tube test method.
2. Weaker reactions may be observed with stored blood than with fresh blood.
3. ABO antigens are not fully developed at birth, weaker reactions may therefore occur with cord or neonatal red cells.
4. ABO blood grouping interpretation on individuals greater than 6 months old should be confirmed by testing serum or plasma of the individual against group A and group B red cells (reverse grouping). If the results obtained with the serum do not correlate with the red cell test, further investigation is required.
5. Return the kit to the agent if it does not function properly.
6. Anti-D IgG/IgM blend Reagent tests conducted on particular weak-D phenotypes, while satisfactory, cannot ensure recognition of all weak variants, due to the variability of antigen patterns.

DIAGNOSTIC PERFORMANCE CHARACTERISTICS

The following tables compare the results in slide and tube techniques of 3 lots of Atlas Medical reagents and the results of a CE marked device.

Slide Technique				
Group A				
Positive with anti-A monoclonal reagent and anti-AB monoclonal reagent Negative with anti-B and Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
232	232	232	232	100%
Tube Technique				
Group A				
Positive with anti-A monoclonal reagent and anti-AB monoclonal reagent Negative with anti-B and Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
212	212	212	212	100%

Slide Technique				
Group B				
Positive with anti-B monoclonal reagent and anti-AB monoclonal reagent Negative with anti-A and Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
61	61	61	61	100%
Tube Technique				
Group B				
Positive with anti-B monoclonal reagent and anti-AB monoclonal reagent Negative with anti-A and Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
61	61	61	61	100%

Slide Technique				
Group O				
Negative with anti-A monoclonal reagent, Anti-B monoclonal reagent and anti-AB monoclonal reagent Negative with Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
241	241	241	241	100%
Tube Technique				
Group O				
Negative with anti-A monoclonal reagent, Anti-B monoclonal reagent and anti-AB monoclonal reagent Negative with Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
243	243	243	243	100%

Slide Technique				
Group AB				
Positive with anti-A monoclonal reagent, Anti-B monoclonal reagent and anti-AB monoclonal reagent Negative with Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
33	33	33	33	100%
Tube Technique				
Group AB				
Positive with anti-A monoclonal reagent, Anti-B monoclonal reagent and anti-AB monoclonal reagent Negative with Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
24	24	24	24	100%

No inversion in diagnosis has been shown: from a qualitative point of view we have observed 100% compliance in direct group testing in slide and tube techniques for determination of A, B, AB and O groups for the three lots of Atlas Medical.

QUALITY CONTROL

The reactivity of all blood grouping reagents should be confirmed by testing known positive and negative red blood cells on each day of use.

To confirm the specificity and sensitivity, Blood grouping reagents should be tested with antigen-positive and antigen-negative red blood cells.

REFERENCES

1. BCSH Blood Transfusion Task Force. Guidlines for microplate techniques in liquid-phase blood grouping and antibody screening. Clin. Lab. Haem 1990: 12, 437-460.
2. Issitt P. D. Applied Blood Group Serology, 3rd ed. Miami: Montgomery Scientific, 1985.
3. Kholer G., Milstein C. Continuous culture of fused cells secreting antibody of predefined specificity, 256, 495-497, 1975
4. Messeter L. et. al. Mouse monoclonal antibodies with anti-A, anti-B and anti-A,B specificities, some superior to human polyclonal ABO reagents, Vox Sang 46, 185-194, 1984
5. Race R.R. and Sanger R. Blood groups in man, 6th ed., Oxford: Blackwell Scientific, 1975.
6. Voak D. ET. al., Monoclonal anti-A and anti-B development as cost effective reagents. Med. Lab. Sci 39, 109-122. 1982.

7. Standards for Blood Banks d Transfusion Service. 11th Ed., Washington D.C., AABB 1984:25.
8. Widmann F.K.ed Technical Manual, 9th Ed., Wahington D.C.: AABB 1985:9.

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CE 0459

LIST OF VARIENTS:

Product Code	Product Name
8.02.00.0.0010	Anti-A Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 1 vial/Carton Box
8.02.00.1.0100	Anti-A Monoclonal Reagent (Titer: 1 /512), 10ml/vial. 10 vials / Plastic Pack
8.02.00.1.0180	Anti-A Monoclonal Reagent (Titer: 1 /512), 10ml/vial. 18 vials / Carton Box
8.02.01.0.0010	Anti-B Monoclonal Reagent (Titer: 1 /512), 10ml/vial, / Carton Box
8.02.01.1.0100	Anti-B Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 10 vials / Plastic Pack
8.02.01.1.0180	Anti-B Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 18 vials / Carton Box
8.02.02.0.0010	Anti-AB Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 1 vial/ Carton Box
8.02.02.1.0100	Anti-AB Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 10 vials/Plastic Pack
8.02.02.1.0180	Anti-AB Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 18 vials/Carton Box
8.02.03.0.0010	Anti-D IgG/IgM Blend Reagent (Titer: 1 /128), 10ml/vial, 1 vial/ Carton Box
8.02.03.1.0100	Anti-D IgG/IgM Blend Reagent (Titer: 1 /128), 10ml/vial, 10 vials / Plastic Pack
8.02.03.1.0180	Anti-D IgG/IgM Blend Reagent (Titer: 1 /128), 10ml/vial, 18 vials / Carton Box
8.02.04.0.0010	Anti-A Monoclonal Reagent (Titer: 1 /256), 10ml/vial, 1 Vial/Carton Box
8.02.04.0.0100	Anti-A Monoclonal Reagent (Titer: 1 /256), 10ml/vial, 10 vials / Plastic Pack
8.02.05.0.0010	Anti-B Monoclonal Reagent (Titer: 1 /256), 10ml/vial, 1vial/Carton Box
8.02.05.0.0100	Anti-B Monoclonal Reagent (Titer: 1 /256), 10ml/vial, 10 vials /Plastic Pack
8.02.05.6.0030	ABO Set (Anti-A (1/256), Anti-B (1 /256), Anti-D (1/64)),3x10ml / plastic Pack
8.02.05.7.0020	ABO Set: Anti-A (1/256), Anti-B (1 /256), 2x10ml /Plastic Pack
8.02.06.0.0010	Anti-AB Monoclonal Reagent (Titer: 1 /256), 10ml/vial, 1vial/Carton Box
8.02.06.1.0100	Anti-AB Monoclonal Reagent (Titer: 1 /256), 10ml/vial,10 vials /Plastic Pack
8.02.06.1.0180	Anti-AB Monoclonal Reagent (Titer: 1 /256), 10ml/vial,18 vials / Carton Box
8.02.07.0.0010	Anti-D IgG/IgM Blend Reagent (Titer: 1 /64), 10ml/vial, 1Vial/ Carton Box
8.02.07.1.0100	Anti-D IgG/IgM Blend Reagent (Titer: 1 /64), 10ml/vial, 10 vials / Plastic Pack
8.02.47.0.0030	ABO Set (Anti-A (1 /512), Anti-B (1 /512), Anti-D (1 /128)),3x10ml/Plastic Pack
8.02.47.1.0030	ABO Set (Anti-A (1 /256), Anti-B (1 /256), Anti-D (1 /64)), 3x10ml /Carton Box.
8.02.47.3.0030	ABO Set (Anti-A (1 /256), Anti-B (1 /256), Anti-D (1 /64)), 3x10ml /Plastic Pack
8.02.47.5.0030	ABO Set (Anti-A (1 /256), Anti-B (1 /256), Anti-D (1 /128)), 3x10ml/Plastic Pack
8.02.49.0.0040	ABO Set (Anti-A (1 /256), Anti-B (1 /256), Anti-AB (1 /256), Anti-D (1 /64)), 4x10ml/Carton Box
8.02.49.2.0040	ABO Set (Anti-A (1 /256), Anti-B (1 /256), Anti-AB (1 /256), Anti-D (1 /128)), 4 x 10ml, 4 vials/Plastic Pack
8.02.53.0.0040	ABO Set (Anti-A (1 /512), Anti-B (1 /512), Anti-AB (1 /512) Anti-D (1 /128)), 4x10ml/Plastic Pack
8.02.53.1.0040	ABO Set (Anti-A (1 /512), Anti-B (1 /512), Anti-AB (1 /512) Anti-D (1 /128)), 4x10ml, 4vials/Plastic Pack
8.02.70.0.0010	Anti-A monoclonal reagent , Titer (1/1024), 10 ml/vial, 1Vial/ Carton Box
8.02.71.0.0010	Anti-B Monoclonal reagent (Titer: 1 /1024) , 10 ml/vial ,1Vial/ Carton Box
8.02.72.0.0010	Anti-AB Monoclonal reagent (Titer: 1 /1024) , 10 ml/vial , 1Vial/ Carton Box
8.02.85.0.0010	Anti-D IgG/IgM Blend reagent (Titer 1 /256), 10ml/vial, 1Vial/ Carton Box

REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry

Atlas Medical

STREPTOCOCCAL GROUPING SLIDE TEST

IVD For *In-Vitro* diagnostic and professional use only

2°C 8°C Store at 2° to 8° C

CE

INTENDED USE:

ATLAS Streptococcus Latex Kit is used for qualitative detection and identification of the Lancefield group of Streptococci. Reagents are provided for groups A, B, C, D, F and G.

INTRODUCTION

ATLAS Streptococcal test uses an enzyme extraction procedure to release Carbohydrate antigen from Streptococcal cell walls.

The antigens are detected using specific antibodies to groups A, B, C, D, F and G Lancefield. These antibodies are coated on latex particles. When the antigen extract is mixed with the latex reagent, agglutination will occur. The agglutination appears as a visible clumping and can be seen macroscopically.

PRINCIPLE

Some well isolated colonies are mixed with chemical extraction reagents to liberate the group antigen. This antigen is spread on different circles of the testing glass slide.

Then latex sensitized with antibodies specific for each group, is added. If the correspondent antigen is present in the sample, the antigen-antibody reaction will cause a visible agglutination (clumping). If a sample shows negative reaction with latex of groups A, B, C, F, and G, select other colonies morphologically similar to the proceeding and threat them with the reagent for enzymatic extraction. Test the obtained antigen with latex for group D. A polyvalent extract of streptococci of the above-mentioned groups is supplied as a control for the reliability of the latex reagents.

MATERIALS

MATERIALS PROVIDED

- **Extracting Reagent 1**: Sodium nitrite solution, ready to use.
- **Extracting Reagent 2:** Acetic acid solution, ready to use.
- **Extracting Reagent 3**: Ammonium carbonate solution, ready to use. Contains sodium azide 0.9 g/L as preservative.
- **Extracting Reagent E:** lyophilized lisozyme in Tris buffer pH 8.2 + 0.2. Contains non-reactive stabilizer and sodium azide 0.9 g/L as preservative. Before use, dissolve with 2.0 mL of sterile distilled water.
- **Latex A: sensitized with antibodies (from rabbit) to streptococci of group A. Ready to use. Contains sodium azide 0.9 g/L as preservative.**
- **Latex B**: sensitized with antibodies (from rabbit) to streptococci of group B. Ready to use. Contains sodium azide 0.9 g/L as preservative.
- **Latex C**: sensitized with antibodies (from rabbit) to streptococci of group C. Ready to use. Contains sodium azide 0.9 g/L as preservative.
- **Latex D**: sensitized with antibodies (from rabbit) to streptococci of group D. Ready to use. Contains sodium azide 0.9 g/L as preservative.
- **Latex F:** sensitized with antibodies (from rabbit) to streptococci of group F. Ready to use. Contains sodium azide 0.9 g/L as preservative.
- **Latex G:** sensitized with antibodies (from rabbit) to streptococci of group G. Ready to use. Contains sodium azide 0.9 g/L as preservative.
- **Positive Control**: Lyophilized. Streptococci antigens of groups A, B, C, D, F and G in physiological saline. Contains non-reactive stabilizer and sodium azide 0.9 g/L as preservative. Before use, dissolve with 1.0 mL of sterile distilled water.
- **Test slide.**
- **Stirring Sticks.**
- **Package Insert.**

NOTE: This package insert is also used for individually packed reagent.

MATERIALS NEEDED BUT NOT PROVIDED

- Water bath.
- Test tube.
- Pipettes.
- Sterile loop.

PACKAGING CONTENT

REF **8.00.13.0.0300 (5x1.5 mL Latex (A, B, C, G, F) ,1x3.0 mL Latex (D), 1x1.0 mL Positive Control, 1x 1.5 mL Extraction Reagent 1 , 1x1.5 mL Extraction Reagent 2 , 2x2.5 mL Extraction Reagent 3, 1x2 mL Extraction Reagent E, Glass Slide, plastic stirring sticks).**

STORAGE CONDITIONS

- The reagents should be stored refrigerated between 2 - 8°C avoiding direct light.
- Never Freeze or expose to elevated temperature.
- The reagent is stable until the expiry date stated on the product label. Do not use the reagents past the expiry date.

PRECAUTIONS

1. The reagents are intended *for in vitro diagnostic and professional* use only.
2. Do not pipette by mouth.
3. Always ensure an acceptable performance of the kit by performing the test on the Positive controls before using the kit.
4. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
5. Test materials and samples should be discarded properly in a biohazard container.
6. Wash hands and the test table top with water and soap once the testing is done.
7. Test specimens may contain pathogenic organisms and must be handled with appropriate precautions.
8. When used in accordance with the principles of Good Laboratory Practice, good standards of occupational hygiene and the instructions in these Instructions for Use, the reagents supplied are not considered to present a hazard to health.
9. Do not use the kit if the kit label is not available or damaged.
10. Don't use the kit if damaged or the vials are leaking and discard the contents immediately.
11. The test should be performed at room temperature in a well let area with very good visibility.
12. Do not use the reagent if it contains particles as this may indicate reagent deterioration or contamination.
13. The Latex Suspensions and Positive Control contain 0.9g/l sodium azide . Azides can react with copper and lead used in some plumbing systems to form explosive salts. The quantities used in this kit are small; nevertheless when disposing of azide-containing materials they should be flushed away with large volumes of water.
14. In accordance with the principles of Good Laboratory Practice it is strongly recommended that extracts at any stage of testing should be treated as potentially infectious and handled with all necessary precautions.
15. Extraction Reagents 2 and 3 contain a weak acid and a mild irritant respectively. Avoid direct contact by wearing suitable protective equipment. If the material comes into contact with the skin, mucous membranes or eyes immediately wash the area by rinsing with plenty of water.

REAGENT PREPARITION

Latex reagents and extracting reagents 1, 2, and 3 and are ready to use. Bring the reagents to room temperature before use, shake the latex reagents gently to obtain a homogenous suspension of particles. After opening, the reagents are stable until the expiry date if kept as indicated in "STORAGE CONDITIONS". Extracting Reagent E and Positive control are lyophilized and must be re-suspended in sterile distilled water before use. If stored at 2-8 ° C and preserved from contamination, reagents are stable for 3 months.

SPECIMEN AND SAMPLE PREPARATION

For a correct identification it is important that the colonies (which must be well isolated on blood agar) are picked up fresh.

Before serological analysis, it is advisable to observe the hemolytic activity and set up a slide with Gram stain to ensure the purity of the strain to be tested.

PROCEDURES

Allow all reagents and samples to reach room temperature (18-30°C) before use.

A. Technique with Chemical Extraction

1. Transfer **30 µL (one drop) of Extracting Reagent 1** into a labelled test tube.
2. Pick up 5-6 colonies with a stirring stick, being careful not to pick up part of the culture medium. Add colonies into the test tube and mix to obtain a homogeneous suspension.
3. Transfer **30 µL (one drop) of Extracting Reagent 2**.
4. Let stand for at least **5 minutes at room temperature**. Do not exceed 10 minutes. A prolonged extraction time decreases the sensitivity of the test.
5. Transfer **60 µL (two drops) of Extracting Reagent 3** and mix. Use within 15 minutes.
6. Re-suspend the latex reagent to be used (i.e. A, B, C, F, and/or G) by shaking the vial.
7. Holding the dropper vertically, add 1 free-falling drop of latex in one circle of the glass slide. Repeat this operation for each latex to be used.
8. Transfer **15 µL of antigenic extract** in each circle.
9. Using a clean stirring stick, mix and spread the reaction mixture carefully. Discard the used stirring stick.
10. Tilt and rotate the glass slide. After one minute, observe each circle for evidence of agglutination (clumping). Later agglutinations should be considered as nonspecific.
    NOTE: If all results are negative, proceed with the technique for identification of Group D Streptococci.

B. Direct Technique

(This procedure is able to identify about 70% of Group D strains).

1. Transfer **30 µL (a drop) of Extracting Reagent 3** in a circle of the slide.
2. Pick up 2-3 colonies with a clean stirring stick, being careful not to pick up part of the culture medium, and carefully mix them in the same circle of the slide.
3. Add a drop of Latex D.
4. Tilt the slide for 1 minute. At the end observe each circle for the presence or absence of agglutination. Later agglutinations should be considered as nonspecific.
   NOTE: If negative results are obtained continues with enzymatic extraction technique.

C. Technique with Enzymatic Extraction

(This procedure is able to identify more than 95% of group D strains)

1. Distribute, after reconstitution, **60 µL (two drops) of Extracting Reagent E** into a labelled test tube.
2. Pick up 2-3 colonies with a clean stirring stick, being careful not to pick up part of the culture medium. Insert colonies into the test tube and mix to obtain a homogeneous suspension.
3. Incubate at **37° C for 10 minutes.**
4. Holding the dropper vertically, add **1 free-falling drop of Latex D** in one circle of the glass slide.
5. Add **15 µL of antigenic extract** in one circle.
6. Using a clean stirring stick, mix and spread the reaction mixture carefully. Discard the used stirring stick.
7. Tilt and rotate the glass slide. After one minute, observe each circle for evidence of agglutination (clumping). Later agglutinations should be considered as nonspecific.

Quality Control

Use the positive control and saline as if they were extracted from a sample. The absence of reactions (respectively positive or negative) is index of alteration of the reagents and / or controls .

READING THE RESULT

A. Technique with Chemical Extraction

Positive: If Agglutination appears in the test circle with latex A, B, C, F or G respectively.
Negative: Fine particles appear in the test circle with latex A, B, C, F or G respectively with **no agglutination or clumping**.

B. Direct Technique

Positive: If Agglutination appears in the test circle with latex D.
Negative: Fine particles appear in the test circle with latex D with **no agglutination or clumping**.

C. Technique with Enzymatic Extraction

Positive: If Agglutination appears in the test circle with latex D.
Negative: Fine particles appear in the test circle with latex D with **no agglutination or clumping**.
NOTE: An insufficient amount of bacterial culture used can cause false negative results.

PERFORMANCE CHARACTERISTICS

Sensitivity

The identification with chemical extraction technique of groups A, B, C, F and G streptococci, performed both on lyophilized collection strains and on clinical isolations, has showed a sensitivity of 98%.
The identification of group D with direct technique has showed a sensitivity of 74.3%.
The identification of group D with enzymatic extraction has showed a sensitivity of 92%.

REFERENCES

1. Arcuri F., Molina A.M., Calegari L., Fontana G (1963). Anticorpi antistreptococcici nei sieri umani. Applicazione della reazione di agglutinazione al latex per la dimostrazione degli anticorpi anti.M. L'Igiene moderna. 56, 147.
2. Fanini A., Vignola D., Strapparava E.
3. Lancefield R.C.(1928). The Antigenic Complex of Streptococcus haemolyticus: I.Demonstration of a type-specific substance in extracts of Streptococcus Haemolyticus. J Exp Med • 47, 91-103.
4. Molina A.M., Saletti M.
5. Pianigiani A. (1965).
6. Pianigiani A., Pianigiani M.
7. Romanzi C.A. (1966). Biology of Streptococcus pyogenes and immunological response to streptococcic antigens in rheumatic disease. Giorn Mal Infett Parass, 18, 375-411,.
8. Rossolini A., Lecchini L., Forte D., Benedetti P.A. (1963) Antibody M in children affected by streptococcal infections. Riv Clin Ped, 72, 268-291.
9. Facklam R.F., Martin D.R., Lovgren M., Johnson D.R., Efstratiou A., Thompson T.A., Gowan S., Kriz P., Tyrrell G.J. Kaplan E. and Beall B. (2002) Extension of the Lancefield classification for group A streptococci by addition of 22 new M protein gene sequence types from clinical isolates: emm 103 to emm 124. Clin. Infect Dis. 34(1):28-38.

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PPI1415A01
Rev G (18.10.2023)

REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry
CONTROL +	Positive control	CONTROL -	Negative control

Atlas D-Dimer Latex Kit

IVD *For In Vitro Diagnostic Use Only.*

Store at 2°C to 8°C.

INTENDED USE

A manual slide latex agglutination test for the qualitative and semi-quantitative detection of circulating derivatives of cross-linked fibrin degradation products (XL-FDP) in human citrated plasma to exclude Venous Thromboembolism (VTE) in patients suspected of Deep Vein Thrombosis (DVP) and Pulmonary Embolism (PE).

INTRODUCTION

During blood coagulation, fibrinogen is converted to fibrin by the activation of thrombin. The resulting fibrin monomers polymerize to form a soluble gel of non-cross-linked fibrin. This fibrin gel is then converted to cross-linked fibrin by thrombin activated Factor XIII to form an insoluble fibrin clot. Production of plasmin, the major clot-lysing enzyme, is triggered when a fibrin clot is formed. Fibrinogen and fibrin are both cleaved by the fibrinolytic enzyme plasmin to yield degradation products, but only degradation products from cross-linked fibrin contain D-Dimer. Therefore, cross-linked fibrin degradation products (XL-FDP) are a specific marker of fibrinolysis.

PRINCIPLE

Atlas D-Dimer Latex is a rapid agglutination assay utilizing latex beads coupled with a highly specific D-Dimer monoclonal antibody. XL-FDP present in a plasma sample bind to the coated latex beads, which results in visible agglutination occurring when the concentration of D-Dimer is above the threshold of detection of the assay.

MATERIALS

MATERIALS PROVIDED

- D-Dimer Latex Reagent: a 0.83% suspension of latex particles coated with murine anti-D-Dimer monoclonal antibody, 10mg/mL BSA and 0.1% sodium azide.
- D-Dimer Positive Control: a solution containing purified human D-Dimer fragment, 5mg/mL BSA and 0.1% sodium azide.
- D-Dimer Negative Control: a buffer solution containing 5mg/mL BSA and 0.1% sodium azide.
- Dilution Buffer
- Reaction slide
- Stirring Sticks
- Instructions for Use.

NOTE: This package insert is also used for individually packed reagent.

MATERIALS NEEDED BUT NOT PROVIDED

- Precision pipettes and tips - 20 µL and 100 µL
- Plastic test tubes and rack
- Stopwatch or timing device
- Disposable gloves
- Tissue (for wiping dropper bottle tips)

PACKAGING CONTENT

REF **8.00.17.0.0025 (D-Dimer Latex 1x0.5mL, 2x0.4mL Controls, 1x10mL Glycine Buffer)**

REF **8.00.17.0.0050 (D-Dimer Latex 1x1mL, 2x0.5mL Controls, 1x10mL Glycine Buffer)**

REF **8.00.17.0.0100 (D-Dimer Latex 1x2mL, 2x1mL Controls, 2x10mL Glycine Buffer)**

REF **8.00.17.2.0100 (D-Dimer Latex 1x2mL, 2x0.5mL Controls, 2x10mL Glycine Buffer)**

REF **8.00.17.0.0200 (D-Dimer Latex 1x4mL, 2x2mL Controls, 1x40mL Glycine Buffer)**

PRECAUTIONS

- For In Vitro Diagnostic Use Only.
- Harmful if swallowed. Avoid contact with skin and eyes. Do not empty into drains.
- Wear suitable protective clothing.
- CAUTION: All reagents in Atlas D-Dimer Latex Kit contain sodium azide (0.1%) as preservative. Do not ingest or allow to contact skin or mucous membranes. Sodium azide may form explosive azides in metal plumbing. Use proper disposal procedures.
- CAUTION: The Positive Control in Atlas D-Dimer Latex Kit contain components of human origin. Each individual blood donation intended for the production of this reagent is tested for HBsAg, anti-HCV, anti-HIV1 and anti-HIV2. Only donations with negative findings are employed. As complete absence of infectious agents can never be assured, all materials derived from human blood should be treated as potentially infectious and handled with due care following the precautions recommended for biohazardous material.
- Do not use the kit if damaged or the glass vials are broken or leaking and discard the contents immediately.
- Do not use these reagents if the label is not available or damaged.
- Test materials and samples should be discarded properly in a biohazard container.

STORAGE AND STABILITY

- Store at 2°C to 8°C.
- DO NOT FREEZE.
- Stability: Refer to outer package and vial labels for expiration date.
- Opened vials are stable until specified expiry date printed on vial label when stored refrigerated (2 - 8°C).
- Indication of Reagent Deterioration
  Reagent deterioration is indicated by failure of the Latex Reagent to agglutinate with the Positive Control, agglutination with the Negative Control, or evidence of microbial contamination.

SPECIMEN COLLECTION AND PREPARATION

- Use fresh plasma prepared by centrifugation of whole blood collected using tube contain sodium citrate anticoagulant. (The use of EDTA and heparin will result in an increased level of false positive reaction).
- The test works best on fresh plasma samples. If testing cannot be done immediately, plasma samples should be stored at -20ºC up to 2 weeks.
- Specimen may be tested directly for the presence of XL-FDP. Defibrination of the plasma is not recommended.
- Frozen specimen should be rapidly thawed at 37 °C and centrifuged before testing.

PROCEDURE

- Equilibrate reagents to room temperature (20°C to 25°C) before use.
- Latex Reagent should be mixed by inversion immediately prior to use.

Qualitative Method

1. Bring reagents and specimens to room temperature before use.
2. Place 20 µL of the reagent within a field on the reaction slide.
3. Accurately pipette 20 µL of undiluted plasma or of control solution next to the drop of Latex Reagent.
4. Mix the Latex Reagent and sample with a stirrer until the Latex is uniformly distributed.
5. Place the slide on a mechanical rotator at 80-100 r.p.m. for three minutes.
6. At exactly 3 minutes, check for agglutination under a strong light source.

NOTE

If test reading is delayed beyond 3 minutes, the latex suspension may dry out **giving a false agglutination pattern. If this is suspected, the specimen must be retested.**

Semi quantitative Method

1. Prepare serial dilutions of the test plasma with Buffer as follows:
1:2 dilution 100 µL plasma plus 100 µL Buffer solution
1:4 dilution 100 µL 1:2 dilution plus 100 µL Buffer solution
1:8 dilution 100 µL 1:4 dilution plus 100 µL Buffer solution
2. Test each dilution as described in the qualitative method.

QUALITY CONTROL

- It is recommended that both Positive and Negative Controls be included in each batch of tests to ensure proper functioning of the system. Control solutions should be tested by the same procedures as patient samples.
- D-Dimer Positive Control consists of a solution of human D-Dimer at a level of approximately ≥ 0.80 mg/L (≥ 800ng/mL).

RESULTS

A. Qualitative Assay

For the qualitative assay protocol, the following pattern of results should be obtained:

Undiluted Plasma D-Dimer (XL-FDP) concentration

- Less than 0.15 mg/L (150ng/mL): Negative result
- Greater than 0.15 mg/L (150ng/mL): Positive result

B. Semiquantitative Assay

Approximate levels of XL-FDP, containing the D-Dimer domain, for specimen dilutions are shown in Table 1. As with all semiquantitative tests, some variability in dose-response can be expected.

Approximate Range of D-Dimer (XL-FDP) mg/L (ng/ml)	Sample Dilution			
	Undil.	1:2	1:4	1:8
< 0.2 (< 200)	-	-	-	-
0.2 – 0.4 (200 – 400)	+	-	-	-
0.4 – 0.8 (400 – 800)	+	+	-	-
0.8 – 1.6 (800 – 1600)	+	+	+	-
1.6 – 3.2* (1600 – 3200*)	+	+	+	+

"+" = agglutination, "-" = no agglutination

* Levels of XL-FDP greater than 3.20 mg/L (3200 ng/mL) can be estimated by further dilutions beyond 1:8.

EXPECTED VALUES

A positive result, indicating active fibrinolysis, should be obtained with D-Dimer Latex Test when XL-FDP (D-Dimer) levels are at or greater than approximately 0.20 mg/L (200ng/mL). Plasma specimens from normal subjects are expected to give negative results because their plasma XL-FDP concentrations are typically less than 0.20 mg/L (200ng/mL). Due to many variables that may affect results, each laboratory should establish its own normal range.

Elevated levels of XL-FDP (containing the D-Dimer domain) have been demonstrated in patients by a combination of immunoprecipitation and gel electrophoresis techniques. Monoclonal antibodies allow the specific detection of the D-Dimer domain. Monoclonal antibody based D-Dimer assay is of diagnostic value in disseminated intravascular coagulation (DIC) and acute vascular diseases, including pulmonary embolism (PE) and deep venous thrombosis (DVT), conditions that are difficult to detect reliably by clinical examination.

The amount of XL-FDP detected in a specimen will depend on several interrelated factors in vivo, such as the severity of the thrombotic episode, the rate of cross linked fibrin formation, and the time elapsed after the thrombotic event until blood is drawn from the patient.

Elevated levels of XL-FDP as an indication of reactive fibrinolysis have also been reported in surgery, trauma, sickle cell disease, liver disease, severe infection, sepsis, inflammation, and malignancy. D-Dimer levels also rise during normal pregnancy but very high levels are associated with complications.

LIMITATIONS

Clinical diagnosis should not be based on the result of D-Dimer Latex alone. Clinical signs and other relevant test information should be included in the diagnostic decision.

SPECIFIC PERFORMANCE CHARACTERISTICS

- Diagnostic Sensitivity: 100.00% (95% CI (97.34% to 100.00%))
- Diagnostic Specificity: 94.38% (95% CI (89.91% to 97.27%)).
- Positive Predictive Value: 93.20% (95% CI (88.24% to 96.16%)).
- Negative Predictive Value : 100%
- Accuracy: 96.83% (95% CI (94.24% to 98.47%)).
- Intra-assay (within run) reproducibility was determined for 10 replicates of 3 plasma samples that contained different levels of XL-FDP. The results were equivalent for all replicates.
- Inter-assay (run-to-run) reproducibility was determined using 10 plasma samples with XL-FDP titers ranging from 1 to 16. In 10 runs, the replicates of these specimens did not vary by more than one titer.
- In an anticoagulant study of 50 parallel citrated, EDTA and heparin plasma samples, the test result showed that the following:
  - Plasma prepared from whole blood anticoagulated with sodium citrate is recommended.
  - The use of EDTA and heparin sodium will result in an increased level of false positive reaction.
- No assay interference was demonstrated with Atlas D-Dimer Latex with spiked specimens containing potential interfering substances at the following concentrations:
  - Bilirubin 0.2 mg/mL
  - Hemoglobin 5.0 mg/mL
  - Lipids (triglycerides) 30 mg/mL
  - Protein (gamma globulin) 0.06 g/mL

REFERENCES

1. Gaffney, P.J. Distinction between Fibrinogen and Fibrin Degradation Products in Plasma. Clin. Chim. Acta. 65 (1): 109-115; 1975.
2. Lane, D.A. et al. Characterisation of Serum Fibrinogen and Fibrin Fragments Produced During Disseminated Intravascular Coagulation. Br. J. Haematol. 40 (4): 609-615; 1978.
3. Whitaker, A.N. et al. Identification of D-Dimer-E complex in Disseminated Intravascular Coagulation. Thromb. Res. 18 (3-4): 453-459; 1980.
4. NCCLS Publication H21-A3 - Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and General Performance of Coagulation Assays; Approved Guideline Third Edition; 1998.
5. Graeff, H. et al. Detection and Relevance of Crosslinked Fibrin Derivatives in Blood. Semin. Thromb. Hemost. 8 (1): 57-68; 1982.
6. Yoshioka, K. et al. Distinction between Fibrinogen and Fibrin Products Produced during Disseminated Intravascular Coagulation in Childhood. Eur. J. Pediatr. 138 (1): 46-48; 1982.
7. Rylatt, D.B. et al. An Immunoassay for Human D-Dimer using Monoclonal Antibodies. Thromb. Res. 31 (6): 767-778; 1983.
8. Elms, M.J. et al. Rapid Detection of Cross-Linked Fibrin Degradation Products in Plasma using Monoclonal Antibody-Coated Latex Particles. Am. J. Clin. Pathol. 85 (3): 360-364; 1986.
9. Whitaker, A.N. et al. Measurement of Cross-Linked Fibrin Derivatives in Plasma: an Immunoassay using Monoclonal Antibodies. J. Clin. Pathol. 37 (8): 882-887; 1984.
10. Hunt, F.A. et al. Serum Crosslinked Fibrin (XDP) and Fibrinogen/Fibrin Degradation Products (FDP) in Disorders Associated with Activation of the Coagulation or Fibrinolytic Systems. Br. J. Haematol. 60 (4): 715-722; 1985.
11. Smith, R.T. et al. Fibrin Degradation Products in the Postoperative Period. Evaluation of a New Latex Agglutination Method. Am. J. Clin. Pathol. 60 (5): 644-647; 1973.
12. Nolan, T.E. et al. Maternal Plasma D-Dimer Levels in Normal and Complicated Pregnancies. Obstet. Gynecol. 81 (2): 235-238, 1993.

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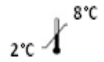
Rev D (06.05.2023)

REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry
CONTROL +	Positive control	CONTROL -	Negative control

Atlas Medical CE

RPR Carbon Antigen

IVD ***For In-Vitro diagnostic and professional use only***



Store at 2 to 8 °C

***INTENDED USE**

A manual rapid plasma reagin carbon test for the qualitative and semi-quantitative detection of non-treponemal antibodies against Syphilis in human serum and plasma to provide serological evidence of past/current Syphilis infections when preceeded by a positive treponemal test. Not to be used as a screening tool for blood or tissue donations.

INTRODUCTION

Syphilis is a disease caused by infection with the spirochete Treponema pallidum. The infection is systemic and the disease is characterized by periods of latency. These features, together with the fact that T pallidum cannot be isolated in culture, mean that serologic techniques play a major role in the diagnosis and follow-up of treatment for syphilis.

