Atlas Medical

MOLDOVA EUROPEANA

# 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%.

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**ATLAS Medical GmbH**
**Ludwig-Erhard Ring 3, 15827**
**Blankenfelde-Mahlow, Germany**
**Tel: +49 - 33708 – 3550 30**
**Email: Info@atlas-medical.com**
**Website: www.atlas-medical.com**



**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

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**ATLAS Medical GmbH**
**Ludwig-Erhard Ring 3**
**15827 Blankenfelde-Mahlow**
**Germany**
**Tel: +49 - 33708 – 3550 30**
**Email: Info@atlas-medical.com**
**Website: www.atlas-medical.com**



**PPI1473A01**
**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

# *H. pylori* Antigen ELISA Test Kit

An enzyme immunoassay (ELISA) for the qualitative and quantitative detection of Helicobacter pylori (*H. pylori*) Antigen in human stool

IVD For *in vitro* diagnostic and professional use only

2°C 8°C Store at (2° to 8°C)

Σ **96 Tests**

CE

## INTENDED USE

The *H. pylori* Antigen ELISA Test Kit is an enzyme immunoassay for the qualitative and quantitative detection of *H. pylori* antigen in human stool. It is intended as an aid in the diagnosis of possible *H. pylori* infection and in the follow-up of patients undergoing antimicrobial therapy.

## INTRODUCTION

*Helicobater pylori* are Gram-negative spiral-shaped bacteria that have adapted to living in the harsh acidic conditions of the stomach. These bacteria can alter their surrounding micro-environment by reducing its acidity so they can survive .Their spherical shape facilitates penetration of the epithelial lining, where the bacteria are protected by mucus against cells of the immune system.

Infections with *H.pylori,* though harmless during childhood*, manifest* as peptic ulcers of the stomach, duodenum and of small intestine, active and chronic gastritis, as well as non-ulcer dyspepsia, in about 60% of the global adult population. The mechanism of bacterial transmission is still unknown, but is thought to be oral and/or fecal borne.

The *H. pylori* Antigen ELISA Test Kit is an immunoassay for the qualitative and quantitative detection of *H. pylori* Antigen in human stool. The test utilizes antibodies to *H. pylori* to selectively detect *H. pylori* Antigen in human stool.

## PRINCIPLE OF THE TEST

The *H. pylori* Antigen ELISA Test Kit is a solid phase enzyme immunoassay based on sandwich principle for the qualitative and quantitative detection of *H. pylori* antigen in human stool. The microwell plate is coated with anti-*H. pylori* antibodies. During testing, the antigens are extracted from the specimen with extraction solution and added onto the antibodies coated microwell plate along with the enzyme- conjugated antibodies to *H. pylori*, and then incubated. If specimens contain *H. pylori* antigens, it will bind to the antibodies coated on the microwell plate and simultaneously bind to the conjugate to form immobilized antibody-H. pylori antigen-conjugate complexes. If specimens do not contain *H.pylori* antigens, the complexes will not be formed. After initial incubation, the microwell plate is washed to remove unbound materials. Substrate A and substrate B are added and then incubated to produce a blue color indicating the amount of *H. pylori* antigens present in the specimens. Sulfuric acid solution is added to the microwell plate to stop the reaction producing a color change from blue to yellow. The color intensity, which corresponds to the amount of *H. pylori* antigens present in the specimens, is measured with a microplate reader at 450/630-700 nm or 450 nm.

## PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- Follow the instructions for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.
- Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- It is important to calibrate all the equipment e.g. micropipettes, and/or the automated instruments used with this device, and to perform routine preventative maintenance.
- Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate.
- Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.
- Avoid cross contamination between reagents to ensure valid test results.
- Follow the wash procedure to ensure optimum assay performance.
- Use Plate Sealer to cover microwell plate during incubation to minimize evaporation.
- Use a new pipette tip for each specimen assayed.
- Do not touch or splash the rim of the well. Do not blow out from micropipettes.
- Do not allow sodium hypochlorite fumes from chlorine bleach or other sources to contact the microwell plate during the assay as the color reaction may be inhibited.

## HEALTH AND SAFETY INFORMATION

- Collect samples in accordance with correct medical practices.
- Some reagents may cause toxicity, irritation, burns or have a carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided but not limited to the following reagents: Stop solution, the Conjugate, and the Wash buffer, Extraction solution, Substrate.
- The Stop solution 0.5M H2SO4 is an acid. Use it with appropriate care. Wipe up spills immediately and wash with water if it comes into contact with the skin or eyes.
- ProClinTM 300 0.1% is used as a preservative; it can cause irritation of the skin. Wipe up spills immediately or wash with water if it comes into contact with the skin or eyes.
- Pipette tips, vials, strips and specimen containers should be collected and autoclaved for not less than 2 hours at 121°C before any further steps of disposal. Solutions containing sodium hypochlorite should NEVER be autoclaved.
- All specimens and materials should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure personal safety.
- Chemicals should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.
- Neutralized acids and other liquids should be decontaminated by adding sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to 1.0% sodium hypochlorite may be necessary to ensure effective decontamination.

## STORAGE AND STABILITY

- Components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8°C, Once opened; all reagents are stable for up to 3 months after the first opening date if stored between 2-8°C. Return reagents to 2-8°C immediately after use.
- Place unused wells in the zip-lock aluminum foiled pouch and return to 2-8 °C, under which conditions the wells will remain stable for 3 months from the opening date.
- Concentrated Wash Buffer may be stored at room temperature to avoid crystallization. If crystals are present, warm up the solution at 37°C. Working Wash Buffer is stable for 2 weeks at room temperature.
- Do not expose reagents especially the Substrate to strong light or hypochlorite fumes during storage or incubation steps.
- Do not store Stop Solution in a shallow dish or return it to the original bottle after use.

## SPECIMEN COLLECTION AND PREPARATION

- This *H. pylori* Antigen ELISA Test can be performed using only human stool.
- Stool samples should be collected in clean containers. Samples can be stored in the refrigerator (2-8 °C) for 1-2 days prior to testing. For longer storage, the specimen must be kept frozen at -20ºC. In this case, the sample should be totally thawed and brought to room temperature before testing.
- The patient has to be asked to collect the specimen avoiding any possible contact with urine or water.
- The patient submitted to the test should not be under antibiotic or anti-bacterial treatments as this pharmaceutical therapy is known to affect *H. pylori* up to a certain extent, depending on the antibiotic used, giving rise to false interpretation.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

## MATERIALS

### MATERIALS PROVIDED

1. ***H.Pylori* Antigen Microwell Plate:** Microwell plate coated with anti-*H. Pylori* antibodies. **(1 plate: 96 wells/plate).**
2. ***H.pylori* Antigen Conjugate:** One red cap vial containing antibodies to *H. pylori* bound to peroxidase; Preservative: 0.1% ProClin™ 300. **(1 x 8 mL).**
3. **Concentrated Wash Buffer (25x):** One white cap bottle containing Tris-HCl buffer containing 0.1% Tween 20; Preservative: 0.1% ProClin™ 300. **(1 x 40 mL).**
4. **Extraction Solution:** One white cap bottle containing 0.9% NaCl buffer containing EDTA; Preservative: 0.1% ProClin™ 300. **(1 x100 mL).**
5. **Substrate A:** One white cap vial containing Citrate-phosphate buffer containing hydrogen peroxide; Preservative: 0.1% ProClin™ 300. **(1 x 8 mL).**
6. **Substrate B:** One blue cap vial containing Buffer containing tetramethylbenzidine (TMB); Preservative: 0.1% ProClin™ 300. **(1 x 8 mL).**
7. **Stop Solution:** One yellow cap vial containing 0.5M Sulfuric acid. **(1 x 8 mL).**

8. ***H.pylori* Antigen Calibrator 1:** One white cap vial containing Buffer non-reactive for *H. pylori* Antigen; Preservative: 0.1% ProClin™ 300. **(1 x 1 mL).**
9. ***H.pylori* Antigen Calibrator 2:** One white cap vial containing Buffer containing 0.1 µg/mL *H. pylori* Antigen; Preservative: 0.1% ProClin™ 300. **(1 x 1 mL).**
10. ***H.pylori* Antigen Calibrator 3:** One white cap vial containing Buffer containing 0.5 µg/mL *H. pylori* Antigen; Preservative: 0.1% ProClin™ 300. **(1 x 1 mL).**
11. ***H.pylori* Antigen Calibrator 4:** One white cap vial containing Buffer containing 1.0 µg/mL *H. pylori* Antigen; Preservative: 0.1% ProClin™ 300. **(1 x 1 mL).**
12. **Plate Sealers (2 pieces).**
13. **Package Insert (1 copy).**

**MATERIALS REQUIRED BUT NOT PROVIDED**

- Freshly distilled or deionized water.
- Sodium hypochlorite solution for decontamination.
- Absorbent paper or paper towel.
- Water bath or incubator capable of maintaining 15°C to 30°C.
- Calibrated automatic or manual microwell plate washer capable of aspirating and dispensing 350 µL/well.
- Disposable gloves.
- Automated processor (optional).
- Calibrated micropipettes with disposable tips capable of dispensing 50 and 100 µL.
- Graduated cylinders for wash buffer dilution.
- Vortex mixer for specimen mixing (optional).
- Disposable reagent reservoirs.
- Calibrated microplate reader capable of reading at 450 nm with a 630-700 nm reference filter, or reading at 450 nm without a reference filter.
- Timer.

**DIRECTIONS FOR USE**

1. Remove unused strips from the microwell plate, and store in the original resealable pouch at 2-8°C.
2. Allow reagents and specimens to reach room temperature (15-30°C) prior to testing.

**WASH PROCEDURE**

a. The wash procedure is critical. Insufficient washing will Result in a poor precision and falsely elevated absorbance readings.

b. Prepare working wash buffer by adding content of wash buffer bottle provided with the kit to distilled or deionized water to reach a final volume of 1 liter. The working wash buffer is stable for 2 weeks at 15-30°C.

3. Dispense 1 mL of Extraction Solution into Specimen Extraction Tube.

**For Solid Stool Specimens:**

i. Take out the cap of the Specimen Extraction Tube
ii. Randomly stab the specimen collection stick into the stool specimen in at least 3 different sites to collect approximately 30 mg of specimen (equivalent to 1/4 of a pea). Do not scoop the stool specimen.
iii. Transfer into Specimen Extraction Tube.

**For Liquid Stool Specimens:**

i. Hold the Liquid Specimen Dropper vertically.
ii. Aspirate stool specimens and then dispense 2 drops (approximately 50 µL) into the Specimen Extraction Tube containing the Extraction Solution.
iii. Screw on and tighten the cap onto the Specimen Extraction Tube.
iv. Shake the Specimen Extraction Tube vigorously to mix the specimen and the Extraction Solution.

4. Leave A1 as Blank well.
5. Dispense 50 µL of Calibrator 1 in wells B1 and C1. (Light Yellow Reagent)
6. Dispense 50 µL of Calibrator 2 in wells D1 and E1. (Green Blue Reagent)
7. Dispense 50 µL of Calibrator 3 in wells F1 and G1. (Light Blue Reagent)
8. Dispense 50 µL of Calibrator 4 in wells H1 and A2. (Dark Blue Reagent)
9. Hold the Specimen Extraction Tube upright and break off the tip of the tube. Invert the Specimen Extraction Tube and dispense 2 drops of the specimen Extraction Solution (approx. 50 µL) to assigned wells starting at B2. (Yellow Reagent)
10. Dispense 50 µL of Conjugate to each well except for the Blank well. (Red Reagent)
11. Mix gently by swirling the microwell plate on a flat bench for 30 seconds.
12. Cover the microwell plate with the Plate Sealer and incubate at room temperature (15-30°C) in a room, a water bath, or an incubator for 60 minutes ± 5 minutes.
13. Remove the Plate Sealer.
14. Wash each well 5 times with 350 µL of Working Wash Buffer per well, and then remove the liquid.
15. Turn the microwell plate upside down on absorbent tissue for a few seconds. Ensure that all wells have been completely washed and dried.
    Note: Improper washing may cause false positive results.
16. Dispense 50 µL of Substrate A to each well. (Clear Reagent)
17. Dispense 50 µL of Substrate B to each well. (Clear Reagent) Then a blue color should develop in wells containing Positive specimens.
18. Mix gently then cover microwell plate with Plate Sealer and incubate at room temperature (15-30°C) in a room, a water bath, or an incubator for 10 minutes ± 1 minute.
19. Remove the Plate Sealer.
20. Dispense 50 µL of Stop Solution to each well. (Clear Reagent) Then a yellow color should develop in wells containing Positive specimens.
21. Read at 450/630-700 nm within 30 minutes.
22. Note: Microwell plate can also be read at 450 nm, but it is strongly recommended to read it at 450/630-700 nm for better results.

**VALIDATION REQUIREMENT AND QUALITY CONTROL**

1. Calculate the Mean Absorbance of Calibrators 1-4 by referring to the table below.

**Example of Calibrator 2 Calculation**

| Item | Absorbent |
|---|---|
| Calibrator 2: Well D1 | 0.469 |
| Calibrator 2: Well E1 | 0.507 |
| Total Absorbance of Calibrator 2 | 0.976 |
| Mean Absorbance of Calibrator 2 | 0.488 |

2. Check the validation requirements below to determine if the test results are valid.

| Item | Validation Requirements |
|---|---|
| Blank Well | Blank Absorbance should be < 0.050 if read at 450/630-700 nm<br>Note: It should be < 0.100 if read at 450 nm |
| Calibrator 1 | Mean Absorbance after subtraction of Blank Absorbance should be < 0.100 |
| Calibrator 2 | Mean Absorbance after subtraction of Blank Absorbance should be > 0.150 |
| Calibrator 3 | Mean Absorbance after subtraction of Blank Absorbance should be > 0.500 |
| Calibrator 4 | Mean Absorbance after subtraction of Blank Absorbance should be > 1.000 |

**NOTE:** The test results are considered invalid if the above validation requirements are not met. Repeat the test or contact your local distributor.

**INTERPRETATION OF RESULTS**

**Qualitative**

Calculate the Index Value to obtain qualitative specimen results.