Syphilis is categorized by an early primary infection in which patients may have non-specific symptoms, and potentially, genital lesions. Patients tested by serology during the primary phase may be negative for antibodies, especially if testing is performed during the first 1 to 2 weeks after symptom onset. As the disease progresses into the secondary phase, antibodies to T pallidum reach peak titers, and may persist indefinitely regardless of the disease state or prior therapy. Therefore, detection of antibodies to nontreponemal antigens, such as cardiolipin (a lipoidal antigen released by host cells damaged by T pallidum) may help to differentiate between active and past syphilis infection. Nontreponemal antibodies are detected by the rapid plasma reagin (RPR) assay, which is typically positive during current infection and negative following treatment or during late/latent forms of syphilis.

PRINCIPLE

RPR utilises carbon particles coated with cardiolipin antigen to detect reagin antibodies present in serum or plasma of syphilitic persons.

Specimens that contain reagin cause aggregation of the carbon particles which appear as dark clumps against a white background. The aggregation can be read macroscopically. Non-reactive samples typically appear as a smooth non-aggregated pattern which may form buttons in the centre of the test area.

MATERIALS

MATERIALS PROVIDED

- **RPR carbon antigen reagent**: A particulate carbon suspension coated with lipid complexes, with 0.95 g/L sodium azide.
- **Positive Control**: Human syphilitic serum reactive with the test reagent, with 0.95 g/L Sodium azide. **(Optional).**
- **Negative control**: non-reactive phosphate buffer containing 5% BSA pH7.4, with 0.1% of Sodium azide. **(Optional).**
- **RPR test cards or white glass slide (Optional).**
- **Plastic sticks (Optional).**
- **Package insert**.

NOTE: This package insert is also used for individually packed reagent.

MATERIALS NEEDED BUT NOT PROVIDED

- Rotator (100rpm).
- Timer.
- Calibrated micropipettes and tips.

PACKAGING CONTENT

REF **8.00.18.0.0100 (2mL Reagent, 1x0.5ml Positive Control, 1x0.5mL Negative Control)**

REF **8.00.18.0.0500 (10mL Reagent, 1x1ml Positive Control, 1x1mL Negative Control)**

REF **8.00.18.3.1000 (2x10ml Reagent, 1x2ml Positive Control, 1x2ml Negative Control)**

STORAGE AND STABILITY

- All components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C.
- *Do Not Freeze.
- *Signs of deterioration:
  - RPR Carbon: Visible agglutination.
  - Controls: Presence of particles and turbidity.

PRECAUTIONS AND WARNINGS

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- *The test is not for near-patient or self-testing.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all negative and positive in the manner as patient specimens .
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- The used test should be discarded according to local regulations.
- Components of different human origin have been tested and found to be negative for the presence of antibodies anti- HIV 1+2 and anti-HCV, as well as for HBsAg. However, the controls should be handled cautiously as potentially infectious.
- *Do not touch, drink, or ingest the reagent.
- *Do not use black glass slides during testing.
- *Perform the test in a well-lit area with good visibility.
- *Failure in following the instructions may give incorrect results or face safety hazards.
- *Wash the area of contact with water immediately if contact occurs.
- *Wash of the hands and the test table top with water and soap.
- *Do not use the reagent if displaying any signs of deterioration.
- *Always use a fresh pipette tip and stirring sticks for each test.
- *Handle the used disinfectant with care.
- *Glass slides should be thoroughly rinsed with water and wiped with lint-free tissue after each use.
- *Do not use the reagents if the label is missing, damaged, or unclear.
- *Do not use leaked vials and making proper disposal of them.
- *Use forceps, scoops, or other mechanical devices for removing broken glass from the working area. A dustpan and brush should be used to clean up shards/small pieces of broken glass. Broken glass must be disposed of in a sharps container.
- *The reagents containing sodium azide may be combined with copper and lead plumbing to form highly explosive metal azides. Dispose of reagents by flushing with large amounts of water to prevent azide bulidup.
- *Any serious incident that occur in relation to the device shall be reported to the manufacturer and the competent authority. (Feedback@atlas-medical.com)

COLLECTION, HANDLING AND PREPARATION OF SPECIMEN

- Fresh serum or plasma. The samples with presence of fibrin should be centrifuged before testing. Do not use highly hemolized or lipemic samples.
- *Samples may be stored at 2-8° C for up to 7 days. For long term storage sera should be stored at -20° C up to 30 days.

REAGENT PREPARATION

RPR reagent is ready to use. No preparation is required.

PROCEDURES

QUALITATIVE PROCEDURE

- **Mix well the RPR reagent before use.**

1. Bring the reagents and samples to room temperature.
2. Dispense **50 µL of each sample** into a separate circle on the card. Use a separate tip for each sample.
3. Dispense **1 drop of each of positive and negative controls** into two additional circles.
4. Gently shake the dispensing vial and slightly press to remove air bubbles from the needle and the drop obtained is correct.

5. Dispense **1 drop (17.5 µl) of RPR antigen** to each circle next to the sample to be tested.
6. *Close the reagent vial tightly.
7. *Spread the specimen evenly over the test circle.
8. Place the card on a mechanical rotator and rotate at 100 r.p.m. for 8 minutes.
9. Observe macroscopically for agglutination within a minute after removing the card from the rotator.

SEMI-QUANTITATIVE PROCEDURE

- **Mix well the RPR reagent before use**.

1. Make doubling dilutions from Undiluted to 1:16 normal saline.
2. Place 50 µl of each dilution in to a separate circle on the test card.
3. Spread each dilution evenly over the test circle.
4. Continue as from Qualitative procedure .
   The titer of the sample is expressed as the final dilution which shows aggregation of the carbon particles.

INTERPRETATION OF TEST RESULTS

1. **Strong Reactive:** Large clumps of carbon particles with a clear background.

2. **Reactive:** Large clumps of carbon particles somewhat more disperse than Strong Reactive pattern.

3. **Weak Reactive:** Small clumps of carbon particles with light grey background.

4. **Trace Reactive:** Slight clumping of carbon particles typically seen as a button of aggregates in the centre of the test circle or dispersed around the edge of the test circle.

5. **Non-Reactive:** Typically a smooth grey pattern or a button of non-aggregated carbon particles in the centre of the test circle.

<u>*LIMITATION OF THE TEST</u>

- Pregnancy may give a false positive reaction.
- Hepatitis and Brucellosis may give a false positive reaction.
- Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

PERFORMANCE CHARACTERISTICS

Sensitivity: 100%.
Specificity: 100%.
Precision: 100%
Hook effect: no prozone effect up to the titer level studied: 1/16.
Interferences: There is no effect from Hemoglobin/Bilirubin and Rheumatoid factor on the results of RPR carbon antigen at the studied concentrations:
Bilirubin: ≤15 mg/dL.
Hemoglobin: ≤10 g/L.
Rheumatoid factor: ≤300 IU/ml.

REFERENCES

- Falcone V.H., Stout G.W. and Moore M.B. Jr., PHR 79: 491-495, 1964.

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REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry
CONTROL +	Positive control	CONTROL -	Negative control

<u>*: Indication of the introduced modifications.</u>

ASO LATEX KIT

IVD For in -*vitro* diagnostic and professional use only

Store at 2-8°C. CE

INTENDED USE

ATLAS ASO latex Test is used for the qualitative and semi-quantitative measurement of antibodies to Antistreptolysin-O in human serum.

INTRODUCTION

The group A ß-hemolytic streptococci produce various toxins that can act as antigens. One of these exotoxins streptolysin-O, was discovered by Todd in 1932.

A person infected with group A hemolytic streptococci produces specific antibodies against these exotoxins, one of which is antistreptolysin-O. The quantity of this antibody in a patient's serum will establish the degree of infection due to the hemolytic streptococcal.

The usual procedure for the determination of the antistreptolysin titer is based on the inhibitory effect that the patient's serum produces on the hemolytic power of a pre-titrated and reduced streptolysin-O. However, the antigen-antibody reaction occurs independently of the hemolytic activity of streptolysin-O. This property enables the establishment of a qualitative and quantitative test for the determination of the antistreptolysin-O by agglutination of latex particles on slide.

PRINCIPLE

ASO test method is based on an immunologic reaction between streptococcal exotoxins bound to biologically inert latex particles and streptococcal antibodies in the test sample. Visible agglutination occurs when increased antibody level is present in the test specimen.

MATERIALS

MATERIALS PROVIDED

- ASO Latex Reagent: Latex particles coated with streptolysin O, pH, 8,2. Preservative**.**
- ASO Positive Control (Red cap): Human serum with an ASO concentration > 200 IU/mL.Preservative.
- ASO Negative Control (Blue cap) Animal serum. Preservative
- Glass Slide.
- Stirring Sticks.

Note: This package insert is also used for individually packed reagent.

MATERIALS REQUIRED BUT NOT PROVIDED

- Mechanical rotator with adjustable speed at 80-100 r.p.m.
- Vortex mixer.
- Pippetes 50 µL.
- Glycine Buffer 20x (1000 mmol/l): add one part to nineteen parts of distilled water before use.

Packaging contents

REF **8.00.02.0.0100 (1x4ml Latex Reagent, 1x1ml positive control, 1x1ml negative control)**

PRECAUTIONS

- All reagents contain 0.1 %(w/v) sodium azide as a preservative.
- Protective clothing should be worn when handling the reagents.
- Wash hands and the test table top with water and soap once the testing is done.
- Reagents containing sodium azide may be combined with copper and lead plumbing to form highly explosive metal azides. Dispose of reagents by flushing with large amounts of water to prevent azide buildup.
- For In Vitro diagnostic use.
- Components prepared using human serum found negative for hepatitis B surface antigen (HBsAg), HCV and antibody to HIV (1/2) by FDA required test. However, handle controls as if potentially infectious.
- Accuracy of the test depends on the drop size of the latex reagent (40µl). Use only the dropper supplied with latex and hold it perpendicularly when dispensing.
- Use a clean pipette tip and stirring stick for each specimen, and glass slides should be thoroughly rinsed with water and wiped with lint-free tissue after each use.
- Check reactivity of the reagent using the controls provided.
- Do not use these reagents if the label is not available or damaged.
- Do not use the kit if damaged or the glass vials are broken or leaking and discard the contents immediately.
- Test materials and samples should be discarded properly in a biohazard container.

REAGENT PREPARATION:

The ASO Latex reagent is ready to use. No preparation is required. Mix gently before use to ensure a uniform suspension of particles.

STORAGE AND STABILITY

- Reagents are stable until specified expiry date on bottle label when stored refrigerated (2-8°C).
- **DO NOT FREEZE.**
- The ASO Latex Reagent, once shaken must be uniform without visible clumping. When stored refrigerated, a slight sedimentation may occur and should be considered normal.
- Do not use the latex reagent or controls if they become contaminated.
- Always keep vials in vertical position. If the position is changed, gently mix to dissolve aggregates that may be present.
- Reagents deterioration: Presence of particles and turbidity.

SAMPLES

- Use fresh serum collected by centrifuging clotted blood.
- If the test cannot be carried out on the same day, store the specimen for 7 days at 2-8°C and for 3 months at -20°C.
- Samples with presence of fibrin should be centrifuged before testing. Do not use highly hemolyzed or lipemic samples.
- **DO NOT USE PLASMA.**

PROCEDURE

Qualitative method

1. Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperatures.
2. Place (40 µL) of the sample and one drop of each Positive and Negative controls into separate circles on the slide test.
3. Mix the ASO-latex reagent vigorously or on a vortex mixer before using and add one drop (40 µL) next to the sample to be tested.
4. Mix the drops with a stirrer, spreading them over the entire surface of the circle. Use different stirrers for each sample.
5. Place the slide on a mechanical rotator at 80-100 r.p.m. for 2 minutes. False positive results could appear if the test is read later than two minutes.

Semi-quantitative method

1. Make serial two-fold dilutions of the sample in 9 g/L saline solution.

2. Proceed for each dilution as in the qualitative method.

QUALITY CONTROL

- Positive and Negative Controls should be included in each test batch.
- Acceptable performance is indicated when a uniform milky suspension with no agglutination is observed with the ASO Negative Control and agglutination with large aggregates is observed with the ASO Positive Control.

CALCULATIONS

The approximate ASO concentration in the patient sample is calculated as follows:

200 x ASO Titer = IU/mL

READING AND INTERPRETATION

Examine macroscopically the presence or absence of visible agglutination immediately after removing the slide from the rotator. The presence of agglutination indicates an ASO concentration equal or greater than 200 IU/mL

The titer, in the semi-quantitative method, is defined as the highest dilution showing a positive result.

REFERENCE VALUES

Up to 200 IU/mL(adults) and 100 IU/mL (children < 5 years old). Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity:

200 (±50) IU/ml.

PROZONE EFFECT

No prozone effect was detected up to 1500 IU/ml.

SENSITIVITY

98%.

SPECIFICITY

97%.

INTERFERENCES

NON-INTERFERING SUBSTANCES:

- Hemoglobin (10 g/L)
- Bilirubin(20 mg/dL)
- Lipids (10 g/L)
- Rheumatoid factors (300 IU/mL)
- Other substances may interfere.

LIMITATIONS

- Reaction time is critical. If reaction time exceeds 2 minutes, drying of the reaction mixture may cause false positive result.
- Freezing the ASO Latex Reagent will result in spontaneous agglutination.
- Intensity of agglutination is not necessarily indicative of relative ASO concentration; therefore, screening reactions should not be graded.
- False positive results may be obtained in conditions such as, rheumatoid arthritis, scarlet fever, tonsilitis, several streptococcal infections and healthy carriers.
- Early infections and children from 6 months to 2 years may cause false negative results. A single ASO determination does not produce much information about the actual state of the disease.
- Titrations at biweekly intervals during 4 or 6 weeks are advisable to follow the disease evolution.
- Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

REFERENCES

1. Haffejee . Quarterly Journal of Medicine 1992. New series 84; 305: 641-658.
2. Ahmed Samir et al. Pediatric Annals 1992; 21: 835-842.
3. Spaun J et al. Bull Wld Hlth Org 1961; 24: 271-279.
4. The association of Clinical Pathologists 1961. Broadsheet 34.
5. Picard B et al. La Presse Medicale 1983; 23: 2-6.
6. Klein GC. Applied Microbiology 1971; 21: 999-1001.
7. Young DS. Effects of drugs on clinical laboratory test, 4th ed. AACC Press, 1995.

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PPI2325A01
Rev A (05.01.2023)

REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry
CONTROL +	Positive control	CONTROL -	Negative control

RF LATEX KIT

IVD For In-*Vitro* diagnostic and professional use only

Store at 2-8°C CE

INTENDED USE

Atlas RF latex test for the qualitative and semi-quantitative measurement of RF in human serum.

INTRODUCTION

Rheumatoid factors (RF) are antibodies directed against antigenic sites in the Fc fragment of human and animal IgG. Their frequent occurrence in rheumatoid arthritis makes them useful for diagnosis and monitoring of the disease.

One method used for rheumatoid factor detection is based on the ability of rheumatoid arthritis sera to agglutinate sensitized sheep red cells, as observed by Waaler and Rose. A more sensitive reagent consisting of biologically inert latex beads coated with human gamma globulin was later described by Singer and Plotz. The RF kit is based on the principle of the latex agglutination assay of Singer and Plotz. The major advantage of this method is rapid performance (2-minutes reaction time) and lack of heterophile antibody interference.

PRINCIPLE

The RF reagent is based on an immunological reaction between human IgG bound to biologically inert latex particles and rheumatoid factors in the test specimen. When serum containing rheumatoid factors is mixed with the latex reagent, visible agglutination occurs.

MATERIALS

MATERIALS PR0VIDED

- RF Latex Reagent: Latex particles coated with human gamma-globulin, pH, 8,2. Preservative.
- RF Positive Control Serum (Red Cap): Human serum with a RF concentration > 30 IU/Ml. Preservative.
- *RF Negative Control (Blue Cap): Non-reactive buffer containing BSA and 0.1% sodium azide.
- *Glycine Buffer 20X (1000 mmol/L) (Optional): add one part to nineteen parts of distilled water before use.
- *Black glass Slide
- Stirring sticks

NOTE: This package insert is also used for individually packed reagent.

MATERIALS REQUIRED BUT NOT PROVIDED

- Mechanical rotator with adjustable speed at 80-100 r.p.m.
- Pipettes 50 µL
- *9 g/L saline.

Packaging contents

REF **8.00.04.0.0100 (1x4ml Latex Reagent, 1x1ml positive control, 1x1ml negative control)**

PRECAUTIONS

- *For *in vitro* diagnostic and professional use only. The test is not for near-patient or self-testing.
- All reagents contain 0.1 %(w/v) sodium azide as a preservative.
- Protective clothing should be worn when handling the reagents.
- Wash hands and the test table top with water and soap once the testing is done.
- Reagents containing sodium azide may be combined with copper and lead plumbing to form highly explosive metal azides. Dispose of reagents by flushing with large amounts of water to prevent azide buildup.
- Components prepared using human serum found negative for hepatitis B surface antigen (HBsAg), HCV and antibody to HIV (1/2) by FDA required test. However, handle controls as if potentially infectious.
- Accuracy of the test depends on the drop size of the latex reagent *(35µL ±5µL). Use only the dropper supplied with latex and hold it perpendicularly when dispensing.
- Use a clean pipette tip and stirring stick for each specimen, and glass slides should be thoroughly rinsed with water and wiped with lint-free tissue after each use.
- Check reactivity of the reagent using the controls provided.
- Do not use these reagents if the label is not available or damaged.
- Do not use the kit if damaged or the glass vials are broken or leaking and discard the contents immediately.
- Test materials and samples should be discarded properly in a biohazard container.
- *Components from human origin have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). However, handle cautiously as potentially infectious.
- *Wash the area of contact with water immediately if contact occurs.
- *Do not drink or ingest the reagent.
- *Do not use the reagent if the label is missing, damaged, or unclear.
- *Do not use white or transparent glass slides during testing.
- *Perform the test in a well-lit area with good visibility.
- *Close the vial after each test.
- *Failure in following the instructions may give incorrect results or face safety hazards.
- *Handle the used disinfectant with care.
- *Any serious incident that occur in relation to the device shall be reported to the manufacturer and the competent authority. (Feedback@atlas-medical.com)

REAGENT PREPARATION:

- The RF Latex reagent is ready to use. No preparation is required. Mix gently before use to ensure a uniform suspension of particles.

STORAGE AND STABILITY

- Reagents are stable until specified expiry date on bottle label when stored refrigerated (2-8°C).
- Do not freeze.
- Always keep vials in vertical position. If the position is changed, gently mix to dissolve aggregates that may be present.
- The RF latex reagent, once shaken must be uniform without visible clumping. When stored refrigerated, a slight sedimentation may occur and should be considered normal.
- Do not use the latex reagent or controls if they become contaminated.
- Reagents deterioration: Presence of particles and turbidity.

SPECIMEN COLLECTION AND STORAGE

- Use fresh serum collected by centrifuging clotted blood.
- If the test cannot be carried out on the same day, store the specimen for 7 days at 2-8°C and for 3 months at -20°C.
- Samples with presence of fibrin should be centrifuged before testing. Do not use highly hemolyzed or lipemic samples.
- Do not use PLASMA.

PROCEDURE

Qualitative method

1. Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperatures.
2. Place (40 µL) of the sample and one drop of each Positive and Negative controls into separate circles on the slide test.
3. *Swirl the reagent gently before use and add one drop (35 µL ±5µL) next to the sample to be tested.
4. *Close the vial tightly after use.
5. Mix the drops with a stirrer, spreading them over the entire surface of the circle. Use different stirrers for each sample.
6. Place the slide on a mechanical rotator at 80-100 r.p.m. for 2 minutes. False positive results could appear if the test is read later than two minutes.

***Semi-quantitative method**

Prepare serial two-fold dilutions of the sample in 9 g/L saline/glycine buffer (1X):

1. Allow the reagents and samples to reach room temperature.
2. Add (40 µL) of 9 g/L saline/glycine buffer (1X) into 6 circles of the black glass slide.
3. Add (40 µL) of the serum sample to the first circle.
4. Mix well using the pipette and then transfer (40 µL) from the first circle to the second circle, repeat until finishing the six circles.
5. Swirl the reagent vial.
6. Add one drop of RF reagent (35µL ±5µL) next to the samples in each circle.
7. Close the reagent vial.
8. Mix the drops with a stirrer, spreading them over the entire surface of the circle.
9. Place the slide on a mechanical rotator at 80-100 r.p.m. for 2 minutes.

READING AND INTERPRETATION

Examine macroscopically the presence or absence of visible agglutination immediately after removing the slide from the rotator. The presence of agglutination indicates a RF concentration equal or greater than 8 IU/mL (Note 1).

The titer, in the semi-quantitative method, is defined as the highest dilution showing a positive result.

CALCULATIONS

The approximate RF concentration in the patient sample is calculated as follows:

8 x RF Titer = IU/mL

QUALITY CONTROL

- Positive and Negative controls are recommended to monitor the performance of the procedure, as well as a comparative pattern for a better result interpretation.
- All result different from the negative control result, will be considered as a positive.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity

8 (6-16) IU/ml, under the described assay conditions.

PROZONE EFFECT

No prozone effect was detected up to 1500 IU/ml.

DIAGNOSTIC SENSITIVITY

100%.

DIAGNOSTIC SPECIFICITY

100%.

The diagnostic sensitivity and specificity have been obtained using 139 samples compared with the same method of a competitor.

***PRECISION**

100%.

INTERFERENCES

NON-INTERFERING SUBSTANCES:

- Hemoglobin (10g/L)
- Bilirubin (20mg/dl)
- Lipids (10g/L)

Other substances may interfere.

LIMITATIONS

- Reaction time is critical. If reaction time exceeds 2 minutes, drying of the reaction mixture may cause false positive result.
- Freezing the RF Latex Reagent will result in spontaneous agglutination.
- Intensity of agglutination is not necessarily indicative of relative RF concentration; therefore, screening reactions should not be graded.
- Increased levels of RF may be found in some diseases other than rheumatoid arthritis such as infectious mononucleosis, sarcoidosis, lupus erythematosus, Sjogren's syndrome.
- Certain patients with rheumatoid arthritis will not have the RF present in their serum.
- The incidence of false positive results is about 3-5 %. Individuals suffering from infectious mononucleosis, hepatitis, syphilis as well as elderly people may give positive results.
- Diagnosis should not be solely based on the results of latex method but also should be complemented with a Waaler Rose test along with the clinical examination.

REFERENCE VALUES

Up to 8 IU/mL. Each laboratory should establish its own reference range.

NOTES

1. Results obtained with a latex method do not compare with those obtained with Waaler Rose test. Differences in the results between methods do not reflect differences in the ability to detect rheumatoid factors.

REFERENCES

1. Robert W Dorner et al. Clinica Chimica Acta 1987; 167: 1 – 21.
2. Frederick Wolfe et al. Arthritis and Rheumatism 1991; 34: 951- 960.
3. Robert H Shmerling et al. The American Journal of Medicine 1991; 91: 528 –534.
4. Adalbert F. Schubart et al. The New England Journal of Medicine 1959; 261: 363 – 368.
5. Charles M. Plotz 1956; American Journal of Medicine; 21:893 – 896.
6. Young DS. Effects of drugs on clinical laboratory test, 4th ed. AACC Press, 1995.

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REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry
CONTROL +	Positive control	CONTROL -	Negative control

***: Indication of the introduced modifications.**

CRP LATEX KIT

IVD For *in vitro* diagnostic and professional use only

2°C 8°C Store at 2-8°C. CE

INTENDED USE

Atlas CRP Latex kit is a manual slide latex agglutination test for the qualitative and semi-quantitative detection of C-reactive protein (CRP) in human serum to aid in the diagnosis of individuals with suspected inflammation.

INTRODUCTION

C-reactive protein (CRP) is an evolutionarily conserved constitutive protein produced primarily by hepatocytes in minute amounts. At baseline levels, CRP mediates important biological functions. Its clinical significance as a component of the acute phase response emerged upon linking elevated blood levels of CRP to trauma, infection and inflammatory non-infectious disorders including autoimmune diseases. Its concentration can increase up to 1000-fold in severe inflammatory insults. CRP quickly rises in blood upon the onset of an acute stimulus (within 6 hours), and may double every 8 hours reaching a peak at 50 hours. Likewise, blood CRP rapidly drops upon cessation of the stimulus in an exponential manner. Although non-discriminatory of the root cause, elevated serum CRP has been established as an important marker of inflammation.

PRINCIPLE

The C-Reactive Protein test is based on the principle of the latex agglutination. When latex particles complexed with human anti-CRP are mixed with a patient's serum containing C- reactive protein, a visible agglutination reaction will take place within 2 minutes.

KIT COMPONENTS

Materials Provided

- **CRP Latex Reagent:** Latex particles coated with goat IgG anti-human CRP (approximately 1 %), pH 7.4. **MIX WELL BEFORE USE.**
- **CRP Positive Control (Red Cap):** Diluted human serum with CRP concentration > 20mg/L.
- **CRP Negative Control (Blue Cap):** Non-reactive buffer containing BSA and 0.1% sodium azide.
- **Glycine Buffer 20X (1000 mmol/L) (Optional):** add one part to nineteen parts of distilled water before use.
- **Black Glass Slide.**
- **Stirring Sticks.**
- **Package insert.**

NOTE: This package insert is also used for individually packed reagent.

Materials Required But Not Provided

- Mechanical rotator with adjustable speed at 80-100 r.p.m.
- Calibrated 50 µL micro-pipette.
- 9 g/L saline.

Packaging Contents

REF **8.00.00.0.0100 (1x4ml Latex Reagent, 1x1ml positive control, 1x1ml negative control)**

REAGENT STORAGE AND STABILITY

- Reagents are stable until specified expiry date on vial label when stored refrigerated (2 - 8°C).
- **DO NOT FREEZE.**
- The CRP latex reagent, once shaken must be uniform without visible clumping. When stored refrigerated, a slight sedimentation may occur and should be considered normal.
- Do not use the latex reagent or controls if they become contaminated.
- Always keep vials in a vertical position. If the position is changed, gently mix to dissolve aggregates that may be present.
- Reagent deterioration: Presence of particles and turbidity.

PRECAUTIONS AND WARNINGS

- For *in vitro* diagnostic and professional use only. The test is not for near-patient or self-testing.
- All reagents contain 0.1% (w/v) Sodium azide as a preservative.
- Protective clothing should be worn when handling the reagents.
- Wash hands and the test table top with water and soap once the testing is done.
- This kit is NOT to be used in CRP-guided therapy.
- Components containing human serum were tested for hepatitis B surface antigen (HBsAg), HCV and antibody to HIV (1/2) as required by FDA; and found to be negative. However, handle controls as if potentially infectious.
- Accuracy of the test depends on the drop size of the latex reagent (35 µL ±5µL). Use only the dropper supplied with latex and hold it perpendicularly when dispensing.
- Use a clean pipette tip and stirring stick for each specimen, and glass slides should be thoroughly rinsed with water and wiped with lint-free tissue after each use.
- Check reactivity of the reagent using the controls provided.
- Do not use these reagents if the label is not available or damaged.
- Do not use the kit if damaged or the glass vials are broken or leaking and discard contents immediately.
- Test materials and samples should be discarded properly in a biohazard container.
- Use forceps, scoops, or other mechanical devices for removing broken glass from the working area. A dustpan and brush should be used to clean up shards/small pieces of broken glass. Broken glass must be disposed of in a sharps container
- Wash the area of contact with water immediately if contact occurs.
- failure in following the instructions may give incorrect results or incur safety hazards
- Handle the used disinfectant with care.
- Close the vial after each test.
- Perform the test in a well-lit area with good visibility.
- Do not use white or transparent glass slides during testing.
- Do not touch, drink, or ingest the reagent.
- Certain nutritional supplements may effect on CRP levels.
- Any serious incident that occur in relation to the device shall be reported to the manufacturer and the competent authority. (Feedback@atlas-medical.com)

COLLECTION, HANDLING AND PREPARATION OF SPECIMEN

- Use fresh serum collected by centrifuging clotted blood.
- Samples with presence of fibrin should be centrifuged before testing. Do not use highly hemolyzed or lipemic samples.
- **Do not use plasma**.

SPECIMEN STORAGE AND STABILITY

If the test cannot be carried out on the same day, store the specimen for 7 days at 2-8°C and for 3 months at -20°C. Frozen samples should be completely thawed and brought to room temperature before testing. Avoid repeated freezing and thawing of the samples.

REAGENT PREPARATION

The CRP Latex reagent is ready to use. No preparation is required. Mix gently before use to ensure a uniform suspension of particles.

PROCEDURE

NOTE: The latex and sample volumes are very critical for correct test performance. Please adhere to the volumes stipulated in this package insert.

QUALITATIVE TEST:

1. Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperatures.
2. Place (40 µL) of the sample and one drop (40 µL ±5µL) of each Positive and Negative controls into separate circles on the slide test.
3. Swirl the CRP latex reagent gently and add one drop (35 µL

±5µL) next to the samples and controls to be tested.
4. Close the reagent vial tightly.
5. Mix the drops with a stirrer, spreading them over the entire surface of the circle. Use different stirrers for each sample and each control.
6. Place the slide on a mechanical rotator at 80-100 r.p.m. for **2 minutes**. False positive results could be obtained if the test is read later than two minutes.

B. SEMI-QUANTITATIVE TEST:

Prepare serial two-fold dilutions of the sample in 9 g/L saline/glycine buffer (1X):

1. Allow the reagents and samples to reach room temperature.
2. Add (40 µL) of 9 g/L saline/glycine buffer (1X) into 6 circles of the black glass slide.
3. Add (40 µL) of the serum sample to the first circle.
4. Mix well using the pipette and then transfer (40 µL) from the first circle to the second circle, repeat until finishing the six circles.
5. Swirl the reagent vial.
6. Add one drop of CRP reagent (35µL ±5µL) next to the samples in each circle.
7. Close the reagent vial.
8. Mix the drops with a stirrer, spreading them over the entire surface of the circle.
9. Place the slide on a mechanical rotator at 80-100 r.p.m. for 2 minutes.

CALCULATIONS

The approximate CRP concentration in the patient sample is calculated as follows:

Sensitivity x CRP Titer = mg/L

(Sensitivity indicated on the label of the latex vial)

INTERPRETATION OF THE RESULT

Examine macroscopically the presence or absence of visible agglutination immediately after stopping the rotator.

The presence of agglutination indicates a CRP concentration equal or greater than the reagent sensitivity (mg/L CRP) (indicated on the label of the latex vial).

The titer, in the semi-quantitative method, is defined as the highest dilution showing a positive result.

REFERENCE VALUES

Each laboratory should establish its own reference range.

QUALITY CONTROL

- Positive and Negative controls are recommended to monitor the performance of the kit, as well as providing a comparative pattern for better result interpretation.
- Any result that differs from the negative control result is considered positive.

LIMITATIONS OF THE TEST

- Reaction time is critical. If reaction time exceeds two (2) minutes, the reaction mixture may dry causing particles, which can be mistaken for false positive results.
- Freezing the CRP Latex Reagent will result in spontaneous agglutination.
- Intensity of agglutination is not necessarily indicative of relative CRP concentration; therefore, reactions should not be graded.
- A false negative can be attributed to a prozone phenomenon (antigen excess). It is recommended, therefore, to check all suspected negative sera by retesting with a 1:10 dilution in 9 g/L saline/glycine buffer (1X).

PERFORMANCE CHARACTERISTICS

- **Sensitivity:** 6 mg/L.
- **Prozone effect:** No prozone effect was detected up to 1600 mg/L.
- **Diagnostic sensitivity: 100 %** in comparison with a commercial latex kit.
- **Diagnostic specificity: 100 %** in comparison with a commercial latex kit.
- **Precision : 100%**
- **Interferences:**

  No interference was observed with the following substances at the concentrations indicated:
  - Hemoglobin (<15 g/dl)
  - Bilirubin (<20 mg/dl)
  - Lipids (<13 g/dL)
  - Other substances interfere, such as RF (>75IU/ml).

NOTES

- Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

REFERENCES

- Pepys, M.B.. Lancet 1:653 (1981).
- Werner, M.. Clin.Chem. Acta 25:299 (1969).
- MacLeod, C.M., et. al.. J. Exp. Med 73:191 (1941).
- Wood, HF., et. al.. J. Clin. Invest. 30: 616 (1951).
- Mancini, G., et. al. Immunochemistry 2:235 (1965).
- Singer, J.M., et. al.. Am. J. Med 21: 888 (1956).
- Fischer, C.L., Gill, C.W.. In Serum Protein Abnormalities. Boston, Little, Brown and Co., (1975).
- Darooghegi M, M; Milajerdi, Aa; .et.al (2019). Garlic Supplementation Reduces Circulating C-reactive Protein, Tumor Necrosis Factor, and Interleukin-6 in Adults: A Systematic Review and Meta-analysis of Randomized Controlled Trials. The Journal of Nutrition; 00:1–14.
- Mazidi, M; Rezaie, P; et.al (2018). Effect of magnesium supplements on serum C-reactive protein: a systematic review and meta-analysis. Archives of Medical Science, 14(4), 707–716.

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REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry
CONTROL+	Positive control	CONTROL-	Negative control

O.K.N.V.I. RESIST-5

Manufacturer:

Coris BioConcept
CREALYS Science Park
Rue Guillaume Fouquet, 11
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Tel.: +32(0)81.719.917
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info@corisbio.com
Produced in BELGIUM

www.corisbio.com
IFU-58R11/EN/06

***In vitro* rapid diagnostic test for the detection of OXA-48, KPC, NDM, VIM and IMP carbapenemases in bacterial culture**

FOR IN VITRO DIAGNOSTIC USE **EN**
FOR PROFESSIONAL USE ONLY
References: K-15R11, 2x20 cassettes, buffer, 20 tubes and transfer pipets

I. INTRODUCTION

Carbapenemase-producing Organisms (CPO), and more specifically, Carbapenem-resistant Enterobacteriaceae (CRE) represent a major public health concern worldwide due to their broad spectrum of resistance to antibiotics including, besides carbapenems, most classes of antimicrobial agents, and thus leaving very few options for the management of infected patients. Besides CREs, CPOs also include nonfermenting Gram-negative bacilli (NFGNB), such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* that exhibit resistance not only to beta lactam and other groups of antibiotics, but also to carbapenems. The rapid spread of CPOs and genes encoding these resistances has led to nosocomial outbreaks and endemic situations worldwide.
Development of new rapid diagnostic tests to track antimicrobial resistance patterns is considered as one of the priority core actions by international experts and health authorities. NDM and KPC represent two of the most increasing and prevalent carbapenemases in many countries. On the other hand, class D OXA-48 type carbapenemases are the most challenging resistance mechanisms to be detected by clinical laboratories. VIM is not only present in Enterobacteriaceae but is also highly prevalent in non-fermenting bacteria. IMP should be regarded as a potential problem since they degrade not only C3G but also carbapenem antimicrobial drug like Imipenem. IMP prevalence is the lowest, apart from Japan where it is more prevalent.
Inhibitor-based phenotypic confirmatory tests exist for the confirmation of class A (KPC) and class B (VIM, IMP, NDM) carbapenemases, Nowadays, definitive confirmation of CPO resistance mechanism relies on molecular assays. These tests are expensive and can only be performed in dedicated environment and by skilled personnel, hence limiting their more generalized usage.
O.K.N.V.I. RESIST-5 test is part of Coris BioConcept RESIST range of antimicrobial resistance diagnostic tests.

II. PRINCIPLE OF THE TESTS

These tests are ready to use and are based on a membrane technology with colloidal gold nanoparticles. Our kit is aimed to detect and identify the carbapenemases from a bacterial colony isolate of Enterobacteriaceae or NFGNB growing on agar plate. Each pouch contains: 2 lateral-flow cassettes for the identification of (i) OXA-48, KPC, NDM and (ii) VIM and IMP.
Identification of OXA-48, KPC and NDM. A nitrocellulose membrane is sensitised with:
(1) a monoclonal antibody directed against OXA-48 carbapenemase and variants (except OXA-163-like enzymes) (“O” line)
(2) a monoclonal antibody directed against KPC carbapenemase (“K” line)
(3) a monoclonal antibody directed against NDM carbapenemase (“N” line)
(4) a control capture reagent (upper “C” line).
Four different colloidal gold nanoparticles conjugates are dried on a membrane: a conjugate directed against a second epitope of the OXA-48 carbapenemase, a conjugate directed against a second epitope of the KPC carbapenemase, a third conjugate specific to NDM carbapenemase and a control conjugate to validate the test conditions.
Identification of VIM and IMP. A nitrocellulose membrane is sensitised with:
(1) a monoclonal antibody directed against VIM carbapenemase (“V” line),
(2) a monoclonal antibody directed against IMP carbapenemase (“I” line)
(3) a control capture reagent (upper “C” line).
Three different colloidal gold nanoparticles conjugates are dried on a membrane: a conjugate directed against VIM carbapenemase, a conjugate directed against IMP carbapenemase and a control conjugate.
When the provided buffer containing the resuspended bacteria comes into contact with the membrane, the solubilised conjugates migrate with the sample by passive diffusion, while conjugates and sample material come into contact with the immobilised respective antibodies that are adsorbed onto the nitrocellulose strip. If the sample contains an OXA-48, KPC, NDM, VIM or IMP carbapenemase, the respective complexes made of the conjugates and either OXA-48, or KPC, or NDM or VIM or IMP will remain bound to their respective specific lines (OXA-48 : “O” line; KPC : “K” line; NDM : “N” line, VIM : “V” line, IMP : “I” line). The migration continues by passive diffusion and both conjugates and sample material come into contact with the (upper) line control reagent that binds a control conjugate (“C” line), thereby producing a red line.
The result is visible within 15 minutes in the form of red lines on the strip.

III. REAGENTS AND MATERIALS

1. O.K.N.V.I. RESIST-5 (2x20 cassettes)
20 sealed pouches containing two lateral-flow cassettes and one desiccant.
Each cassette contains one sensitised strip.
2. LY-D buffer vial (7 mL)
Tris-EDTA solution containing NaN_3 (<0.1%) and a detergent.
3. Instruction for use (1)
4. Disposable collection tubes (20)
5. Disposable transfer pipettes (20)

Materials to be ordered separately:
- RESIST-BC (S-1001): reagents kit for use with blood culture
- ReSCape (S-1002): reagents kits for use with rectal swab

IV. SPECIAL PRECAUTIONS

- All operations linked to the use of the test must be performed in accordance with good laboratory practices.
- All reagents are for *in vitro* diagnostic use only.
- Pouch must be opened with care.
- Avoid touching nitrocellulose with your fingers.
- Wear gloves when handling samples.
- Never use reagents from another kit.
- Green lines indicate immunoreagents adsorption sites. Green colour disappears during the test.
- The quality of the reagents cannot be guaranteed beyond their shelf-life dates or if reagents are not stored under required conditions as indicated in the insert.

V. WASTE DISPOSAL

- Dispose of gloves, swabs, test tubes and used devices in accordance with GLP.
- Each user is responsible for the management of any waste produced, and must ensure that it is disposed of in accordance with the applicable legislation.

VI. STORAGE

- An unopened pouch may be kept at between 4 and 30°C and used until the shelf-life date indicated on the packaging. Once the pouch is opened, run the test immediately.
- Avoid freezing devices and buffer.

VII. SPECIMEN HANDLING AND COLLECTION

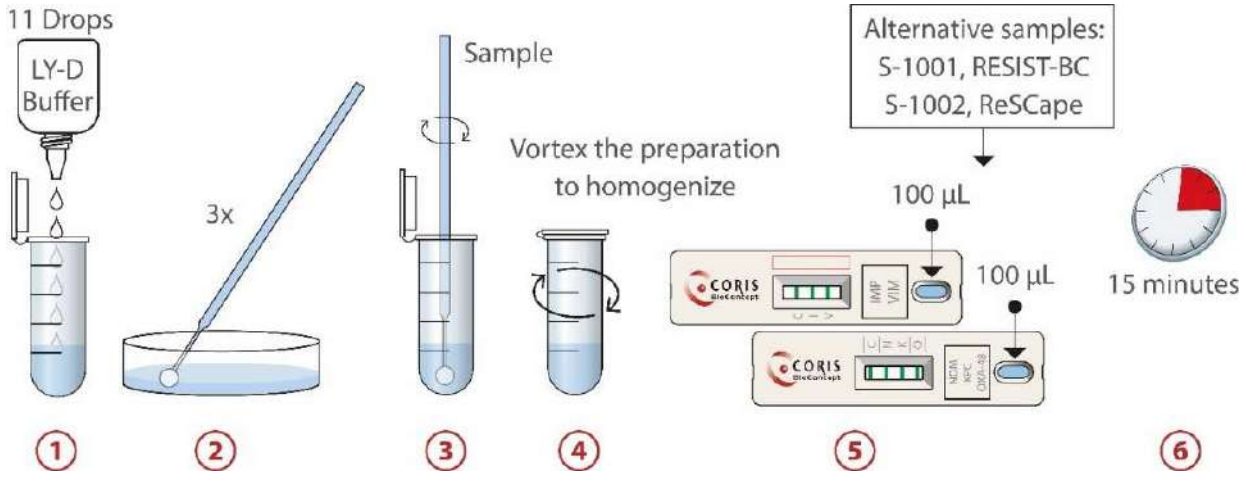
Specimens to be tested should be obtained and handled by standard microbiological methods.
Make sure that the specimens are not treated with solutions containing formaldehyde or its derivatives.
Culture media tested and validated with Coris BioConcept RESIST kits are listed on the website: https://www.corisbio.com/products/oknvi-resist-5/faq

VIII. PROCEDURE

PREPARATIONS OF THE TEST:
Allow kit components, in unopened packaging, and specimens (in the event that the plate containing colony to be tested was kept at 4°C) to equilibrate at room temperature (15-30°C) before performing a test.
Open the pouch and remove the device. Once opened, run the test immediately. Indicate the patient’s name or specimen number on the device (one device per sample).

SPECIMEN PREPARATION PROCEDURE:
Performance claims with regard to sample types other than bacterial colonies have been established for rectal swabs and blood cultures.
With rectal swabs and blood cultures, the preparation procedure has to be followed as described in the respective kits (S-1002, ReSCape and S-1001, RESIST-BC)
With bacterial colonies, we recommend the use of fresh agar cultures for optimal test performance and as followed:

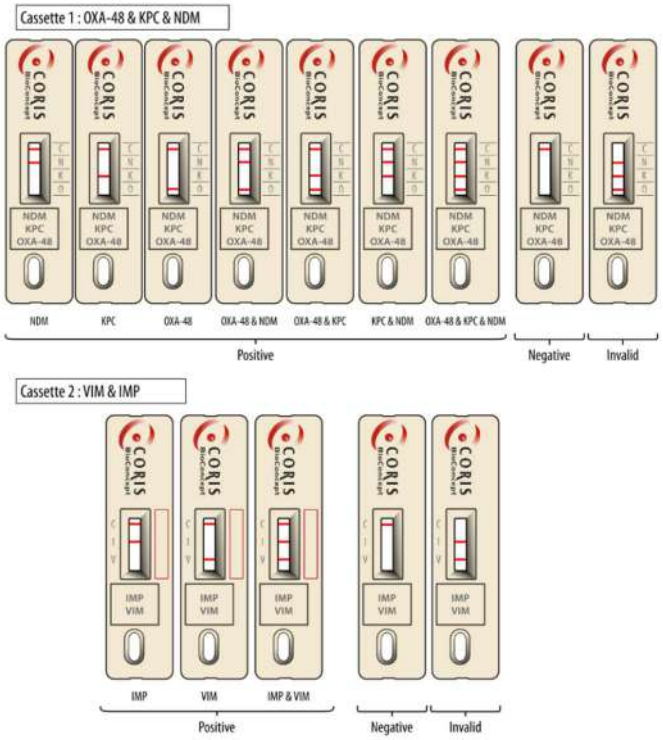
1. Prepare one collection tube and add **11 drops** of LY-D buffer in the tube
2. Harvest bacteria by taking **3 colonies** with a disposable bacteriological loop and dip the loop in the bottom of the tube containing the buffer. The same bacteriological loop can be used to collect the 3 colonies.
3. Stir thoroughly before removing the loop.
4. Close de tube and vortex the preparation to homogenize.
5. Use the transfer pipette provided in the kit and add 100 µL of diluted sample into the sample well of each of the two cassettes labelled (i) NDM, KPC and OXA-48 and (ii) IMP and VIM (**diluted sample must reach the black line indicated on the transfer pipette to accurately aspirate 100 µL**).
6. Allow to react for 15 minutes and read the result.



Positive results may be reported as soon as the test and control lines become visible.
Do not take the appearance of new lines into account after the reaction time has passed.
The result must be read on still wet strip.

IX. INTERPRETING RESULTS

The results are to be interpreted as follows for each of the two cassettes:
Negative test result: a reddish-purple line appears across the central reading window at the Control line (C) position. No other line is present.
Positive test result: in addition to a reddish-purple line at the Control line (C), a visible reddish-purple line appears at one of the Test lines position ("N" or "K" or "O") on cassette labelled (i) NDM, KPC, OXA-48 or at one of the Test lines position ("I" or "V") on cassette labelled (ii) IMP and VIM. Intensity of the test line may vary according to the quantity of antigens as well as of the variant type present in the sample. Any reddish-purple test line (OXA-48, KPC, NDM, VIM and IMP), even weak, should be considered as a positive result.
If a positive test line appears beside of the "O" mark, the sample contains OXA-48 or OXA-48-like variants. If it appears beside the "K" mark, the sample contains KPC variants; beside the "N" mark, the sample contains NDM; the "V" mark, the sample contains VIM; and beside of the "I" mark, IMP is present in the sample. Combinations of positive test lines can occur.
In this case the sample contains several carbapenemases.
Invalid test result: The absence of a Control line indicates a failure in the test procedure. Repeat invalid tests with a new test device.
Note: during the drying process, a very faint shadow may appear at the Test line positions. It should not be regarded as a positive result.



X. PERFORMANCE

A. Detection Limit

The detection limit determined with purified recombinant proteins of OXA-48, KPC, NDM, VIM and IMP have been evaluated at 0.25 ng/mL, 0.5 ng/mL, 0.0625 ng/mL, 0.23 ng/mL and 0.781 ng/mL, respectively.

B. Retrospective study

The test cassettes were validated by comparison with reference molecular method (validated in house multiplex PCR including sequencing) in a retrospective study performed on 180 non duplicated, consecutive suspected CPE clinical isolates collected between 2012 and 2021 from Belgian hospitals.

Molecular method / OXA-48 test	Positive	Negative	Total
Positive	41	0	41
Negative	0	139	139
Total	41	139	180

95 % Confidence Interval [1]

Sensitivity:	100 %	(89.3 to 100 %)
Specificity:	100 %	(96.6 to 100 %)
Positive Predictive value:	100 %	(89.3 to 100 %)
Negative predictive value:	100 %	(96.7 to 100 %)
Agreement:	100 %	(180/180)

Molecular method / KPC test	Positive	Negative	Total
Positive	24	0	24
Negative	0	156	156
Total	24	156	180

95 % Confidence Interval [1]

Sensitivity:	100 %	(82.8 to 100 %)
Specificity:	100 %	(97.0 to 100 %)
Positive Predictive value:	100 %	(82.8 to 100 %)
Negative predictive value:	100 %	(97.0 to 100 %)
Agreement:	100 %	(180/180)

Molecular method / NDM test	Positive	Negative	Total
Positive	40	0	40
Negative	0	140	140
Total	40	140	180

95 % Confidence Interval [1]

Sensitivity:	100 %	(89.1 to 100 %)
Specificity:	100 %	(96.7 to 100 %)
Positive Predictive value:	100 %	(89.1 to 100 %)
Negative predictive value:	100 %	(96.7 to 100 %)
Agreement:	100 %	(180/180)

Molecular method / VIM test	Positive	Negative	Total
Positive	43	0	43
Negative	3	134	137
Total	46	134	180

95 % Confidence Interval [1]

Sensitivity:	93.5 %	(81.1 to 98.3 %)
Specificity:	100 %	(96.5 to 100 %)
Positive Predictive value:	100 %	(89.8 to 100 %)
Negative predictive value:	97.8 %	(93.2 to 99.4 %)
Agreement:	98.3 %	(177/180)

Molecular method / IMP test	Positive	Negative	Total
Positive	19	0	19
Negative	0	161	161
Total	19	161	180

95 % Confidence Interval [1]

Sensitivity:	100 %	(79.1 to 100 %)
Specificity:	100 %	(97.1 to 100 %)
Positive Predictive value:	100 %	(79.1 to 100 %)
Negative predictive value:	100 %	(97.1 to 100 %)
Agreement:	100 %	(180/180)

The O.K.N.V.I. RESIST-5 kit was also validated with rectal swabs and blood cultures.

C. Repeatability and reproducibility

To check intra-batch accuracy (repeatability), the same positive samples and a buffer solution were processed 15 times on kits of the same production batch in the same experimental conditions. All observed results were confirmed as expected.
To check inter-batch accuracy (reproducibility), some samples (positive and buffer) were processed on kits from three different production batches. All results were confirmed as expected.

XI. LIMITS OF THE KIT

The test is qualitative and cannot predict the quantity of antigens present in the sample. Clinical presentation and other test results must be taken into consideration to establish diagnosis. A positive test does not rule out the possibility that other antibiotic resistance mechanisms may be present.

XII. TECHNICAL PROBLEMS / COMPLAINTS

If you face a technical problem or if performances do not correspond with those indicated in this package insert:

1. Record the lot number of the kit concerned.
2. If possible, keep the sample in the appropriate storage condition during the complaint management.
3. Contact Coris BioConcept (client.care@corisbio.com) or your local distributor.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

XIII. BIBLIOGRAPHIC REFERENCES

A. **J. Wesley MacDonald and V. Chibabhai Evaluation of the RESIST-4 O.K.N.V immunochromatographic lateral flow assay for the rapid detection of OXA-48, KPC, NDM and VIM carbapenemases from cultured isolates** Access Microbiology 2019;1
B. T. Pilate, S. Desmet Detection of carbapenemase production in pseudomonas aeruginosa in a tertiary care centre Annual Meeting of the Royal Belgian Society of Laboratory Medicine November 15th, 2019 Belgium
C. **Oueslati S, Iorga BI, Tlili L, Exilie C, Zavala A, Dortet L, Jousset AB, Bernabeu S, Bonnin RA, Naas T.** Unravelling ceftazidime/avibactam resistance of KPC-28, a KPC-2 variant lacking carbapenemase activity. **J Antimicrob Chemother. 2019 Aug 1;74(8):2239-2246**
D. **Brolund A, Lagerqvist N, Byfors S, Struelens MJ, Monnet DL, Albiger B, Kohlenberg A.** Worsening epidemiological situation of carbapenemase-producing Enterobacteriaceae in Europe, **assessment by national experts from 37 countries, July 2018. Euro Surveill. 2019 Feb; 24 (9) 1560-7917**
E. **Oliveira J, Reygaert WC.** Gram Negative Bacteria. **StatPearls Publishing; 2019 Jan-2019**
F. **Baeza LL, Pfennigwerth N, Greissl C, Göttig S, Saleh A, Stelzer Y, Gatermann SG, Hamprecht A.** Comparison of five methods for detection of carbapenemases in Enterobacterales with proposal of a new algorithm. **Clin Microbiol Infect. 2019 Mar 18. pii: S1198-743X(19)30104-1**
G. **Rösner S, Kamalanabhaiah S, Küsters U, Kolbert M, Pfennigwerth N, Mack D.** Evaluation of a novel immunochromatographic lateral flow assay for rapid detection of OXA-48, NDM, KPC and VIM carbapenemases in multidrug-resistant Enterobacteriaceae. **J Med Microbiol. 2019 Mar;68(3):379-381.**
H. **Glupczynski Y, Evrard S, Huang TD, Bogaerts P.** Evaluation of the RESIST-4 K-SeT assay, a multiplex immunochromatographic assay for the rapid detection of OXA-48-like, KPC, VIM and NDM carbapenemases. **J Antimicrob Chemother. 2019 Feb 6. doi: 10.1093**

Last update : 20 FEBRUARY 2023

REF	Catalogue number		Manufacturer
IVD	In vitro diagnostic medical device		Temperature limits
Σ	Contains sufficient for <n> tests	LOT	Batch code
	Consult instructions for use		Do not reuse
	Keep dry		Use by
DIL SPE	Diluent specimen	CONT NaN_3	Contains Sodium azide
UDI	Unique device identifier		

[1] Newcombe, Robert G. "Two-Sided Confidence Intervals for the Single Proportion: Comparison of Seven Methods," *Statistics in Medicine,* **17**, 857-872 (1998).

Cardiac Markers

Coagulation Markers

Diabetes Mellitus

Inflammation

Thyroid Function

Metabolic Marker

Renal Function

Tumor Markers

Reproduction /Fertility

Infectious Disease

Getein Biotech, Inc.

Stock Code: 603387

OPTIMIZED POINT-OF-CARE SOLUTION

MAKING TEST EASY

Getein 1100

Immunofluorescence Quantitative Analyzer

Getein 1100

Immunofluorescence Quantitative Analyzer

HIGHLY EFFICIENT & ACCURATE

Advanced fluorescence immunoassay

Multiple quality control

REAL-TIME AND RAPID TEST

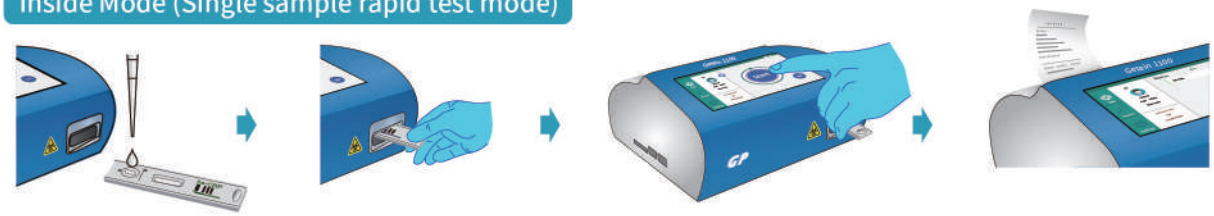
One-step test

3-15 min/test

5 sec/test for multiple tests

OPERATION MODES

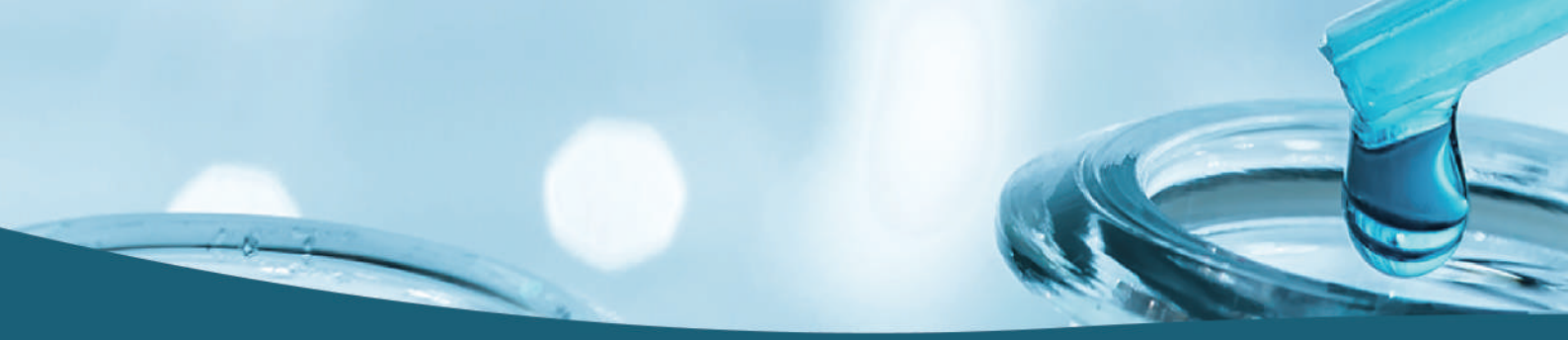
Inside Mode (Single sample rapid test mode)



Sample Transfer | Test Card Insert | Click “Start” Icon | Result Show and Print

Outside Mode (Mass samples rapid test mode)

Sample Transfer | Timing the Reaction Manually | Click “Start” Icon | Result Show and Print



CONVENIENT OPERATION

RFID card calibration

Keyboard and mouse connectivity through USB port

Handwriting input available

Continuous test for 3 hours with optional lithium battery

USER-FRIENDLY INTERFACE

Android system

7-inch touch screen

1. 7-inch Touch Screen
2. SD Card Recognition Zone
3. Test Card Slot
4. SD Card Slot
5. USB Slot
6. Built-in Thermal Printer

PORTABLE DESIGN

Small in size: 261*241*115 mm

Light in weight: 2.0 kg

LARGE MEMORY

Up to 10, 000 results storage capacity

TECHNICAL PARAMETERS

Methodology	Immunofluorescence
Result	Quantitative
Sample Type	WB, Plasma, Serum, Urine, Stool, Nasal swab, Saliva, Capillary blood
Storage Capacity	10, 000 data
Language	English/Chinese/Spanish/Portuguese
Screen	7-inch touch screen
Power Supply	100-240 V ~ 50 Hz/60 Hz, 60 VA
Working Environment	Temperature: 10-35°C Relative humidity ⩽ 70% Air pressure 70.0 ~ 106.0 kpa
Dimensions	261 mm*241 mm*115 mm (D*W*H)
Weight	2.0 kg

TEST ITEMS

Cat. #	TEST ITEMS	DISEASES	CUT-OFF VALUE	SAMPLE TYPES	MEASURING RANGE	SAMPLE VOLUME	REACTION TIME	QUALIFICATION
Cardiac Markers								
IF1001	cTnI	Myocardial infarction	0.10 ng/mL	S/P/WB	0.10-50.00 ng/mL	100 µL	10 min	NMPA CE
IF1098	TnT	Myocardial infarction	14.0 pg/mL	S/P/WB	10.0-10000.0 pg/mL	100 µL	15 min	NMPA CE
IF1089	BNP	Heart failure	100.0 pg/mL	P/WB	5.0-5000.0 pg/mL	100 µL	10 min	NMPA CE
IF1002	NT-proBNP	Heart failure	300 pg/mL	S/P/WB	100-35000 pg/mL	100 µL	10 min	NMPA CE
IF1004	NT-proBNP/cTnI	Heart failure/acute myocardial infarction/acute coronary syndrome	NT-proBNP: 185 pg/mL cTnI: 0.10 ng/mL	S/P/WB	100-15000 pg/mL 0.10-50.00 ng/mL	100 µL	10 min	NMPA CE
IF1005	CK-MB/cTnI/Myo	Myocardial damage /infarction	CK-MB: 5.00 ng/mL cTnI: 0.10 ng/mL Myo: 70.0 ng/mL	S/P/WB	2.50-80.00 ng/mL 0.10-50.00 ng/mL 30.0-600.0 ng/mL	100 µL	10 min	NMPA CE
IF1012	CK-MB/cTnI	Myocardial damage /infarction	CK-MB: 5.00 ng/mL cTnI: 0.10 ng/mL	S/P/WB	2.50-80.00 ng/mL 0.10-50.00 ng/mL	100 µL	10 min	NMPA CE
IF1014	H-FABP	Myocardial damage	6.36 ng/mL	S/P/WB	1.00-120.00 ng/mL	100 µL	3 min	NMPA CE
IF1016	CK-MB/cTnI/H-FABP	Myocardial damage /infarction	CK-MB: 5.00 ng/mL cTnI: 0.10 ng/mL H-FABP: 6.36 ng/mL	S/P/WB	2.50-80.00 ng/mL 0.10-50.00 ng/mL 2.00-100.00 ng/mL	100 µL	10 min	NMPA CE
IF1018	CK-MB	Myocardial injury	5.00 ng/mL	S/P/WB	2.50-80.00 ng/mL	100 µL	10 min	NMPA CE
NEW IF1087	ST2	AMI and chronic heart failure	35.0 ng/mL	S/P/WB	3.0-200.0 ng/mL	100 µL	15 min	CE
Coagulation Marker								
IF1006	D-Dimer	Venous thromboembolism	0.50 mg/L	P/WB	0.10-10.00 mg/L	100 µL	10 min	NMPA CE
Inflammation								
IF1003	hs-CRP+CRP	Cardiovascular inflammation /normal inflammation	3.0 mg/L 10.0 mg/L	S/P/WB/ Fingertip blood	0.5-200.0 mg/L	10 µL	3 min	NMPA CE
IF1007	PCT	Sepsis, bacterial infection	0.10 ng/mL	S/P/WB	0.05-50.00 ng/mL	100 µL	15 min	NMPA CE
IF1015	PCT/CRP	Sepsis, bacterial infection	PCT: 0.10 ng/mL CRP: 3.0 mg/L	S/P/WB	0.10-50.00 ng/mL 0.5-200.0 mg/L	20 µL	15 min	NMPA CE
IF1044	SAA	Bacterial/Virus infection	10.0 mg/L	S/P/WB/ Fingertip blood	5.0-200.0 mg/L	10 µL	5 min	NMPA CE
IF1090	SAA/CRP	Neonatal sepsis, Bacterial/virus infection	SAA: 10.0 mg/L CRP: 10.0 mg/L	S/P/WB/ Peripheral blood	5.0-200.0 mg/L 0.5-200.0 mg/L	10 µL	5 min	NMPA CE
IF1088	IL-6	Acute inflammation	7.0 pg/mL	S/P/WB/ Peripheral blood	1.5-4000.0 pg/mL	40 µL	15 min	NMPA CE
IF1096	hs-CRP	Cardiovascular disease, routine inflammation	Refer to user manual	S/P	0.5-200.0 mg/L	100 µL	3 min	NMPA CE
NEW IF1139	Calprotectin	Inflammatory bowel disease	<50.0 µg/g	Fecal specimen	10.0-600.0 µg/g	100 µL	15 min	CE
Renal Function								
IF1008	CysC	Acute and chronic renal diseases	0.51-1.09 mg/L	S/P/WB	0.50-10.00 mg/L	10 µL	3 min	NMPA CE
IF1009	mAlb	Diabetic nephropathy, hypertensive nephropathy	20.0 mg/L	Urine	10.0-200.0 mg/L	100 µL	3 min	NMPA CE
IF1010	NGAL	Acute kidney injury	Serum: 200.0 ng/mL Urine: 100.0 ng/mL	S/Urine	50.0-5000.0 ng/mL	10 µL	10 min	NMPA CE
IF1011	β_2-MG	Acute and chronic kidney diseases/tumours	0.80-3.00 mg/L	S/P/WB	0.50-20.00 mg/L	10 µL	3 min	NMPA CE
Diabetes Mellitus								
IF1017	HbA1c	Diabetes mellitus	3.80%-5.80%	WB	2.00%-14.00%	10 µL	5 min	NGSP IFCC NMPA CE
Metabolic Marker								
IF1031	25-OH-VD	Osteomalacia, osteoporosis	30.00-50.00 ng/mL	S/P/WB/ Capillary blood	8.00-70.00 ng/mL	20 µL	20 min	NMPA CE
NEW IF1112	Osteocalcin	Osteoporosis	Male: 14-70 ng/mL Female:11-48 ng/mL	S/P/WB	1.5-300.0 ng/mL	100 µL	15 min	CE
Thyroid Function								
IF1024	TSH	Thyroid malfunction	0.27-4.20 µIU/mL	S/P	0.10-50.00 µIU/mL	100 µL	15 min	NMPA CE
IF1022	T3	Hyperthyroidism, hypothyroidism	1.30-3.10 nmol/L	S/P	0.30-10.00 nmol/L	100 µL	15 min	NMPA CE
IF1023	T4	Hyperthyroidism, hypothyroidism	59.00-154.00 nmol/L	S/P	5.40-320.00 nmol/L	100 µL	15 min	NMPA CE
IF1067	fT3	Hyperthyroidism, hypothyroidism	3.10-6.80 pmol/L	S/P/WB	0.60-50.00 pmol/L	100 µL	15 min	NMPA CE
IF1068	fT4	Hyperthyroidism, hypothyroidism	12.00-22.00 pmol/L	S/P/WB	0.30-100.00 pmol/L	100 µL	15 min	NMPA CE

Cat. #	TEST ITEMS	DISEASES	CUT-OFF VALUE	SAMPLE TYPES	MEASURING RANGE	SAMPLE VOLUME	REACTION TIME	QUALIFICATION
Reproduction/Fertility								
IF1013	HCG+β	Fertility	5.1 mIU/mL	S/P	5.0-100000.0 mIU/mL	100 μL	10 min	NMPA CE
IF1055	LH	PCOS, infertility evaluation	Refer to User Manual	S/P	0.20-150.00 mIU/mL	100 μL	15 min	NMPA CE
IF1056	FSH	PCOS, infertility evaluation and pituitary disorders	Refer to User Manual	S/P	0.20-150.00 mIU/mL	100 μL	15 min	NMPA CE
IF1066	AMH	Fertility, PCOS, gonadal function, precocious/late puberty	Refer to User Manual	S/P	0.10-20.00 ng/mL	100 μL	15 min	NMPA CE
IF1048	PRL	Infertility, gonadal disorders	Refer to User Manual	S/P	0.50-200.00 ng/mL	100 μL	15 min	NMPA CE
IF1071	Prog	Infertility, evaluation of ovulation	Refer to User Manual	S/P	0.10-40.00 ng/mL	100 μL	15 min	CE
NEW IF1073	Testosterone	Female polycystic ovary syndrome, male testosterone insufficiency	Male: 1.75-7.81 ng/mL Female: 0.10-0.75 ng/mL	S/P	0.10-16.00 ng/mL	100 μL	15 min	CE
NEW IF1138	Estradiol	Ovarian function	Refer to User Manual	S/P	40.0-4800.0 pg/mL	100 μL	15 min	CE
Tumor Markers								
IF1053	tPSA	Prostate cancer	4.00 ng/mL	S/P	0.40-100.00 ng/mL	100 μL	15 min	NMPA
IF1072	fPSA	Prostate cancer	1.00 ng/mL	S/P	0.03-30.00 ng/mL	100 μL	10 min	NMPA
IF1050	AFP	Liver cancer, cancer of ovaries or testicles, etc.	7.0 ng/mL	S/P	2.0-500.0 ng/mL	100 μL	15 min	NMPA CE
IF1051	CEA	Cancer marker: colon cancer etc.	4.7 ng/mL	S/P	2.0-500.0 ng/mL	100 μL	15 min	NMPA CE
NEW IF1079	CA125	Ovarian cancer	35.0 U/mL	S/P/WB	2-500.0 U/mL	100 μL	15 min	CE
NEW IF1080	CA19-9	Pancreatic cancer	27.0 U/mL	S/P/WB	2-1000.0 U/mL	100 μL	15 min	CE
NEW IF1081	CA15-3	Breast cancer	26.2 U/mL	S/P/WB	1.5-300.0 U/mL	100 μL	15 min	CE
Infectious Disease								
IF1057	Anti-HCV	Hepatitis C	1.00 S/CO	S/P	1.00-20.00 S/CO	100 μL	15 min	
IF1058	Anti-TP	Syphilis	1.00 S/CO	S/P	1.00-50.00 S/CO	100 μL	15 min	CE
IF1059	Anti-HIV	AIDS	1.00 S/CO	S/P	1.00-1000.00 S/CO	100 μL	15 min	
IF1064	HBsAg	Hepatitis B	1.00 IU/mL	S/P	1.00-100.00 IU/mL	100 μL	15 min	
IF1063	Anti-HBs	Hepatitis B	10.00 mIU/mL	S/P/WB	10.00-1000.00 mIU/mL	100 μL	15 min	
IF1091	SARS-CoV-2 Antigen	COVID-19	1.00 COI	Nasal swab		100 μL	15 min	CE
IF1047	*H. pylori*	*H. pylori* infection	5.0 ng/mL	Stool	1.0-200.0 ng/mL	10-50 mg	10 min	CE
IF1086	Influenza A/B	Respiratory viral infection	1.00 COI	Nasal swab		100 μL	15 min	CE
IF1136	Dengue NS1 Ag	Dengue virus infection	1.00 S/CO	S/P/WB	1.00-50.00 S/CO	100 μL	15 min	CE
Specific Protein and Rheumatism								
IF1075	RF	Rheumatoid arthritis	15.9 IU/mL	S/P/WB	10.0-640.0 IU/mL	10 μL	10 min	NMPA CE
IF1076	ASO	Rheumatic fever, acute glomerulonephritis, group A streptococcal infection	408.0 IU/mL	S/P/WB	60.0-1370.0 IU/mL	10 μL	10 min	NMPA CE
IF1029	Anti-CCP	Rheumatoid arthritis	25.0 U/mL	S/P/WB	10.0-400.0 U/mL	10 μL	15 min	CE
Others								
IF1077	Ferritin	Anemia/tumors	Male: 30.00-400.00 ng/mL Female: 13.00-150.00 ng/mL	S/P	0.50-1000.00 ng/mL	10 μL	15 min	NMPA CE
IF1069	Total IgE	Allergic disorders	Refer to User Manual	S/P/WB	1.00-2000.00 IU/mL	100 μL	15 min	CE
NEW IF1052	PG I/PG II	Atrophic gastritis, stomach cancer	PG I < 70.0 ng/mL PG I/PG II < 3.0 ng/mL	S/P	PG I: 1.0-200.0 ng/mL PG II: 1.0-100.0 ng/mL	100 μL	15 min	CE
IF1110	Cortisol	Adrenal cortex function	Refer to User Manual	S/P	11-1655 nmol/L	100 μL	15 min	CE

Coming soon: VB12, Folate...