1. If the test is valid, obtain Cut-Off Value by subtracting the Blank Absorbance from the Mean Absorbance of 1/2× (Calibrator 2+Calibrator 1). See an example of Cut-Off Value calculation below.

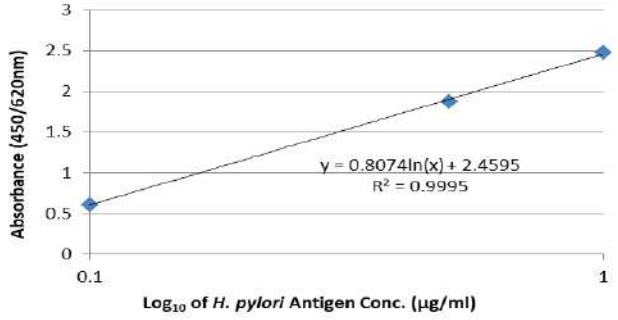
| Item | Absorbent |
|---|---|
| Blank Absorbance: Well A1 | 0.011 |
| Cut-Off Value: 1/2× (Mean Absorbance of Calibrator 2+ Mean Absorbance of Calibrator 1) – Blank Absorbance | 1/2× (0.488+0.012)-0.011=0.239 |

2. Calculate the Index Value by dividing the Specimen Absorbance by the Cut-Off Value, and then read the results by referring to the Interpretation of Results table below.

| Item | Absorbent |
|---|---|
| Specimen: Well F2 | 0.968 |
| Blank Absorbance: Well A1 | 0.011 |
| Cut-Off Value | 0.239 |
| Index Value: Specimen/Cut-Off Value | (0.968-0.011)/0.239=4.0 |

**Quantitative**

Draw the calibration curve and obtain quantitative specimen results.



1. Subtract the Blank Absorbance from the Mean Absorbance of each Calibrator, and then plot them on the Y-axis against their $Log_{10}$ of the corresponding concentration in µg/mL on the X-axis on a linear graph paper and draw the calibration curve. Draw the best fitted line through data points to obtain a standard curve. Refer to an example of the calibration curve at right.
   **NOTE:** Do not use the calibration curve at right to make any calculation. A calibration curve must be performed for each run.
2. Obtain quantitative specimen results from their absorbance by using the calibration curve.
   **NOTE:** Specimens that have absorbance above Calibrator 4 should be pre-diluted using Extraction Solution and retested. The concentration must be multiplied by the dilution factor. Automated reading and calculation may also be performed using linear regression function on suitable computer programs.

**Interpretation of Results - Qualitative and Quantitative**

| Results | Qualitative | Quantitative |
|---|---|---|
| | Index Value | Concentration |
| Negative | < 0.9 | < 0.045 µg/mL |
| Positive | > 1.1 | > 0.055 µg /mL |
| Equivocal* | ≥ 0.9 and ≤ 1.1 | 0.045 – 0.055 µg/mL |

***NOTE:** For Equivocal results, the specimen should be retested. Specimens that are repeatedly Equivocal after retest should be confirmed using an alternate method. If the results remain Equivocal, collect a new specimen in two weeks. If the new specimen is Positive, the specimen is presumed to be Positive.

**LIMITATIONS**

1. The *H. pylori* Antigen ELISA Test Kit is used for the detection of *H. pylori* antigen in human stool. Diagnosis of an infectious disease should not be established based on a single test result. Further testing, including confirmatory testing, should be performed before a specimen is considered positive. A negative test result does not exclude the possibility of exposure. Specimens containing precipitate may give inconsistent test results.
2. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
3. As with other sensitive immunoassays, a false positive result may arise due to inadequate washing from the initial test. The results may be affected due to procedural or instrument error.

**PERFORMANCE CHARACTERISTICS**

**Sensitivity and Specificity**

The *H. pylori* Antigen ELISA Test Kit has been compared to a leading commercial *H. Pylori* Antigen ELISA test using clinical specimens. The results show that the clinical sensitivity of the *H. pylori* Antigen ELISA Test Kit is 98.6%, and the clinical specificity is 95.4%.

***H. pylori* Antigen ELISA vs. Other ELISA**

| Method | | Other ELISA | | Total Results |
|---|---|---|---|---|
| *H. pylori* Antigen ELISA | Results | Positive | Negative | |
| | Positive | 70 | 6 | 76 |
| | Negative | 1 | 125 | 126 |
| Total Results | | 71 | 131 | 202 |

**Clinical Sensitivity: 98.6% (92.4-100.0%) ***
**Clinical Specificity: 95.4% (90.3-98.3%)***
**Overall Agreement: 96.5% (93.0-98.6%)***
***95% Confidence Interval**

**Reproducibility**

**Intra-Assay:** Within-run precision has been determined by using 10 replicates of two specimens: a low positive and a high positive.
**Inter-Assay:** Between-run precision has been determined by using 10 replicates on the same two specimens: a low positive and a high positive. Three different lots of the *H. pylori* Antigen ELISA Test Kit have been tested using these specimens.

| Specimen | Intra-Assay | | | Inter-Assay | | |
|---|---|---|---|---|---|---|
| | Mean Absorbance/ Cut-Off | Standard Deviation | Coefficient of Variation (%) | Mean Absorbance/ Cut-Off | Standard Deviation | Coefficient of Variation (%) |
| 1 | 1.741 | 0.156 | 8.96 | 1.723 | 0.133 | 7.72 |
| 2 | 4.726 | 0.252 | 5.33 | 4.861 | 0.252 | 5.18 |

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**ATLAS MEDICAL**
**Ludwig-Erhard Ring 3**
**15827 Blankenfelde-Mahlow**
**Germany**
**Tel: +49 - 33708 – 3550 30**
**Email: Info@atlas-medical.com**
**Website: www.atlas-medical.com**



**PPI1532A01**
**Rev B (21.10.2021)**


| | | | |
|---|---|---|---|
| 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 |

# Urinalysis Strips

## SUMMARY AND INTENDED USE

The content of the instruction includes usage, reaction principle, and notification.
Urinalysis strips are intended for qualitative and semi-quantitative urinalysis and for *in vitro* diagnostics use.
The strips are for professional use only.
The strips may be read visually or instrumentally. Please read the instruction carefully before use.
There are the test items of every products type.

| Products type | Test item |
|---|---|
| URS 14 | Urobilinogen, Bilirubin, Ketone (acetoacetic acid), Blood, Protein, Nitrite, Leukocytes, Glucose, Specific Gravity, pH, and Ascorbic Acid, Microalbumin, Creatinine, Calcium |
| URS 13, URS 12, URS 11, URS 10, URS 9, URS 8, URS 7, URS 6, URS 5, URS 4, URS 3, URS 2, URS 1 | The items of product type from URS 2 to URS 13 can be combined randomly from URS 14 test items mentioned above.<br>US1 item can be any item from URS 14. |

## SPECIMEN COLLECTION AND PREPARATION

Collect fresh urine in a clean dry container. Use uncentrifuged urine and mix the sample before testing. The sample should not be more than 2 hours old at the time of testing. Always handle specimens under sanitary conditions.
**Note:** Water should not be used as negative control. Preservatives will not prevent the deterioration of ketones, bilirubin or urobilinogen. Bacterial growth from contaminating organisms may affect glucose, pH, nitrite and blood test results.

## VISUAL READING TECHNIQUE

1. Immerse all reagent areas in specimen and remove strip immediately.
2. Run edge of strip against the rim of the container to remove excess urine.
3. Hold strip horizontally and compare test areas closely with color chart on bottle label. Record the results.

For a semi-quantitative result read the reagent areas at the time specified on the color chart. The pH and Protein areas may also be read immediately or at any time up to 60 seconds after dipping. For a qualitative result read the reagent areas between 1 and 2 minutes. If a positive result is obtained, repeat the test, reading each reagent at the time specified on the color chart.
Color changes after 2 minutes are of no diagnostic value.


## INSTRUMENT READING TECHNIQUE

Follow the directions given in the appropriate instrument-operating manual.

## STORAGE AND HANDLING PROCEDURES

Store only in original bottle. Do not use after expiry date. Every strip can be used only once. Do not remove desiccant(s). Do not remove strip from the bottle until immediately before it is to be used for testing. Replace cap immediately and tightly after removing reagent strip. Shelf life – 24 months, 3 months shelf life for strips in opened canisters.
Store at temperatures between 2℃-30℃.Don't store in refrigerator. Keep away from direct sunlight.
Do not touch test areas of reagent strips. PROTECTION AGAINST AMBIENT MOISTURE, LIGHT AND HEAT IS ESSENTIAL TO GUARD AGAINST ALTERED REAGENT REACTIVITY. Deterioration may result in discoloration or darkening of the reagent areas. If this is evident or if test results are questionable or inconsistent with expected results, confirm that strips are within the expiry dates and compare with control urine. Please deal with the waste strips according to "Treatment Regulations of Lab Biohazard Materials".

## LIMITATIONS OF PROCEDURES

As with all laboratory tests, definitive diagnostic or therapeutic decisions should not be made or based on any single result or method.

## TEST PRINCIPLES

**Glucose:** One enzyme, glucose oxidize catalyzes the formation of gluconic acid and hydrogen peroxide from the oxidation of glucose. Hydrogen peroxide releases neo-ecotypes oxide [O] under the function of peroxidase, [O] oxidates potassium. iodides chromogen, so that it makes the color change.
**Bilirubin:** This test is based on coupling the direct bilirubin with diazotized dichloroaniline in a strongly acid medium, which produce the diazotizing colors.
**Ketone:** This test is based on that acetoacetate acid react with sodium nitroprusside in an alkaline medium, producing violet color.
**Specific gravity:** Electrolyte (M+X-) in the form of salt in urine reacts with poly (methyl vinyl ether/maleic anhydride (-COOH), which is weakly acid ionic exchanger. And then hydrogenous ion is replaced from it and reacts with pH indicator in order to make the color change.
**Blood:** This test is based on the peroxidase activity of hemoglobin and myoglobin, which makes peroxide release neo-ecotypic Oxide [O]. Indicator is oxidized by [O] and shows color change subsequently.
**pH:** This test is based on a double indicator principle.
**Protein:** The test is based on the protein-error-of-indicators principle. Anion on the specific pH indicator is absorbed by cation on protein molecule, which make the indicator ionize and present color change at critical point of color.
**Urobilinogen:** This test is based on coupling the urobilinogen with diazotized salt in a strongly acid medium, which produce the pink-red azo dye.
**Nitrite:** This test depends upon the nitrite diazotize with aromatic amino sulphanilamide to form a diazonium compound. This diazonium compound in turn couples with 1,2,3,4-tetrahydro-benzo(h)quinolin-3-phenol to produce a pink color.
**Leucocytes:** Granulocytic leukocytes in urine contain esterase's that catalyze the hydrolysis of the privatized pyrrole amino acid ester to liberate 3-hydroxy-5-pheny pyrrole. This pyrrole then reacts with a diazonium salt to form a purple color.
**Ascorbic acid:** Ascorbic acid, with 1,2-dihydroxy alkenes, under alkaline condition, deoxidizes blue 2,6-dichloroindophenolate form into colorless N-(P-phenol)-2,6-dichloro–P- amine phenol.
**Microalbumin:** Based on the protein deviation method, utilizing the sulfone phthalein dyestuff only specific to Microalbumin.
**Creatinine：** Creatinine can act with 3, 5-2 nitro benzoic acid in strong alkalinity, generating colored compound.
**Calcium：** Calcium can with o-Cresol phthalein complex one in an alkaline medium, producing violet color.

**Note:**

**GLUCOSE:** The test is specific for glucose, no substance excreted in urine other than glucose is known to give a positive result. In dilute urine containing less than 0.28 mmol/L ascorbic acid, as little as 2.2 mmol/L glucose may produce a color change that might be interpreted as positive. Ascorbic acid concentrations of 2.8 mmol/L or greater and/or high acetoacetic concentrations (1.0 mmol/L) may influence test. Small amounts of glucose may normally be excreted by the kidney. These amounts are usually below the sensitivity of this test.
**BILIRUBIN:** Normally no bilirubin is detectable in urine by even the most sensitive methods. Even trace amounts of bilirubin are sufficiently abnormal to require further investigation. Medicines that color the urine red or that are themselves red in an acid medium, e.g., phenazopyridine may influence the test. Large ascorbic acid concentration may cause false negatives.
**KETONES:** The test reacts with acetoacetic acid in urine. It does not react with acetone or β-hydroxybutyric acid. Normal urine specimens usually yield negative results with this reagent. False positive results may occur with highly pigmented urine specimens or those containing large amounts or levodopa metabolites.
**SPECIFIC GRAVITY:** The specific gravity permits determination of urine specific gravity between 1.000 and 1.030. In general, it correlates within 0.005 with values obtained with the refractive index method. For increased accuracy, 0.005 may be added to readings from urines with pH equal to or greater than 6.5. Strips read instrumentally are automatically adjusted for pH by the instrument. The SG test is not affected by certain nonionic urine constituents such as glucose or by the presence of radiopaque dye. Highly buffered alkaline urines may cause low readings relative to other methods. Elevated specific gravity readings may be obtained in the presence of moderate quantities (1-7.5 g/L) of protein.
**BLOOD:** The significance of the 'Trace' reaction may vary among patients, and clinical judgment is required for assessment in an individual case. Development of green spots (intact erythrocytes) or green color (free hemoglobin/ myoglobin) on the reagent area within 60 seconds indicates the need for further investigation. Blood is often found in the urine of menstruating females. Hemoglobin concentration of 150-620 μg/L is approximately equivalent to 5-15/μL intact red blood cells per microlite.
This test is highly sensitive to hemoglobin and thus complements the microscopic examination. The sensitivity of this test may be reduced in urines with high specific gravity. This test is equally sensitive to myoglobin as to hemoglobin. Certain oxidizing contaminants, such as hypochlorite, may produce false positive results. Microbial peroxidase associated with urinary tract infection may cause a false positive reaction. Levels of 5.0 mmol/L ascorbic acid normally found in urine do not interfere with this test.
**pH:** The pH test area measures pH values generally to within 1 unit in the range of 5.0-8.5 visually and 5.0-9.0 instrumentally.

**PROTEIN and MICROALBUMIN:**

The Protein reagent area can detect albumin in urine and has low sensitivity to mucoprotein, generally up to 0.6 g/L concentration.

The Microalbumin reagent area is to detect the microalbumin. Beyond 0.15 g/L indicate albuminuria clinically. The Microalbumin reagent can detect specifically microalbumin, and 9 times more sensitive than other protein.
Visible blood urine (⩾0.05 g/L) can be false negative action.

**UROBILINOGEN:** This test area will detect urobilinogen in concentrations as low as 3 μmol/L (approximately 0.2 Ehrlich unit/dL) in urine. The normal range with this test is 3-16 μmol/L. a result of 33 μmol/L represents the transition from normal to abnormal, and the patient and/or urine specimen should be evaluated further. The absence of urobilinogen cannot be determined with this test.

**NITRITE:** This test depends upon the conversion of nitrate (derived from the diet) to nitrite by the action of principally Gram-negative bacteria in the urine. The test is specific for nitrite and will not react with any other substance normally excreted in urine. Pink spots or pink edges should not be interpreted as a positive result. Any degree of uniform pink color development should be interpreted as a positive nitrite test suggesting the presence of $10^5$ or more organisms per mL, but color development is not proportional to the number of bacteria present. A negative result does not prove that there is no significant bacteriuria. Negative results may occur ① when urinary tract infections are caused by organisms which do not contain reductase to convert nitrate to nitrite; ② when urine has not been retained in the bladder long enough (four hours or more) for reduction of nitrate to occur, ③ or when dietary nitrate is absent. Sensitivity of the nitrite test is reduced for urines with high specific gravity. It may resist 2.8mmol/L Ascorbic Acid.

**LEUKOCYTES:** Test area react with esterase in leucocytes (granulocytic leukocytes). Normal urine specimens generally yield negative result; positive results (+ or greater) are clinically significant. Individually observed 'Trace' results may be of questionable clinical significance; however, 'Trace' results observed repeatedly may be clinically significant. 'Positive' results may occasionally be found with random specimens from females due to contamination of the specimen by vaginal discharge. Elevated glucose concentrations (160 mmol/L) or high specific gravity may cause decreased test results.

**ASCORBIC ACID:** The test area can detect the ascorbic acid in urine. Through the ascorbic acid detection, we will know the level of ascorbic acid in the body and the effect degree that the ascorbic acid brings to the test on glucose, bilirubin, blood and nitrite. It will reduce the sensitivity when the oxidant (such as potassium permanganate, hypochlorite) in the urine.

**Creatinine:** Adult normal urine creatinine is 0.6-2.0 g/24 hour (the Creatinine reagent area results is about 50-200 mg/dL). Result of random urine sample differ largely, from 10 mg/dL to 300 mg/dL. Concentrated urine and morning urine have high concentration (possibly over 200 mg/dL). Because of diuresis, excessive drinking water, or other urine dilution, resulting in testing analyte concentration decrease (result can be less than 50 mg/dL).

## SPECIFIC PERFORMANCE CHARACTERISTICS

Specific performance characteristics are based on clinical and analytical studies. In clinical specimens, the sensitivity depends upon several factors; the variability of color perception, the presence or absence of inhibitory factors typically found in urine, specific gravity, pH, and the lighting conditions when the product is read visually. Each color block or instrumental display value represents a range of values. Because of specimen and reading variability, specimens with analyte concentrations that fall between nominal levels may give results at either level. Results at levels greater than the second positive level for the Protein, Glucose, Ketone, and Urobilinogen tests will usually be within one level of the true concentration. Exact agreement between visual results and instrumental results might not be found because of the inherent differences between the perception of the human eye and the optical system of the instruments.

### Sensitivity and test range of urinalysis strips

| Item | Sensitivity | Instrumental test range | Visual test range |
|---|---|---|---|
| Glucose (mmol/L) | 2.8-5.5 | Neg-55 | |
| Protein (g/L) | 0.15-0.3 | Neg -3.0 | Neg – 20.0 |
| Microalbumin (g/L) | 0.08-0.15 | 0-0.15 | |
| Ketone (acetoacetic acid) (mmol/L) | 0.5-1.0 | Neg-7.8 | Neg-16 |
| Blood (Ery/uL) | 5-15 | Neg.- 200 | |
| Bilirubin (μmol /L) | 3.3-8.6 | Neg.-100 | |
| Nitrite (μmol /L) | 13-22 | Neg. or Pos. | |
| Leukocytes (cells/μL) | 5-15 | Neg. - 500 | |
| Urobilinogen (μmol /L) | 3.3-16 | 3.3-131 | |
| Ascorbic acid (mmol/L) | 0.3-0.6 | 0-5.0 | |
| Creatinine (mg/dL) | 25-75 | 10-300 | |
| pH | —— | 5.0-9.0 | 5.0-8.5 |
| Specific Gravity | —— | 1.005-1.030 | 1.000-1.030 |
| Calcium (mmol/L) | 2.5-3.5 | 2.5-10.0 | 2.5-10.0 |

## REACTIVE INGREDIENTS (based on dry weight at time of impregnation)

**Protein:** 0.1% m/m tetrabromphenol blue; 97.4% w/w buffer; 2.5% w/w nonreactive ingredients

**Blood:** 26.0% w/w diisopropylbenzene dihydro peroxide; 1.5% w/w tetramethylbenzidine; 35.3% w/w buffer; 37.2 % nonreactive ingredients.

**Glucose:** 1.7% w/w glucose oxidase (microbial.123U); 0.2 % w/w peroxidase (horseradish. 203 IU); 0.1% w/w potassium iodide; 71.8% w/w buffer; 26.2% w/w nonreactive ingredients.

**Ketone:** 5.7% w/w sodium nitroprusside; 29.9% w/w nonreactive ingredients;64.4% w/w buffer;

**Leukocytes:** 4.3% w/w pyrrole amino acid ester; 0.4% w/w diazonium salt; 92.6% w/w buffer; 2.7% w/w nonreactive ingredients.

**Nitrite:** 1.3% w/w1,2,3,4-Tetrahydrobenzo(h)quinolin-3-ol; 89.6% w/w buffer; 9.1% w/w nonreactive ingredients**.**

**Specific Gravity:** 4.8% w/w bromothymol blue; 90.2% w/w poly (methyl vinyl ether co maleic anhydride); 5.0% w/w sodium hydroxide.

**pH:** 3.3% w/w bromocresol green; 55.0% w/w bromothymol blue; 41.7% w/w nonreactive ingredients.

**Bilirubin**: 0.6% w/w 2.4-dichlorbenzene amine diazonium salt; 57.3% w/w buffer; 42.1% w/w nonreactive ingredients.

**Urobilinogen:** 0.2% w/w fast B blue; 98.0% w/w buffer; 1.8% w/w nonreactive ingredients.

**Ascorbic acid:** 0.8% w/w 2,6-dichloroindophenolate hydrate; 40.7% w/w buffer; 58.5% w/w nonreactive ingredients.

**Microalbumin:** 2.2% w/w sulfone phthalein dyestuff; 96.0% w/w buffer; 1.8 w/w nonreactive ingredients.

**Creatinine:** 4.8% w/w 3, 5-2 nitro benzoic acid; 85.2% w/w buffer; 10% w/w nonreactive ingredients.

**Calcium:** 2.5% W/W o-Cresol phthalein Complex one; 87.5% w/w buffer; 10% w/w nonreactive ingredients.

## 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 |
| 30°C 2°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 URS-1T, URS-2T, URS-3T, URS-4T, URS-5T, URS-6T, URS-7T, URS-8T, URS-9T, URS-10T, URS-11T, URS-12T, URS-13T & URS-14T

Revision Date：2023-02-28
B20952-05

# 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

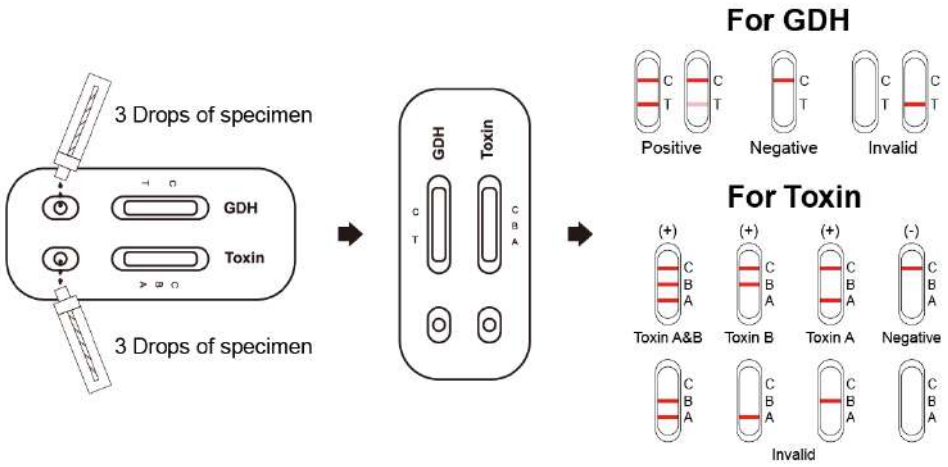
assay is performed within 6 hours after collection.

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

# One Step Multi-Drug Screen Cassette Test (Urine) Package Insert

CE

Package insert for testing of any combination of the following drugs: Amphetamine, Barbiturates, Benzodiazepines, Buprenorphine, Cocaine, Cotinine, Ecstasy, Ethyl Glucuronide, Fentanyl, Lysergic acid diethylamide, Marijuana, Methadone, EDDP (Methadone Metabolites), Ketamine, Methamphetamine, Methaqualone, Methylenedioxypyrovalerone, 6-Monoacetylmorphine, Morphine, Oxycodone, Phencyclidine, Propoxyphene, K2 (Synthetic Cannabinoid), Tramadol and Tricyclic Antidepressants.

*A rapid, one step screening test for the simultaneous, qualitative detection of Amphetamine, Barbiturates, Benzodiazepines, Buprenorphine, Cocaine, Cotinine, Ecstasy, Ethyl Glucuronide, Fentanyl, Lysergic acid diethylamide, Marijuana, Methadone, EDDP (Methadone Metabolites), Ketamine, Methamphetamine, Methaqualone, Methylenedioxypyrovalerone, 6-Monoacetylmorphine, Morphine, Oxycodone, Phencyclidine, Propoxyphene, K2 (Synthetic Cannabinoid), Tramadol and Tricyclic Antidepressants and the metabolites in human urine.*

*For healthcare professional in vitro diagnostic use only.*

## INTENDED USE

Urine based Drug tests for multiple drugs of abuse range from simple immunoassay tests to complex analytical procedures. The speed and sensitivity of immunoassays have made them the most widely accepted method to screen urine for multiple drugs of abuse.

The **One Step Multi-Drug Screen Cassette Test (Urine)** is a lateral flow chromatographic immunoassay for the qualitative detection of multiple drugs, drug metabolites and alcohol at the following cut-off concentrations in urine:[1]

| Test | Calibrator | Cut-off (ng/mL) |
|---|---|---|
| Amphetamine (AMP) | D-Amphetamine | 1,000 |
| Amphetamine (AMP) | D-Amphetamine | 500 |
| Amphetamine (AMP) | D-Amphetamine | 300 |
| Barbiturates (BAR) | Secobarbital | 300 |
| Barbiturates (BAR) | Secobarbital | 200 |
| Benzodiazepines (BZO) | Oxazepam | 300 |
| Benzodiazepines (BZO) | Oxazepam | 200 |
| Buprenorphine (BUP) | Buprenorphine | 10 |
| Cocaine (COC ) | Benzoylecgonine | 300 |
| Cocaine (COC ) | Benzoylecgonine | 150 |
| Cotinine (COT) | Cotinine | 200 |
| MDMA (Ecstasy) | D,L-3,4-Methylenedioxymethamphetamine (MDMA) | 500 |
| Ethyl Glucuronide (ETG) | Ethyl Glucuronide | 500 |
| Ethyl Glucuronide (ETG) | Ethyl Glucuronide | 300 |
| Fentanyl (FEN) | Fentanyl | 300 |
| Fentanyl (FEN) | Fentanyl | 200 |
| Fentanyl (FEN) | Fentanyl | 100 |
| Fentanyl (FEN) | Norfentanyl | 20 |
| Ketamine (KET) | Ketamine | 1,000 |
| Ketamine (KET) | Ketamine | 100 |
| Lysergic acid diethylamide (LSD) | D-lysergic acid diethylamide | 20 |
| Marijuana (THC ) | 11-nor-$\Delta^9$-THC-9 COOH | 50 |
| Marijuana (THC ) | 11-nor-$\Delta^9$-THC-9 COOH | 25 |
| Marijuana (THC ) | 11-nor-$\Delta^9$-THC-9 COOH | 20 |
| Methadone (MTD) | Methadone | 300 |
| EDDP (Methadone Metabolites) | 2-Ethylidene-1,5-dimethyl-3,3-dipheylpyrrolidine (EDDP) | 300 |
| EDDP (Methadone Metabolites) | 2-Ethylidene-1,5-dimethyl-3,3-dipheylpyrrolidine (EDDP) | 100 |
| Methamphetamine (MET, mAMP ) | D-Methamphetamine | 1,000 |
| Methamphetamine (MET, mAMP ) | D-Methamphetamine | 500 |
| Methamphetamine (MET, mAMP ) | D-Methamphetamine | 300 |
| Methaqualone (MQL) | Methaqualone | 300 |
| Methylenedioxypyrovalerone | 3,4-Methylenedioxypyrovalerone | 1,000 |
| (MDPV) | | |
| 6-Monoacetylmorphine (6-MAM) | 6-Monoacetylmorphine | 10 |
| Morphine (MOP 300) | Morphine | 300 |
| Morphine (OPI, MOP 2000 ) | Morphine | 2,000 |
| Oxycodone (OXY ) | Oxycodone | 100 |
| Phencyclidine (PCP) | Phencyclidine | 25 |
| Propoxyphene (PPX) | Propoxyphene | 300 |
| K2 Synthetic Cannabinoid | JWH-073/JWH-018 | 50 |
| K2 Synthetic Cannabinoid | JWH-073/JWH-018 | 25 |
| Tramadol (TRA) | Tramadol | 200 |
| Tricyclic Antidepressants (TCA) | Nortriptyline | 1,000 |
| Alcohol (ALC) | Ethanol | >0.04% B.A.C |

This test will detect other related compounds, please refer to the Analytical Specificity table in this package insert.