Getein Biotech, Inc.
Add.: No.9 Bofu Road, Luhe District, Nanjing, 211505, China
Tel.: +86-25-68568508/68568594
Fax: +86-25-68568500
E-mail: overseas@getein.com.cn; marketing@getein.com.cn
Web.: www.getein.com

ISO 9001:2015 ISO13485 FSC CE NMPA MDSAP BGMP NGSP IFCC IVD

WPT3-11-12

CE IVD

D-Dimer

Fast Test Kit

(Immunofluorescence Assay)

User Manual

Getein1100: Cat.# IF1006
Getein1600: Cat.# IF2006

INTENDED USE

D-Dimer Fast Test Kit (Immunofluorescence Assay) is intended for *in vitro* quantitative determination of D-Dimer in plasma or whole blood. The test is used as an aid in the assessment and evaluation of patients suspected of deep-vein thrombosis or pulmonary embolism.

SUMMARY

Deep-vein thrombosis is a common condition, with a lifetime cumulative incidence of 2 to 5 percent. Untreated deep-vein thrombosis can result in pulmonary embolism, a potentially fatal outcome. Anticoagulant therapy reduces both morbidity and mortality from venous thromboembolism, and early diagnosis is therefore important. Accurate diagnosis of deep-vein thrombosis minimizes the risk of thromboembolic complications and averts the exposure of patients without thrombosis to the risks of anticoagulant therapy.

D-Dimer is a marker of endogenous fibrinolysis and should therefore be detectable in patients with deep-vein thrombosis. In recent years, an increasing number of studies have shown the D-Dimer assay has a high negative predictive value and D-Dimer is a sensitive but nonspecific marker of deep-vein thrombosis. Negative D-Dimer can exclude deep-vein thrombosis and pulmonary embolism.

PRINCIPLE

The test uses an anti-human D-Dimer monoclonal antibody conjugated with fluorescence latex and another anti-human D-Dimer monoclonal antibody coated on the test line. After the sample has been applied to the test strip, the fluorescence latex-labelled anti-human D-Dimer monoclonal antibody binds with the D-Dimer in sample and forms a marked antigen-antibody complex. This complex moves to the test card detection zone by capillary action. Then marked antigen-antibody complex is captured on the test line by another anti-human D-Dimer monoclonal antibody. The fluorescence intensity of the test line increases in proportion to the amount of D-Dimer in sample.

Then insert test card into Getein1100 Immunofluorescence Quantitative Analyzer/Getein1600 Immunofluorescence Quantitative Analyzer (hereinafter referred to as Getein1100 and Getein1600), the concentration of D-Dimer in sample will be measured and displayed on the screen. The value will be stored in Getein1100/Getein1600 and available for downloading. The result can be easily transmitted to the laboratory or hospital information system.

CONTENTS

1. A kit for Getein1100 contains:
   Getein D-Dimer test card in a sealed pouch with desiccant 25
   Disposable pipet 25
   Sample diluent 25
   SD card 1
   User manual 1
2. A kit for Getein1600 contains:
   Sealed cartridge with 24/48 Getein D-Dimer test cards 2
   User manual 1
   Package specifications:
   2×24 tests/kit, 2×48 tests/kit
   Materials required for Getein1600:
   Sample diluent 1
   Box with pipette tips 1
   Mixing plate 1
3. Sample diluent composition:
   Phosphate buffered saline, proteins, detergent, preservative, stabilizer.
4. A test card consists of:
   A plastic shell and a reagent strip which is composed of a sample pad, nitrocellulose membrane (one end of the membrane is coated with a fluorescence latex-labelled anti-human D-Dimer monoclonal antibody, the test line is coated with another anti-human D-Dimer monoclonal antibody and the control line is coated with rabbit anti-mouse IgG antibody), absorbent paper and liner.

Note: Do not mix or interchange different batches of kits.

APPLICABLE DEVICE

Getein1100 Immunofluorescence Quantitative Analyzer
Getein1600 Immunofluorescence Quantitative Analyzer

STORAGE AND STABILITY

Store the test card at 4~30℃ with a valid period of 24 months. Use the test card for Getein1100 within 1 hour once the foil pouch is opened.

For test card of Getein1600: if the cartridge is opened, it could be stable within 24 hours once exposed to air. If the test cards can't be used up at a time, please put the cartridge back to the foil pouch and reseal along the entire edge of zip-seal. The remaining test cards should be used up within 7 days.

Store the sample diluent/whole blood buffer at 0~30℃ with a valid period of 24 months.

Store the sample diluent/whole blood buffer at 2~8℃ for better results.

PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. For professional use only.
3. Do not use the kit beyond the expiration date.
4. Do not use the test card if the foil pouch or the cartridge is damaged.
5. Do not open pouches or the cartridge until ready to perform the test.
6. Do not reuse the test card.
7. Do not reuse the pipet.
8. Handle all specimens as potentially infectious. Proper handling and disposal methods should be followed in accordance with local regulations.
9. Carefully read and follow user manual to ensure proper test performance.

SPECIMEN COLLECTION AND PREPARATION

1. This test can be used for ***plasma and whole blood samples.*** ***Sodium citrate*** can be used as the anticoagulant for plasma and whole blood. Samples should be free of hemolysis.
2. Suggest using plasma for better results.
3. If testing will be delayed, plasma sample may be stored up to 3 days at 2~8℃ or stored at -20℃ for 1 month before testing (whole blood sample may be stored up to 3 days at 2~8℃).
4. Refrigerated or frozen sample should reach room temperature

and be homogeneous before testing. Avoid multiple freeze-thaw cycles.
5. Do not use heat-inactivated samples.
6. SAMPLE VOLUME *(for Getein1100): 100 µl*.

TEST PROCEDURE

1. Collect specimens according to user manual.
2. Test card, sample and reagent should be brought to room temperature before testing.

For Getein1100:

3. Confirm SD card lot No. in accordance with test kit lot No.. Perform “SD Card Calib” calibration when necessary (Details refer to 8.5.2 of Getein1100 User Manual).
4. On the main interface of Getein1100, press "ENT" button to enter testing interface.
5. Remove the test card from the sealed pouch immediately before use. Label the test card with patient or control identification.
6. Put the test card on a clean table, horizontally placed.
7. Using sample transfer pipette, deliver *100 µl* of sample into one tube of sample diluent, mix gently and thoroughly. Then drop 100 µl of sample mixture (or 3~4 drops of sample when using disposable pipet) into the sample port on the test card.
8. *Reaction time: 10 minutes.* Insert the test card into Getein1100 and press "ENT" button after reaction time is elapsed. The result will be shown on the screen and printed automatically.

For Getein1600:

9. Each cartridge for Getein1600 contains a specific RFID card which can calibrate automatically.
10. Place samples in the designed area of the sample holder, insert the holder and select the right test item, Getein1600 will do the testing and print the result automatically.

Notes:

1. It is required to perform “SD Card Calib” calibration when using a new batch of kits.
2. It is suggested to calibrate once for one batch of kits for Getein1100.
3. Make sure the test card and the sample insertion is correct and complete.

TEST RESULTS

Getein1100/Getein1600 can scan the test card automatically and display the result on the screen. For additional information, please refer to the user manual of Getein1100/Getein1600.

EXPECTED VALUE

The expected normal value for D-Dimer was determined by testing samples from 500 apparently healthy individuals. The 95th percentile of the concentration for D-Dimer is 0.5 mg/L. (The probability that value of a normal person below 0.5 mg/L is 95%.)

It is recommended that each laboratory establish its own expected values for the population it serves.

PERFORMANCE CHARACTERISTICS

Measuring Range	0.1~10.0 mg/L
Lower Detection Limit	≤0.1 mg/L
Within-Run Precision	≤10%
Between-Run Precision	≤15%

Method Comparison:

The assay was compared with SIEMENS CA-7000 and its matching D-Dimer test kits with 200 plasma samples (60 positive samples and 140 negative samples). The correlation coefficient (r) for D-Dimer is 0.978.

LIMITATIONS

1. As with all diagnostic tests, a definitive clinical diagnosis should not be made based on the result of a single test. The test results should be interpreted considering all other test results and clinical information such as clinical signs and symptoms.
2. Samples containing interferents such as rheumatoid factor, human anti-mouse antibody and heterophile antibody may influence the results. In this case, results of this test should be used in conjunction with clinical findings and other tests. The table below listed the maximum allowance of these potential interferents.

Interferent	Hemoglobin	Triglyceride	Bilirubin
Concentration (Max)	5 g/L	25 g/L	0.1 g/L

REFERENCES

1. Sarig G, Klil-Drori AJ, Chap-Marshak D, Brenner B, Drugan A. Activation of coagulation in amniotic fluid during normal human pregnancy.Thromb Res. 2011 Apr 18.
2. Roldán V, Marín F, Muiña B, Torregrosa JM, Hernández-Romero D, Valdés M, Vicente V, Lip GY. Plasma von Willebrand Factor Levels Are an Independent Risk Factor for Adverse Events Including Mortality and Major Bleeding in Anticoagulated Atrial Fibrillation Patients. J Am Coll Cardiol. 2011 Apr 11.
3. Sakamoto K, Yamamoto Y, Okamatsu H, Okabe M. D-dimer is helpful for differentiating acute aortic dissection and acute pulmonary embolism from acute myocardial infarction. Hellenic J Cardiol. 2011 Mar-Apr; 52(2):123-127.
4. EN ISO 18113-1:2009 *In vitro* diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 1: Terms, definitions and general requirements.
5. EN ISO 18113-2:2009 *In vitro* diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 2: *In vitro* diagnostic reagents for professional use (ISO 18113-2:2009).

DESCRIPTION OF SYMBOLS USED

The following graphical symbols used in or found on D-Dimer Fast Test Kit (Immunofluorescence Assay) are the most common ones appearing on medical devices and their packaging. They are explained in more details in the European Standard EN 980:2008 and International Standard ISO 15223-1:2007.

Key to symbols used			
	Manufacturer		Expiration date
	Do not reuse		Date of manufacture
	Consult instructions for use	LOT	Batch code
	Temperature limitation	IVD	*In vitro* diagnostic medical device
	Sufficient for	EC REP	Authorized representative in the European Community
CE	CE mark		Do not use if package is damaged

Thank you for purchasing D-Dimer Fast Test Kit (Immunofluorescence Assay). Please read this user manual carefully before operating to ensure proper use.

Version: WIF05-S-02

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D-Dimer Control

REF QC006

User Manual

PRODUCT NAME

D-Dimer Control

PRODUCT SPECIFICATION

Package: 3(Level)*2(Vial)*1(ml), 3(Level)*1(Vial)*1(ml)
D-Dimer Control - Level 1/2/3

INTENDED USE

This product is intended for *in vitro* diagnostic use in the quality control of D-Dimer on the Getein Platforms.

PRINCIPLE

The lyophilized D-Dimer control is prepared from dissolving stable and high quality recombinant D-Dimer antigen into calf serum. With matching equipments and reagents, it can fulfill value transfer work. As different equipments and reagents have uncertainty to some extent, different control results may appear.

CONTENTS

The kit for FIA8000/FIA8600/Getein1100 contains:

1. D-Dimer Control - Level 1
   D-Dimer Control - Level 2
   D-Dimer Control - Level 3
2. User manual: 1 piece/box
3. Target value sheet: 1 piece/box

The kit for Getein1600 contains:

1. D-Dimer Control - Level 1
   D-Dimer Control - Level 2
   D-Dimer Control - Level 3
2. User manual: 1 piece/box
3. Target value sheet: 1 piece/box
4. Quality control holder - Level 1
   Quality control holder - Level 2
   Quality control holder - Level 3

Note: Each quality control holder is labelled with barcode which contains target value and level of different items.

MATCHING EQUIPMENTS

FIA8000/8600 Quantitative Immunoassay Analyzer
Getein1100/1600 Immunofluorescence Quantitative Analyzer

STORAGE AND STABILITY

UNOPENED: The product is stable for 18 months at -20℃ and for 90 days at 2 ~ 8℃ to avoid light.
OPENED: The product is stable for 15 days at 2 ~ 8℃ if kept capped in orginal container and free from contamination. Only the required amount of product should be removed. Any residual product should NOT BE RETURNED to the original vial after using. It is recommended to be dispensed into smaller vials after dilution and stable for 30 days at -20 ~ -70℃.

MATERIALS REQUIRED BUT NOT PROVIDED

1. 1 ml pipette
2. Distilled water
3. Getein test kit
4. Getein instrument

TEST PROCEDURE

1. The product should be brought to room temperature (15 ~ 30℃) prior to use.
2. Open the vial carefully in case of the loss of content.

3. Dissolve each control material with 1 ml distilled water.
4. Close the vial and mix gently until all contents are dissolved completely. Avoid violent shaking or foam formation.
5. Keep it at room temperature for 5 ~ 10 minutes before use.

For FIA8000/FIA8600/Getein1100:

6. Treat the control in the same manner as patient specimen in the assay procedure. Follow the directions of test kit and the instrument application instruction.

For Getein1600:

7. Insert quality control holder into sample holder.
8. Insert sample holder with a constant speed and barcode facing the scanner, refer to the User Manual of Getein1600 to start QC testing.

ASSIGNED VALUES

Refer to values listed on the target value sheet.
If the result is beyond the range, it indicates the existence of some unreliable factors in the testing system. Referring to the control graph helps judge the accuracy and stability of the testing system.
The expected range of the mean is provided to aid laboratory until it has established its own mean and SD for its methods.

PERFORMANCE CHARACTERISTICS

1. Homogeneity: ≤ 15%
2. Accuracy range: Refer to the target value sheet

LIMITATIONS

1. This product can only be used on the Getein Platforms.
2. Variation exists between different equipments developed by different methods even using the same control product.
3. This product is not intended to be used as standard material.

NOTES

1. For *in vitro* diagnostic use only.
2. Do not use the product beyond the expiration date.
3. Avoid multiple freeze-thaw cycles.
4. Do not use the product if it is contaminated with bacteria.
5. Proper handling and disposal methods should be followed in accordance with local regulations.

DESCRIPTION OF SYMBOLS USED

The following graphical symbols used in or found on D-Dimer control are the most common ones appearing on medical devices and their packaging. They are explained in more details in the European Standard EN 980:2008 and EN ISO15223-1:2016.

Key to symbols used			
	Manufacturer		Expiration date
REF	Catalogue number		Date of manufacture
	Consult instructions for use	LOT	Batch code
	Temperature limitation	IVD	*In vitro* diagnostic medical device
Σ	Sufficient for		Biological risk
CE	CE mark	EC REP	Authorized representative in the European Community

Please read this user manual carefully before operating to ensure proper use.

Version: WZK04-S-04

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Please contact Getein if you have any questions.

Getein
Biotech, Inc.

CE

Getein 1100

Immunofluorescence Quantitative Analyzer

User Manual

Getein Biotech, Inc.

Warnings, Precautions and Limitations

a. Read this user manual carefully to obtain optimum performance from your analyzer.
b. Only used for in vitro diagnostic analysis of human whole blood, plasma, serum, urine, stool and swabs.
c. To avoid fire, electric shock or personal injuries, please turn off the power immediately and disconnect the power plug when any liquid seeps into the instrument, or the instrument leaks, emits smoke or a smell.
d. Take proper safeguard measures in accordance with health and safety standards in the local country.
e. Specimens and reagents may have potentially biological risks of infection. Operators should wear laboratory protective clothing and gloves required by the operation regulations of laboratory safety to avoid potential biological infection or contamination.
f. All the test kits and consumables should be disposed of after a single use. Proper handling and disposal methods should be established by the laboratory director in accordance with local, status and federal regulations.
g. Operators or person in charge shall be trained on cautions and operation instructions before operating the analyzer.
h. If the instrument is used in a manner not specified by the manufacturer, the protection provided by the instrument may be impaired.

Symbols & Description

	Manufacturer
	Date of manufacture
	Consult *instructions for use* or consult electronic *instructions for use*
	Serial number
	*In Vitro* diagnostic medical device
	Catalogue number
	CE Mark
	Authorized representative in the European Community/European Union
	Warning
	Warning; Biological hazard
	This way up
	Fragile, handle with care
	Keep away from sunlight
	Keep dry
	Stacking limit by number
	Atmospheric pressure limitation
	Humidity limitation
	Temperature limit

Content

1.Introduction

1.1 Intended Use

Getein 1100 Immunofluorescence Quantitative Analyzer (hereinafter called Getein 1100) is an analyzer for processing Getein test kits and analysis of markers for cardiovascular diseases, renal diseases, inflammation, fertility, diabetes mellitus, bone metabolism, tumor and thyroid. This manual contains instructions for the use of Getein 1100 and general instructions for testing specimens and quality control materials.

1.2 Product Description

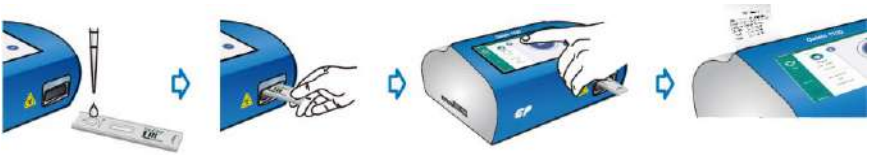
Getein 1100 is used to measure concentration of biomarkers in human whole blood, serum, plasma, urine, stool and swabs. The results can be used as an aid in clinical diagnosis of laboratory and point of care testing.

1.3 Product Principle

1.3.1 Overview: Running a Test

Apply sample (for example, serum) to the test card, insert the test card into Getein 1100 after a certain time (outside mode) or immediately (inside mode) and click the "Start" icon. Then the concentration of biomarkers in the sample will be measured and the result will be displayed (Fig.1-1). The test results can be transmitted to the lab or hospital information system (LIS or HIS) when the analyzer is connected to a computer.

Inside Mode (single sample rapid test mode)



Dispense sample Insert test card Click "Start" Show and print result

Quick mode (mass samples rapid test mode)

Dispense sample Time the reaction manually Click "Start" Show and print result

Fig.1-1 Running a Test

1.3.2 Working Principle

The detection element scans the binding area and converts the optical signal to electrical signal. The voltage variation between test line and background has a linear relationship with the antigen concentration which can be used to calculate the concentration. In conclusion, the antigen concentration in whole blood, plasma, serum, urine, stool and swabs can be calculated quantitatively according to the optical signal of the test line.

1.4 List of Icons

Icon	Name	Function
	Patient Information	Name, Gender, Age, Sample ID and Barcode can be edited here.
	Power Connection	Power is connected
	Battery	Built-in lithium battery.
	Search	Search results by name, sample ID or time.
	ON	Indicate the function is on.
	OFF	Indicate the function is off.

2.Installation

2.1 Unpacking

Check the analyzer and accessories with the packing list (Table 2-1). If you find any parts missing or any damages caused by improper transportation, contact your local agent or Getein's after-sales support immediately.

Table 2-1 Getein 1100 Packing List

No.	Description	Model	Unit	Quantity
1	Device	Getein 1100	set	1
2	Power Adapter	12 V 5 A	pc	1
3	Printing Paper	57 × 35 mm	pc	1
4	Data Cable		pc	1
5	User Manual (Device)		pc	1
6	Qualification Certificate & Warranty Card		pc	1
7	Lithium Battery	6.4 Ah	pc	Optional
8	Barcode Scanner		pc	Optional

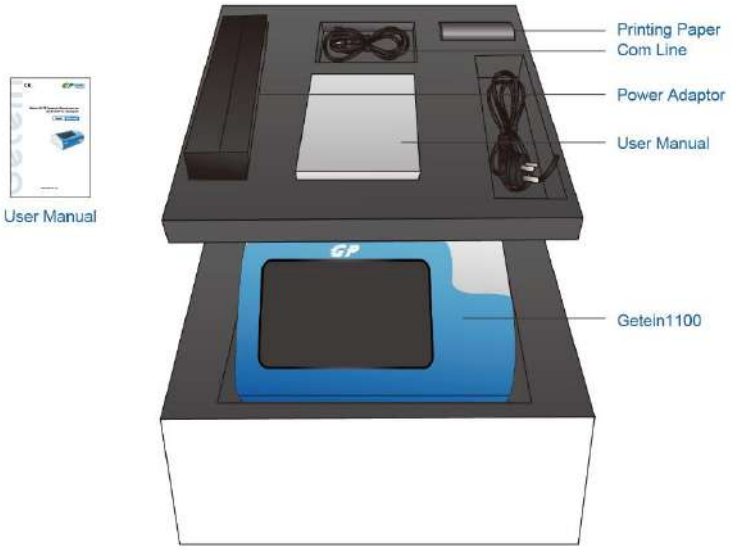


Fig.2-1 Getein 1100 and Main Accessories

2.2 Analyzer Configuration

Getein 1100 is composed of control system, optical system, display unit, analog signal acquisition system, mechanical drive system, etc.

Front and rear view of Getein 1100 are shown in Fig.2-2 and Fig.2-3.

Fig.2-2 Front View of Getein 1100

Fig.2-3 Rear View of Getein 1100

2.3 Main Interface

1 Status Information

It mainly includes the status of power, battery and SD card, current date and time.

2 Test Information

It mainly includes patient information, test card information and result information.

3 System Menu

It mainly includes Test, Search and Settings.

Test: see details in 3.4.

Search: see details in 3.5.

Settings: see details in 4.1~4.7.

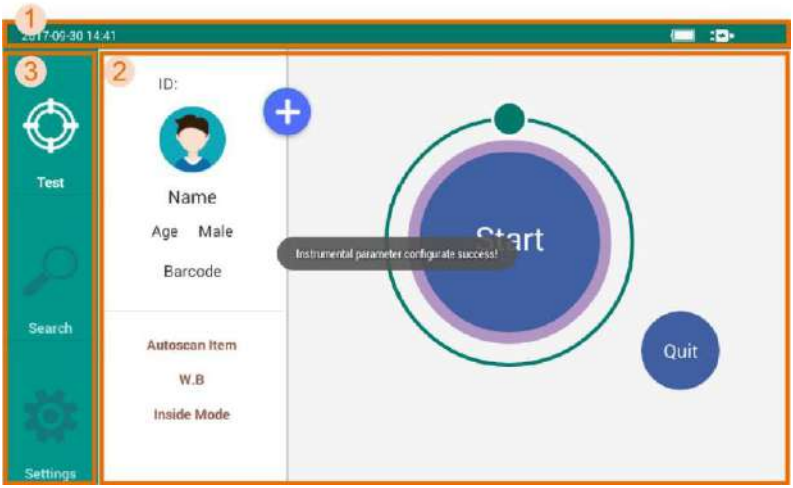


Fig.2-4 Main Interface

2.4 Performance Summary

2.4.1 Basic Parameters

Model	Operating Wavelength (nm)	Detection Range (mV)	Resolution (mV)
Getein 1100	635 ± 5	0 ~ 15000	1

2.4.2 Performance Indexes

Blank Count	Voltage of the blank QC card should be less than 100 mV
Linearity	r ≥ 0.95 in the detection range from 0 mV to 15000 mV
Repeatability	CV ≤ 2% within range [100-15000] mV; CV ≤ 10% within range [0-100) mV
Stability	The voltage variation of the same standard card with a fixed concentration tested within 1 hour should be within ±10%

2.4.3 Technical Specifications

Touch Screen	7-inch LCD touch screen, 1024 × 600
Communications	Two USB ports for barcode scanning and software update COM port for PC Ethernet port for LIS

Data Storage	10,000 data	
Dimensions	261mm × 241mm × 115mm	
Weight	2.0 kg	
Operating Environment	Temperature	10℃ ~ 35℃
	Relative humidity	≤ 70%
	Air pressure	70.0kPa ~ 106.0kPa
Storage	Temperature	-40℃ ~ +55℃
	Relative humidity	≤ 93%
	Air pressure	50.0kPa ~ 106.0kPa
Power Supply	100 - 240V~ 50/60Hz 60VA	

2.5 Installation Requirements

2.5.1 Environment

Dry, clean, flat and horizontal surface away from direct sunlight, wind, hot source, noise source, power interferences, electronic brush type engine and mechanical vibration.

2.5.2 Space Requirement

Place Getein 1100 at a horizontal position and reserve enough space for the reagents.

2.6 Setup

2.6.1 Loading Paper

1) Open the printer cover.
2) Place the printing paper into the printer with carbon sensitive surface facing the screen.
3) Pull out approximate 5 cm of paper from the roll, and then close the cover.

a). Open the printer cover b). Load paper c). Close the printer cover

Fig.2-5 Loading Paper

2.6.2 Barcode Scanner Connection (Optional)

Connect the barcode scanner via USB port in the lateral of the analyzer (see details in the instruction of scanner).

2.6.3 Lithium Battery (Optional)

1) The battery capacity can be shown by 4 icons. They are  which represent low, medium, high and full power respectively.
2) The charging time of lithium battery is 6 hours and the battery can work for at least 3 hours (Note: The charging time and working time will change over time).
3) To avoid being affected by the low power and extend battery lifetime, please charge the analyzer when a low battery is indicated .

2.6.4 Power Connection

1) Use the adapter packed together with Getein 1100.
   Power supply: 100 - 240V~, Frequency: 50/60Hz
2) Connect power with Getein 1100 through the AC/DC adapter.
3) Press the power switch to turn on the analyzer.

3. Operation

3.1 Preparations before Power On

Please confirm whether the analyzer is ready for use according to the following steps before turning on the power switch.

1) Check whether the power supply is ready or connected safely.

2) Check whether the printing paper is enough and correctly loaded.

3.2 Power On

Press the switch to turn on the analyzer, and then system will start its self-checking and then enter the Test Interface.

Test Interface mainly includes Patient Information, Test Card Information and Result Information. Users can input patient information and select test item, sample and test mode as required (Fig.3-1). Barcode can be scanned through the scanner or inputted by the user; No. is generated automatically and cannot be modified.

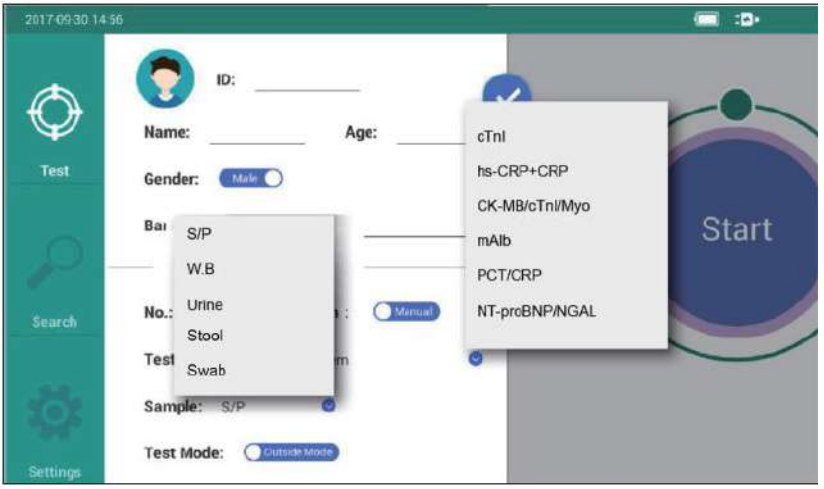


Fig.3-1 Test Interface

3.3 SD Card Calibration

To guarantee the accuracy of measurement and the comparability of data, calibration is required before patient samples testing. Please use the corresponding SD card to calibrate the analyzer before testing different batches of kits (Note: SD card for different batches cannot be exchanged).

Steps: Attach the SD card to the SD card recognition zone, and the analyzer would show the prompt. Click “OK” to import the test card parameters (Fig.3-2).

Note: User can also insert the SD card into the SD card slot to do the calibration.

Fig.3-2 SD Card Calibration

3.4 Sample Test

User can perform sample test by the following steps (Fig.3-3):

1) Edit patient's information if needed.
2) Click "Start" after inserting the card. Test item will be auto-recognized and the result will be shown on the screen after the test is completed. Users can also see the voltage waveform by sliding to the left.
3) Normally, the test card will auto-quit after testing. If not, click the "Quit" icon.

Fig.3-3 Sample Test

Note:

1) Do not switch the interfaces during the measurement.
2) Test items vary in sample volume and reaction time. Refer to theuser manual of the specific item for accurate information.

3.5 Result Query

3.5.1 Query

Click the “Search” icon to switch to result query interface. There are three ways to query results (Fig.3-4).

Fig.3-4 Query Interface

1) Input full or part of the Name or Sample ID (Fig.3-5).

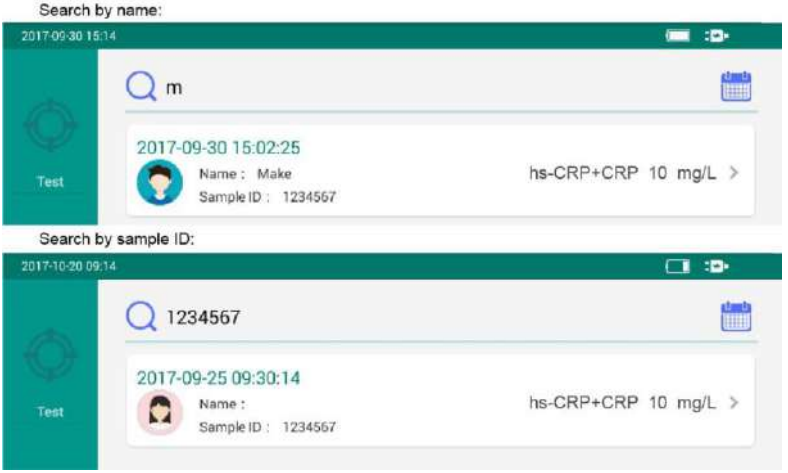


Fig.3-5 Search by Name and Sample ID

2) Select required Date & Time (Fig.3-6).

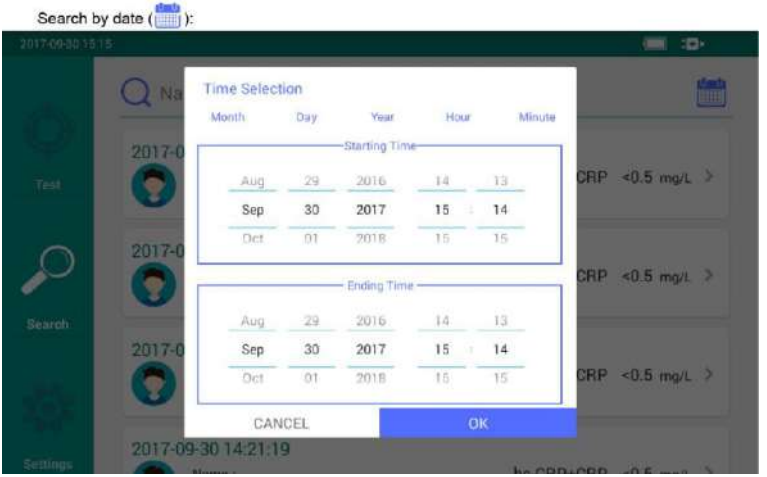


Fig.3-6 Search by Date & Time

3.5.2 Result Deletion

Select a result and slide it to the left. Click “Delete” and a prompt will be shown in the following interface (Fig.3-7). Then Click “OK” to delete it.

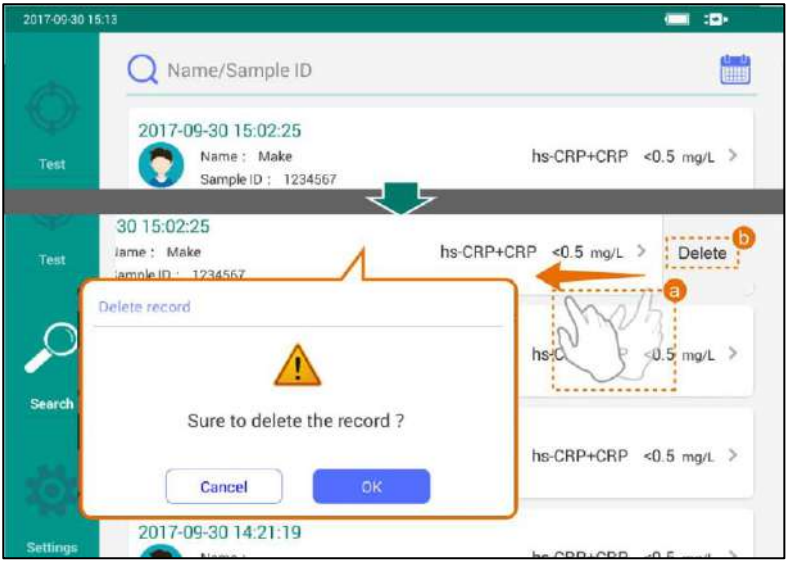


Fig.3-7 Result Deletion

3.5.3 Test Report

Click on the test result for the detailed test report (Fig.3-8). In the report interface, three icons “Print”, “Save” and “Upload” are listed. Slide to the left to view the test voltage waveform.

Print: click to print the test result.

Save: click to save the modifications.

Upload: click to transmit data to the information management system.