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

## SUMMARY

### AMPHETAMINE (AMP)

Amphetamine is a Schedule II controlled substance available by prescription (Dexedrine®) and is also available on the illicit market. Amphetamines are a class of potent sympathomimetic agents with therapeutic applications. They are chemically related to the human body's natural catecholamines: epinephrine and norepinephrine. Acute higher doses lead to enhanced stimulation of the central nervous system and induce euphoria, alertness, reduced appetite, and a sense of increased energy and power. Cardiovascular responses to Amphetamines include increased blood pressure and cardiac arrhythmias. More acute responses produce anxiety, paranoia, hallucinations, and psychotic behavior. The effects of Amphetamines generally last 2-4 hours following use, and the drug has a half-life of 4-24 hours in the body. About 30% of Amphetamines are excreted in the urine in unchanged form, with the remainder as hydroxylated and deaminated derivatives.

### BARBITURATES (BAR)

Barbiturates are central nervous system depressants. They are used therapeutically as sedatives, hypnotics, and anticonvulsants. Barbiturates are almost always taken orally as capsules or tablets. The effects resemble those of intoxication with alcohol. Chronic use of barbiturates leads to tolerance and physical dependence. Short acting Barbiturates taken at 400 mg/day for 2-3 months can produce a clinically significant degree of physical dependence. Withdrawal symptoms experienced during periods of drug abstinence can be severe enough to cause death. Only a small amount (less than 5%) of most Barbiturates are excreted unaltered in the urine.

The approximate detection time limits for Barbiturates are:

Short acting (e.g. Secobarbital) 100 mg PO (oral) 4.5 days

Long acting (e.g. Phenobarbital) 400 mg PO (oral) 7 days.

### BENZODIAZEPINES (BZO)

Benzodiazepines are medications that are frequently prescribed for the symptomatic treatment of anxiety and sleep disorders. They produce their effects via specific receptors involving a neurochemical called gamma aminobutyric acid (GABA). Because they are safer and more effective, Benzodiazepines have replaced barbiturates in the treatment of both anxiety and insomnia. Benzodiazepines are also used as sedatives before some surgical and medical procedures, and for the treatment of seizure disorders and alcohol withdrawal. Risk of physical dependence increases if Benzodiazepines are taken regularly (e.g., daily) for more than a few months, especially at higher than normal doses. Stopping abruptly can bring on such symptoms as trouble sleeping, gastrointestinal upset, feeling unwell, loss of appetite, sweating, trembling, weakness, anxiety and changes in perception. Only trace amounts (less than 1%) of most Benzodiazepines are excreted unaltered in the urine; most of the concentration in urine is conjugated drug. The detection period for the Benzodiazepines in the urine is 3-7 days.

### BUPRENORPHINE (BUP)

Buprenorphine is a semisynthetic opioid analgesic derived from thebain, a component of opium. It has a longer duration of action than morphine when indicated for the treatment of moderate to severe pain, peri-operative analgesia, and opioid dependence. Low doses buprenorphine produces sufficient agonist effect to enable opioid-addicted individuals to discontinue the misuse of opioids without experiencing withdrawal symptoms. Buprenorphine carries a lower risk of abuse, addiction, and side effects compared to full opioid agonists because of the "ceiling effect", which means no longer continue to increase with further increases in dose when reaching a plateau at moderate doses. However, it has also been shown that Buprenorphine has abuse potential and may itself cause dependency. Subutex® and a Buprenorphine/Naloxone combination product, Suboxone® are the only two forms of Buprenorphine that have been approved by FDA in 2002 for use in opioid addiction treatment. Buprenorphine was rescheduled from Schedule V to Schedule III drug just before FDA approval of Suboxone and Subutex.

### COCAINE (COC)

Cocaine is a potent central nervous system (CNS) stimulant and a local anesthetic. Initially, it brings about extreme energy and restlessness while gradually resulting in tremors, over-sensitivity and spasms. In large amounts, cocaine causes fever, unresponsiveness, difficulty in breathing and unconsciousness.

Cocaine is often self-administered by nasal inhalation, intravenous injection and free-base smoking. It is excreted in the urine in a short time primarily as Benzoylecgonine.[1,2] Benzoylecgonine, a major metabolite of cocaine, has a longer biological half-life (5-8 hours) than cocaine (0.5-1.5 hours), and can generally be detected for 24-48 hours after cocaine exposure.[2]

### COTININE (COT)

Cotinine is the first-stage metabolite of nicotine, a toxic alkaloid that produces stimulation of the autonomic ganglia and central nervous system when in humans. Nicotine is a drug to which virtually every member of a tobacco-smoking society is exposed whether through direct contact or second-hand inhalation. In addition to tobacco, nicotine is also commercially available as the active ingredient in smoking replacement therapies such as nicotine gum, transdermal patches and nasal sprays.

In a 24-hour urine, approximately 5% of a nicotine dose is excreted as unchanged drug with 10% as cotinine and 35% as hydroxycotinine; the concentrations of other metabolites are believed to account for less than 5%.[1] While cotinine is thought to be an inactive metabolite, it's elimination profile is more stable than that of nicotine which is largely urine pH dependent. As a result, cotinine is considered a good biological marker for determining nicotine use. The plasma half-life of nicotine is approximately 60 minutes following inhalation or parenteral administration.[2] Nicotine and cotinine are rapidly eliminated by the kidney; the window of detection for cotinine in urine at a cutoff level of 200 ng/mL is expected to be up to 2-3 days after nicotine use.

### MDMA (ECSTASY)

Methylenedioxymethamphetamine (ecstasy) is a designer drug first synthesized in 1914 by a German drug company for the treatment of obesity. Those who take the drug frequently report adverse effects, such as increased muscle tension and sweating. MDMA is not clearly a stimulant, although it has, in common with amphetamine drugs, a capacity to increase blood pressure and heart rate. MDMA does produce some perceptual changes in the form of increased sensitivity to light, difficulty in focusing, and blurred vision in some users. Its mechanism of action is thought to be via release of the neurotransmitter serotonin. MDMA may also release dopamine, although the general opinion is that this is a secondary effect of the drug (Nichols and Oberlender, 1990). The most pervasive effect of MDMA, occurring in virtually all people who took a reasonable dose of the drug, was to produce a clenching of the jaws.

### ETHYL GLUCURONIDE (ETG)

Ethyl Glucuronide (EtG) is a direct metabolite of ethanol alcohol. The presence of EtG in the urine can be used to detect recent alcohol consumption, even after the ethanol alcohol is no longer measurable. Consequently, the presence of EtG in the urine is a definitive indicator that alcohol has been ingested. Traditional laboratory practices typically measure the amount of alcohol present in the body. Depending on the amount of alcohol that has been consumed, this method usually reveals alcohol ingestion within the past few hours.

The presence of EtG in the urine, on the other hand, demonstrates that ethanol alcohol was ingested within the past three or four days, or roughly 80 hours after the ethanol alcohol has been metabolized by the body. As a result, it can be determined that a urine alcohol test employing EtG is a more accurate indicator of the recent consumption of alcohol as opposed to simply measuring for the existence of ethanol alcohol.

### FENTANYL (FEN)

Fentanyl is a synthetic opioid. It has the brand names of Sublimaze, Actiq, Durogestic, Fentora and others. The Fentanyl drug is approximately 100 times more potent than morphine, with 100 micrograms of fentanyl approximately equivalent to 10 mg. of morphine or 75 mg. of meperidine in analgesic activity. The Fentanyl drug is a potent narcotic analgesic with rapid onset and short duration of action. Historically, the fentanyl drug has been used to treat chronic breakthrough pain and is commonly used pre-procedures. Illicit use of pharmaceutical fentanyl drugs first appeared in the mid-1970s. Because the effects of the fentanyl drug last for only a very short time, it is even more addictive than heroin. Regular users may become addicted very quickly. The Fentanyl drug is much more potent than heroin, and tends to produce significantly worse respiratory depression, making it somewhat more dangerous than heroin to users. Overdose of the fentanyl drug has caused death. In the United States, the fentanyl drug is classified as a Schedule II controlled substance.

### KETAMINE (KET)

Ketamine is a short-acting "dissociative" anesthetic due to its ability to separate perception from sensation. It also has hallucinogenic and painkilling qualities that seem to affect people in very different ways. Ketamine is chemically related to PCP ('Angel Dust'). Ketamine is occasionally administered to people but, more commonly, is used by vets for pet surgery. Generally street K is

most often diverted in liquid form from vets' offices or medical suppliers. Ketamine generally takes 1-5 minutes to take effect. Snorted ketamine takes a little longer at 5-15 minutes. Depending on how much and how recently one has eaten, oral ketamine can take between 5 and 30 minutes to take effect. The primary effects of ketamine last approximately a 30-45 minutes if injected, 45-60 minutes when snorted, and 1-2 hours if used orally. The Drug Enforcement Administration reports that the drug can still affect the body for up to 24 hours.

**LYSERGIC ACID DIETHYLAMIDE (LSD)**

D-lysergic acid diethylamide (LSD) is the most potent hallucinogenic substance known to man. Dosages of LSD are measured in micrograms, or millionths of a gram. By comparison, dosages of cocaine and heroin are measured in milligrams, or thousandths of a gram. Compared to other hallucinogenic substances, LSD is 100 times more potent than psilocybin and psilocin and 4,000 times more potent than mescaline. The dosage level that will produce a hallucinogenic effect in humans generally is considered to be 25 micrograms. Over the past several years, the potency of LSD obtained during drug law enforcement operations has ranged between 20 and 80 micrograms per dosage unit. The Drug Enforcement Administration (DEA) recognizes 50 micrograms as the standard dosage unit equivalency.

**MARIJUANA (THC)**

THC ($\Delta^9$-tetrahydrocannabinol) is the primary active ingredient in cannabinoids (marijuana). When smoked or orally administered, it produces euphoric effects. Users have impaired short term memory and slowed learning. They may also experience transient episodes of confusion and anxiety. Long term relatively heavy use may be associated with behavioral disorders. The peak effect of smoking marijuana occurs in 20-30 minutes and the duration is 90-120 minutes after one cigarette. Elevated levels of urinary metabolites are found within hours of exposure and remain detectable for 3-10 days after smoking. The main metabolite excreted in the urine is 11-nor-$\Delta^9$-tetrahydrocannabinol-9-carboxylic acid ($\Delta^9$-THC-COOH).

**METHADONE (MTD)**

Methadone is a narcotic analgesic prescribed for the management of moderate to severe pain and for the treatment of Morphine dependence (heroin, Vicodin, Percocet, Morphine). The pharmacology of Oral Methadone is very different from IV Methadone. Oral Methadone is partially stored in the liver for later use. IV Methadone acts more like heroin. In most states you must go to a pain clinic or a Methadone maintenance clinic to be prescribed Methadone. Methadone is a long acting pain reliever producing effects that last from twelve to forty-eight hours. Ideally, Methadone frees the client from the pressures of obtaining illegal heroin, from the dangers of injection, and from the emotional roller coaster that most opiates produce. Methadone, if taken for long periods and at large doses, can lead to a very long withdrawal period. The withdrawals from Methadone are more prolonged and troublesome than those provoked by heroin cessation, yet the substitution and phased removal of methadone is an acceptable method of detoxification for patients and therapists.

**EDDP**

EDDP is the primary metabolite of methadone. Methadone is a controlled substance and is used for detoxification and maintenance of opiate-dependent patients. Patients on methadone maintenance may exhibit methadone (parent) levels that account for 5-50% of the dosage and 3-25% of EDDP in urinary excretion during the first 24 hours. The tampering of specimens by spiking the urine with methadone can be prevented. Also, renal clearance of EDDP is not affected by urinary pH; therefore the EDDP test provides a more accurate result of methadone ingestion than the methadone test. Methadone is an unusual drug in a sense that its primary urinary metabolites (EDDP and EMDP) are cyclic in structure. Thus, they are very difficult to detect with immunoassays targeted to the native compound. Exacerbating this problem, there is a subsection of the population classified as "extensive metabolizers" of methadone. In these individuals, a urine specimen may not contain enough parent methadone to yield a positive drug screen even if the individual is in compliance with their methadone maintenance.

**METHAMPHETAMINE (MET, mAMP)**

Methamphetamine is an addictive stimulant drug that strongly activates certain systems in the brain. Methamphetamine is closely related chemically to amphetamine, but the central nervous system effects of Methamphetamine are greater. Methamphetamine is made in illegal laboratories and has a high potential for abuse and dependence. The drug can be taken orally, injected, or inhaled. Acute higher doses lead to enhanced stimulaDtion of the central nervous system and induce euphoria, alertness, reduced appetite, and a sense of increased energy and power. Cardiovascular responses to Methamphetamine include increased blood pressure and cardiac arrhythmias. More acute responses produce anxiety, paranoia, hallucinations, psychotic behavior, and eventually, depression and exhaustion. The effects of Methamphetamine generally last 2-4 hours and the drug has a half-life of 9-24 hours in the body. Methamphetamine is excreted in the urine as amphetamine and oxidized and delaminated derivatives. However, 10-20% of Methamphetamine is excreted unchanged. Thus, the presence of the parent compound in the urine indicates Methamphetamine use.

**METHAQUALONE (MQL)**

Methaqualone (Quaalude, Sopor) is a quinazoline derivative that was first synthesized in 1951 and found clinically effective as a sedative and hypnotic in 1956. It soon gained popularity as a drug of abuse and in 1984 was removed from the US market due to extensive misuse. It is occasionally encountered in illicit form, and is also available in Europeon countries in combination with diphenhydramine (Mandrax). Methaqualone is extensively metabolized in vivo principally by hydroxylation at every possible position on the molecule. At least 12 metabolites have been identified in the urine.

**METHYLENEDIOXYPYROVALERONE (MDPV)**

Bath salts', a form of designer drugs, also promoted as 'plant food' or 'research chemicals', is sold mainly in head shops, on the Internet, and at other retail locations. Designer drugs were developed in recent years to subvert law enforcement and drug testing agencies and are advertised a'legal'highs. The technical term for 'bath salts' is substituted cathinone. Substituted cathinone is synthetic, concentrated version of the stimulant chemical in Khat. Khat is a plant that is cultivated and used in East Africa and the Middle East. It has a stimulant effect on the user and can be quite dangerous. The white crystals resemble legal bathing salts, thus the name of 'bath salts'. In 2009 and 2010 there was a significant rise in the abuse of synthetic cathinone, initially in the United Kingdom and the rest of Europe, and subsequently in the US and Canada,

Established as one of the main ingredients for 'bath salts', among other synthetic stimulants like Mephedrone, Methylone, Butylone and Methedrone, MDPV started appearing around 2004 when it was popularized as a club drug, often used in combination with alcohol, GHB, cannabis and other abused drugs, for its desired effects such as euphoria, alertness, talkativeness, and sexual arousal. There are currently no prescribed used for the synthetic stimulants.

While synthetic stimulants appear to affect users in ways similar to amphetamines, ecstasy and cocaine, reports concerning aggression, tachycardia, paranoia and suicide suggest that they may be more acutely toxic. These negative effects have resulted in an increase of ER visits and hospitalizations, severe psychotic and violent episodes, self-inflicted wounds, suicide and an alarming increase in abuse-related deaths. U.S. Poison Control and National Drug Intelligence have all issued health warnings, noting nationwide emergency room visits related to these drugs. In October 2011, the DEA announced an emergency ban on MDPV, Methylone and Mephedrone, making testing for these substances more vital than ever.

**6-MONOACETYLMORPHINE (6-MAM)**

6-Monoacetylmorphine (6-MAM) is one of three active metabolites of heroin (diacetylmorphine), the others being morphine and the much less active 3-acetylmorphine (3-ACM). 6-MAM is rapidly created from heroin in the body, and then is either metabolized into morphine or excreted in the urine. Since 6-ACM is a unique metabolite to heroin, its presence in the urine confirms that heroin was the opioid used. This is significant because on a urine immunoassay drug screen, the test typically tests for morphine, which is a metabolite of a number of legal and illegal opiates/opioids such as codeine, morphine sulphate, and heroin. 6-MAM remains in the urine for no more than 24 hours so a urine specimen must be collected soon after the last heroin use, but the presence of 6-MAM guarantees that heroin was in fact used as recently as within the last day.

**MORPHINE (MOP)**

Opiate refers to any drug that is derived from the opium poppy, including the natural products, morphine and codeine, and the semi-synthetic drugs such as heroin. Opioid is more general, referring to any drug that acts on the opioid receptor. Opioid analgesics comprise a large group of substances which control pain by depressing the central nervous system. Large doses of morphine can produce higher tolerance levels, physiological dependency in users, and may lead to substance abuse. Morphine is excreted unmetabolized, and is also the major metabolic product of codeine and heroin. Morphine is detectable in the urine for several days after an opiate dose.[4]

**OXYCODONE (OXY)**

Oxycodone, [4,5-epoxy-14-hydroxy-3-methoxy-17-methyl-morphinan-6-one, dihydrohydroxycodeinone] is a semi-synthetic opioid agonist derived from thebaine, a constituent of opium. Oxycodone is a Schedule II narcotic analgesic and is widely used in clinical medicine. The pharmacology of oxycodone is similar to that of morphine, in all respects, including its abuse and dependence liabilities. Pharmacological effects include analgesia, euphoria, feelings of relaxation, respiratory depression, constipation, papillary constriction, and cough suppression. Oxycodone is prescribed for the relief of moderate to high pain under pharmaceutical trade names as OxyContin® (controlled release), OxyIR®, OxyFast® (immediate release formulations), or Percodan® (aspirin) and Percocet® (acetaminophen) that are in combination with other nonnarcotic analgesics. Oxycodone's behavioral effects can last up to 5 hours. The controlled-release product, OxyContin®, has a longer duration of action (8-12 hours).

**PHENCYCLIDINE (PCP)**

Phencyclidine, also known as PCP or Angel Dust, is a hallucinogen that was first marketed as a surgical anesthetic in the 1950's. It was removed from the market because patients receiving it became delirious and experienced hallucinations. Phencyclidine is used in powder, capsule, and tablet form. The powder is either snorted or smoked after mixing it with marijuana or vegetable matter. Phencyclidine is most commonly administered by inhalation but can be used intravenously, intra-nasally, and orally. After low doses, the user thinks and acts swiftly and experiences mood swings from euphoria to depression. Self-injurious behavior is one of the devastating effects of Phencyclidine. PCP can be found in urine within 4 to 6 hours after use and will remain in urine for 7 to 14 days, depending on factors such as metabolic rate, user's age, weight, activity, and diet.[5] Phencyclidine is excreted in the urine as an unchanged drug (4% to 19%) and conjugated metabolites (25% to 30%).

**PROPOXYPHENE (PPX)**

Propoxyphene (PPX) is a mild narcotic analgesic found in various pharmaceutical preparations, usually as the hydrochloride or napsylate salt. These preparations typically also contain large amounts of acetaminophen, aspirin, or caffeine. Peak plasma concentrations of propoxyphene are achieved from 1 to 2 hours post dose. In the case of overdose, propoxyphene blood concentrations can reach significantly higher levels. In human, propoxyphene is metabolized by N-demethylation to yield norpropoxyphene. Norpropoxyphene has a longer half-life (30 to 36 hours) than parent propoxyphene (6 to 12 hours). The accumulation of norpropoxyphene seen with repeated doses may be largely responsible for resultant toxicity.

**SYNTHETIC MARIJUANA (K2)**

Synthetic Marijuana or K2 is a psychoactive herbal and chemical product that, when consumed, mimics the effects of Marijuana. It is best known by the brand names K2 and Spice, both of which have largely become genericized trademarks used to refer to any synthetic Marijuana product. The studies suggest that synthetic marijuana intoxication is associated with acute psychosis, worsening of previously stable psychotic disorders, and also may have the ability to trigger a chronic (long-term) psychotic disorder among vulnerable individuals such as those with a family history of mental illness.

Elevated levels of urinary metabolites are found within hours of exposure and remain detectable for 72 hours after smoking (depending on usage/dosage).

As of March 1, 2011, five cannabinoids, JWH-018, JWH-073, CP-47, JWH-200 and cannabicyclo hexanol are now illegal in the US because these substances have the potential to be extremely harmful and, therefore, pose an imminent hazard to the public safety. JWH-018 was developed and evaluated in basic scientific research to study structure activity relationships related to the cannabinoid receptors. JWH-073 has been identified in numerous herbal products, such as "Spice", "K2", K3" and others. These products may be smoked for their psychoactive effects.

**TRAMADOL (TRA)**

Tramadol is a quasi-narcotic analgesic used in the treatment of moderate to severe pain. It is a synthetic analog of codeine, but has a low binding affinity to the mu-opioid receptors. It has been prescribed off-label for the treatment of diabetic neuropathy and restless leg syndrome.[2] Large doses of Tramadol could develop tolerances and physiological dependency and lead to its abuse. Both Δ (d) and L forms of the isomers are controlled substances. Approximately 30% of the dose is excreted in the urine as unchanged drug, whereas 60% is excreted as metabolites. The major pathways appear to be N- and O- demethylation, glucoronidation or sulfation in the liver.

**TRICYCLIC ANTIDEPRESSANTS (TCA)**

TCA (Tricyclic Antidepressants) are commonly used for the treatment of depressive disorders. TCA overdoses can result in profound central nervous system depression, cardiotoxicity and anticholinergic effects. TCA overdose is the most common cause of death from prescription drugs. TCAs are taken orally or sometimes by injection. TCAs are metabolized in the liver. Both TCAs and their metabolites are excreted in urine mostly in the form of metabolites for up to ten days.

**ALCOHOL (ALC)**

Excess or inappropriate consumption of alcohol is a common and pervasive social problem. It is a contributory factor to many accidents, injuries and medical conditions. Screening of individuals for alcohol consumption is an important method for the identification of individuals who might be at risk due to alcohol use or intoxication. Screening is also an important deterrent against inappropriate alcohol consumption. The blood alcohol concentration at which a person becomes impaired is variable dependent on the individual. Parameters specific to the individual such as physical size, weight, activity level, eating habits and alcohol tolerance all affect the level of impairment. Determination of ethyl alcohol in urine, blood and saliva is commonly used for measuring legal impairment, alcohol poisoning, etc. Gas chromatography techniques and enzymatic methods are commercially available for the determination of ethyl alcohol in human fluids. Alcohol Test is designed to detect ethyl alcohol in urine specimens.

**ADULTERANT TESTS (SPECIMEN VALIDITY TESTS) SUMMARY**

The Adulterant Test Strip contains chemically treated reagent pads. Observation of the color change on the strip compared to the color chart provides a semi-quantitative screen for Oxidants, Specific Gravity, pH, Creatinine, Nitrite and Glutaraldehyde in human urine which can help to assess the integrity of the urine specimen.

Adulteration is the tampering of a urine specimen with the intention of altering the test results. The use of adulterants in the urine specimen can cause false negative results by either interfering with the test and/or destroying the drugs present in the urine. Dilution may also be used to produce false negative drug test results. To determine certain urinary characteristics such as specific gravity and pH, and to detect the presence of oxidants, Nitrite, Glutaraldehyde and Creatinine in urine are considered to be the best ways to test for adulteration or dilution.

- **Oxidants (OX)**: Tests for the presence of oxidizing agents such as bleach and peroxide in the urine.
- **Specific Gravity (S.G.)**: Tests for sample dilution. Normal levels for specific gravity will range from 1.003 to 1.030. Specific gravity levels of less than 1.003 or higher than 1.030 may be an indication of adulteration or specimen dilution.
- **pH:** tests for the presence of acidic or alkaline adulterants in urine. Normal pH levels should be in

the range of 4.0 to 9.0. Values below pH 4.0 or above pH 9.0 may indicate the sample has been altered.

- **Nitrite (NIT)**: Tests for commercial adulterants such as Klear and Whizzies. Normal urine specimens should contain no trace of nitrite. Positive results for nitrite usually indicate the presence of an adulterant.
- **Glutaraldehyde (GLU):** Tests for the presence of an aldehyde. Glutaraldehyde is not normally found in a urine specimen. Detection of glutaraldehyde in a specimen is generally an indicator of adulteration.
- **Creatinine (CRE):** Creatinine is one way to check for dilution and flushing, which are the most common mechanisms used in an attempt to circumvent drug testing. Low creatinine may indicate dilute urine.

## PRINCIPLE

(1) The **One Step Multi-Drug Screen Cassette Test (Urine)** is an immunoassay based on the principle of competitive binding. Drugs which may be present in the urine specimen compete against their respective drug conjugate for binding sites on their specific antibody.

During testing, a urine specimen migrates upward by capillary action. A drug, if present in the urine specimen below its cut-off concentration, will not saturate the binding sites of its specific antibody coated on the particles. The antibody coated particles will then be captured by the immobilized drug conjugate and a visible colored line will show up in the test line region of the specific drug strip. The colored line will not form in the test line region if the drug level is above its cut-off concentration because it will saturate all the binding sites of the antibody coated on the particles.

A drug-positive urine specimen will not generate a colored line in the specific test line region of the strip because of drug competition, while a drug-negative urine specimen or a specimen containing a drug concentration less than the cut-off will generate a line in the test line region. To serve as a procedural control, a colored line will always appear at the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

(2) Alcohol Test: A pad coated with enzymes, turns to color shades of green and blue on contact with alcohol in urine. The alcohol pad employs a solid phase chemistry which uses the following highly specific enzymatic reaction:

$$CH_3CH_2OH + O_2 \xrightarrow{\text{Alcohol Oxidase}} CH_3CHO + H_2O_2$$

$$H_2O_2 + DH_2 \xrightarrow[\text{Blue}]{\text{Peroxidase}} D + 2H_2O$$

## REAGENTS

Each test line in the test panel contains mouse monoclonal antibody-coupled particles and corresponding drug-protein conjugates. A goat antibody is employed in each control line.

## ADULTERANT TESTS (SPECIMEN VALIDITY TEST) REAGENTS

| Adulteration Pad | Reactive Indicator | Buffers and Non-reactive Ingredients |
|---|---|---|
| Oxidants (OX) | 0.30% | 99.70% |
| Specific Gravity (S.G.) | 0.21% | 99.79% |
| pH | 0.06% | 99.94% |
| Nitrite (NIT) | 0.06% | 99.94% |
| Glutaraldehyde (GLU) | 0.02% | 99.98% |
| Creatinine (CRE) | 0.03% | 99.97% |

## PRECAUTIONS

- For healthcare professional *in vitro* diagnostic use only.
- Do not use after the expiration date.
- The test dip card should remain in the sealed pouch until use.
- All specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The used test dip card should be discarded according to local regulations.

## STORAGE AND STABILITY

Store as packaged in the sealed pouch either at room temperature or refrigerated (2-30°C). The test dip card is stable through the expiration date printed on the sealed pouch. The test dip card must remain in the sealed pouch until use. Keep away from direct sunlight, moisture and heat. **DO NOT FREEZE.** Do not use beyond the expiration date.

## SPECIMEN COLLECTION AND PREPARATION

### Urine Assay

The urine specimen must be collected in a clean and dry container. Urine collected at any time of the day may be used. Urine specimens exhibiting visible precipitates should be centrifuged, filtered, or allowed to settle to obtain a clear supernatant for testing.

### Specimen Storage

Urine specimens may be stored at 2-8°C for up to 48 hours prior to testing. For prolonged storage, specimens may be frozen and stored below -20°C. Frozen specimens should be thawed and mixed well before testing.