Fig.3-8 Test Report

Patient information is editable in case that user forgets to input or input wrong information. No., test item and result are not editable (Fig.3-9). Click the “Save” button after editing.

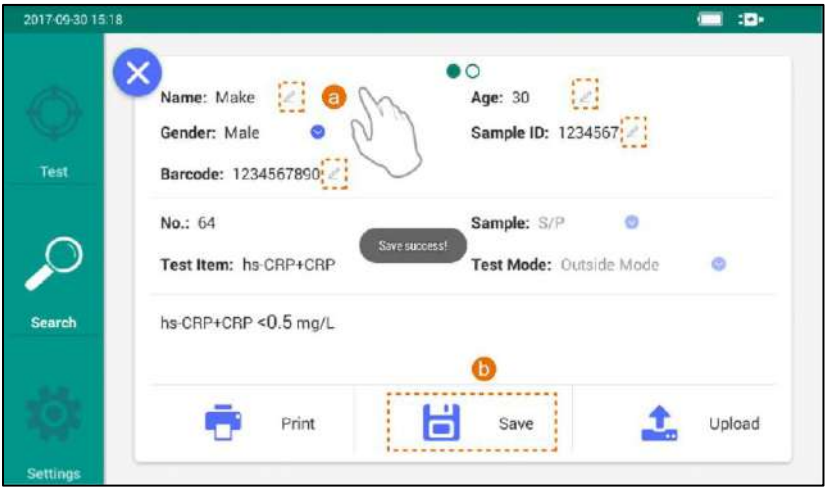


Fig.3-9 Edit Patient Information

3.6 Shutdown

In any interface, user can press the switch to shut off the analyzer directly.

3.7 Waste Disposal

Liquid waste, used test cards, consumables and other wastes, including instrument at the end of life, are considered as medical waste, industrial waste or source of infection. Please handle them properly in accordance with local regulations.

Biological hazard

- Follow and obey lab safety rules and guideline. Wear protective goggles, surgery gloves and laboratory coat to avoid the potential biological pollution risks.
- Disposal of medical wastes should be in accordance with the local regulations.

4.Settings

The installation and debugging of analyzer are performed before it leaves the factory. Operators can reset certain parameters in Settings to meet your laboratory's specific requirements.

Click the "Settings" icon to switch to the setting interface (Fig.4-1). There are 7 icons: Communications, Print Setting, Test Setting, Reaction Time, System Setting, System Version and Debug Mode.

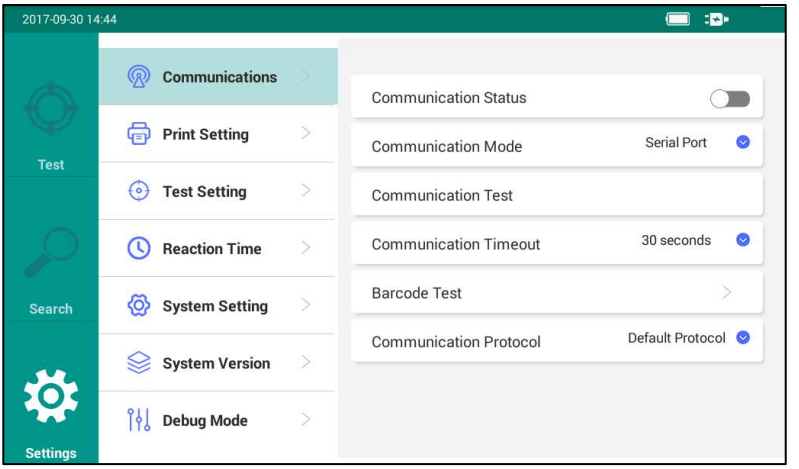


Fig.4-1 Settings

4.1 Communications Setting

It mainly includes Serial Port Status, Communication Mode, Communication Test, Communication Protocol, Communication Timeout and Barcode Test (Fig.4-2).

Note:

- Click " " to the right of Communication Status to enable communication function; the system baud rate is 9600.
- Communication Mode includes Serial Port and WiFi.
- Click Communication Test to send test data through the selected serial port or Ethernet port
- Communication Timeout (10s, 30s, 1min and 5min) can be selected as the disconnection standard of Getein 1100 with the host computer.
- Click Barcode Test to perform barcode testing with a barcode scanner. The test result will be displayed in Barcode Testing.
- Communication Protocol should be selected by or with the assistance of after-sales personnel.

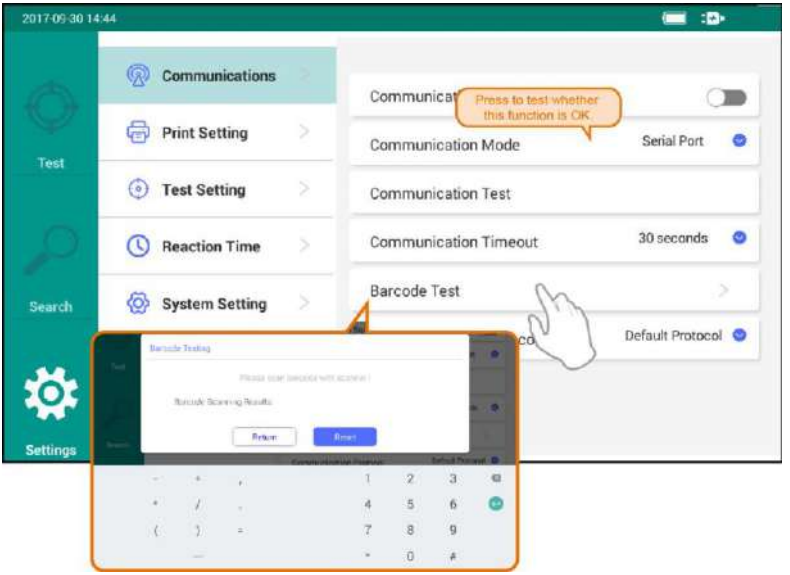


Fig.4-2 Communications Setting

4.2 Print Setting

It mainly includes Auto Print Status and Print Test (Fig.4-3).

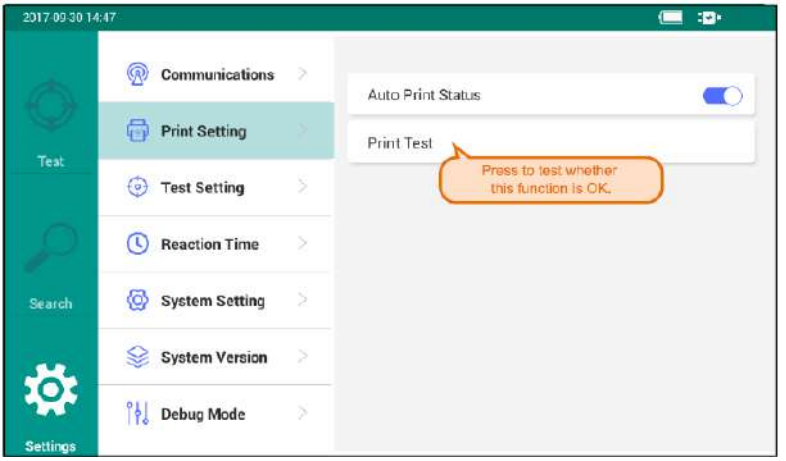


Fig.4-3 Print Setting

4.3 Test Setting

It mainly includes Sample and Test Mode (Fig.4-4).

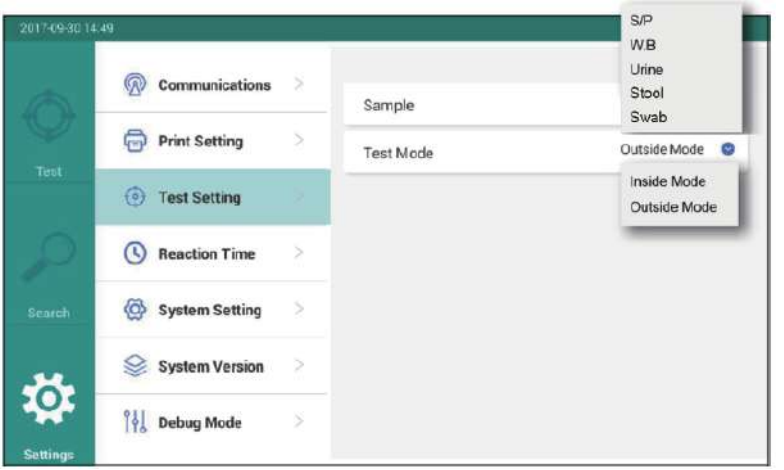


Fig.4-4 Test Setting

4.4 Reaction Time

This interface displays the reaction time imported from SD card (Fig.4-5).

Note: Do not change the reaction time manually unless it is incorrect.

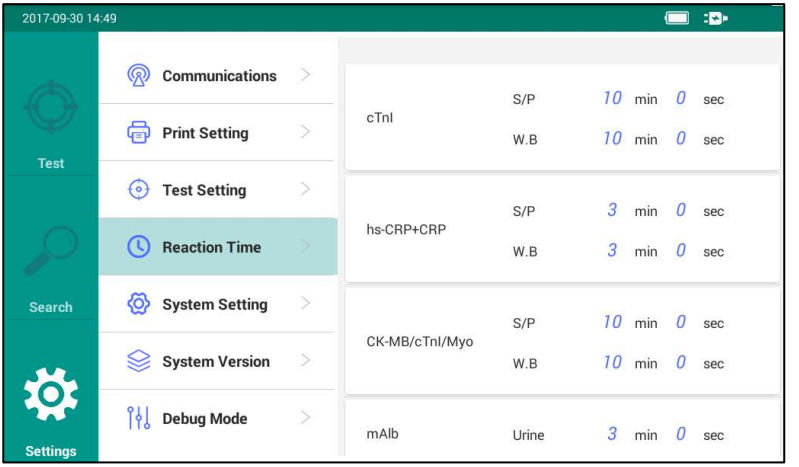


Fig.4-5 Reaction Time

4.5 System Setting

It mainly includes Screen Saver, Language, System Date/Time and Factory Reset (Fig.4-6).

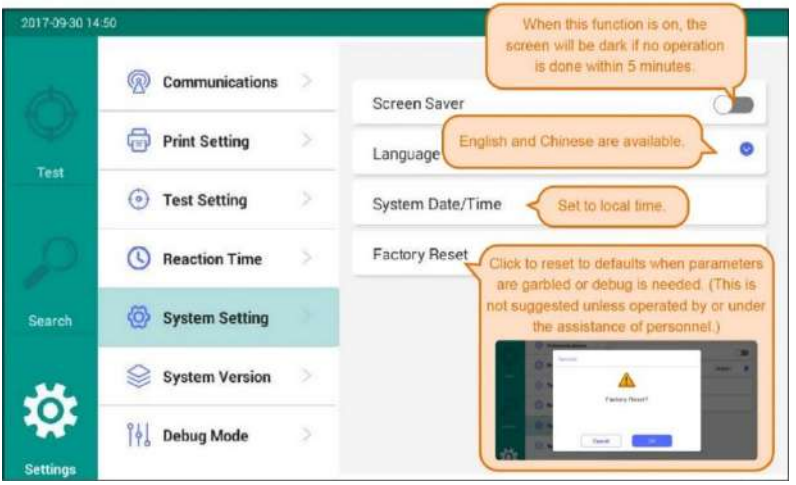


Fig.4-6 System Setting

4.6 System Version

In this interface, user can check the version of analyzer, serial number (SN) and the number of compatible assays (Fig.4-7).

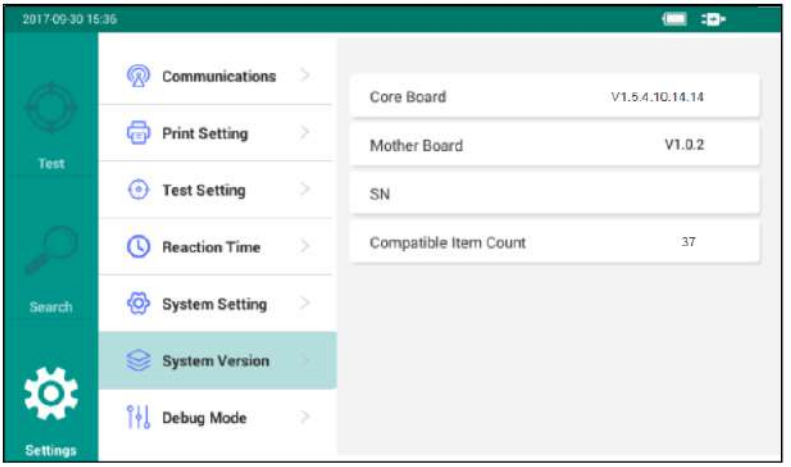


Fig.4-7 System Version

4.7 Debug Mode

Debugging functions are for Getein's after-sales support to debug the instrument. To avoid system parameters being modified by accident, users are not granted the access to the debugging interface.

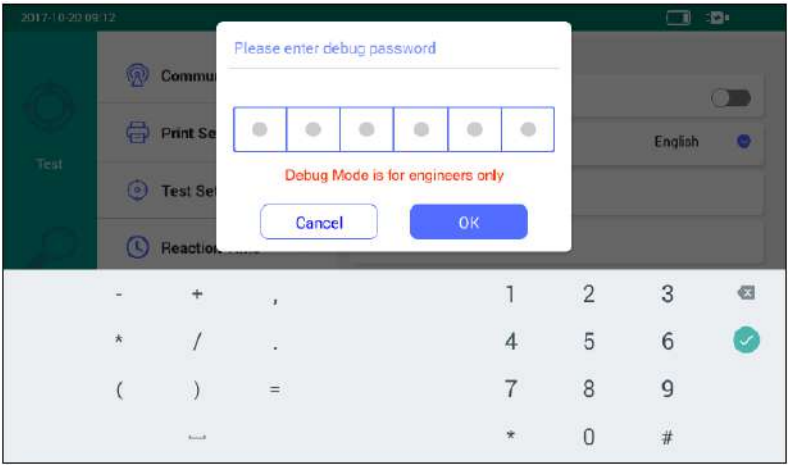


Fig.4-8 Debug Mode

5.Maintenance and Troubleshooting

5.1 Maintenance

Getein 1100 requires minimal maintenance. Clean the surface with wet cloth and 70% ethanol (Turn off the analyzer and ensure the power plug is unconnected before cleaning in case of short circuit and electric shock). Do not clean any internal parts or inner surface. Strong bleach solution (0.5% or higher) is forbidden as oxidant solvent may damage the surface or the touch screen of analyzer.

Maintenance Item	Every Day	Every Week	Every Month	When needed
Dedusting		√		
SD Calibration				A new batch used
Replace Printing Paper				Printing paper used up
Replace LED Lamp				Light intensity weakened
Replace Lithium Battery				Battery damaged

5.2 Precautions

1) Please place the analyzer at a horizontal position for good operation.
2) Under power outage situation, please wait for 30 seconds before restarting the analyzer.
3) Only reagents supplied by Getein can be used on Getein 1100. Refer to the specific user manual for more details.
4) Preheat the analyzer for 20 minutes before testing to ensure the accuracy and reliability of results.
5) Dispose of the used test cards in accordance with the local regulations, as the sample and reagents may have potential risk of biological infections.
6) Please operate the analyzer according to the requirements of the instruction for long-term reliable work.
7) The personnel who operate the PC software should be familiar with the Windows XP, Windows 7 system together with the software installation and uninstallation.
8) Results will be stored automatically in the analyzer and can be recovered automatically after the analyzer is powered off. All data will be cleared if users select "Factory Reset" function.
9) Do not disassemble the analyzer. Operation done by laypeople may damage analyzer.
10) Please charge the analyzer when low battery is indicated.

5.3 Troubleshooting

If there is a malfunction during operating, alarm prompts will pop up.

Error	Cause	Solution
Invalid test card	No C line or shallow C line	Check the shelf life and test again with a new card
Sample type selection error	Inconsistent test item and sample type	Correct the sample mode, and re-test
Test item recognition error	SD card calibration not performed or barcode recognition failure	Do SD card calibration. Change a new card with a clear barcode
Lot error	Inconsistent test card with information stored in the analyzer	Re-calibration with the SD card (same Lot No. with the test card)

6. Appendix

6.1 Copyright

Getein Biotech, Inc.
Instrument Name: Immunofluorescence Quantitative Analyzer
Model: Getein 1100
Version: V3.1
Issue Date: 2023.11

6.2 Statement

- Getein Biotech Inc. owns the copyright to this non-published manual and has the right to take it as confidential information. This manual is provided for operation, maintenance and repair for Getein 1100 only. Anyone has no right to make this manual public.
- This manual contains proprietary information which is protected by copyright law. Copyright of this manual belongs to Getein Biotech Inc. Any content in this manual cannot be copied, reproduced or translated into other languages without the written consent of Getein.
- No warranties of any kind are made by Getein regarding this manual. Getein takes no responsibility for any consequential damages caused by errors in this manual.
- Getein holds the authority of the modification for contents of the manual without informing prior to it.

6.3 Manufacturer Responsibility

- Getein will only be responsible for instrument safety, reliability and performance in following cases: installation, upgrade, calibration, repair and maintenance are done by personnel assigned by Getein; users develop a regular maintenance plan and perform strictly.
- Hospitals or institutions who use this instrument should make a regular maintenance plan and perform strictly, otherwise inappropriate operations may lead to instrument failure or even endanger people's health.
- Getein will conditionally provide circuit diagram, calibration specifications and other documents required to assist the appropriate personnel to finish maintenance or repair under situations users can do themselves.
- Use only as directed. Getein will take no responsibility for protection failure of the analyzer caused by the analyzer being used in a manner not consistent with the instructions in this manual.

6.4 Analyzer Lifespan

The lifespan of Getein 1100 is 8 years (continuous working time no more than eight hours every day) under standardized operation and proper maintenance.

Getein Biotech, Inc.
Add: No.9 Bofu Road, Luhe District, Nanjing, 211505, China
Tel: +86-25-68568508
Fax: +86-25-68568500
E-mail: tech@getein.com.cn, overseas@getein.com.cn
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CMC Medical Devices & Drugs S.L.
Add: C/ Horacio Lengo Nº 18, CP 29006, Málaga, Spain
Tel: +34951214054

Pursue excellence

Deliver health

SVE6600052-RC

Clostridium difficile GDH & Toxin A/B Rapid Test Cassette (Feces)

CE

INTENDED USE

The Clostridium difficile GDH & Toxin A/B Rapid Test Cassette (Feces) a rapid visual immunoassay for the simultaneous detection and differentiation of Clostridium difficile Glutamate Dehydrogenase (GDH), Toxin A and Toxin B in human fecal specimens, as a screening test and as an aid in the diagnosis of Clostridium difficile infection.

INTRODUCTION

Clostridium difficile (C. difficile), a Gram-positive spore bearing anaerobic bacterium is the major aetiological agent of diarrhoea and colitis associated with antibiotics. C. difficile is the most common cause of health care-associated diarrhoea in developed countries and is a major source of nosocomial morbidity and mortality worldwide.

Disease due to C. difficile develops when the organism is allowed to proliferate in the colon, most commonly after antibiotic use has eliminated competing flora. C. difficile can release two high-molecular-weight toxins, toxin A and toxin B, which are responsible for the clinical manifestations, which range from mild, self-limited watery diarrhoea to fulminant pseudomembranous colitis, toxic megacolon and death.

Clostridium difficile Glutamate Dehydrogenase (GDH) is an enzyme produced in large quantities by all toxigenic and non-toxigenic strains, making it an excellent marker for the organism.

The toxigenic culture (TC) is used as the gold standard technique to determine Clostridium difficile infection. This method consists in culture and isolation of C. difficile from feces, followed by toxin testing of the isolate, a labour-intensive assay to obtain a result.

The Clostridium difficile GDH & Toxin A/B Rapid Test Cassette (Feces) is a rapid test to qualitatively detect Clostridium difficile Glutamate Dehydrogenase (GDH), Toxin A and Toxin B in human feces in 10 minutes. The test can be performed by untrained or minimally skilled personnel, without cumbersome laboratory equipment.

PRINCIPLE

The Clostridium difficile GDH & Toxin A/B Rapid Test Cassette (Feces) is a qualitative lateral flow immunoassay for the detection of Clostridium difficile GDH, Toxin A&B in human feces samples.

For the Clostridium difficile GDH Rapid Test Cassette (Feces), the membrane is pre-coated with monoclonal antibodies against GDH on the test line region. During testing, the sample reacts with the particle coated with anti-GDH antibodies, which were pre-dried on the test strip. The mixture moves upward on the membrane by capillary action. If there is sufficient Clostridium difficile GDH in the specimen, a colored band will form at the test region of the membrane. The presence of this colored band indicates a positive result, while its absence indicates a negative result. The appearance of a colored band at the control region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking has occurred. If the control line does not appear, the test result is not valid.

For the Clostridium difficile Toxin A/B Rapid Test Cassette (Feces), the membrane is pre-coated with monoclonal antibodies against Toxin A on the A test line region and monoclonal antibodies against Toxin B on the B test line region. During testing, the sample reacts with the particle coated with anti-Toxin A and anti-Toxin B antibodies, which were pre-dried on the test strip. The mixture moves upward on the membrane by capillary action. If there is sufficient Clostridium difficile Toxin or Toxin B in the specimen, a colored band will form at the test region of the membrane. The presence of this colored band indicates a positive result, while its absence indicates a negative result. The appearance of a colored band at the control region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking has occurred. If the control line does not appear, the test result is not valid.

PRODUCT CONTENTS

The Clostridium difficile Antigen GDH Rapid Test Cassette (Feces) containing Clostridium difficile GDH-specific antibodies coated particles and GDH-specific antibodies coated on the membrane.

The Clostridium difficile Toxin A&B Rapid Test Cassette (Feces) containing Clostridium difficile Toxin A and Toxin B antibodies coated particles and Toxin A-specific antibodies and Toxin B-specific antibodies coated on the membrane.

MATERIALS SUPPLIED

20 Test cassettes
20 Extraction tubes with buffer
1 Package insert

MATERIAL REQUIRED BUT NOT PROVIDED

Timer

STORAGE AND STABILITY

The kit can be stored at room temperature or refrigerated (2-30℃). The test cassette is stable through the expiration date printed on the sealed pouch. The test cassette must remain in the sealed pouch until use. DO NOT FREEZE. Do not use beyond the expiration date.

WARNINGS AND PRECAUTIONS

1. For professional *in vitro* diagnostic use only.
2. Do not use after the expiration date indicated on the package. Do not use the test if the foil pouch is damaged.
3. Test is for single use only. Do not re- use under any circumstances.
4. Avoid cross-contamination of specimens by using a new extraction tube for each specimen obtained.
5. Read the entire procedure carefully prior to testing.
6. Do not eat, drink or smoke in any area where specimens and kits are handled.
7. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow standard procedures for the proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
8. Do not interchange or mix reagents from different lots. Do not mix solution bottle caps.
9. Humidity and temperature can adversely affect results.
10. Do not perform the test in a room with strong air flow, ie. electric fan or strong airconditioning.

SPECIMEN COLLECTION AND PREPARATION

- The Clostridium difficile GDH & Toxin A/B Rapid Test Cassette (Feces) is intended for use with human fecal specimens only.
- Stool samples should be collected in clean containers. The samples can be stored in the refrigerator (2-8℃) for 7 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at -20℃. In this case, the sample will be totally thawed and brought to room temperature before testing. Ensure only the amount needed is thawed because of freezing and defrosting cycles are not recommended. Homogenise stool samples as thoroughly as possible prior to preparation.

SPECIMEN PREPARATION

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

1. Collect a random sample of feces in a clean, dry receptacle. Best results will be obtained if the

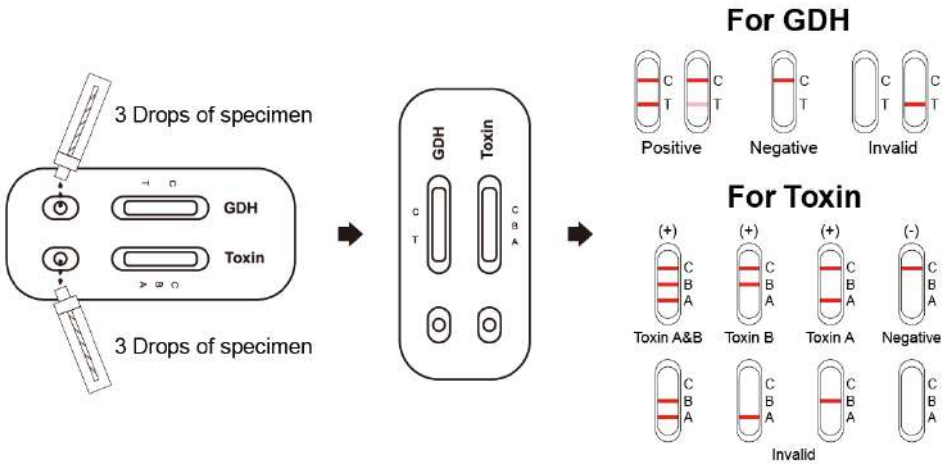
assay is performed within 6 hours after collection.

2. Unscrew and remove the dilution tube applicator. Be careful not to spill or spatter solution from the tube. Collect specimens by inserting the applicator stick into at least 5 different sites of the feces to collect approximately 50 mg of feces (equivalent to 1/4 of a pea).
3. **For liquid specimens:** Hold the pipette vertically, aspirate fecal specimens, and then transfer 3 drops (approximately 80 μL) into the specimen collection tube containing the extraction buffer.
4. Replace the stick in the tube and tighten securely.
5. Shake the specimen collection tube vigorously to mix the specimen and the extraction buffer. Specimens prepared in the specimen collection tube may be stored for 6 months at -20℃ if not tested within 1 hour after preparation.

TEST PROCEDURE

Bring tests, specimens, reagents and/or controls to room temperature (15-30℃) prior to testing.

1. Remove the test from the sealed pouch and place it on a clean, level surface. Label the device with patient or control identification. For best results, the assay should be performed immediately after opening the foil pouch.
2. Hold the specimen collection tube upright and then unscrew and open the upper cap.
3. Squeeze 3 drops (~90 μL) of the sample solution in each sample well of the device and start the timer.
4. Wait for the colored line(s) to appear. Read results in 10 minutes. Do not interpret the result after 10 minutes.



INTERPRETATION OF RESULTS

(Please refer to the illustration above)

For the GDH test:

1. **Positive:** Two lines appear. One colored line should be in the control line region (C) and another apparent colored line should be in the test line region (T).

2. **Negative:** One colored line appears in the control line region (C). No line appears in the test line region (T).

3. **Invalid:** Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test Cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

For the Toxin A&B test:

1. Positive:

1.1 Toxin A Positive:

The presence of two lines as control line (C) and A test line within the result window indicates a positive result for Toxin A.

1.2 Toxin B Positive:

The presence of two lines as control line (C) and B test line within the result window indicates a positive result for Toxin B.

1.3 Toxin A & B Positive:

The presence of three lines as control line (C), A test line and B test line within the result window indicates a positive result for both Toxin A and Toxin B.

2. Negative:

One colored line appears in the control line region (C). No line appears in the test line region (T).

3. Invalid:

If the control band (C) is not visible within the result window after performing the test, the result is considered invalid. Some causes of invalid results are because of not following the directions correctly or the test may have deteriorated beyond the expiration date. It is recommended that the specimen be re-tested using a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

QUALITY CONTROL

A procedural control is included in the test. A red line appearing in the control region (C) is the internal procedural control. It confirms sufficient specimen volume and correct procedural technique. Control standards are not supplied with this test. However, it is recommended that positive and negative controls are sourced from a local competent authority and tested as a good laboratory practice, to confirm the test procedure and verify the test performance.

LIMITATIONS

1. The Clostridium difficile GDH & Toxin A/B Rapid Test Cassette (Feces) will only indicate the presence of parasites in the specimen (qualitative detection) and should be used for the detection of Clostridium difficile GDH, Toxin A&B in feces specimens only. Neither the quantitative value nor the rate of increase in antigen concentration can be determined by this test.
2. An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
3. The Clostridium difficile GDH & Toxin A/B Rapid Test Cassette (Feces) should be used only with samples from human feces. The use of other samples has not been established. The quality of the test depends on the quality of the sample; proper fecal specimens must be obtained
4. 4.. A negative result is not meaningful because of it is possible the antigen concentration in the stool samples is lower than the detection limit value. If the symptoms or situation still persist, a Clostridium difficile determination should be carried out, on a sample from an enrichment culture.
5. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

PERFORMANCE CHARACTERISTICS

1. Clinical Sensitivity, Specificity and Accuracy

The Clostridium difficile GDH & Toxin A/B Rapid Test Cassette (Feces) has been evaluated with specimens obtained from patients. ELISA method was used as the reference method. The results show that the Clostridium difficile GDH & Toxin A/B Rapid Test Cassette (Feces) has a high overall relative accuracy.

Table 1: The Clostridium difficile GDH Rapid Test vs ELISA

Method		ELISA		Total Results
Clostridium difficile Antigen GDH Rapid Test Cassette	Results	Positive	Negative	
	Positive	62	1	63
	Negative	0	50	50
Total Results		62	51	113

Relative Sensitivity: 100%
Relative Specificity: 98.0%
Accuracy: 99.1%

Table 2: The Clostridium difficile Toxin A Rapid Test vs ELISA

Method		ELISA		Total Results
Clostridium difficile Toxin A&B Rapid Test Cassette	Results	Positive	Negative	
	Positive	43	1	44
	Negative	0	69	69
Total Results		43	70	113

Relative Sensitivity: 100%
Relative Specificity: 98.6%
Accuracy: 99.1%

Table 3: The Clostridium difficile Toxin B Rapid Test vs ELISA

Method		ELISA		Total Results
Clostridium difficile Toxin A&B Rapid Test Cassette	Results	Positive	Negative	
	Positive	36	1	37
	Negative	0	76	76
Total Results		36	77	113

Relative Sensitivity: 100%
Relative Specificity: 98.6%
Accuracy: 99.1%

2. Analytical Sensitivity

The Clostridium difficile GDH & Toxin A/B Rapid Test Cassette (Feces) was determined by testing serial dilutions of recombinant antigen. Detection limit values of Clostridium difficile GDH & Toxin A/B are 1 ng/mL for GDH, 2 ng/mL for Toxin A and 1 ng/mL for Toxin B.

3. Cross-Reactivity

Cross-reactivity to samples positive for the following pathogens was tested and found to be negative:

Campylobacter coli	*Salmonella enteritidis*	*Shigella dysenteriae*
Campylobacter jejuni	*Salmonella paratyphi*	*Shigella flexneri*
E. Coli O157: H7	*Salmonella typhi*	*Shigella sonnei*
H. Pylori	*Salmonella typhimurium*	*Staphliococcus aureus*
Listeria monocytogenes	*Shigella boydii*	*Yersinia enterocolitica*

REFERENCE

1. Knoop, F.C. et al.: Clostridium difficile: Clinical disease and diagnosis. Clin. Microbiol. Rev. (1993); 6: 251-265.
2. Kelly, C.P. et al.: Clostridium difficile Colitis. New Engl. J. Med. (1994); 330: 257-262.
3. Sullivan, N.M. et al.: Purification and characterization of toxins A and B of Clostridium difficile. Infect. Immun. (1982); 35: 1032-1040.
4. McDonald, L.C. et al.: An epidemic, toxin gene-variant strain of Clostridium difficile. N. Engl. J. Med. (2005); 353: 23.
5. Loo, V.G. et al.: A predominantly clonal multi-institutional outbreak of Clostridium difficile-associated diarrhea with high morbidity and mortality. N. Engl. J. Med. (2005); 353. 23.
6. Bartlett, J.G., Gerding, D.N.: Clinical recognition and diagnosis of Clostridium difficile infection. CID (2008); 46 (Suppl. 1): 12-18.

INDEX OF SYMBOLS

	Consult instructions for use		Tests per kit	EC REP	Authorized Representative
IVD	For *in vitro* diagnostic use only		Use by		Do not reuse
2°C 30°C	Store between 2~30°C	LOT	Lot Number	REF	Catalog#

Zhejiang Orient Gene Biotech Co.,Ltd
Address: 3787#, East Yangguang Avenue, Dipu Street,
Anji 313300, Huzhou, Zhejiang, China
Tel: +86-572-5226111 Fax: +86-572-5226222
Website: www.orientgene.com

EC REP CMC Medical Devices & Drugs S.L
C/Horacio Lengo Nº 18 CP 29006, Málaga-Spain
Tel: +34951214054 Fax: +34952330100
Email-info@cmcmedicaldevices.com

REF GCCD-602a

Revision Date: 2022-12-06
B22719-02

H. pylori Ag Rapid Test Cassette (Feces)

CE

INTENDED USE

H. pylori Ag Rapid Test Cassette (Feces) is a sandwich lateral flow chromatographic immunoassay for the qualitative detection of H.Pylori antigen in feces.It is for professional *in vitro* diagnostic use only.

INTRODUCTION

H.Pylori is associated with a variety of gastrointestinal diseases included non-ulcer dyspepsia, duodenal and gastric ulcer and active, chronic gastritis.[1,2] The prevalence of H.pylori infection could exceed 90% in patients with signs and symptoms of gastrointestinal diseases. Recent studies indicate an association of H. Pylori infection with stomach cancer.[3]H. Pylori colonizing in the gastrointestinal system elicits specific antibody responses[4,5,6] which aids in the diagnosis of H. Pylori infection and in monitoring the prognosis of the treatment of H. Pylori related diseases. Antibiotics in combination with bismuth compounds have been shown to be effective in treating active H. Pylori infection. Successful eradication of H. pylori is associated with clinical improvement in patients with gastrointestinal diseases providing a further evidence.[7]

PRINCIPLE

H. pylori Ag Rapid Test Cassette (Feces) is a lateral flow chromatographic immunoassay based on the principle of the double antibody–sandwich technique. The test cassette consists of: 1) a burgundy colored conjugate pad containing H. Pylori antibodies conjugated with color particles (H. Pylori conjugates. 2) a nitrocellulose membrane strip containing a test band (T band) and a control band (C band). The T band is pre-coated with non-conjugated H. Pylori antibodies.