## MATERIALS

### Materials Provided

1. 25 Sealed pouches each containing a test dip card and a desiccant
2. 1 Package insert
3. 2 Color Chart Cards for Adulterant Interpretation (when applicable)
4. 2 Color Chart Cards for Alcohol (when applicable)

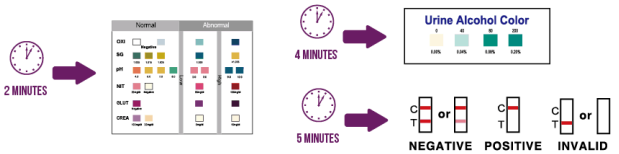
### Materials Required But Not Provided

- Timer

## DIRECTIONS FOR USE

**Allow the test dip card, and urine specimen to come to room temperature [15-30°C (59-86°F)] prior to testing.**

1) Remove the test dip card from the foil pouch.
2) Remove the cap from the test dip card. Label the dip card with patient or control identifications.
3) Immerse the absorbent tip into the urine sample for 10-15 seconds. Urine sample should not touch the plastic dip card.
4) Replace the cap over the absorbent tip and lay the dip card flatly on a non-absorptive clean surface.
5) Read the adulteration strip at 2 minutes by comparing the colors on the adulteration strip to the enclosed color chart. If the result indicates adulteration, do not interpret the drug test results. Either retest the urine or collect another specimen.
6) Read the alcohol strip in 4 minutes by comparing the colors on the alcohol strip to the enclosed color chart.
7) Read the drug strip results at 5 minutes. **DO NOT INTERPRET RESULT AFTER 5 MINUTES.**




## INTERPRETATION OF RESULTS

(Please refer to the illustration above)

**NEGATIVE:*** Two lines appear. One red line should be in the control region (C), and another apparent red or pink line adjacent should be in the test region (Drug/T). This negative result indicates that the drug concentration is below the detectable level.

*NOTE: The shade of red in the test line region (Drug/T) will vary, but it should be considered negative whenever there is even a faint pink line.

**POSITIVE:** One red line appears in the control region (C). No line appears in the test region (Drug/T). This positive result indicates that the drug concentration is above the detectable level.

**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 using a new test panel. If the problem persists, discontinue using the lot immediately and contact your manufacturer.

**Note**: There is no meaning attributed to line color intensity or width.

A preliminary positive test result does not always mean a person took illegal drugs and a negative test result does not always mean a person did not take illegal drugs. There are a number of factors that influence the reliability of drug tests. Certain drugs of abuse tests are more accurate than others.

**IMPORTANT**: The result you obtained is called preliminary for a reason. The sample must be tested by laboratory in order to determine if a drug of abuse is actually present. Send any sample which does not give a negative result to a laboratory for further testing.

***What Is A False Positive Test?***

The definition of a false positive test would be an instance where a substance is identified incorrectly by One Step Multi-Drug Screen Test Dip Card (Urine). The most common causes of a false positive test are cross reactants. Certain foods and medicines, diet plan drugs and nutritional supplements may cause a false positive test result with this product.

***What Is A False Negative Test?***

The definition of a false negative test is that the initial substance is present but isn't detected by One Step Multi-Drug Screen Test Dip Card (Urine). If the sample is diluted, or the sample is adulterated that may cause false negative result.

## ALCOHOL/ADULTERANT INTERPRETATION

(Please refer to the color chart)

Semi-quantitative results are obtained by visually comparing the reacted color blocks on the strip to the printed color blocks on the color chart. No instrumentation is required.

## QUALITY CONTROL

A procedural control is included in the test. A colored line appearing in the control line region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

## LIMITATIONS

1. The One Step Multi-Drug Screen Cassette Test (Urine) provides only a qualitative, preliminary analytical result. A secondary analytical method must be used to obtain a confirmed result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.
2. There is a possibility that technical or procedural errors, as well as other interfering substances in the urine specimen may cause erroneous results.
3. Adulterants, such as bleach and/or alum, in urine specimens may produce erroneous results regardless of the analytical method used. If adulteration is suspected, the test should be repeated with another urine specimen.
4. A positive result does not indicate level or intoxication, administration route or concentration in urine.
5. A negative result may not necessarily indicate drug-free urine. Negative results can be obtained when drug is present but below the cut-off level of the test.
6. The test does not distinguish between drugs of abuse and certain medications.
7. A positive result might be obtained from certain foods or food supplements.

## PERFORMANCE CHARACTERISTICS

### Accuracy

80 clinical urine specimens were analyzed by GC-MS and by the **One Step Multi-Drug Screen Cassette Test (Urine)**. Each test was performed by three operators. Samples were divided by concentration into five categories: drug-free, less than half the cutoff, near cutoff negative, near cutoff positive, and high positive. Results were as follows:

| Specimen | AMP | AMP 500 | AMP 300 | BAR | BAR 200 | BUP | BZO |
|---|---|---|---|---|---|---|---|
| Positive | 91.7% | 95.8% | 96.7% | 95.0% | 94.2% | 93.3% | 91.7% |
| Negative | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| Total | 95.8% | 97.9% | 98.3% | 97.5% | 97.1% | 96.7% | 95.8% |

| Specimen | BZO 200 | COC | COC 150 | COT | EDDP | EDDP 100 | ETG |
|---|---|---|---|---|---|---|---|
| Positive | 92.5% | 95.8% | 95.0% | 92.5% | 94.2% | 93.3% | 95.0% |
| Negative | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| Total | 96.3% | 97.9% | 97.5% | 96.3% | 97.1% | 96.7% | 97.5% |

| Specimen | ETG 300 | FEN | FEN 200 | FEN 100 | FEN 20 | K2 | K2 25 |
|---|---|---|---|---|---|---|---|
| Positive | 95.8% | 97.5% | 95.8% | 93.3% | 97.5% | 93.3% | 95.8% |
| Negative | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| Total | 97.9% | 98.8% | 97.9% | 96.7% | 98.8% | 96.7% | 97.9% |

| Specimen | KET | KET 100 | LSD | MET | MET 500 | MET 300 | MDMA |
|---|---|---|---|---|---|---|---|
| Positive | 95.8% | 91.7% | 91.7% | 95% | 95.8% | 95.8% | 95.0% |
| Negative | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| Total | 97.9% | 95.8% | 95.8% | 97.5% | 97.9% | 97.9% | 97.5% |

| Specimen | MOP | MQL | 6-MAM | MTD | OPI | OXY | PCP |
|---|---|---|---|---|---|---|---|
| Positive | 96.7% | 91.7% | 92.5% | 95.0% | 88.3% | 93.3% | 91.7% |
| Negative | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| Total | 98.3% | 95.8% | 96.3% | 97.5% | 94.2% | 96.7% | 95.8% |

| Specimen | PPX | THC | THC 25 | THC 20 | TCA | TRA | MDPV |
|---|---|---|---|---|---|---|---|
| Positive | 95.0% | 95.8% | 94.2% | 91.7% | 95.0% | 93.3% | 94.2% |
| Negative | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| Total | 97.5% | 97.9% | 97.1% | 95.8% | 97.5% | 96.7% | 97.1% |

### Analytical Sensitivity

Total 150 samples equally distributed at concentrations of -50% Cut-Off; -25% Cut-Off; Cut-Off; +25% Cut-Off; +50% Cut-Off were tested using three different lots of each dip card by three different operators. Results were all positive at and above +25% Cut-off and all negative at and below -25% Cut-off for Methamphetamine, Amphetamine, Cocaine, Morphine, Ecstasy, EDDP (Methadone Metabolites), Tricyclic Antidepressants, Oxycodone, Barbiturates, Buprenorphine, Phencyclidine, K2 (Synthetic Cannabinoid), Ketamine, Methaqualone, Methadone, Fentanyl, Tramadol, Ethyl Glucuronide, Cotinine, 6-Monoacetylmorphine, Methylenedioxypyrovalerone, Lysergic acid diethylamide, Marijuana and Benzodiazepines. The cut-off value for the dip card is verified.

### Analytical Specificity

The following table lists compounds that are positively detected in urine by the **One Step Multi-Drug Screen Cassette Test (Urine)** at 5 minutes.

| Drug | Concentration (ng/mL) |
|---|---|
| **AMPHETAMINE (AMP)** | |
| D-Amphetamine | 1,000 |
| D,L - Amphetamine (Amphetamine Sulfate) | 1,000 |
| Phentermine | 1,250 |
| (+/-)-4-Hydroxyamphetamine HCL | 600 |
| L-Amphetamine | 20,000 |
| 3,4-Methylenedioxyamphetamine HCl (MDA) | 1,500 |
| d-Methamphetamine | >100,000 ng/mL |
| 1-Methamphetamine | >100,000 ng/mL |
| ephedrine | >100,000 ng/mL |
| 3,4-Methylenedioxyethylamphetamine (MDE) | >100,000 ng/mL |
| 3,4-methylenedioxy-methamphetamine (MDMA) | >100,000 ng/mL |
| | |
| **AMPHETAMINE (AMP 500)** | |
| D-Amphetamine | 500 |
| D,L-Amphetamine | 750 |
| L-Amphetamine | 16,000 |
| Phentermine | 650 |
| (+/-)-Methylenedioxyamphetamine (MDA) | 800 |
| d-Methamphetamine | >100,000 |
| 1-Methamphetamine | >100,000 |
| ephedrine | >100,000 |
| 3,4-Methylenedioxyethylamphetamine (MDE) | >100,000 |
| 3,4-methylenedioxy-methamphetamine (MDMA) | >100,000 |
| | |
| **AMPHETAMINE (AMP 300)** | |
| D-Amphetamine | 300 |
| D,L-Amphetamine | 450 |
| L-Amphetamine | 9,000 |
| Phentermine | 450 |
| (+/-)-Methylenedioxyamphetamine (MDA) | 600 |
| | |
| **BARBITURATES (BAR)** | |
| Secobarbital | 300 |
| Amobarbital | 300 |
| Alphenal | 750 |
| Aprobarbital | 250 |
| Butabarbital | 2,500 |
| Butethal | 2,500 |
| Cyclopentobarbital | 500 |
| Pentobarbital | 2,500 |
| Phenobarbital | 25,000 |
| | |
| **BARBITURATES (BAR 200)** | |
| Secobarbital | 200 |
| Amobarbital | 200 |
| Alphenal | 500 |
| Aprobarbital | 200 |
| Butabarbital | 2,000 |
| Butethal | 2,000 |
| Butalbital | 2,000 |
| Cyclopentobarbital | 300 |
| Pentobarbital | 2,000 |
| | |
| **BENZODIAZEPINES (BZO)** | |
| Alprazolam | 200 |
| Bromazepam | 1,560 |
| Chlordiazepoxide HCL | 1,560 |
| Clobazam | 100 |

| Drug | Concentration (ng/mL) |
|---|---|
| Clonazepam | 780 |
| Clorazepate Dipotassium | 200 |
| Delorazepam | 1,560 |
| Desalkylflurazepam | 400 |
| Diazepam | 200 |
| Estazolam | 2,500 |
| Flunitrazepam | 400 |
| a-Hydroxyalprazolam | 1260 |
| (±) Lorazepam | 1,560 |
| RS-Lorazepam glucuronide | 160 |
| Midazolam | 12,500 |
| Nitrazepam | 100 |
| Norchlordiazepoxide | 200 |
| Nordiazepam | 400 |
| Oxazepam | 300 |
| Temazepam | 100 |
| Triazolam | 2,500 |
| | |
| **BENZODIAZEPINES (BZO200)** | |
| Alprazolam | 200 |
| Bromazepam | 1,000 |
| Chlordiazepoxide HCL | 1,000 |
| Clobazam | 80 |
| Clonazepam | 500 |
| Clorazepate Dipotassium | 100 |
| Delorazepam | 1,000 |
| Desalkylflurazepam | 300 |
| Diazepam | 100 |
| Estazolam | 2,000 |
| Flunitrazepam | 300 |
| a-Hydroxyalprazolam | 840 |
| (±) Lorazepam | 1,000 |
| RS-Lorazepam glucuronide | 100 |
| Midazolam | 10,000 |
| Nitrazepam | 100 |
| Norchlordiazepoxide | 100 |
| Nordiazepam | 300 |
| Oxazepam | 200 |
| Temazepam | 800 |
| Triazolam | 2,000 |
| | |
| **BUPRENORPHINE (BUP)** | |
| Buprenorphine | 10 |
| Norbuprenorphine | 20 |
| | |
| **COCAINE (COC)** | |
| Benzoylecogonine | 300 |
| Cocaethylene | 300 |
| Cocaine HCl | 300 |
| | |
| **COCAINE (COC 150)** | |
| Benzoylecogonine | 150 |
| Cocaethylene | 2,500 |
| Cocaine | 500 |
| Ecgonine | 12,500 |
| Ecgonine methylester | 50,000 |
| | |
| **COTININE (COT)** | |
| Cotinine | 200 |

| Drug | Concentration (ng/mL) |
|---|---|
| Nicotine | 6,250 |
| | |
| **MDMA (ECSTASY)** | |
| D,L-3,4-Methylenedioxymethamphetamine (MDMA) | 500 |
| 3,4-Methylenedioxyamphetamine HCl (MDA) | 3,000 |
| 3,4-Methylenedioxyethyla-amphetamine (MDEA) | 300 |
| d-methamphetamine | 2500 |
| d-amphetamine | >100,000 |
| l-amphetamine | >100,000 |
| l-methamphetamine | >100,000 |
| | |
| **ETHYL GLUCURONIDE (EtG 500)** | |
| Ethyl-β-D-glucuronide | 500 |
| Ethyl-β-D-glucuronide-D5 | 500 |
| | |
| **ETHYL GLUCURONIDE (EtG 300)** | |
| Ethyl-β-D-glucuronide | 300 |
| Ethyl-β-D-glucuronide-D5 | 300 |
| | |
| **FENTANYL (FEN)** | |
| Norfentanyl | 20 |
| Fentanyl | 300 |
| | |
| **FENTANYL (FEN20)** | |
| Norfentanyl | 20 |
| Fentanyl | 300 |
| | |
| **FENTANYL (FEN200)** | |
| Norfentanyl | 15 |
| Fentanyl | 200 |
| Sufentanyl | 50,000 |
| Fenfluramine | 50,000 |
| | |
| **FENTANYL (FEN 100)** | |
| Norfentanyl | 10 |
| Fentanyl | 100 |
| Buspirone | >100,000 |
| Sufentanyl | 25,000 |
| Fenfluramine | 25,000 |
| | |
| **KETAMINE (KET)** | |
| Ketamine | 1,000 |
| Norketamine | 3,000 |
| Methoxy-amphetamine | 12,500 |
| Promethazine | 25,000 |
| 4-hydroxyphenyl cyclohexyl piperidine | 50,000 |
| | |
| **KETAMINE (KET 100)** | |
| Ketamine | 100 |
| Norketamine | 100 |
| Methoxy-amphetamine | 1,250 |
| Promethazine | 2,500 |
| 4-hydroxyphenyl cyclohexyl piperidine | 5,000 |
| | |
| **LYSERGIC ACID DIETHYLAMIDE (LSD)** | |
| D-lysergic acid diethylamide | 20 |
| Fentanyl | 75 |
| Norfentanyl | 300 |
| | |

| Drug | Concentration (ng/mL) |
|---|---|
| **MARIJUANA (THC)** | |
| Delta-9-Tetrahydrocannabinol | 50,000 |
| 11-nor-delta-9-THC-carboxyglucuronide | 75 |
| (-)-11-nor-9-carboxy-delta9-THC | 75 |
| 11-Nor-$\Delta^9$-Tetrahydrocannabinol | 50 |
| 11-Hydroxy-$\Delta^9$-Tetrahydrocannabinol | 5,000 |
| 11-Nor-$\Delta^8$-Tetrahydrocannabinol | 50 |
| $\Delta^8$-THC-COOH | 50,000 |
| | |
| **MARIJUANA (THC 25)** | |
| Delta-9-Tetrahydrocannabinol | 25,000 |
| 11-nor-delta-9-THC-carboxyglucuronide | 37.5 |
| (-)-11-nor-9-carboxy-delta9-THC | 37.5 |
| 11-Nor-$\Delta^9$-Tetrahydrocannabinol | 25 |
| 11-Hydroxy-$\Delta^9$-Tetrahydrocannabinol | 2,500 |
| 11-Nor-$\Delta^8$-Tetrahydrocannabinol | 25 |
| $\Delta^8$-THC-COOH | 25,000 |
| | |
| **MARIJUANA (THC 20)** | |
| Delta-9-Tetrahydrocannabinol | 20,000 |
| 11-nor-delta-9-THC-carboxyglucuronide | 30 |
| (-)-11-nor-9-carboxy-delta9-THC | 30 |
| 11-Nor-$\Delta^9$-Tetrahydrocannabinol | 20 |
| 11-Hydroxy-$\Delta^9$-Tetrahydrocannabinol | 2,000 |
| 11-Nor-$\Delta^8$-Tetrahydrocannabinol | 20 |
| $\Delta^8$-THC-COOH | 20,000 |
| | |
| **METHADONE (MTD)** | |
| Methadone | 300 |
| Doxylamine | 5,000 |
| | |
| **EDDP (Methadone Metabolites)** | |
| EDDP | 300 |
| Disopyramide | 50,000 |
| Methadone | >100,000 |
| EMDP | 500 |
| | |
| **EDDP100 (Methadone Metabolites)** | |
| EDDP | 100 |
| Disopyramide | 20,000 |
| Methadone | >100,000 |
| EMDP | 200 |
| | |
| **METHAMPHETAMINE (mAMP)** | |
| D-Methamphetamine | 1,000 |
| (+/-) 3,4-Methylenedioxy-n-ethylamphetamine (MDEA) | 20,000 |
| Procaine (Novocaine) | 60,000 |
| Trimethobenzamide | 20,000 |
| Methamphetamine | 1,000 |
| Ranitidine (Zantac) | 50,000 |
| (+/-) 3,4-Methylenedioxymethamphetamine (MDMA) | 2,500 |
| Chloroquine | 50,000 |
| Ephedrine | 100,000 |
| Fenfluramine | 50,000 |
| p-Hydroxymethamphetamine | 10,000 |
| | |
| **METHAMPHETAMINE (MET 500)** | |
| p-Hydroxymethamphetamine | 15,000 |
| l-Methamphetamine | 4,000 |

| Drug | Concentration (ng/mL) |
|---|---|
| Mephentermine | 25,000 |
| d,l-Amphetamine | 75,000 |
| (1R,2S)-(-)-Ephedrine | 50,000 |
| β-Phenylethylamine | 75,000 |
| d-Methamphetamine | 500 |
| 3,4-Methylenedioxymethamphetamine (MDMA) | 1,000 |
| d-Amphetamine | 50,000 |
| Chloroquine | 12,500 |
| (+/-) 3,4-Methylenedioxy-n-ethylamphetamine (MDEA) | 20,000 |
| Procaine (Novocaine) | 50,000 |
| Trimethobenzamide | 20,000 |
| Ranitidine (Zantac) | 50,000 |
| Fenfluramine | 50,000 |
| | |
| **METHAMPHETAMINE (MET 300)** | |
| p-Hydroxymethamphetamine | 10,000 |
| l-Methamphetamine | 3,000 |
| Mephentermine | 15,000 |
| d,l-Amphetamine | 50,000 |
| (1R,2S)-(-)-Ephedrine | 50,000 |
| β-Phenylethylamine | 50,000 |
| d-Methamphetamine | 300 |
| 3,4-Methylenedioxymethamphetamine (MDMA) | 1,000 |
| d-Amphetamine | 30,000 |
| Chloroquine | 7,500 |
| (+/-) 3,4-Methylenedioxy-n-ethylamphetamine (MDEA) | 12,000 |
| Procaine (Novocaine) | 30,000 |
| Trimethobenzamide | 12,000 |
| Ranitidine (Zantac) | 30,000 |
| Fenfluramine | 30,000 |
| | |
| **METHAQUALONE (MQL)** | |
| Methaqualone | 300 |
| | |
| **METHYLENEDIOXYPYROVALERONE (MDPV)** | |
| 3,4-Methylenedioxypyrovalerone | 1,000 |
| Ethylone HCl | 1,200 |
| Methylone | 50,000 |
| Pyrovalerone | 50,000 |
| | |
| **6-MONOACETYLMORPHINE (6-MAM)** | |
| 6-Moonacetylmorphine | 10 |
| Morphine | >500,000 |
| Codeine | >600,000 |
| Dextromethorphan | >100,000 |
| Dihydrocodeine | >100,000 |
| Heroin HCl | 250 |
| Hydrocodone | >100,000 |
| Hydromorphone | >100,000 |
| Imipramine | >100,000 |
| Levorphanol | >10,000 |
| NorMeperidine | >10,000 |
| Normorphine | >100,000 |
| Nalorphine | >100,000 |
| Naloxone | >100,000 |
| Naltrexone | >100,000 |
| Norcodeine | >100,000 |
| Oxycodone | >100,000 |
| Oxymorphone | >100,000 |

| Drug | Concentration (ng/mL) |
|---|---|
| | |
| **MORPHINE (MOP)** | |
| Morphine | 300 |
| O6-Acetylmorphine | 400 |
| Codeine | 300 |
| EthylMorphine | 100 |
| Heroin | 600 |
| Hydromorphone | 500 |
| Hydrocodone | 50,000 |
| Levorphanol | 1,500 |
| Oxycodone | 30,000 |
| Procaine | 15,000 |
| Thebaine | 6,240 |
| | |
| **MORPHINE (OPI, MOP2000)** | |
| Morphine | 2,000 |
| O6-Acetylmorphine | 2,500 |
| Codeine | 1,000 |
| EthylMorphine | 250 |
| Heroin | 5,000 |
| Hydromorphone | 2,500 |
| Hydrocodone | 5,000 |
| Oxycodone | 75,000 |
| Thebaine | 13,000 |
| | |
| **OXYCODONE (OXY)** | |
| Naloxone hydrochloride | 10,000 |
| Naltrexone hydrochloride | 50,000 |
| Oxycodone | 100 |
| Hydrocodone | 5,000 |
| Hydromorphone | 5,000 |
| Oxymorphone-D3 | 5,000 |
| Oxymorphone | 200 |
| N-Benzylisopropylamine | 2,500 |
| | |
| **PHENCYCLIDINE (PCP)** | |
| Phencyclidine | 25 |
| 4-Hydroxy Phencyclidine | 90 |
| | |
| **PROPOXYPHENE (PPX)** | |
| Norpropoxyphene | 300 |
| d-Propoxyphene | 300 |
| | |
| **K2 (SYNTHETIC CANNABINOID)** | |
| JWH-018 5-Pentanoic acid metabolite | 50 |
| JWH-018 5-Hydroxypentyl metabolite | 500 |
| JWH-018 4-Hydroxypentyl metabolite | 400 |
| JWH-018 N-(4-hydroxypentyl) metabolite solution | 5,000 |
| JWH-019 5-hydroxyhexylmetabolite | <10,000 |
| JWH-019 6-Hydroxyhexyl | 5,000 |
| JWH-073 4-butanoic acid metabolite | 50 |
| JWH-073 4-Hydroxybutyl metabolite | 500 |
| JWH-210 5-Hydroxypentyl metabolite solution | <10,000 |
| JWH-122 5-Hydroxypentyl metabolite solution | <10,000 |
| Spice Cannabinoid Mix 3 solution | <10,000 |
| JWH-122 4-Hydroxypentyl metabolite solution | <10,000 |
| JWH-122 4-Hydroxypentyl metabolite-D5 solution | <10,000 |
| JWH-019 5-hydroxyhexylmetabolite | <10,000 |
| JWH-018 N-(4-hydroxypentyl) metabolite solution | <10,000 |

| Drug | Concentration (ng/mL) |
|---|---|
| JWH-073 N-(3-Hydroxybutyl) metabolite solution | <10,000 |
| | |
| **K2 (SYNTHETIC CANNABINOID) 25 ng/mL** | |
| JWH-018 5-Pentanoic acid metabolite | 25 |
| JWH-018 5-Hydroxypentyl metabolite | 250 |
| JWH-018 4-Hydroxypentyl metabolite | 200 |
| JWH-018 N-(4-hydroxypentyl) metabolite solution | 2,500 |
| JWH-019 5-hydroxyhexylmetabolite | <10,000 |
| JWH-019 6-Hydroxyhexyl | 2,500 |
| JWH-073 4-butanoic acid metabolite | 25 |
| JWH-073 4-Hydroxybutyl metabolite | 250 |
| JWH-210 5-Hydroxypentyl metabolite solution | <10,000 |
| JWH-122 5-Hydroxypentyl metabolite solution | <10,000 |
| Spice Cannabinoid Mix 3 solution | <10,000 |
| JWH-122 4-Hydroxypentyl metabolite solution | <10,000 |
| JWH-122 4-Hydroxypentyl metabolite-D5 solution | <10,000 |
| JWH-019 5-hydroxyhexylmetabolite | <10,000 |
| JWH-018 N-(4-hydroxypentyl) metabolite solution | <10,000 |
| JWH-073 N-(3-Hydroxybutyl) metabolite solution | <10,000 |
| | |
| **TRAMADOL (TRA )** | |
| Tramadol | 200 |
| N-desmethyl-tramadol | 500 |
| O-desmethyl-tramadol | 20,000 |
| | |
| **Tricyclic Antidepressants (TCA)** | |
| Nortriptyline | 1,000 |
| Amitriptyline | 1,500 |
| Clomipramine | 50,000 |
| Desipramine | 5,000 |
| Doxepine | 10,000 |
| Imipramine | 10,000 |
| Maprotiline | 100,000 |
| Nordoxepin | 10,000 |
| Promazine | 50,000 |
| Promethazine | 2,500 |
| Trimipramine | 50,000 |
| Cyclobenzaprine Hydrochloride | 5,000 |
| Norclomipramine | 50,000 |

**Precision**

This study is performed 2 runs/day and lasts 25 days for each format with three lots. Three operators who don't know the sample number system participate in the study. Each of the 3 operators tests 2 aliquots at each concentration for each lot per day (2 runs/day). A total of 50 determinations by each operator, at each concentration, were made. The results are given below:


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 GBDOA-1X5

| Drug Conc. (Cut-off range) | AMP | | AMP 500 | | AMP 300 | | BAR | | BAR 200 | | BZO | | BZO 200 | | BUP | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | - | + | - | + | - | + | - | + | - | + | - | + | - | + | - | + |
| 0% Cut-off | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 |
| -75% Cut-off | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 |
| -50% Cut-off | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 |
| -25% Cut-off | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 |
| Cut-off | 20 | 30 | 20 | 30 | 22 | 28 | 23 | 27 | 23 | 27 | 18 | 32 | 24 | 26 | 28 | 22 |
| +25% Cut-off | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 |
| +50% Cut-off | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 |
| +75% Cut-off | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 |
| +100% Cut-off | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 |

| Drug Conc. (Cut-off range) | COC | | COC150 | | COT | | EDDP | | EDDP 100 | | ETG | | ETG 300 | | FEN | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | - | + | - | + | - | + | - | + | - | + | - | + | - | + | - | + |
| 0% Cut-off | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 |
| -75% Cut-off | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 |
| -50% Cut-off | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 |
| -25% Cut-off | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 41 | 9 | 44 | 6 | 42 | 8 | 50 | 0 |
| Cut-off | 20 | 30 | 24 | 26 | 20 | 30 | 21 | 29 | 30 | 20 | 23 | 27 | 23 | 27 | 22 | 28 |
| +25% Cut-off | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 3 | 47 | 8 | 42 | 4 | 46 | 0 | 50 |
| +50% Cut-off | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 |
| +75% Cut-off | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 |
| +100% Cut-off | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 |

| Drug Conc. (Cut-off range) | FEN 200 | | FEN 100 | | FEN 20 | | K2 | | K2 25 | | KET | | KET 100 | | MET | | MET 500 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | - | + | - | + | - | + | - | + | - | + | - | + | - | + | - | + | - | + |
| 0% Cut-off | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 |
| -75% Cut-off | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 |
| -50% Cut-off | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 |
| -25% Cut-off | 46 | 4 | 43 | 7 | 50 | 0 | 50 | 0 | 50 | 0 | 45 | 5 | 44 | 6 | 50 | 0 | 50 | 0 |
| Cut-off | 28 | 22 | 20 | 30 | 22 | 28 | 18 | 32 | 22 | 28 | 18 | 32 | 30 | 20 | 24 | 26 | 25 | 25 |
| +25% Cut-off | 5 | 45 | 2 | 48 | 0 | 50 | 0 | 50 | 0 | 50 | 6 | 44 | 3 | 47 | 0 | 50 | 0 | 50 |
| +50% Cut-off | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 |
| +75% Cut-off | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 |
| +100% Cut-off | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 |

| Drug Conc. (Cut-off range) | OXY | | MET 300 | | MDMA | | MOP | | MQL | | 6-MAM | | MTD | | OPI | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | - | + | - | + | - | + | - | - | - | + | - | + | - | + | - | + |
| 0% Cut-off | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 50 | 50 | 0 | 50 | 0 | 50 | 0 |
| -75% Cut-off | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 50 | 50 | 0 | 50 | 0 | 50 | 0 |
| -50% Cut-off | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 50 | 50 | 0 | 50 | 0 | 50 | 0 |
| -25% Cut-off | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 48 | 50 | 50 | 0 | 50 | 0 | 50 | 0 |
| Cut-off | 24 | 26 | 25 | 25 | 24 | 26 | 22 | 28 | 24 | 22 | 22 | 27 | 28 | 22 | 22 | 28 |
| +25% Cut-off | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 6 | 0 | 5 | 45 | 0 | 50 | 0 | 50 |
| +50% Cut-off | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 0 | 0 | 50 | 0 | 50 | 0 | 50 |
| +75% Cut-off | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 0 | 0 | 50 | 0 | 50 | 0 | 50 |
| +100% Cut-off | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 0 | 0 | 50 | 0 | 50 | 0 | 50 |

| Drug Conc. (Cut-off range) | PCP | | PPX | | THC | | THC 25 | | THC 20 | | TCA | | TRA | | LSD | | MDPV | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | - | + | - | + | - | + | - | + | - | + | - | + | - | + | - | + | - | + |
| 0% Cut-off | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 |
| -75% Cut-off | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 |
| -50% Cut-off | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 |
| -25% Cut-off | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 47 | 3 | 44 | 6 | 48 | 2 |
| Cut-off | 22 | 28 | 26 | 24 | 20 | 30 | 23 | 27 | 25 | 25 | 22 | 28 | 25 | 25 | 21 | 29 | 24 | 26 |
| +25% Cut-off | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 1 | 49 | 5 | 45 | 7 | 43 |
| +50% Cut-off | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 |
| +75% Cut-off | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 |
| +100% Cut-off | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 |