When an adequate volume of test specimen is dispensed into the sample well of the cassette, the specimen migrates by capillary action across the cassette. The antigen of H. Pylori if present in the specimen will bind to the H. Pylori antibodies conjugates. The immunocomplex is then captured on the membrane by the pre-coated H. Pylori antibodies, forming a burgundy colored T band, indicating a H. Pylori antigen positive test result. To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred. Otherwise, the test result is invalid and the specimen must be retested with another device.

PRODUCT CONTENTS

H. pylori Ag Rapid Test Cassette (Feces) containing anti- H.pylori antibodies particles and anti-H.pylori antibodies coated on the membrane.

MATERIALS SUPPLIED

20 Sealed pouches each containing a test cassette and a desiccant
20 Specimen collection tubes with extraction buffer, 2.0 mL
1 Package insert

MATERIAL REQUIRED BUT NOT PROVIDED

1. Clock or timer
2. Specimen collection containers.

STORAGE AND STABILITY

All reagents are ready to use as supplied. Store unused test device unopened at 2°C-30°C. If stored at 2°C-8°C, ensure that the test device is brought to room temperature before opening. The test is not stable out off the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit over 30°C.

WARNINGS AND PRECAUTIONS

1. For professional *in vitro* diagnostic use only.
2. Do not use it if the tube/pouch is damaged or broken.
3. Test is for single use only. Do not re- use under any circumstances.
4. Handle all specimens as if they contain infectious agents. Observe established standard procedure for proper disposal of specimens
5. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assay.
6. Humidity and temperature can adversely affect results

SPECIMEN COLLECTION

Collect sufficient quantity of feces (1-2 mL or 1-2 g) in a clean, dry specimen collection container to obtain maximum antigens (if present). Best results will be obtained if the assay is performed within 6 hours after collection. Specimen collected may be stored for 3 days at 2-8˚C if not tested within 6 hours. For long term storage, specimens should be kept below -20˚C.

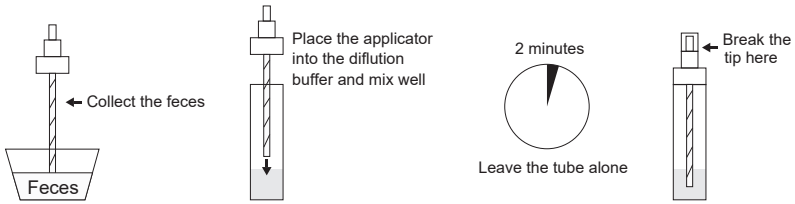
To process fecal specimens:

◦ For Solid Specimens:

Unscrew the cap of the specimen collection tube， then randomly stab the specimen collection applicator into the fecal specimen in at least 3 different sites to collect approximately 50 mg of feces (equivalent to 1/4 of a pea). Do not scoop the fecal specimen.

◦ For Liquid Specimens:

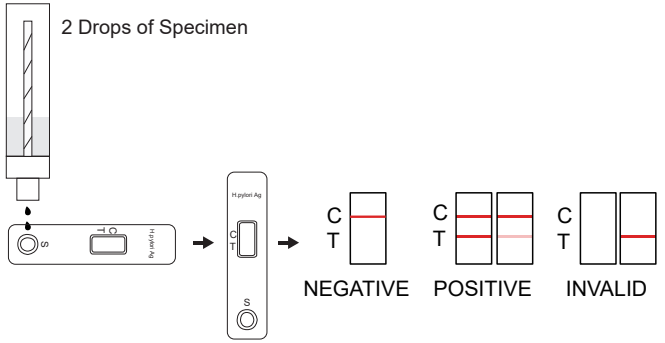
Hold the dropper vertically, aspirate fecal specimens, and then transfer 2 drops (approximately 80 µL) into the specimen collection tube containing the dilution buffer. Screw on and tighten the cap onto the specimen collection tube, then shake the specimen collection tube vigorously to mix the specimen and the dilution buffer. Leave the tube alone for 2 minutes.



TEST PROCEDURE

1. Remove the test device from its foil pouch by tearing along the notch and use it as soon as possible.
2. Specimen collection. See also specimen collection.
3. Holding the sample collection device upright, carefully break off the tip of collection device.
4. Squeeze 2 drops (~80 µL) of the sample solution in the sample well of the cassette, as in the illustration.
5. Read the test results in 10 minutes. It is important that the background is clear before the result is read. Do not read results after 10 minutes. To avoid confusion, discard the test device after interpreting the result.

INTERPRETATION OF RESULTS



H. pylori Ag Rapid Test Cassette (Feces)

Positive: Two lines appear. One colored line should be in the control line region (C) and another apparent colored line should be in the test line region (T).
Negative: One colored line appears in the control line region(C). No line appears in the test line region (T).
Invalid: Control line fails to appear.

QUALITY CONTROL

A procedural control is included in the test. A colored line appearing in the control line region (C) is an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.
Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS

1. The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of H. Pylori antigen in feces from individual subjects. Failure to follow the procedure may give inaccurate results.
2. H. pylori Ag Rapid Test Cassette (Feces) is limited to the qualitative detection of H. Pylori antigen in feces. The intensity of the test band does not have linear correlation with the antigen titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable H. Pylori antigen. However, a negative test result does not preclude the possibility of exposure to or infection with H. Pylori.
4. A negative result can occur if the quantity of the H. Pylori angtigen present in the specimen is below the detection limits of the assay, or the antigen that are detected are not present during the stage of disease in which a sample is collected.
5. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

PERFORMANCE CHARACTERISTICS

A study was performed with 165 patient feces samples including both symptomatic gastrointestinal disorders and samples from non-symptomatic patients and 100 normal feces samples.Comparison for all subjects with H. pylori Ag Rapid Test Cassette (Feces) and reference ELISA kit is showed in the following table:

Method		EIA		Total Results
H.P Test Cassette	Results	Positive	Negative	
	Positive	163	0	163
	Negative	2	100	102
Total Results		165	100	265

Relative sensitivity: 98.8%
Relative specificity: 100%
Accuracy:98.9%

REFERENCE

1. Marshall,B.J.et.al. Pyloric Campylobacter infection and gastroduodenal disease. Med. J. Australia.149:439-44, 1985.
2. Marshall,B.J.et.al. Prospective double-blind trial of duodenal ulcer relapse after eradication of Campylobacter pylori. Lancet. Dec.1437-42,1988.
3. Megraud,F.et.al. Seroepidemiology of Campylobacter pylori infection in virious populations J.Clin.Microbiology. 27:1870-3,1989.
4. Soll,A.H. Pathogenesis of peptic ulcer and implications for therapy. New England J. Med.322:909-916,1990.
5. Parsonnet,J.et.al. Helicobacter pylori infection and the risk of gastric carcinoma. New England J.Med. 325:1127-31,1991.
6. Ansong,R. et.al. Evaluation of techniques for isolation, subcultivation and preservation of Helicobacter pylori. J.Clin.Micro. 29:51-53,1991.
7. Pronovost,A.P.et.al. Evaluation of a new immunodiagnostic assay for Helicobacter pylori antibody detection: Correlation with histopathological and microbiological results. J.Clin.Microbiol.32:46-50,1994.

INDEX OF SYMBOLS

	Consult instructions for use	Σ	Tests per kit	EC REP	Authorized Representative
IVD	For *in vitro* diagnostic use only		Use by		Do not reuse
2°C 30°C	Store between 2~30°C	LOT	Lot Number	REF	Catalog#

Zhejiang Orient Gene Biotech Co.,Ltd
Address: 3787#, East Yangguang Avenue, Dipu Street, Anji 313300, Huzhou, Zhejiang, China.
TEL: +86-572-5226111 FAX: +86-572-5226222
Website: www.orientgene.com

EC REP Shanghai International Holding Corp. GmbH (Europe)
Add: Eiffestrasse 80, 20537 Hamburg, Germany

REF GCHP-602a

Revision Date: 2022-03-08
B20435-03

X+V Factor discs

DD022

Used for the presumptive identification of *Haemophilus* species on the basis of their requirements for X or V factors or both.

Directions

Inoculate the surface of a Blood Agar (M073) plate or Brain Heart Infusion Agar (M211) plate with the test organisms by either streaking or surface spreading. Aseptically place the X (DD020), V (DD021) and X+V (DD022) factor discs on the plate, in the following positions:

Disc Position on the Agar plate

X factor disc 12 O' clock

V factor disc 4 O' clock

X+V factor disc 8 O' clock

Incubate the plates at 35 - 37°C for 24 - 48 hours. Observe for the growth in the neighbourhood of the discs.

Principle And Interpretation

Both X and V factors are growth factors that are essential for certain organisms like *Haemophilus* species and also enhance growth of organisms like *Neisseria* species.

X+V factor discs are the sterile filter paper discs impregnated with growth factors x <(>&<)> V which are used for differentiating *Haemophilus* species in conjuction of X factor & V factor discs. *Bordetella* and *Haemophilus* species can also be identified on the basis of the requirement of X and V growth factors in the basal medium.

The X factor (hemin) and V factor (Coenzyme- Nicotinamide adenine dinucleotide NAD+) are impregnated on the sterile filter paper discs of diameter 6 mm.

The test organism requiring X factor alone, grows only in the vicinities of X and X+V factor discs. Those which require V factor alone grow in the vicinities of V and X+V factor discs. If both X and V factors are required, then the organism will grow only in the vicinity of the X+V factor discs. This satellite growth is seen around the disc promoting growth (1).

Quality Control

Appearance

Filter paper discs of 6 mm diameter bearing letters "X+V" in continuous printing style.

Cultural response

Cultural characteristics observed on Brain Heart Infusion Agar (M211)or Blood Agar Base (M073) after an incubation of 24-48 hours at 35-37°C.

Organism	Growth with X +V factor	Growth without growth factor
Bordetella pertussis ATCC 8467	Positive(initial isolation on Bordet Gengou Agar (M175))	Positive(initial isolation on Bordet Gengou Agar (M175))
Haemophilus influenzae ATCC 35056	Positive	Negative
Haemophilus parainfluenzae ATCC 7901	Positive	Negative

Haemophilus haemoglobinophilus ATCC19416	Positive	Negative
Haemophilus ducreyi	Positive	Negative

Storage and Shelf Life

Store below -10°C. Use before the expiry date on the label.

Reference

1.Murray PR, Baron EJ, Jorgensen J.H., Pfaller M A, Yolken R.H(Eds.),8th ed, 2003, Manual of Clinical Microbiology, ASM, Washington D.C.

Note:
Use known strains of *Haemophilus influenzae* to monitor the performance of the differentiation discs and the medium.
Do not use too heavy suspension of the test organisms as X or V factor carryover from the primary growth medium may take place

Revision : 1 / 2011

Bile Esculin Discs

DD024

Bile Esculin Discs are used for detection of esculin hydrolysis in the presence of bile, for differentiating Group D streptococci from other Streptococcal groups.

Directions

Esculin impregnated disc is placed on the seeded Bile Esculin Agar Base (M340) plate and is incubated at 35-37°C for 18-24 hours.

Principle And Interpretation

Group D streptococci hydrolyze esculin to esculetin and dextrose. Esculetin reacts with an iron salt such as ferric citrate to form a blackish brown coloured complex (4).

Rochaix found that esculin hydrolysis is an important criteria in the identification of enterococci (1). Meyer and Schonfeld (2) observed that when bile was added to esculin medium, around 60% enterococci were able to grow and split the esculin while other streptococci could not. When a comparative study was performed by Facklam and Moody (3) for presumptive identification of Group D streptococci, they found the bile esculin test as a reliable means of identifying Group D streptococci and differentiating them from other streptococci groups.

Quality Control

Appearance

Plain filter paper discs of 6mm diameter

Cultural response

Cultural response observed by placing Bile Esculin disc (DD024) on seeded Bile Esculin Agar Base(M340) plate, incubated at 35-37°C for 18-24 hours.

Organism	Growth	Esculin hydrolysis
Enterococcus faecalis ATCC 29212	luxuriant	positive: blackening of media around the disc.
Streptococcus agalactiae ATCC 13813	luxuriant	negative: no blackening
Listeria monocytogenes ATCC 19118	luxuriant	positive: blackening of media around the disc.
Streptococcus pyogenes ATCC 19615	luxuriant	negative: no blackening

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Reference

1. Rochaix, 1924, C. R. Soc. Biol., 90:771.
2. Meyer and Schonfeld, 1926, Zentralbl. Bacteriol. Parasitenkd. Infectionskr. Hyg. Abt. I Orig., 99:402.
3. Facklam and Moody, 1970, Appl. Microbiol., 20:245.
4.MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd ed., Philadelphia: Lippincott. Williams and Wilkins.

Revision : 1 / 2011

CE

Disclaimer :

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DMACA Indole Discs

DD040

The DMACA Indole Discs are used for Indole test to determine the ability of an organism to split indole from the tryptophan molecule, and thus to aid differentiation between *Escherichia coli* from *Klebsiella.*

Directions

Place the DMACA Indole Disc on suspected colony from HiCrome UTI Agar (M1353) or HiCrome UTI Agar, Modified (M1418) plate. Observe for appearance of blue-purple colour within 10 - 30 seconds.

Principle And Interpretation

In the presence of oxygen, some bacteria are able to split tryptophan into indole and alpha-aminopropionic acid. The presence of indole can be detected by the addition of DMACA (p-Dimethylaminocinnamaldehyde) reagent indicated by formation of bluish-purple colour (1).

Quality Control

Appearance

Filter paper discs of 6 mm diameter bearing letters 'Dm' in continuous printing style.

Cultural response

The indole production by organisms was tested after an incubation of 18-24 hours at 35-37°C, using HiCrome UTI Agar (M1353).

Cultural Response

Organism	**Indole production**
Cultural response	
Escherichia coli ATCC 25922	Positive reaction, blue-purple colour formation
Klebsiella pneumoniae ATCC 13883	Negative reaction.
Pseudomonas aeruginosa ATCC 27853	Negative reaction.

Storage and Shelf Life

Store at 2-8°C. Use before the expiry date on the label.

Reference

1.MacFaddin J. F., 1980, Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins, Baltimore.

Revision : 1 / 2011

HiMedia Laboratories Pvt. Ltd. A-516,Swastik Disha Business Park,Via Vadhani Ind. Est., LBS Marg, Mumbai-400086, India. Customer care No.: 022-6147 1919 Email: techhelp@himedialabs.com

Colistin Ezy MIC™ Strip (CL) (0.016-256 mcg/ml) EM020

Antimicrobial Susceptibility Testing
For *In Vitro* Diagnostic use

It is a unique MIC determination paper strip which is coated with Colistin on a single paper strip in a concentration gradient manner, capable of showing MICs in the range of 0.016mcg/ml to 256 mcg/ml, on testing against the test organism.

Introduction:
Ezy MIC™ strip is useful for quantitative determination of susceptibility of bacteria to antibacterial agents. The system comprises of a predefined quantitative gradient which is used to determine the Minimum Inhibitory Concentration (MIC) in mcg/ml of different antimicrobial agents against microorganisms as tested on appropriate agar media, following overnight incubation.

Ezy MIC™ Strip FEATURES AND ADVANTAGES
Ezy MIC™ strip exhibits several advantages over existing plastic strip.

1. Ezy MIC™ strip is made up of porous paper material unlike plastic non-porous material.
2. Ezy MIC™ strip has MIC values printed on both sides identically.
3. The antimicrobial agent is evenly distributed on either side of the Ezy MIC™ strip and hence it can be placed by any side on the agar surface.
4. For Ezy MIC™ strips, MIC values can be read without opening the lid of the plate as most commonly translucent medium such as Mueller Hinton Agar is employed.
5. Once placed, Ezy MIC™ strip is adsorbed within 60 seconds and firmly adheres to the agar surface.
6. Unlike the plastic material, it does not form air bubbles underneath and hence there is no need to press the strip once placed.

METHOD AND USE OF EZY MIC™ STRIPS

- **Type of specimen**

Pure cultures should be derived from specimens obtained from patients prior to the initiation of antimicrobial therapy. Specimens can be of bacterial or fungal isolates derived from blood, urine, faeces, pus, CSF etc. Direct specimens should not be employed in this test. Refer procedure, which includes preparation of inoculum (1,3).

- **Clinical specimen collection, handling and processing**

Follow appropriate techniques for handling specimens as per established guidelines. After use, contaminated materials must be sterilized by autoclaving before discarding (1,3).

- **Guidelines for preparation of the medium**

Prepare the medium of choice from dehydrated powder according to the directions specified on the label. Cool the sterilized molten medium to 45-50°C and pour in sterile, dry Petri plates on a leveled surface, to a depth of 4 ± 0.2 mm and allow to solidify. Few droplets appearing on the surface of the medium following cooling do not matter. Hence, once poured, Petri plates containing media should not be dried on laminar flow and can be used immediately for swabbing.

- **Preparation of Inoculum**

Use only pure cultures. Confirm by Gram-staining before starting susceptibility test. Transfer 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for 2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 620 nm).
Also direct colony suspension method can be used. Prepare a direct colony suspension, from 18-24 hour old non-selective media agar plate in broth or saline. Adjust the turbidity to that of standard 0.5 McFarland. This method is recommended for testing fastidious organisms like *Haemophilus* spp., *Neisseria* spp, *Bacteroides* spp, streptococci and for testing staphylococci for potential Methicillin or Oxacillin resistance.

- **Test Procedure**

1. Prepare plates with suitable make of Mueller Hinton Agar for rapidly growing aerobic organisms as mentioned above.

2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking.
3. Remove Ezy MIC™ strip container from cold and keep it at room temperature for 15 minutes before opening.
4. Remove one applicator from the self sealing bag stored at room temperature.
5. Hold the applicator in the middle and gently press its broader sticky side on the centre of Ezy MIC™ strip.
6. Lift the applicator along with attached Ezy MIC™ strip.
7. Place the strip at a desired position on agar plate pre-spread with test culture. Gently turn the applicator clockwise with fingers. With this action, the applicator will detach from the strip.
8. DO NOT PRESS EZY MIC™ STRIP. Within 60 seconds, Ezy MIC™ strip will be adsorbed and will firmly adhere to the agar surface.
9. Ezy MIC™ strip should not be repositioned or adjusted once placed.
10. Transfer plates in the incubator under appropriate conditions.

MIC Reading:

1. Read the plates only when sufficient growth is seen.
2. Read the MIC where the ellipse intersects the MIC scale on the strip.
3. For bactericidal drugs such as Colistin, Amikacin, Vancomycin, Gentamicin, β-lactams class of drugs always read the MIC at the point of complete inhibition of all growth, including hazes, microcolonies and isolated colonies. If necessary, use magnifying glass.
4. Isolated colonies, microcolonies and hazes appearing in the zone of inhibition are indicative of hetero nature of the culture having resistant subpopulation in it. In such cases, consider reading for MIC determination at a point on the scale above which no resistant colonies are observed close to MIC strip (within 1-3 mm distance from the strip).
5. Since Ezy MIC™ strip has continuous gradient, MIC values “in-between” two fold dilutions can be obtained.
6. Always round up these values to the next two-fold dilution before categorization. For example: Colistin showing reading of 0.75 mcg/ml should be rounded up to next concentration i.e. 1.0 mcg/ml.
7. If the ellipse intersects the strip in between 2 dilutions, read the MIC as the value which is nearest to the intersection.
8. When growth occurs along the entire strip, report the MIC as $\geq$ the highest values on the MIC strip. When the inhibition ellipse is below the strip (does not intersect the strip), report the MIC < the lowest value on the MIC scale.

Warning and Precautions:

1. Ezy MIC™ Strip is intended for *In vitro* diagnostic use only.
2. Although based on simple procedure, Ezy MIC™ Strip should only be used by at least semi-trained personnel.
3. This strip is intended only for agar diffusion method and not for broth dilution method.
4. Ezy MIC™ Strip should be used strictly according to procedures described herein.
5. Performance of Ezy MIC™ Strips depends on use of proper inoculum and control cultures, recommended test medium and proper storage temperature.
6. Follow aseptic techniques and precautions against microbiological hazards should be used when handling bacterial or fungal specimen throughout the testing procedure.
7. Before using Ezy MIC™ Strips, ensure that the strips is at room temperature.
8. When applying strips be steady. Do not move the strip once in contact with agar surface, since the antibiotic instantaneously diffuse on contact with agar.
9. Place the unused strips back to recommended temperature.

INTERPRETATION & QUALITY CONTROL :

Interpretation:

Table 1: Use following interpretive criteria for susceptibility categorization as per CLSI.

When testing	Incubation	Interpretative Criteria (mcg/ml)		
		≤ S	I	≥ R
Other non-*Enterobacterales*	35-37°C for 18 hrs.	2	4	8
Enterobacterales , Acinetobacter spp., *P.aeruginosa,*	35-37°C for 18 hrs.	-	≤ 2	4

Quality control:

Quality control of Ezy MIC™ Strip is carried out by testing the strips with standard ATCC Cultures recommended by CLSI on a suitable medium incubated appropriately.

Following are the reference MIC values (mcg/ml) range for Colistin.

Organism	Medium used	Incubation	Std. Quality Control limits (mcg/ml)
E.coli ATCC 25922[a]	Mueller Hinton Agar	35-37°C for 18 hrs.	0.25 - 0.5 – 1.0 - 2.0
P. aeruginosa ATCC 27853	Mueller Hinton Agar	35-37°C for 18 hrs.	0.25 - 0.5 – 1.0 - 2.0
E. coli NCTC 13846	Mueller Hinton Agar	35-37°C for 18 hrs.	2.0 - 4.0 – 8.0

[a] : Quality Control Limit deleted in CLSI 2024.

In-house Quality Control for Resistant Clinical Isolates :

Organism	Medium used	Incubation	MIC values obtained by repeated Microbroth dilution (mcg/ml)	MIC values obtained by Ezy MIC™ Strip (mcg/ml)
Col-Res Clinical Isolate 1	Mueller Hinton Agar	35-37°C for 18 hrs.	**32** (**Range:** 16.0 -32.0-64.0)	32, 32, 24, 32, 24
Col-Res Clinical Isolate 2	Mueller Hinton Agar	35-37°C for 18 hrs.	**16** (**Range:** 8.0 - 16.0 - 32.0)	16, 16, 8, 8, 12
Col-Res Clinical Isolate 3	Mueller Hinton Agar	35-37°C for 18 hrs.	**8** (**Range:** 4.0 - 8.0 - 16.0)	8, 8, 8, 4, 4

Storage & Shelf Life:

1. Once the consignment is received, store applicators at Room Temperature and Ezy MIC™ strips container at 2-8°C, for prolonged use store below -20°C.
2. Use before expiry date on the label.
3. Ezy MIC Strip left over from opened package must be kept dry.
4. Moisture should be prevented from penetrating into or forming within the package or storage container.
5. Check whether the batch number and expiry date are marked on the storage container.
6. Product performance is best within stated expiry period if correctly stored and handled.

Disposal:

After use, Ezy MIC™ Strips and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Limitation of Test

Ezy MIC™ Strips provides *In vitro* MIC values, which provides only a possible insinuation of pathogens potential in *In vivo* susceptibility. These values can be considered as a guide to therapy selection only after taking into consideration several other factors; and must be the sole decision and responsibility of the physician along with the clinical experience in treating the infection. These tests are comparable to the standards as per the given specifications and set of experiment standards as far as possible. Please refer to CLSI standards for detailed limitation of susceptibility test on the clinical use of an antibiotic in various therapeutic conditions.

References:

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 1, Section 2.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 3, Section 15.
3. Jorgensen, J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. Performance Standards of Antimicrobial Susceptibility Testing; 34th Edition. M100-Ed34, Vol.44, No.5, Jan-2024.

Packing:

Each Pack contains following material packed in air-tight plastic container with a desiccator capsule.

1) Colistin Ezy MIC™ strips (10/30/60/90/120/150 Strips per pack)
2) Applicator sticks
3) Package insert

Revision: 05/2024

CE

On receipt store at -20°C / 8°C

IVD In vitro diagnostic medical device

Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Estate, Thane (W) - 400604, Maharashtra, India

Indicates a single sterile barrier system with protective packaging outside

Do not re-use

EC REP CEpartner4U, ESDOORNLAAN 13, 3951DB MAARN, NL www.cepartner 4u.eu

Do not use if package is damaged

Technical Data

Tinsdale Selective Supplement (Part A & Part B) FD073

A selective supplement recommended for the isolation and presumptive identification of *Corynebacterium diphtheriae*.

Composition

Per vial sufficient for 1000 ml medium

Ingredients	Concentration
Part A	
Horse serum	100ml
Part B	
Potassium tellurite	1ml

Directions:

Warm up the refrigerated contents of Part B vial and aseptically add 29 ml sterile distilled water. Mix thoroughly. Aseptically add warmed up (to 50°C) contents of Part A and B vials to sterile, molten, cooled (45-50°C) Tinsdale Agar Base M314 / Tinsdale HiVeg™ Agar Base MV314 as required. Mix well and pour into sterile petri plates.

For 10 ml of M314 : 1.0 ml of Part A and 0.3 ml of Part B, is recommended.

Type of specimen

Clinical samples- Throat swab, nasal swab, wound swab, pus, etc.; Food samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,2).
For food samples follow appropriate techniques for handling specimens as per established guidelines (3).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning & Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1.Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.

*** Not For Medicinal Use**

Revision : 02/2022

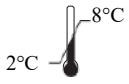
HiMedia Laboratories Pvt. Limited, Plot No.C-40, Road No.21Y, MIDC, Wagle Industrial Area, Thane (W) -400604, MS, India

EC REP

CEpartner4U, Esdoornlaan 13, 3951DB Maarn, NL
www.cepartner4u.eu

IVD ***In vitro* diagnostic medical device**

CE Marking



Storage temperature

Do not use if package is damaged

Disclaimer :

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Bismuth Sulphite Agar (BS)

M027

Intended Use:

Recommended for selective isolation and enumeration of *Salmonella* species from food samples. The composition and performance criteria of this medium are as per specifications laid down in ISO 6579-1:2017.

Composition**

ISO 6579-1 Specification -Bismuth Sulphite Agar

Ingredients	g/ L
Enzymatic digest of animal tissues Meat	10.000
extract	5.000
Dextrose	5.000
Disodium hydrogen phosphate, anhydrous	4.000
Ferrous sulphate, anhydrous	0.300
Bismuth sulphite indicator	8.000
Brilliant green	0.025
Agar	20.000
Final pH (at 25°C)	7.7±0.2

Bismuth Sulphite Agar

(BS) Ingredients	g/ L
Peptone #	10.000
HM extract ##	5.000
Dextrose (Glucose)	5.000
Disodium hydrogen phosphate, anhydrous	4.000
Ferrous sulphate, anhydrous	0.300
Bismuth sulphite indicator	8.000
Brilliant green	0.025
Agar	20.000
Final pH (at 25°C)	7.7±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Enzymatic digest of animal tissues ##-Equivalent to Meat extract

Directions

Suspend 52.33 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. **DO NOT STERILIZE IN AUTOCLAVE** or by fractional sterilization since overheating may destroy the selectivity of the medium. The sensitivity of the medium depends largely upon uniform dispersion of precipitated bismuth sulphite in the final gel, which should be dispersed before pouring into sterile Petri plates.

Principle And Interpretation

The Salmonellae constitute the most taxonomically complex group of bacteria among*Enterobacteriaceae* (1). Human *Salmonella* infections are most commonly caused by ingestion of food, water or milk contaminated by human or animal excreta. Humans are the only reservoirs of *S.*Typhi (2). Of the various media employed for the isolation and preliminary identification of *Salmonellae*, particularly *Salmonella* Typhi; Bismuth Sulphite Agar is the most productive. Bismuth Sulphite Agar is a modification of original Wilson and Blair Medium (3-5). It is also recommended by various Associations (2,6-8) for the isolation and preliminary identification of *Salmonella* Typhi and other Salmonellae from pathological materials, sewage, water, food and other products. Bismuth Sulphite Agar (M027I) is recommended for selective isolation and enumeration of *Salmonella* species in accordance with ISO Committee (8). *S.*Typhi, *S.*Enteritidis and *S.*Typhimurium typically grow as black colonies with or without a surrounding metallic sheen resulting from hydrogen sulphide production and reduction of sulphite to black ferric sulphide. *Salmonella* Paratyphi A grows as light green colonies. Bismuth Sulphite Agar may be inhibitory to some strains of *Salmonella* species and therefore should not be used as the sole selective medium for these organisms. Also this medium favors use of larger inoculum as compared to other selective media, as it has unique inhibitory action towards gram-positive organisms and coliforms.

Peptone and HM extract serve as sources as carbon, nitrogen, long chain amino acids, vitamins and essential growth factors. Dextrose is the carbon source. Disodium phosphate maintains the osmotic equilibrium. Bismuth sulphite indicator along with brilliant green inhibits the intestinal gram-positive and gram-negative bacteria. Ferrous sulphate aids in detection of hydrogen sulphide production. In case of food samples, pre-enrichment of the sample is done prior to inoculation.

Type of specimen

Clinical samples- faeces, Food and meat samples. milk and milk products, animal feed, animal faeces, environmental samples.

Specimen Collection and Handling

Processesing : (8)

Pre-enrichment : Samples (25 grams in 225 ml) are pre-enriched in Buffered Peptone Water (M1494I) and incubated at 34°C to 38°C for 18 h ± 2 hours.

Selective enrichment: 0.1 ml of pre- enriched sample is inoculated in 10 ml RVS Broth (M1448I) or MSRV Agar (M1428) and incubated at 41.5 ± 1°C for 24 ± 3 hours and 1 ml of culture is inoculated in MKTT broth (M1496I) and incubated at 37± 1°C for 24 ± 3 hours. In-case of *Salmonella* Typhi and *Salmonella* Paratyphi A selective enrichment is carried out in Selenite Cystine broth and then incubated at 37± 1°C for 24 h ± 3 h and 48 h ± 3 h.

Isolation : The culture thus obtained is then plated on Bismuth Sulphite Agar (BS) (M027) and incubated at 37± 1°C for 24 ± 3 hours. An additional incubation of 24 ± 3 hours is recommended. Simultaneously plating on isolation agar XLD Agar, Modified (M031I) is carried out.

Confirmation : Biochemical and serological tests are performed for confirmation.

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,9).

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. **DO NOT AUTOCLAVE OR OVERHEAT THE MEDIUM,** as it destroys the selectivity of the medium.
2. *S.*Typhi and *S.*Arizonae exhibit typical brown colonies , with or without metallic sheen.
3. This medium is highly selective and must be used in parallel with less selective media for isolation.
4. With certain *Salmonella* species, typical black colonies with metallic sheen is observed near heavy inoculation and isolated colonies may show green colonies.
5. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to greenish yellow homogeneous free flowing powder.

Gelling

Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium

Greenish yellow coloured, opalescent with flocculent precipitate forms in Petri plates.

Reaction

Reaction of 5.23% w/v aqueous solution at 25°C. pH : 7.7±0.2.

pH

7.50-7.90

Cultural Response

Cultural response was observed after an incubation at 37 ± 1°C for 24 ± 3 hours. The plates are further incubated for an additional 24 ± 3 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Productivity				
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	good	>=50 %	Brown, grey or black colonies usually with a metallic sheen after 24 hours becoming uniformly black after 48 hours.
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	good	>=50 %	Brown, grey or black colonies usually with a metallic sheen after 24 hours becoming uniformly black after 48 hours.
Selectivity & Specificity				
Escherichia coli ATCC 8739 (00012*)	>=10^4	growth or partial inhibition		Dull green or brown colonies without metallic sheen
Escherichia coli ATCC 25922 (00013*)	>=10^4	growth or partial inhibition		Dull green or brown colonies without metallic sheen

Selectivity				
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0 %	-
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	inhibited	0 %	-
Additional testing				
Salmonella Typhi ATCC 6539	50 -100	good	>=50 %	Brown, grey or black colonies usually with a metallic sheen after 24 hours becoming uniformly black after 48 hours.
Salmonella Paratyphi A ATCC 9150	50 -100	good	>=50 %	Brown, grey or black colonies usually with a metallic sheen after 24 hours becoming uniformly black after 48 hours.

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (7, 9).

Reference

1. Tindall B. J., Crimont P. A. D., Gorrity G. M., EUZESY B. P., 2005, Int. J. Sys. Evol. Microbiol., 55:521.
2. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
3. Wilson and Blair, 1926, J. Pathol. Bateriol., 29:310.
4. Wilson and Blair, 1927, J. Hyg., 26:374.
5. Wilson and Blair, 1931, J. Hyg., 31:138.
6. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, D.C.
7. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
8. Microbiobiology of the food chain- Horizontal method for the detection, enumeration and serotyping of *Salmonella*- Part I Detection of *Salmonella* . International Organization for Standardization (ISO), ISO/ DIS 6579-1:2017.
9. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

Revision : 02/2024

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IVD *In vitro* diagnostic medical device

10°C–30°C Storage temperature

EC REP CEpartner4U, Esdoornlaan 13, 3951DB Maarn, NL www.cepartner4u.eu

CE CE Marking

Do not use if package is damaged

Tinsdale Agar Base

M314

Intended Use:

Recommended for selective isolation and differentiation of *Corynebacterium diphtheriae.*

Composition**

Ingredients	Gms / Litre
Peptone	20.000
Sodium chloride	5.000
L-Cystine	0.240
Sodium thiosulphate	0.430
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.67 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add Tinsdale Selective Supplement (FD073, Part A and Part B). Mix well and pour into sterile Petri plates.

Principle And Interpretation

The Corynebacteria are gram-positive, non-sporulating, non-motile rods. They are often club-shaped and frequently banded or beaded with irregularly stained granules. These bacteria are generally aerobic or facultative, but microaerophilic species do occur. *Corynebacterium diphtheriae* produces a powerful exotoxin that causes diphtheria in humans. In nature, *C. diphtheriae* occurs in nasopharyngeal area of infected persons or healthy carriers.

The three biotypes of *C. diphtheriae* are *mitis*, *intermedius* and *gravis* (1). The signs and symptoms of diphtheria are sore throat, malaise, headache and nausea (2). Tinsdale Agar Base Medium was developed by Tinsdale (3,4) for the selective isolation and differentiation of *C. diphtheriae* from diphtheroids. This medium was modified by Billings (2), which improved the recovery and differential qualities of *C. diphtheriae*. The present medium is according to the modified Billings Medium. Moore and Parsons (3) confirmed the halo formation as a characteristic property of *C. diphtheria* with the exception of *C. ulcerans*, which forms colony with similar features as *C. diphtheriae*.

Peptone provides nitrogenous compounds. L-cystine and sodium thiosulphate form the H_2S indicator system. Potassium tellurite from the supplement inhibits all gram-negative bacteria and most of the upper respiratory tract normal flora.

C. diphtheriae forms grayish black colonies surrounded by a dark brown halo while diphtheroids commonly found in the upper respiratory tract do not form such colonies. Dark brown halo around the colony is due to H_2S production from cystine combining with the tellurite salt. Moore and Parsons (3) found Tinsdale Medium as an ideal medium for the routine cultivation and isolation of *C. diphtheriae*. They also confirmed the stability of halo formation on clear medium and its specificity for *C. diphtheriae* and *C. ulcerans*. *C. ulcerans* found in nasopharynx form colonies same as *C. diphtheriae* and require further biochemical confirmation (5).

Do not incubate the plates in 5-10% CO_2 as it retards the development of characteristic halos (6). Tinsdale Agar is not suitable as a primary plating medium, since it may not support the growth of some strains of *C. diphtheriae* (1). *C. ulcerans* , *C. pseudotuberculosis* and (rarely) *Staphylococcus* species may produce a characteristic halo on Tinsdale Agar (1).Several organisms may exhibit slight browning on Tinsdale Agar in 18 hours; therefore the plates should be read after complete incubation period (48 hours) (1).

Type of specimen

Clinical samples - Throat swab

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Do not incubate the plates in 5-10% CO_2 as it retards the development of characteristic halos (6).

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light amber coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 4.07% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 40-48 hours with added Tinsdale Selective Supplement (FD073, Part A and Part B).

Organism	Inoculum (CFU)	Growth	Recovery	Colony characteristics
Corynebacterium diphtheriae type gravis	50-100	good-luxuriant	>=50%	brown-black with halo
Corynebacterium diphtheriae type intermedius	50-100	good-luxuriant	>=50%	brown-black with halo
Corynebacterium diphtheriae type mitis	50-100	good-luxuriant	>=50%	brown-black with halo
Klebsiella pneumoniae ATCC 13883 (00097*)	>=10^4	inhibited	0 %	
Streptococcus pyogenes ATCC 19615	50-100	good	40-50%	black pin point, without halo

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

1. Isenberg, (Eds.), 1992, Clinical Microbiology Procedures Handbook, Vol. 1, American Society for Microbiology, Washington, D.C.
2. Billings E., 1956, An investigation of Tinsdale Tellurite Medium: its usefulness and mechanisms of halo-formation, M.S. thesis, University of Michigan, Ann Arbor, Mich.
3. Moore M. S. and Parsons E. I., 1958, J. Infect. Dis., 102:88.
4. Tinsdale G. F. W., 1947, J. Pathol. Bacteriol., 59:461.
5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
6. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
8. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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IVD ***In vitro* diagnostic medical device**

CE Marking

10°C – 30°C **Storage temperature**

Do not use if package is damaged

Disclaimer :

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HiCrome™ UTI Agar

M1353

Intended use

Recommended for presumptive identification and confirmation of microorganisms mainly causing urinary tract infections, can also be used for testing water, food, environmental and other clinical samples.

Composition**

Ingredients	g / L
Peptone, special	15.000
Chromogenic mixture	2.450
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 32.45 gram in 1000 ml purified /distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Urinary tract infections are bacterial infections affecting parts of urinary tract. The common symptoms of urinary tract infection are urgency and frequency of micturition, with associated discomfort or pain. The common condition is cystitis, due to infection of the bladder with a uropathogenic bacterium, which most frequently is *Escherichia coli,* but sometimes *Staphylococcus saprophyticus* or especially in hospital-acquired infections, *Klebsiella* species, *Proteus mirabilis*, other coliforms, *Pseudomonas aeruginosa* or *Enterococcus faecalis* (1). HiCrome™ UTI Agar is formulated on basis of work carried out by Pezzlo (2) Wilkie et al (3), Friedman et al (4), Murray et al (5), Soriano and Ponte (6) and Merlino et al (7). These media are recommended for the detection of urinary tract pathogens where HiCrome™ UTI Agar has broader application as a general nutrient agar for isolation of various microorganisms. It facilitates and expedites the identification of some gram-negative bacteria and some gram-positive bacteria on the basis of different contrasted colony colours produced by reactions of genus or species specific enzymes with two chromogenic substrates. The chromogenic substrates are specifically cleaved by enzymes produced by *Enterococcus* species, *E.coli* and coliforms. Presence of amino acids like phenylalanine and tryptophan from peptones helps for detection of tryptophan deaminase activity, indicating the presence of *Proteus* species, *Morganella* species and *Providencia* species.

One of the chromogenic substrate is cleaved by ß-glucosidase possessed by Enterococci resulting in formation of blue colonies. *E.coli* produce pink colonies due to the enzyme ß-D-galactosidase that cleaves the other chromogenic substrate. Further confirmation of *E.coli* can be done by performing the indole test. Coliforms produce purple coloured colonies due to cleavage of both the chromogenic substrate. Colonies of *Proteus, Morganella* and *Providencia* species appear brown because of tryptophan deaminase activity. Peptone special provides nitrogenous, carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. This medium can be made selective by supplementation with antibiotics for detecting microorganisms associated with hospital borne infections.

Type of specimen

Clinical samples : urine, faeces, etc.; Food samples, Water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (10,11). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (12).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Since it is an enzyme-substrate based reaction, the intensity of colour may vary with isolates.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% Agar gel
Colour and Clarity of prepared medium
Light amber coloured, clear to slightly opalescent gel forms in Petri plates
Reaction
Reaction of 3.24% w/v aqueous solution at 25°C. pH : 6.8±0.2
pH
6.60-7.20
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 16-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=70%	Purple to magenta
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=70%	blue-green (small)
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	>=70%	blue to purple, mucoid
Proteus mirabilis ATCC 12453	50-100	luxuriant	>=70%	light brown
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	luxuriant	>=70%	colourless (greenish pigment may be observed)
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	luxuriant	>=70%	golden yellow

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

1. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone.
2. Pezzlo M., 1998, Clin. Microbiol. Rev., 1:268-280.
3. Wilkie M. E., Almond M. K., Marsh F. P., 1992, British Medical Journal 305:1137-1141.
4. Friedman M. P. et al, 1991, J. Clin. Microbiol., 29:2385-2389.

5. Murray P., Traynor P. Hopson D., 1992, J. Clin. Microbiol., 30:1600- 1601.
6. Soriano F., Ponte C., 1992, J. Clin. Microbiol., 30:3033-3034.
7. Merlino et al, 1995, Abstr. Austr. Microbiol. 16(4):17-3.
8. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
9. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
10. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
11. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
12. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

Revision : 07/2024

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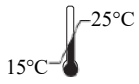
IVD *In vitro* diagnostic medical device



Storage temperature

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CE CE Marking

Do not use if package is damaged

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Gram's Crystal Violet

S012

Intended use

Gram's crystal violet is used as staining solution for Gram's staining and monochrome staining of microbes.

Composition**

Ingredients	
Solution A	-
Crystal violet	2.0 gm
Ethyl alcohol	20.0 ml
Solution B	-
Ammonium oxalate,monohydrate	0.8 gm
Distilled water	80.0 ml

Note: Mixed solution A and B, stored for 24 hours. The resulting stain is stable and ready to use.
**Formula adjusted, standardized to suit performance parameters

Directions

1. Prepare a thin smear on clear, dry glass slide.
2. Allow it to air dry and fix by gentle heat.
3. Flood with Gram's Crystal Violet (S012) for 1 minute. (If over staining results in improper decolourization of known gram-negative organisms, use less crystal violet).
4. Wash with tap water.
5. Flood the smear with Gram’s Iodine (S013). Allow it to remain for 1 minute.
6. Decolourize with Gram's Decolourizer (S032) until the blue dye no longer flows from the smear. (Acetone may be used as a decolourizing agent with caution, since this solvent very rapidly decolourized the smear).
7. Wash with tap water.
8. Counter stain with 0.5% w/v Safranin (S027) for 20 seconds and rinses off with water.
9. Wash with tap water.
10. Allow the slide to air dry or blot dry between sheets of clean bibulous paper and examine under oil immersion objective.

Principle And Interpretation

The Gram stain is a differential staining technique most widely applied in all microbiology disciplines laboratories. It is one of the most important criteria in any identification scheme for all types of bacterial isolates. Different mechanisms have been proposed to explain the gram reaction. There are many physiological differences between gram-positive and gram-negative cell walls. Ever since Christian Gram has discovered Gram staining, this process has been extensively investigated and redefined. In practice, a thin smear of bacterial cells is stained with crystal violet, then treated with an iodine containing mordant to increase the binding of primary stain. A decolourizing solution of alcohol or acetone is used to remove the crystal violet from cells which bind it weakly and then the

.
counterstain (like safranin) is used to provide a colour contrast in those cells that are decolourized. Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50–90% of cell envelope), and as a result are stained purple by crystal violet, whereas gram-negative bacteria have a thinner layer (10% of cell envelope), so do not retain the purple stain and are counter-stained pink by safranin. In a properly stained smear by gram staining procedure, the gram-positive bacteria appear blue to purple and gram-negative cells appear pink to red.

Type of specimen

Any isolated colony on primary or subculture plates can be isolated from following specimens. Clinical specimen: Blood, urine, CSF, pus, wounds, lesions, body tissues, sputum etc. From environment: Air, water, soil, sludge, waste water, food, dairy samples etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines.
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines.
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.
Generally, the smear is made in laboratory; however, when there is a concern that transport will be delayed or that the preservation for culture will alter the specimen, prepare smear and submit slides to the laboratory.

Warning and Precautions

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets

Limitations

1. Use results of Gram stains in conjunction with other clinical and laboratory findings. Use additional procedures (e.g., special stains, inclusion of selective media, etc.) to confirm findings suggested by gram-stained smears
2. Proper smear preparation is key to obtaining good gram staining results. Avoid excessive material or thick smears which may interfere with the passage of light and lead to distortion of images.
3. Overheating slides during heat fixation can distort the appearance of the organisms.
4. Only fresh cultures and specimens should be gram stained since cell wall integrity of older cells may give improper gram-staining characteristics. Gram positive organisms that have lost cell wall integrity because of old age or antibiotic treatment may appear pink.
5. The decolorization step is the most important step in the gram-staining process. Over decolorization results in an abundance of bacteria that appear gram negative, while under decolorization results in too many bacteria that appear to be gram-positive.
6. The procedure given is based on an ideal thin smear of cells. Staining and decolorization times may vary depending on the sample and its thickness
7. False Gram stain results may be related to inadequately collected specimens or delay in transit.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature

Quality Control

- **Appearance :** Purple coloured solution.
- **Clarity :** Clear without any particles.
- **Microscopic Examination :** Gram staining is carried out where Gram's Crystal Violet is used as one of the stains and staining characteristics of organisms are observed under microscope by using oil immersion lens.
- **Results :** Gram-positive microorganisms : violet
  Gram-negative microorganisms : pinkish red

Storage and Shelf Life

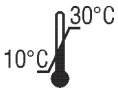
Store between 10-30°C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

Reference

1. Downes F. P. and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
2. Rice E.W., Baird, R.B., Eaton A. D., Clesceri L. S. (Eds.), 2012, Standard Methods for the Examination of Water and Wastewater, 22nd ed., APHA, Washington, D.C.
3. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. Shanhooltzer, C.J.,P. Schaper ,and L.R. Peterson 1982. Concentrated Gram stain smear prepared with a cytospin centrifuge. J.clin. Microbiol.16:1052-1056
7. Thorpe, J.E., R.P.Banghman,P.T. Frame,T.A. Wessler,and J.L. Staneck.1987.Bronchoalveolar lavage for diagnosing acute bacterial pneumoniae.J.Infect.Dis.155:855-861
8. Brown,M.S.,and T.C. Wu. 1986. The Gram stain morphology of fungi, mycobacteria, and Pneumocytis carinii. J.Med Techno l3:495-499
9. Washington, J.A.1986.Rapid diagnosis by microscopy. Clin. Microbiol. Newsl.8:135-137
10. Lamanna and Mallette, 1965, Basic Bacteriology, 3rd ed., Williams and Wilkins Co., Baltimore.
11. Salton, 1964, The Bacterial Cell Wall, Elsevier, Amsterdam.



Storage temperature

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Revision 01/2022

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User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications.The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory,diagnostic, research or further manufacturing use only, unless otherwise specified.Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

Gram's Iodine

S013

Intended use

Grams's Iodine is used as mordant in Gram's staining method.

Composition**

Ingredients

Iodine	1.0 ml
Potassium iodide	2.0 ml
Distilled water	300.0 ml

**Formula adjusted, standardized to suit performance parameters

Directions

1. Prepare a thin smear on clear, dry glass slide.
2. Allow it to air dry and fix by gentle heat.
3. Flood with Gram's Crystal Violet (S012) for 1 minute. (If over staining results in improper decolourization of known gram-negative organisms, use less crystal violet).
4. Wash with tap water.
5. Flood the smear with Gram’s Iodine (S013). Allow it to remain for 1 minute.
6. Decolourize with Gram's Decolourizer (S032) until the blue dye no longer flows from the smear. (Acetone may be used as a decolourizing agent with caution, since this solvent very rapidly decolourized the smear).
7. Wash with tap water.
8. Counter stain with 0.5% w/v Safranin (S027) for 20 seconds and rinses off with water.
9. Wash with tap water.
10. Allow the slide to air dry or blot dry between sheets of clean bibulous paper and examine under oil immersion objective.

Principle And Interpretation

The Gram stain is a differential staining technique most widely applied in all microbiology disciplines laboratories. It is one of the most important criteria in any identification scheme for all types of bacterial isolates. Different mechanisms have been proposed to explain the gram reaction. There are many physiological differences between gram-positive and gram negative cell walls Ever since Christian Gram has discovered Gram staining, this process has been extensively investigated and redefined In practice, a thin smear of bacterial cells is stained with crystal violet, then treated with an iodine containing mordant to increase the binding of primary stain A decolourizing solution of alcohol or acetone is used to remove the crystal violet from cells which bind it weakly and then the counterstain (like safranin) is used to provide a colour contrast in those cells that are decolourized. Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50–90% of cell envelope), and as a result are stained purple by crystal violet, whereas gram-negative bacteria have a thinner layer (10% of cell envelope), so do not retain the purple stain and are counter-stained pink by safranin. In a properly stained smear by gram staining procedure, the gram-positive bacteria appear blue to purple and gram negative cells appear pink to red.

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Type of specimen

Any isolated colony on primary or subculture plates can be isolated from following specimens. Clinical specimen: Blood, urine, CSF, pus, wounds, lesions, body tissues, sputum etc. From environment: Air, water, soil, sludge, waste water, food, dairy samples etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.
Generally, the smear is made in laboratory; however, when there is a concern that transport will be delayed or that the preservation for culture will alter the specimen, prepare smear and submit slides to the laboratory.

Warning and Precautions

In Vitro diagnostic use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Use results of Gram stains in conjunction with other clinical and laboratory findings. Use additional procedures (e.g., special stains, inclusion of selective media, etc.) to confirm findings suggested by gram-stained smears.
2. Proper smear preparation is key to obtaining good gram staining results. Avoid excessive material or thick smears which may interfere with the passage of light and lead to distortion of images.
3. Overheating slides during heat fixation can distort the appearance of the organisms.
4. Only fresh cultures and specimens should be gram stained since cell wall integrity of older cells may give improper gram staining characteristics. Gram positive organisms that have lost cell wall integrity because of old age or antibiotic treatment may appear pink.
5. The decolorization step is the most important step in the gram-staining process. Over decolorization results in an abundance of bacteria that appear gram negative, while under decolorization results in too many bacteria that appear to be gram-positive.
6. The procedure given is based on an ideal thin smear of cells. Staining and decolorization times may vary depending on the sample and its thickness.
7. False Gram stain results may be related to inadequately collected specimens or delay in transit.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature

Quality Control

- **Appearance :** Yellow to dark brown coloured solution.
- **Clarity :** Clear without any particles.

- **Microscopic Examination :** Gram staining is carried out where Gram's Iodine is used as one of the stains and staining characteristics of organisms are observed under microscope by using oil immersion lens.
- **Results :** Gram-positive microorganisms : violet
  Gram-negative microorganims : pinkish red

Storage and Shelf Life

Store between 10 - 30 °C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques .

Reference

1. Downes F. P. and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
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3. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
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6. Shanhooltzer, C. J., P. Schaper ,and L.R. Peterson 1982 .Concentrated Gram stain smear prepared with a cytospin centrifuge. J.clin. Microbiol.16:1052-1056
7. Thorpe, J.E., R.P.Banghman,P.T. Frame,T.A. Wessler,and J.L. Staneck.1987.Bronchoalveolar lavage for diagnosing acute bacterial pneumoniae .J.Infect.Dis.155:855-861
8. Brown,M.S.,and T.C. Wu. 1986. The Gram stain morphology of fungi, mycobacteria, and Pneumocytis carinii. J.Med .Techno l3:495-499
9. Washington, J.A.1986.Rapid diagnosis by microscopy. Clin.Microbiol. Newsl.8:135-137
10. Lamanna and Mallette, 1965, Basic BActeriology,3rd ed.,Williams and Wilkins Co.,Baltimore.
11. Salton,1964,The Bacterial cell Wall, Elsevier, Amsterdam.

Storage temperature

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Cloxacillin COX 5 mcg SD075

Cloxacillin COX 5 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

Composition

*Ingredients	Concentration
Cloxacillin	5 mcg/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "COX 5" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
S.aureus (25923)	18-30

Storage and Shelf-life:

On receipt discs should always be stored at -20°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

*** Not for Medicinal Use**

Revision : 1 / 2012

CE

Netillin (Netilmicin Sulphate) NET 10 mcg SD085

Netillin (Netilmicin Sulphate) NET 10 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

Composition

*Ingredients	Concentration
Netillin (Netilmicin Sulphate)	10 mcg/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "NET 10" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
E. coli (25922)	17-25
S.aureus (25923)	20-29
P.aeruginosa (27853)	13-19

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :

Use following media to carry out susceptibility test

For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)

For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)

For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood

For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

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Storage temperature

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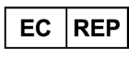

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Revision : 02 / 2022

Miconazole MIC 30 mcg SD273

Miconazole MIC 30 mcg discs are used for antimicrobial susceptibility testing of fungal cultures

Composition

*Ingredients	Concentration
Miconazole	30 mcg/disc

Susceptibility Test Procedure:

Preparation of Inoculum:

1. Inoculum is prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on Sabouraud Dextrose Agar (M063) and incubated at 35 ± 2°C. Colonies are suspended in 5ml of sterile 0.85% Saline.
2. Vortex the resulting suspension and adjust the turbidity to yield 1 x 10^6 - 5 x 10^6 cells /ml (i.e. 0.5 McFarland standard).

Test Procedure:

1. Prepare plates with Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye (M1825) for carrying out susceptibility of antifungal discs. The medium in the plates should be sterile and have a depth of about 4 mm.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum (turbidity so adjusted, as to obtain semi confluent growth on the petri plate) and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
3. Apply the discs using aseptic technique. Deposit the discs with centers at least 24 mm apart.
4. Invert the plates and place in an incubator set to 35 ± 2°C within 15 minutes after the discs are applied.
5. Examine each plate after 20 - 24 hours of incubation. If plate was satisfactorily streaked the resulting zones of inhibition will be uniformly circular and there will be a semi-confluent lawn of growth. Read at 48 hours only when insufficient growth is observed after 24 hours incubation.

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "MIC 30" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye after 24-48 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
C.albicans (90028)*	22-26
C.parapsilosis (22019)*	13-17
C.tropicalis (750)*	14-20
C.krusei(6528)*	19-26
C.albicans(10231)	20-27
S.cerevisiae(9763)	20-28

* = Q.C. Strains recommended by CLSI

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guidelines-Second edition Vol.29 No.17, August- 2009 CLSI document M44-A2. For more details refer to this volume

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Revision : 1 / 2012

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MASTDISCS®

Leading the field with a complete solution for AST and Identification disc testing

- Comprehensive range
- Premium quality products
- Compatible with EUCAST and CLSI standards
- Bespoke service available

Mast Group Ltd., has been a manufacturer of antibiotic susceptibility test products since 1957, and continues to be at the forefront of developments in this field. With an ever-expanding portfolio of antibiotic susceptibility, combination and identification discs, Mast Group Ltd. supplies the most comprehensive range available worldwide. Mast Group Ltd. also offers a range of services including **mast**pharma® development and **mast**pharma® stability for the evaluation of novel antimicrobial compounds for the pharmaceutical industry.

Disc diffusion is still the most popular method for determining bacterial antibiotic susceptibility in the treatment of infectious disease, as highlighted by the variety of international standardised procedures including The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and The Clinical and Laboratory Standards Institute (CLSI). From January 2016 The British Society for Antimicrobial Chemotherapy (BSAC) guidelines were superseded by EUCAST. Mast Group Ltd. continues to support laboratories with the supply of BSAC concentrations until January 2020.

MASTDISCS® are available as an extensive range of test discs presented in dispensing cartridges or vials, with quality assurance guaranteed in accordance to standardised test protocols as issued by the above national and international organisations.

Disc Diffusion Susceptibility Testing

Order No	Product		Packsize
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MASTDISCS®	**in Cartridges (5 × 50 discs per pack)**		
STOCKCART	Stock Susceptibility Cartridge Discs of a single type	EUCAST Compliant	1 pack
FUNGCART	Stock Antifungal Cartridge Discs of a single type		1 pack
SPECIALCART	Cartridge Discs made to special order*		Min 18 packs
VOLCART	Cartridge Discs made to special order*	EUCAST Compliant	Min 98 packs
MASTDISCS®	**(100 discs per vial)**		
STOCKDISC	Stock Susceptibility Discs of a single type		1 vial
FUNGDISC	Stock Antifungal Discs of a single type		1 pack
SPECIALDISC	Discs made to special order*		Min 22 packs
VOLDISC	Discs made to special order*		Min 97 packs
TOOL/C	*Set up charge for Special Discs in vials or cartridges A one off charge for customisation of each new specification		per new specification

Disc Diffusion Susceptibility Testing

MASTDISCS® *AST*

ANTIBIOTIC SUSCEPTIBILITY DISCS IN CARTRIDGES - STOCK RANGE

Description **Antibiotic & Content µg per disc** (unless otherwise stated)	**STANDARD**			**Order Code** **Cartridges** **5 × 50 discs**
	BSAC	**EUCAST**	**CLSI**	
Amikacin 30	✓	✓	✓	AK30C
Amoxicillin 2	✓	-	-	A2C
Amoxicillin 10	✓	-	-	A10C
Amoxicillin 25	✓	-	-	A25C
Amoxicillin/clavulanic acid 2-1	✓	✓	-	AUG3C
Amoxicillin/clavulanic acid 20-10	✓	✓	✓	AUG30C
Ampicillin 2	✓	✓	-	AP2C
Ampicillin 10	✓	✓	✓	AP10C
Ampicillin 25	✓	-	-	AP25C
Ampicillin/Sulbactam 10-10	-	-	✓	SAM20C
Azithromycin 15	✓	✓	✓	ATH15C
Aztreonam 30	✓	✓	✓	ATM30C
Bacitracin 10 units	-	-	-	BA10C
Carbenicillin 100 ◆	✓	-	✓	PY100C
Cefachlor 30	✓	✓	✓	CFC30C
Cefadroxil 30	✓	✓	-	CDX30C
Cefalexin 30	✓	-	-	CFX30C
Cefalothin 30	✓	-	✓	KF30C
Cefamandole 30	✓	-	✓	CMD30C
Cefazolin 30	-	-	✓	CZ30C
Cefepime 30	✓	✓	✓	CPM30C
Cefixime 5	✓	✓	✓	CFM5C
Cefoperazone 30	✓	-	-	CPZ30C
Cefotaxime 5	✓	✓	-	CTX5C
Cefotaxime 30	✓	-	✓	CTX30C
Cefoxitin 10	✓	-	-	FOX10C
Cefoxitin 30	✓	✓	✓	FOX30C
Cefpodoxime 10	✓	✓	✓	CPD10C
Cefradine 30	✓	-	-	CRD30C
Ceftaroline 5	-	✓	-	CPT5C
Ceftaroline 30	-	-	✓	CPT30C
Ceftazidime 10	-	✓	-	CAZ10C
Ceftazidime 30	✓	✓	✓	CAZ30C
Ceftazidime/avibactam 10-4	-	✓	-	CZA14C
Ceftazidime/avibactam 30-20	-	-	✓	CZA50C
Ceftibuten 30	-	✓	✓	CFB30C
Ceftolozane/tazobactam 30-10	-	✓	✓	CT40C
Ceftriaxone 5	✓	-	✓	CRO5C
Ceftriaxone 30	✓	✓	✓	CRO30C
Cefuroxime 5	✓	-	-	CXM5C
Cefuroxime 30	✓	✓	✓	CXM30C
Chloramphenicol 10	✓	-	-	C10C
Chloramphenicol 30	✓	✓	✓	C30C
Ciprofloxacin 1	✓	-	-	CIP1C
Ciprofloxacin 5	✓	✓	✓	CIP5C
Clarithromycin 2	✓	-	-	CLA2C
Clarithromycin 5	✓	-	-	CLA5C
Clarithromycin 15	-	✓	✓	CLA15C
Clindamycin 2	✓	✓	✓	CD2C
Delafloxacin 5 NEW	-	-	✓	DLX5C
Doripenem 10	-	✓	✓	DOR10C
Doxycycline 30	✓	-	✓	DXT30C
Eravacycline 20 NEW	-	✓	✓	ERV20C
Ertapenem 10	✓	✓	✓	ETP10C
Erythromycin 5	✓	-	-	E5C
Erythromycin 15	-	✓	✓	E15C
Florfenicol 30	-	-	-	FFC30

◆ Selected individually priced discs

Disc Diffusion Susceptibility Testing

MASTDISCS® *AST*

ANTIBIOTIC SUSCEPTIBILITY DISCS IN CARTRIDGES - STOCK RANGE

Description **Antibiotic & Content µg per disc** (unless otherwise stated)	**STANDARD**			**Order Code** **Cartridges** **5 × 50 discs**
	BSAC	**EUCAST**	**CLSI**	
Fosfomycin 200/Glucose-6-Phosphate 50	✓	-	✓	FOT200C
Fusidic Acid 10	✓	✓	-	FC10C
Gentamicin 10	✓	✓	✓	GM10C
Gentamicin 30	-	✓	-	GM30C
Gentamicin 120	-	-	✓	GM120C
Gentamicin 200	✓	-	-	GM200C
Imipenem 10	✓	✓	✓	IMI10C
Imipenem/Relebactam 10-25 Coming Soon	-	-	✓	IMR35C
Kanamycin 30	-	-	✓	K30C
Levofloxacin 1	✓	-	-	LEV1C
Levofloxacin 5	✓	✓	✓	LEV5C
Linezolid 10	✓	✓	-	LZD10C
Linezolid 30	-	-	✓	LZD30C
Mecillinam 10	✓	✓	✓	MEC10C
Meropenem 10	✓	✓	✓	MEM10C
Meropenem/Vaborbactam 20-10 NEW	-	✓	✓	MEV30C
Metronidazole 5	✓	-	-	MZ5C
Minocycline 30	✓	-	✓	MN30C
Moxifloxacin 1	✓	-	-	MFX1C
Moxifloxacin 5	✓	✓	✓	MFX5C
Mupirocin 5	✓	-	-	MUP5C
Mupirocin 20	✓	-	-	MUP20C
Mupirocin 200	-	✓	-	MUP200C
Nalidixic Acid 30	✓	✓	✓	NA30C
Neomycin 10	✓	-	-	NE10C
Neomycin 30	-	-	-	NE30C
Netilmicin 10	-	✓	-	NET10C
Netilmicin 30	✓	✓	-	NET30C
Nitrofurantoin 100	-	✓	-	NI100C
Nitrofurantoin 200	✓	-	-	NI200C
Nitrofurantoin 300	-	-	✓	NI300C
Nitroxoline 30	-	-	✓	NIB30C
Norfloxacin 2	✓	-	-	NOR2C
Norfloxacin 10	-	✓	✓	NOR10C
Novobiocin 5	-	-	-	NO5C
Ofloxacin 5	✓	✓	✓	OFX5C
Oxacillin 1	✓	✓	✓	OX1C
Pefloxacin 5	-	✓	-	PEF5C
Penicillin G 1 unit	✓	✓	-	PG1C
Penicillin G 10 units	-	-	✓	PG10C
Piperacillin 30	-	-	-	PRL30C
Piperacillin 75	✓	-	-	PRL75C
Piperacillin 100	-	-	✓	PRL100C
Piperacillin/tazobactam 30-6	-	✓	-	PTZ36C
Piperacillin/tazobactam 75-10	✓	-	-	PTZ85C
Piperacillin/tazobactam 100-10	-	-	✓	PTZ110C
Polymyxin B 300 units	-	-	-	PB300C
Quinupristin/dalfopristin 15	✓	-	✓	SYN15C
Rifampicin 2	✓	-	-	RP2C
Rifampicin 5	✓	✓	✓	RP5C
Spectinomycin 25	✓	-	-	SPC25C
Streptomycin 10	✓	-	✓	S10C
Streptomycin 300	-	✓	✓	S300C
Teicoplanin 30	✓	-	✓	TEC30C
Temocillin 30 ❇	-	-	-	TEM30C
Tetracycline 10	✓	-	-	T10C
Tetracycline 30	-	✓	✓	T30C

❇ Temocillin 30µl is included in CA-SFM standard

Disc Diffusion Susceptibility Testing

MASTDISCS® *AST*

ANTIBIOTIC SUSCEPTIBILITY DISCS IN CARTRIDGES - STOCK RANGE

Description **Antibiotic & Content µg per disc** (unless otherwise stated)	**STANDARD**			**Order Code** **Cartridges** **5 × 50 discs**
	BSAC	**EUCAST**	**CLSI**	
Ticarcillin 75	✓	-	✓	TC75C
Tigecycline 15	✓	✓	✓	TGC15C
Ticarcillin/clavulanic acid 75-10	✓	-	✓	TIM85C
Tobramycin 10	✓	✓	✓	TN10C
Trimethoprim 2.5	✓	-	-	TM2.5C
Trimethoprim 5	✓	✓	✓	TM5C
Trimethoprim/sulfamethoxazole 1.25-23.75	✓	✓	✓	TS25C
Vancomycin 30	-	-	✓	VA30C
Blank discs	-	-	-	BD0680W/C

MASTDISCS® *AST*

NON CLINICAL ANTIBIOTIC SUSCEPTIBILITY DISCS IN CARTRIDGES - STOCK RANGE

Description **Antibiotic & Content µg per disc** (unless otherwise stated)	**Order Code** **Cartridges** **5 × 50 discs**
Cefperazone/sulbactam 75-30	SPZ105C/NCE
Colistin Sulphate 10	CO10C/NCE
Colistin Sulphate 25	CO25C/NCE
Enrofloxacin 5	ENF5C/NCE

MASTDISCS® *AST*

SPECIALIST VETERINARY SUSCEPTIBILITY DISCS - STOCK RANGE

Description **Antibiotic & Content µg per disc** (unless otherwise stated)	**Order Code**	
	Cartridges **5 × 50 discs**	**Vials** **100 discs**
Cefquinone 30	CEQ30C/NCE	-
Gamithromycin 15	GAM15C/NCE	-
Pradofloxacin 5	PRA5/NCE	-
Marbofloxacin 5	MAR5/NCE	-
Tildipirison 60	TIP60/NCE	-
Tylosin 30	TY30C/NCE	TY30/NCE

Disc Diffusion Susceptibility Testing

Order No Product Mechanism Packsize (tests)

MASTDISCS® AST

ANTIBIOTIC SUSCEPTIBILITY DISCS IN VIALS - STOCK RANGE

Description Antibiotic & Content µg per disc (unless otherwise stated)	STANDARD BSAC	 EUCAST	 CLSI	Order Code Cartridges 5 × 50 discs
Amikacin 30	✓	✓	✓	AK30
Bacitracin 8 units	-	-	-	BA8
Bacitracin 10 units	-	-	-	BA10
Cefpodoxime 10	✓	✓	✓	CPD10
Chloramphenicol 10	✓	-	-	C10
Chloramphenicol 30	✓	✓	✓	C30
Gentamicin 10	✓	✓	✓	GM10
Meropenem 10	✓	✓	✓	MEM10
Metronidazole 2.5	-	-	-	MZ2.5
Metronidazole 5	✓	-	-	MZ5
Nalidixic Acid 30	✓	✓	✓	NA30
Novobiocin 5	-	-	-	NO5
Oxacillin 1	✓	✓	✓	OX1
Penicillin G 1 unit	✓	✓	-	PG1
Penicillin G 2 units	-	-	-	PG2
Rifampicin 5	✓	✓	✓	RP5
Tylosin 30	-	-	-	TY30
Vancomycin 5	✓	✓	-	VA5
Blank discs	-	-	-	BD0638W

MASTDISCS® AST

ANTIFUNGAL SUSCEPTIBILITY DISCS IN CARTRIDGES AND VIALS - STOCK RANGE

Description Antibiotic & Content µg per disc (unless otherwise stated)	Order Code Cartridges 5 × 50 discs	 Vials 100 discs
Amphotericin B 20	AMB20C	-
Clotrimazole 10	CTM10C	-
Econazole 10	ECN10C	-
Fluconazole 10	FCN10C	-
Fluconazole 25	FCN25C	FCN25
Flucytosine 1	FY1C	-
Ketoconazole 10	KCA10C	-
Miconazole 10	MCL10C	-
Nystatin 100	NY100C	-

Disc Diffusion Susceptibility Testing

Order No	Product	Mechanism	Packsize (tests)

MASTDISCS® *Combi*

COMBINATION DISC SETS FOR THE DETECTION OF ANTIBIOTIC RESISTANCE

D52C	Extended Spectrum ß Lactamase Set	ESßL	50 tests
D62C	Cefotaxime 30 & Cefotaxime 30/Clavulanic Acid 10	ESßL	150 tests
D63C	Cefepime 30 & Cefepime 30/Clavulanic Acid 10	ESßL	150 tests
D64C	Ceftazidime 30 & Ceftazidime 30/Clavulanic Acid 10	ESßL	150 tests
D66C	Cefpodoxime 10 & Cefpodoxime 10/Clavulanic Acid 1	ESßL	150 tests
D67C	Extended Spectrum ß Lactamase Set (CPD10)	ESßL	50 tests
D68C	AmpC & ESBL Detection Set	AmpC/ES?L	50 tests
D69C	AmpC Detection Set	AmpC	50 tests
D72C	AmpC, ESßL & Carbapenemase Detection Set	AmpC/ESßL/Carbapenemase	50 tests
D73C	MAST® *Carba plus*	MBL/KPC/OXA	50 tests

Additional carbapenemase screening and identification tests

PACE-ID NEW	Colorimetric test for the rapid detection of carbapenemase producing *Pseudomonas* spp., *Acinetobacter* spp.and Enterobacterales.	48 tests
D71C	MAST® CAT-ID - For presumptive identification of carbapenemase production	250 tests
D74	MAST® ICT - screening test for the detection of carbapenemase production in Enterobacterales, *Pseudomonas* and *Acinetobacter* spp.	25 tests
TEM30C	To aid presumptive identification of OXA-48 when used with D70C	5 × 50 discs

MASTDISCS® *AST*

PENICILLIN DISCS

For detecting the emergence of penicillin resistance. (vials of 100 discs or 5 × 50 discs in cartridges)

PG1	Penicillin G	1 unit (vials)	1 vial
PG1C	Penicillin G	1 unit (cartridges)	1 pack

HIGH CONTENT AMINOGLYCOSIDE DISCS

The following discs are available to special order and subject to 6 weeks lead time.
For detecting high level aminoglycoside resistance. (vials of 100 discs or 5 × 50 discs in cartridges)

GM500C/NCE	500µg	Min order
K1000C	1000µg	18 packs
K1000	1000µg	Min order 22 vials

Disc Diffusion Susceptibility Testing

Order No	Product	Packsize

DISCMASTER DISPENSER

Robust and reliable antimicrobial cartridge disc dispensers designed for use with MASTDISCS®
Antimicrobial Susceptibility Test Cartridges.

MDD65	MAST® DISCMASTER Dispenser - 6 place	1
SILICA63	Silica Gel Capsule for MAST® DISCMASTER	4
SHD5	Single Cartridge Hand Dispenser	5
CANISTER	Canister for MDD64 models and below	1
CANISTER63	Canister for MDD65 MAST® DISCMASTER Dispenser	1

ZT147 v6.0 CEA 07/19

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MASTDISCS®

Antimicrobial Susceptibility Test Discs

MASTDISCS®**.** An extensive range of individual antimicrobial susceptibility test discs in glass vials and plastic dispensing cartridges.

FOR IN VITRO DIAGNOSTIC USE ONLY

Contents: 100 discs in a vial or a pack of 5 cartridges each cartridge containing 50 discs.

Formulation*
6mm diameter filter paper discs printed with an appropriate identification code of letters and/or numbers and impregnated with accurately assayed quantities of antimicrobial agent.

Storage and shelf life
Store at 2 to 8°C in the containers provided until the expiry date shown on the pack label. Allow to equilibrate to room temperature before opening. Return to the refrigerator promptly after use.

Precautions
For *in vitro* diagnostic use only. Observe approved biohazard precautions and aseptic techniques. To be used only by adequately trained and qualified laboratory personnel. Sterilise all biohazard waste before disposal. Refer to Product Safety Data sheet.

Materials required but not provided
Standard microbiological supplies and equipment such as loops, MAST® culture media, swabs, applicator sticks, incinerators and incubators, etc., as well as serological and biochemical reagents and additives such as blood. Suitable interpretive criteria from standardised reference methods. MAST® DiscMaster Dispenser.

Procedure
MASTDISCS® should be used according to an appropriate standardised susceptibility test method. Various alternative methodologies are available and **MAST**DISCS® are compatible with these.

1. Remove the **MAST**DISCS® container from the refrigerator and allow to equilibrate to room temperature before opening.
2. Using a sterile needle or forceps, transfer each disc required onto the surface of a suitable plate of MAST® susceptibility test medium e.g. Mueller-Hinton Agar (DM170) dried and pre-inoculated with test organism according to the methodology followed.
3. If using **MAST**DISCS® in cartridges, load each cartridge required into a MAST® DiscMaster Dispenser.
4. Place the loaded DiscMaster Dispenser over the Petri Dish and dispense discs (see DiscMaster instructions for full details).
5. Incubate plates in air at 35 to 37°C for 18 to 24 hours (or alternative incubation conditions according to the methodology followed).
6. Measure (to the nearest whole mm) and record the diameter of any zones of inhibition that are observed around the antibiotic impregnated discs.

Interpretation of results
Interpret measured zones of inhibition by reference to published tables of critical zone diameter breakpoints provided by appropriate authorities and classify test isolate as Susceptible (S), Intermediate (I) or Resistant (R).

Quality control
Check for signs of deterioration. Quality control must be performed with at least one organism to demonstrate a correct susceptibility pattern. Do not use the product if the reactions with the control organisms are incorrect. The list below illustrates a range of performance control strains which the end user can easily obtain.

Test Organisms	
Escherichia coli ATCC® 25922	Correct susceptibility pattern*
Pseudomonas aeruginosa ATCC® 27853	Correct susceptibility pattern*
Staphylococcus aureus ATCC® 25923	Correct susceptibility pattern*

*See appropriate quality control table

Limitations
Any deviation from the prescribed method may produce incorrect results.
The latest published version of the method used should be consulted for complete details of test procedures and interpretive criteria.

References
Bibliography available on request.

IFU112 GB 01/18 V4

*Formulation may change to meet performance criteria

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MAST® CARBA PAcE

Intended Use

PACE-ID. For the rapid detection of carbapenemase producing Enterobacterales, *Pseudomonas*, OXA 48 and 23-like enzyme production in *Acinetobacter.*

FOR IN VITRO DIAGNOSTIC USE ONLY

Contents

- **Vial PEL.** Freeze dried pellet* - 4 vials containing inhibitors and lysis components, each designed for 12 tests.
- **Vial RB.** Reconstitution buffer* - 4 vials containing chromogenic indicator resuspension buffer, each sufficient for 12 tests.
- Plastic 0.5 ml tubes, sufficient for 48 tests.

Storage and shelf life

Store at 2 to 8°C in the containers provided until the expiry date shown on the pack label. Allow to equilibrate to room temperature before opening. Once reconstituted, test solution stored at 2 to 8°C, must be used within 4 weeks.

Precautions

For *in vitro* diagnostic use only. Observe approved biohazard and aseptic techniques. To be used by only trained and qualified laboratory personnel. Sterilise all biohazard waste before disposal. Refer to product safety data sheets.

Materials required but not provided

Standard microbiological supplies and equipment such as loops, MAST Group Ltd. culture media, table top vortexes, pipettes, incinerators and incubators, etc.

Procedure

1. Reconstitute the pellet by tipping the entire contents of vial RB into vial PEL.
2. Allow the pellet to fully dissolve at room temperature for 1 minute and mix contents by gently vortexing for 10 seconds. Reconstituted solution should be yellow, if the solution is any other colour do not use.
3. Dispense 250µl of reconstituted solution into the tubes provided. One tube per test.
4. Using a pure, fresh culture of the test organism, take an approximate 1 to 5µl loopful of organism, and add to the tube containing test solution. Mix well by vortexing for 20 seconds.
   Note: to obtain distinct results, ensure that the bacterial resuspension is similar to the turbidity of a 3.0 to 3.5 McFarland standard; Approx. 10^9 CFU/ml.
5. Incubate at 35±1°C for 10 minutes.
6. Record the colour of the test solution immediately or up to 20 minutes after incubation.

Please refer to corresponding steps on the image page.

Interpretation of results

If a colour change is recorded; from yellow to orange/red, record the organism as demonstrating carbapenemase activity.
If no colour change is recorded; solution remains yellow, record the organism as negative for carbapenemase activity.

Quality control

Check for signs of deterioration. Quality control must be performed with at least one organism to demonstrate a positive reaction and another to demonstrate a negative reaction. Do not use the product if the reactions with the control organisms are incorrect. The list below illustrates a range of performance control strains which the end user can easily obtain.

Test Organism	Result
Acinetobacter baumanii NCTC 13301	Orange/Red Carbapenemase positive
Pseudomonas aeruginosa NCTC 13437	Orange/Red Carbapenemase positive
Acinetobacter lwoffi ATCC® 15309	Remains Yellow Carbapenemase negative
Pseudomonas aeruginosa ATCC® 25668	Remains Yellow Carbapenemase negative
Klebsiella pneumoniae NCTC 13438	Orange/Red Carbapenemase positive

Limitations

1. Colonies isolated from indicator media are not recommended.
2. This product only detects the presence of a carbapenemase, differentiation can be carried out by using a suitable genotypic or phenotypic test (for example **MAST**DISCS® *Combi Carba Plus*; D73C).
3. Some GES-type carbapenemases might be difficult to detect.
4. To avoid potentially erroneous results, ensure that equipment used for testing is free of contamination.
5. Test results must be recorded within 20 minutes following the initial 10 minute incubation.
6. Results obtained with this kit must be considered alongside other clinically relevant data when diagnosing an infection.

References

Bibliography available on request.

Acknowledgement

HMRZ compound used in this product was developed by Dr. Hideaki Hanaki of Kitasato, Institute, Japan.

IFU159 GB 05/19 V2

*Formulation may change to meet performance criteria

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Mast Group

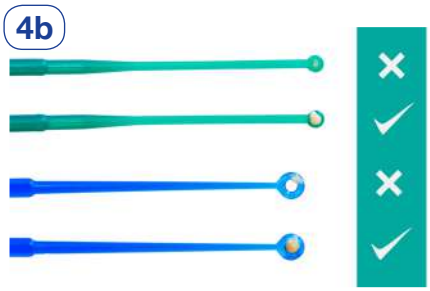
1

2

3

4a

4b



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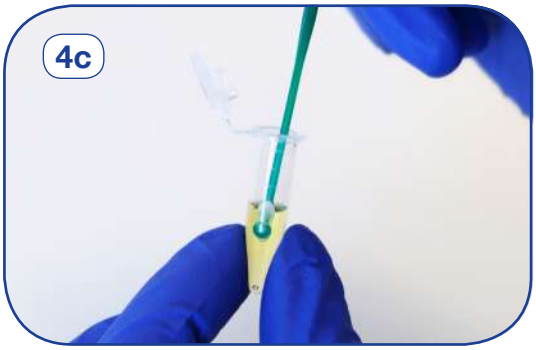

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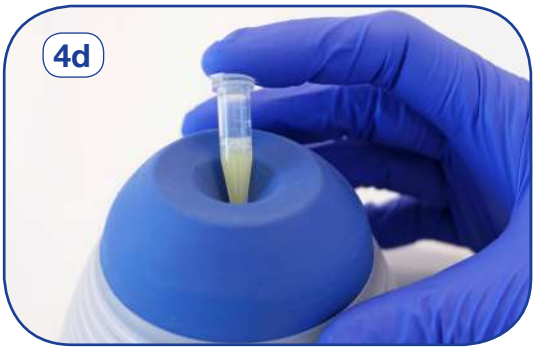

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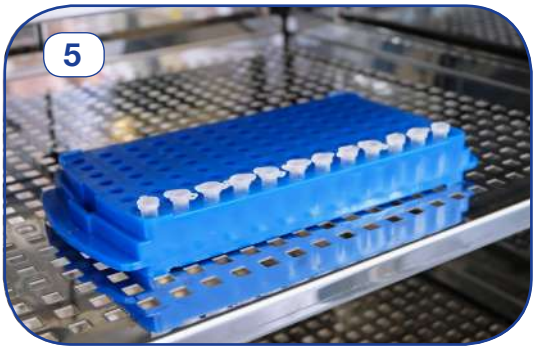

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SELF DECLARATION OF CONFORMITY

We declare under our sole responsibility in accordance with MHRA account number 0000009546 that the following CE marked products:

GMDN Term	TCS product code and description
Culture medium base ingredient IVD	Horse Blood Defbrinated HB001, HB001B, HB001L, HB002, HB002B, HB003B, HB004B, HB005B, HB008B, HB009B, HB011B, HB012, HB014B, HB015B, HB028B, HB029, HB030, HB031, HB032, HB033, HB034, HB035, HB035B, HB035T, HB051B, HB057B, HB058B, HB061, HB062B, HB063B, HB064B, HB065, HB068B, HB071B, HB074B, HB075B, HB077B, HB079, HB079B, HB095B, HB099B Horse Blood Lysed HB024, HB025, HB026, HB036, HB037, HB038, HB038B, HB052B, HB056, HB056B, HB059, HB063B, HB069B, HB070B, HB072B, HB080B, HB093B, HB098L Horse Blood in EDTA HB073, HB075, HB076 Horse Blood Oxalated HB039, HB040, HB041, HB108

conform to the relevant provisions of the In-vitro Diagnostic Medical Devices Directive 98/79/EC and The Medical Devices Regulations 2002 (SI 2002 No.618) and The Medical Devices (Amendment) Regulations 2003 (SI 2003 No.1697) for in-vitro diagnostic medical devices.

This declaration is made on the basis of meeting the requirements of Annexes I and III of the In-Vitro Diagnostic Medical Devices Directive 98/79/EC and continued maintenance of an approved Quality Management System meeting the requirements of ISO 9001, as certified by BSi, certificate number FS 28907.

Signed by: Sue Brown Date: February 2021

Name: Sue Brown
Position: Quality Assurance & Regulatory Affairs Manager

Signed by: Lynda Preston Date: February 2021

Name: Lynda Preston
Position: Managing Director

1

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Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of total IgE in human serum or plasma

Total IgE EIA

Catalogue number REF **K200**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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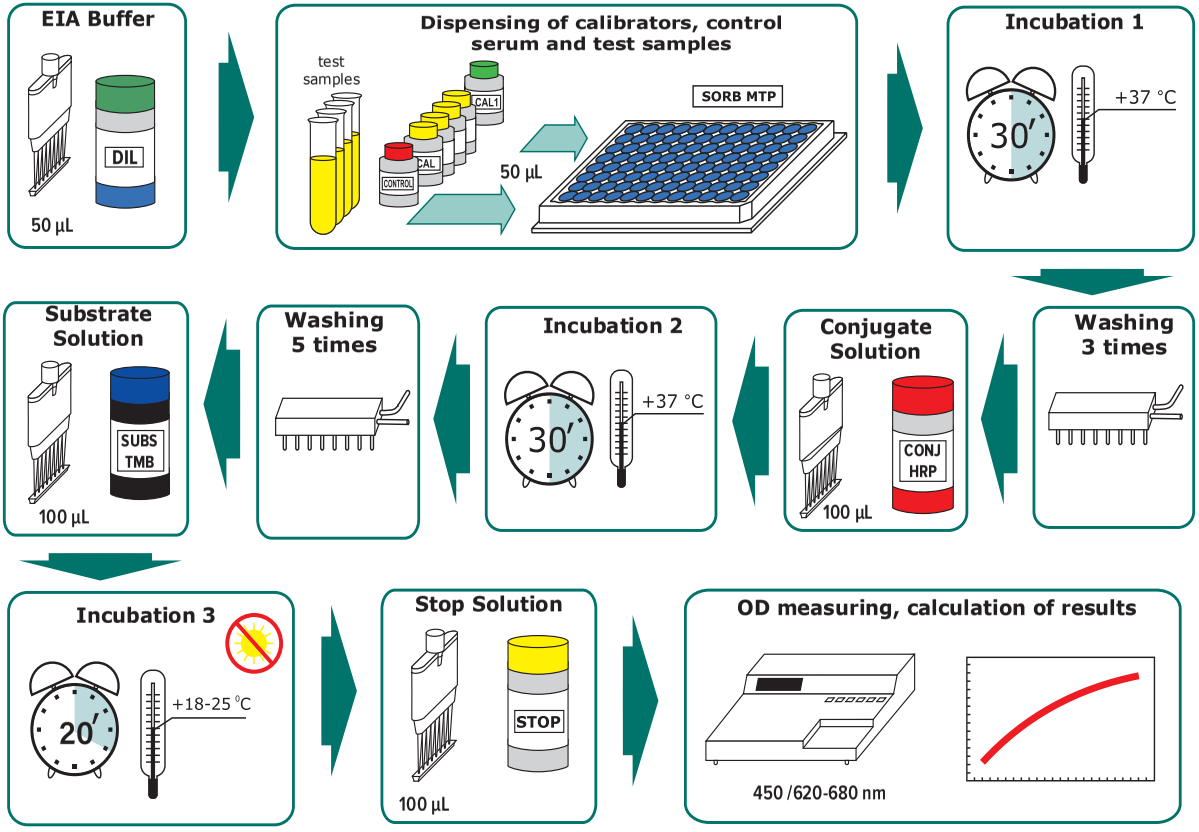
ASSAY PROCEDURE



K200

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of total IgE in human serum or plasma
Total IgE EIA

1. INTENDED USE

The Total IgE EIA kit is an enzyme immunoassay, intended for the quantitative determination of total IgE concentration in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Total immunoglobulin E (IgE) serum level is widely reported as the laboratory marker of atopic diseases such as atopic asthma, atopic dermatitis, and pollenosis. An atopic (IgE-dependent) mechanism can also underlie gastroenterocolitis, urticaria, other forms of vasculitis (including systemic), cholecystitis, vulvovaginitis, and cystitis. Part of the drug allergy (mainly to penicillin and protein drugs) also develops according to the IgE-dependent mechanism. In all of the conditions listed above, the production of high titers of specific IgE antibodies can lead to an increase in the level of total IgE in the serum. A particularly high level of total IgE is characteristic of atopic dermatitis. In addition to atopic diseases, total serum IgE is significantly increased in parasitic infestations and mycoses (especially systemic), rarely in systemic autoimmune diseases and immunodeficiency states (especially in hyper-IgE syndrome), as well as in mastocytosis (mast cell tumor) and extremely rare IgE-myeloma. A decrease in the level of total IgE in serum (below 15 IU/ml in adults) is a rare and little-studied phenomenon described in hypogammaglobulinemia, some autoimmune diseases, ulcerative colitis, and primary biliary cirrhosis.

3. TEST PRINCIPLE

The determination of the total IgE is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to human IgE. Second antibodies – rabbit polyclonal antibodies to IgE conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes tree stages of incubation:

- during the first stage the total IgE from the specimen is captured by the monoclonal antibodies coated onto the microwell surface;
- during the second stage horseradish peroxidase-conjugated with rabbit polyclonal antibodies bind to free epitopes of immobilized total IgE, fixed in the formed at the previous stage complexes;
- during the third stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured total IgE in the serum specimen (plasma).

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of total IgE in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P200Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to total IgE; ready to use
C200Z	CAL 1	**Calibrator C1**	0.8 mL	1	Solution based on phosphate buffer, free of total IgE, with preservative, ready to use (yellow liquid)
C200Z	CAL 1-5	**Calibrators**	0.8 mL	5	Solutions based on phosphate buffer, containing 50; 200; 500 and 1000 IU/mL of total IgE, ready to use (red liquids)
Q200Z	CONTROL	**Control serum**	0.8 mL	1	Solution based on human serum, containing of known total IgE content, with preservative, ready to use (colourless liquid)
T200Z	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of rabbit polyclonal antibodies to human total IgE conjugated to the horseradish peroxidase; ready to use (red liquid)
S011Z	DIL	**EIA Buffer**	14 mL	1	Buffer solution with detergent and preservative, ready to use (blue liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	30 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (3 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

- microplate photometer with 450 nm wavelength or 450\620-680 nm;
- dry thermostat for 37 °C±2 °C;
- automatic plate washer (optional);
- micropipettes with variable volume, range volume 5-1000 µL;
- graduated cylinder of 1000 mL capacity;
- distilled or deionized water;
- timer;
- vortex mixer;
- disposable gloves;
- absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below; do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The Total IgE EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The Total IgE EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- EIA Buffer, Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing solution preparation

Add the contents of the 30 mL washing solution concentrate vial to 750 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the washing solution concentrate, mL	2.5	5	7.5	10	12.5	15	17.5	20	22.5	25	27.5	30
Volume of water, mL	62.5	125	187.5	250	312.5	375	437.5	500	562.5	625	687.5	750

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 12 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-5) and 2 wells for Control Serum (Q)).

10.2 Dispense **50 µL of EIA Buffer** to all wells.

10.3 Dispense **50 µL of Calibrators and Control Serum as well as 50 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP3	SAMP3	SAMP11	SAMP11						
B	CAL2	CAL2	SAMP4	SAMP4	SAMP12	SAMP12						
C	CAL3	CAL3	SAMP5	SAMP5	SAMP13	SAMP13						
D	CAL4	CAL4	SAMP6	SAMP6	SAMP14	SAMP14						
E	CAL5	CAL5	SAMP7	SAMP7	SAMP15	SAMP15						
F	Q	Q	SAMP8	SAMP8								
G	SAMP1	SAMP1	SAMP9	SAMP9								
H	SAMP2	SAMP2	SAMP10	SAMP10								

10.4 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **30 minutes at +37 °C**.

10.5 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 3 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.6 Add **100 µL of Conjugate Solution** to all wells.

10.7 Cover strips with a plate sealing tape and incubate for **30 minutes at +37 °C**.

10.8 At the end of the incubation period, aspirate and wash each well 5 times as described in 10.5.

10.9 Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 20 minutes**.

10.10 Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.11 Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the stop solution. Set photometer blank on CAL1.

10.12 Plot a calibration curve in linear coordinates: (x) is the concentration of total IgE in the Calibrators IU/mL, (y) – OD versus concentration of total IgE (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.13 Determine the corresponding concentration of total IgE in tested samples from the calibration curve.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

12.1. Therapeutical consequences should not be based on the results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for total IgE. Based on data obtained by XEMA LLC, the following normal range is recommended (see below).

NOTE: values of total IgE concentrations in the tested samples that are below the LoD (3 IU/mL) and also exceed the value of the upper calibrator (1000 IU/mL) should be provided in the following form : «the total IgE concentration of tested sample X is «lower than 3 IU/mL» or «higher than 1000 IU/mL».

12.2. The calibrators concentration values of the Total IgE EIA kit are expressed in IU/mL. To calculate concentrations in ng/mL, the received concentration value in IU/mL shall be multiplied by 2.4.

1 IU/mL = 2.4 ng/mL.

Sex, age	Units, IU/mL		Units alternative, ng/mL	
	Lower limit	Upper limit	Lower limit	Upper limit
< 6 months	-	12	-	28.8
6-12 months	-	30	-	72.0
1-3 yrs	-	45	-	108.0
4-6 yrs	-	70	-	168.0
7-9 yrs	-	90	-	216.0
10-15 yrs	-	120	-	288.0
>15 yrs	-	130	-	312.0

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, IU/mL	CV, %
1	10.6	4.33
2	116.2	5.47

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, IU/mL	CV, %
1	12.5	8.36
2	113.4	1.47

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, IU/mL	Concentration2, IU/mL	Concentration3, IU/mL	CV, %
1	12.7	13.3	12.3	3.66
2	115.5	117.8	115.1	1.25

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known total IgE concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 50-1000 IU/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest total IgE concentration in the serum or plasma sample that is detected by the Total IgE EIA kit is no lower than 3 IU/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for Total IgE EIA kit is 50IU/mL.

13.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of total IgE with other analytes is shown in the table:

Analyte	Concentration, IU/mL	Cross-reactivity, %
IgA	1000	Not detected
IgM	1000	Not detected
IgG	1000	Not detected

14. REFERENCES

1. Zetterstrom and Hohansson S.G.O. Allergy 1981; 36:537.
2. Buckley R. H. Immunopharmacology of Allergic Disease 1979; 117.
3. Michel f. B., Bousquet J. and Greilier P. J. Allergy Clin. Immunol. 1980; 64:422.
4. Ishizaka T. Ann Allergy 1982; 48: 313.
5. Kulczyski A. Jr. J. Allergy Clin. Immunol. 1981; 68:5.
6. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
7. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
8. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

XEMA

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

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CE IVD

zybio

Instructions for Use of Lipase (LPS) Kit (Colorimetric Method)

Package Specification

REF	Reagent	Systems
01.09.0B.01.EC.01	R1 30 mL × 3 R2 10 mL × 3 Calibrator 1 Level × 1.0 mL × 1 Control 2 Levels × 1.0 mL × 1	Zybio EXC200/220
01.09.0B.01.EC.02	R1 45 mL × 2 R2 15 mL × 2 Calibrator 1 Level × 1.0 mL × 1 Control 2 Levels × 1.0 mL × 1	Hitachi 7180 Zybio EXC400/420

Intended Use

In vitro test for the quantitative determination of the catalytic activity concentration of lipase (LPS) in human samples (serum or plasma). Clinically, it is used as the most important evaluation indicator for differential diagnosis of pancreatic diseases.

Summary

Lipases are glycoproteins with a molecular weight of 47000 Da. They are defined as triglyceride hydrolases which catalyze the cleavage of triglycerides to diglycerides with subsequent formation of monoglycerides and fatty acids. In addition to α-amylase, pancreatic lipases have for many years been undeniably the most important clinical chemistry parameters for the differential diagnosis of diseases of the pancreas. The lipase activity determination has gained increasing international recognition because of its high specificity and rapid response. After acute pancreatitis the lipase activity increases within 4 - 8 hours, reaches a peak after 24 hours and decreases after 8 - 14 days.

Numerous methods have been described for the determination of lipase which determine the decrease in substrate turbidimetrically or nephelometrically or determine degradation products.

The methylresorufin substrate method is based on the cleavage of a specific chromogenic lipase substrate 1,2-dilauryl glycerol-3-glutaric acid-(6-methylresorufin)-ester emulsified with bile acids. The pancreatic enzyme activity is determined specifically by the combination of bile acid and colipase used in this assay.

Virtually no lipase activity is detected in the absence of colipase. Colipase only activates pancreatic lipase, but not other lipolytic enzymes found in serum. The high amount of cholate ensures that the esterases present in the serum do not react with the chromogenic substrate due to the highly negative surface charge.

Principle

Lipase, in the presence of co-lipase and bile acids, can hydrolyze 1,2-Di-O-Lauryl-Rac-Glycero-3-Glutaric Acid-(6-Methylresorufin)-Ester to generate 1,2-O-Dilauryl-Rac-Glycerol and Glutaric Acid-(6-Methylresorufin)-Ester, and the latter continues to decompose under alkaline conditions to form glutaric acid and red methylresorufin. The increase in absorbance caused by this red dye is directly proportional to the lipase catalytic activity concentration in the sample.

1. 1,2-Di-O-Lauryl-Rac-Glycero-3-Glutaric Acid-(6-Methylresorufin)-Ester + H_2O $\xrightarrow{LPS}$ 1,2-O-Dilauryl-Rac-Glycerol + Glutaric Acid-(6-Methylresorufin)-Ester

2. Glutaric Acid-(6-Methylresorufin)-Ester + H_2O $\xrightarrow{OH^-}$ glutaric acid + Methylresorufin

Reagents Components and Concentration

Components	Main Constituents	Concentration
R1	N,N-Bis(2-hydroxyethyl)glycine	> 130 mmol/L
	Co-lipase	> 0.5 mg/L
R2	Tartaric acid buffer	9 mmol/L
	Lipase substrate	> 0.10 g/L
Calibrator	Lipase Bovine Serum	Refer to the label for marked value
Control	Lipase Bovine Serum	Refer to the label for marked value

The components in different batches are non-interchangeable.

The measurement system can be traceable to enterprise standard.

The target value of control has batch specificity.

Storage and Validity

1. The reagents should be stored at 2 - 8 ℃ and kept away from direct light and freezing. The unopened reagents are valid for 18 months.
2. Once opened, the reagents are stable for 30 days at 2 - 8 ℃. For reagents not in use, the cap should be tightened to avoid contamination.
3. In order to ensure the accuracy of test results, calibrator and control are stable for 7 days at 2 - 8 ℃ after reconstitution.
4. The production date and expiration date are available on package insert.

System Information

Hitachi 7180, Zybio EXC400/420, Zybio EXC200/220 Chemistry Analyzer. Other models can be used after verification.

Specimen Information

Serum or plasma (heparin for anticoagulation) is suitable for samples, which are stable for 3 days at 2 - 8 ℃ and for 90 days at - 20 ℃. Avoid repeated freezing and thawing.

Warnings and Precautions

1. For in vitro diagnostic use only. In case of contact, immediately flush the affected area with plenty of water.
2. The dosage of reagents and samples can be appropriately increased or decreased according to various instruments, under the condition that the volume proportion of reagents and samples is invariable.
3. The necessary precautions should be taken to use the reagents. The bottle cap should be tightened immediately after use to avoid contamination.
4. When reagent becomes turbid or the blank absorbance > 0.800, the reagent is failed and should be discarded.
5. All samples and reaction wastes should be treated as sources of infection. The waste liquid generated during the experiment and the used packaging materials shall be collected and disposed in accordance with relevant regulations.
6. Dedicated calibrator and control are recommended for use to ensure the accuracy of test values.
7. The same sample tested with reagents from different manufacturers may lead to different measured values.
8. Warning: This product contains ingredients of human or animal origin. At present, there is no way to completely guarantee that it is free from infectious substances and may be contaminated during use. This product and samples shall be considered as potential sources of infection, and operators should take protective measures and comply with laboratory safety regulations. All waste shall be disposed of in accordance with local regulations.

Test Process

1. Parameters

Method	Rate Method	**Sample/Reagent**	1/100
Main Wavelength	570 nm	**Reaction Temperature**	37 ℃
Sub Wavelength	700 nm	**Reaction Time**	10 min
Reaction Direction	+		

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Tel: +86 (0)23 6865 5509 Fax: +86 (0)23 6869 9779 E-mail: info@zybio.com Web: www.zybio.com

2. Operation

Addition	Blank	Calibration	Detection
Sample (μL)	/	/	3
Calibrator (μL)	/	3	/
Purified Water (μL)	3	/	/
Reagent 1 (μL)	225	225	225
Mix well, incubate at 37 ℃ for 5 min			
Reagent 2 (μL)	75	75	75
Mix well, incubate at 37 ℃ for 1.5 min, then continuously monitor the absorbance change rate (ΔA/min) within 2 min.			

3. Calibration

Use Zybio matched calibrator or Zybio Clinical Chemistry Multi-analyte Calibrator.
Calibration cycle: Re-calibration is needed in case of replacement of the reagent batch, deviation of quality control, or the major repair and maintenance of equipment.
Calibrator reconstitution: Reconstitution with the amount of purified water labeled on the bottle accurately absorbed, leave for 30 minutes, and mix well before use.

4. Quality Control

To ensure the reliability of the test results, quality control is required after each calibration, and the quality control value should be within the specified range. If it is out of the specified range, the user should check the measurement system and recalibrate it if necessary. It is recommended that users conduct regular quality control according to their own needs to ensure the accuracy of the measurement system and the reliability of the test results.
Control reconstitution: Reconstitution with the amount of purified water labeled on the bottle accurately absorbed, leave for 30 minutes, and mix well before use.

5. Calculation

Linear calibration was used to draw the working curve. The catalytic activity concentration of lipase (LPS) in the sample can be calculated on the working curve based on its absorbance change rate.

Reference Intervals

≤ 60 U/L

This reference interval is determined based on 95% distribution interval obtained from 200 healthy human specimens without related diseases, and is for reference only. It is recommended that each laboratory establish its own reference range.

Explanation of Results

If the catalytic activity concentration of LPS in the sample exceeds 300 U/L, the reduction mode of the chemistry analyzer will be used, or the high concentration sample shall be diluted with normal saline, with its report result multiplied by the dilution factor.
The professional is responsible for the review of the test result, which may be affected by the subject's age, sex, or weight. The test values within the critical range should be confirmed by remeasuring, if it is obviously beyond the reference value range or if it is still beyond the reference range after confirmation detection, the target concentration in the sample is considered to be abnormal. If the test result is inconsistent with or even contrary to the clinical situation, cause analysis should be performed correspondingly.

Limitations

1. The deviation of test results caused by interferents is ≤ 10% if the concentrations of the following interferents are at or below the given values:

Substances	Concentrations
Hemoglobin	1 g/L
Chyle	0.30%
Bilirubin	342 μmol/L

2. The test results of this product are only for clinical reference and cannot be used as the basis for diagnosis or exclusion of cases. The clinical diagnosis and treatment of patients should be combined with their symptoms/signs, medical history, other laboratory tests and treatment response. For achieve diagnostic purposes, the test results should be combined with clinical tests, medical history and other test results.

Performance Characteristics

1. The reagent blank absorbance ≤ 0.800, the reagent blank absorbance change rate (Δ*A*/min) ≤ 0.010.
2. Analytical sensitivity: at the test catalytic activity concentration of 100 U/L, the reagent absorbance change rate (Δ*A*/min) ≥ 0.015.
3. Accuracy: the relative deviation ≤ 10%.
4. Precision: within-run *CV* ≤ 5%; between-run relative range ≤10%.
5. Linear range:
[8, 300] U/L, the correlation coefficient (*r*) ≥ 0.990.
[8, 40] U/L, the absolute deviation ≤ 8 U/L;
(40, 300] U/L, the relative deviation ≤ 10%.
6. Calibrator accuracy: the relative deviation ≤ 10%.
7. Calibration homogeneity: between-vial *CV* shall be ≤ 10%.
8. Control accuracy: test value is within the allowable range of the marked value.
9. Control homogeneity: between-vial *CV* shall be ≤ 10%.

Materials Required (but not provided)

Chemistry analyzer, General lab equipment and consumable.

References

[1] Kazmierczak S, Catrou P, Lente F. Diagnostic accuracy of pancreatic enzymes evaluated by use of multivariate data analysis[J]. Clin Chem, 1993, 39:1960-1965.

Symbol Interpretation

IVD	In Vitro Diagnostic Medical Device	LOT	Batch Code
	Consult Instructions for Use		Use-By Date
REF	Catalogue Number		Manufacturer
	Temperature Limit		Date of Manufacture
CE	CE marking of conformity	EC REP	Authorized Representative in the European Community

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