**Effect of Urinary Specific Gravity**

Twelve (12) urine samples of normal, high, and low specific gravity from 1.000 to 1.035 were spiked with drugs at 25% below and 25% above cut-off levels respectively. The **One Step Multi-Drug Screen Cassette Test (Urine)** was tested in duplicate using drug-free urine and spiked urine samples. The results demonstrate that varying ranges of urinary specific gravity do not affect the test results.

**Effect of Urinary pH**

The pH of an aliquot of negative urine pool is adjusted in the range of 4.00 to 9.00 in 1 pH unit increment and spiked with the target drug at 25% below and 25% above Cutoff levels. The spiked, pH-adjusted urine was tested with The **One Step Multi-Drug Screen Cassette Test (Urine)**. The results demonstrate that varying ranges of pH do not interfere with the performance of the test.

**Cross-Reactivity**

A study was conducted to determine the cross-reactivity of the test with compounds in either drug-free urine or Methamphetamine, Amphetamine, Cocaine, Morphine, Ecstasy, EDDP (Methadone Metabolites), Tricyclic Antidepressants, Oxycodone, Barbiturates, Buprenorphine, Phencyclidine, K2(Synthetic Cannabinoid), Ketamine, Methaqualone, Methadone, Fentanyl, Tramadol, Ethyl Glucuronide, Cotinine, 6-Monoacetylmorphine, Methylenedioxypyrovalerone, Lysergic acid diethylamide, Marijuana and Benzodiazepines positive urine. The following compounds show no cross-reactivity when tested with the **One Step Multi-Drug Screen Cassette Test (Urine)** at a concentration of 100 μg/mL.

**Non Cross-Reacting Compounds**

| | | | |
|---|---|---|---|
| Acetophenetidin | Cortisone | Pseudoephedrine | Quinidine |
| N-Acetylprocainamide | Creatinine | Kynurenic Acid | Quinine |
| Acetylsalicylic acid | Dexamethasone | Labetalol | Salicylic acid |
| Amiloride | Dextromethorphan | Loperamide | Serotonin |
| Amoxicillin | Desipramine | Meprobamate | Sulfamethazine |
| Ampicillin | Diflunisal | Methoxyphenamine | Sulindac |
| l-Ascorbic acid | Digoxin | Methylphenidate | Tetracycline |
| Apomorphine | Droperidol | Nalidixic acid | Tetrahydrocortisone, |
| Aspartame | Ethyl-p-aminobenzoate | Naproxen | 3-Acetate |
| Atropine | Ethopropazine | Niacinamide | Theobromine |
| Benzilic acid | Estrone-3-sulfate | Nifedipine | Tolazamide |
| p-Aminobenzoic Acid | Erythromycin | Norethindrone | Tetrahydrozoline |
| Bilirubin | Fenoprofen | Noscapine | Thiamine |
| Beclomethasone | Furosemide | Octopamine | Thioridazine Hydrochloride |
| Caffeine | Gentisic acid | Oxalic acid | D/L-Tyrosine |
| Cannabidiol | Hemoglobin | Oxyphenbutazone | Tolbutamide |
| Carbamazepine | Hydralazine | Oxymetazoline | Triamterene |
| Chloramphenicol | Hydrochlorothiazide | Papaverine | Trifluoperazine |
| Chlorothiazide | Hydrocortisone | Paclitaxel | Trimethoprim |
| Chlorpheniramine | α-Hydroxyhippuric acid | Perphenazine | D,L-Tryptophan |
| Chlorpromazine | Hydroxyprogesterone | Phenelzine | Uric acid |
| Cholesterol | Isoproterenol-(+/-) | Prednisone | Verapamil |
| Clonidine | Isoxsuprine | Prilocaine | Zomepirac |

**BIBLIOGRAPHY**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2. *Ambre J. J. Anal. Toxicol. 1985; 9:241.*
3. *Hawks RL, CN Chiang. Urine Testing for Drugs of Abuse. National Institute for Drug Abuse (NIDA), Research Monograph 73, 1986.*
4. *Tietz NW. Textbook of Clinical Chemistry. W.B. Saunders Company. 1986; 1735.*
5. *FDA Guidance Document: Guidance for Premarket Submission for Kits for Screening Drugs of Abuse to be Used by the Consumer, 1997.*


**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# |

Revision Date: 2022-05-31
B21769-03

# Troponin I

## Troponin I Rapid Test Cassette (Whole Blood/Serum/Plasma)

## Package Insert

*A rapid visual immunoassay for the qualitative presumptive detection of cardiac Troponin I in human whole blood, serum, or plasma specimens. For professional in vitro diagnostic use only.*

**INTENDED USE**

The Troponin I Rapid Test Cassette (Whole Blood/Serum/Plasma) is a rapid visual immunoassay for the qualitative presumptive detection of cardiac Troponin I in human whole blood, serum, or plasma specimens. This kit is intended to be used as an aid in the diagnosis of myocardial infarction (MI).

**SUMMARY**

Cardiac Troponin I (cTnI) is a protein found in cardiac muscle with a molecular weight of 22.5 kDa.[1] Troponin I is part of a three subunit complex comprising of Troponin T and Troponin C. Along with tropomyosin, this structural complex forms the main component that regulates the calcium sensitive ATPase activity of actomyosin in striated skeletal and cardiac muscle.[2] After cardiac injury occurs, Troponin I is released into the blood 4-6 hours after the onset of pain. The release pattern of cTnI is similar to CK-MB, but while CK-MB levels return to normal after 72 hours, Troponin I remains elevated for 6-10 days, thus providing for a longer window of detection for cardiac injury. The high specificity of cTnI measurements for the identification of myocardial damage has been demonstrated in conditions such as the perioperative period, after marathon runs, and blunt chest trauma.[3] cTnI release has also been documented in cardiac conditions other than acute myocardial infarction (AMI) such as unstable angina, congestive heart failure, and ischemic damage due to coronary artery bypass surgery.[4] Because of its high specificity and sensitivity in the myocardial tissue, Troponin I has recently become the most preferred biomarker for myocardial infarction.[5]

**PRINCIPLE**

The Troponin I Rapid Test Cassette (Whole Blood/Serum/Plasma) has been designed to detect cardiac Troponin I through visual interpretation of color development in the strip. The membrane was immobilized with anti-cTnI antibodies on the test region. During the test, the specimen is allowed to react with colored anti-cTnI antibodies colloidal gold conjugates, which were precoated on the sample pad of the test. The mixture then moves on the membrane by a capillary action, and interact with reagents on the membrane. If there were enough cTnI in specimens, a colored band will form at the test region of the membrane.

Presence of this colored band indicates a positive result, while its absence indicates a negative result. Appearance of a colored band at the control region serves as a procedural control. This indicates that proper volume of specimen has been added and membrane wicking has occurred.

**PRECAUTIONS**

1. For professional *in vitro* diagnostic use only.
2. Warning: the reagents in this kit contain sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
3. Do not use it if the tube/pouch is damaged or broken.
4. Test is for single use only. Do not re- use under any circumstances.
5. Handle all specimens as if they contain infectious agents. Observe established standard procedure for proper disposal of specimens
6. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assay.
7. Humidity and temperature can adversely affect results.

**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.

**SPECIMEN COLLECTION AND PREPARATION**

- The Troponin I Rapid Test Cassette (Whole Blood/Serum/Plasma) is intended only for use with human whole blood, serum, or plasma specimens.
- Only clear, non-hemolyzed specimens are recommended for use with this test.
- Serum or plasma should be separated with soonest possible opportunity to avoid hemolysis.
- Perform the testing immediately after the specimen collection. Do not leave the specimens at room temperature for prolonged periods. Specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be kept below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Avoid repeated freezing and thawing of specimens.
- Pack the specimens in compliance with applicable regulations for transportation of etiological agents, in case they need to be shipped.
- Icteric, lipemic, hemolysed, heat treated and contaminated sera may cause erroneous results.
- There is a slight possibility that some whole blood specimens with very high viscosity or which have been stored for more than 2 days may not run properly on the test device. Repeat the test with a serum or plasma specimen from the same patient using a new test device.

**Materials Provided**

1. Test cassettes 2.Disposable Droppers 3. Package insert

**Materials Required But Not Provided**

1. Specimen collection containers 2.Centrifuge (for plasma only)
3. Clock or Timer

**DIRECTIONS FOR USE**

**Allow test device, specimen, buffer and/or controls to equilibrate to room temperature (15-30°C) prior to testing.**

1. Remove the test from its sealed pouch, and place it on a clean, level surface. Label the device with patient or control identification. To obtain a best result, the assay should be performed within one hour.
2. Transfer **2-3 drops of serum or plasma** to the specimen well(S) of the device with a disposable pipette provided in the kit, and then start the timer.

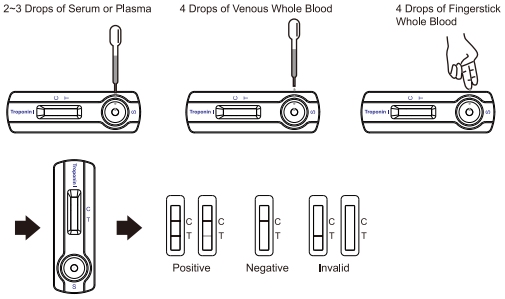
**OR**

Transfer **4 drops of whole blood** specimen to the specimen well(S) of the device with a disposable pipette provided in the kit, and then start the timer.

**OR**

Allow **4 hanging drops of fingerstick whole blood** specimen to fall into the center of the specimen well (S) of the device, and then start the timer. Avoid trapping air bubbles in the specimen well (S), and do not drop any solution in observation window.As the test begins to work, you will see color move across the membrane.

2. Wait for the colored band(s) to appear. The result should be read at 15 minutes. Do not interpret the result after 20 minutes.




**INTERPRETATION OF RESULTS**

(Please refer to the illustration above)

**POSITIVE**:Two colored bands appear on the membrane. One band appears in the control region (C) and another band appears in the test region (T).

**NEGATIVE**:Only one colored band appears in the control region (C). No apparent colored band appears in the test region (T).

**INVALID**:Control band fails to appear. Results from any test which has not produced a control band at the specified reading time must be discarded.

Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

***NOTE:*** Insufficient specimen volume, incorrect operation procedure, or performing expired tests are the most likely reasons for control band failure.

**QUALITY CONTROL**

Internal procedural controls are included in the test. A colored band appearing in the control region (C) is considered an internal positive procedural control. It confirms sufficient specimen volume and correct procedural technique.

External controls are not supplied with this kit. 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 Troponin I Rapid Test Cassette (Whole Blood/Serum/Plasma) is for professional in vitro diagnostic use, and should be used for the qualitative detection of cardiac Troponin I only. There is no meaning attributed to linen color intensity or width.
2. The Troponin I Rapid Test Cassette (Whole Blood/Serum/Plasma) will only indicate the presence of Troponin I in the specimen and should not be used as the sole criteria for the diagnosis of tuberculosis.
3. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. The test cannot detect less than 0.5 ng/mL of cTnI in specimens. Thus, a negative result does not at anytime rule out the existence of Troponin I in blood, because the antibodies may be absent or below the minimum detection level of the test.
4. Like with all diagnostic tests, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.
5. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor (RF) may affect expected results. Even if the test results are positive, further clinical evaluation should be considered with other clinical information available to the physician.

**PERFORMANCE CHARACTERISTICS**

**Table: Troponin I Rapid Test vs. EIA**

| Method | | Troponin I Rapid Test Cassette | | Total Results |
|---|---|---|---|---|
| | | Positive | Negative | |
| EIA | Positive | 138 | 2 | 140 |
| | Negative | 1 | 315 | 316 |
| Total Results | | 139 | 317 | 456 |

Relative Sensitivity: 98.6% (94.9%-99.8%)* Relative Specificity: 99.7% (98.3%-99.9%)*

Overall Agreement: 99.3% (98.1%-99.9%)* *95% Confidence Interval

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2. Mehegan JP, Tobacman LS. Cooperative interaction between troponin molecules bound to the cardiac thin filament. J.Biol.Chem. 266:966, 1991.
3. Adams, et al. Diagnosis of Perioperative myocardial infarction with measurements of cardiac troponin I. N.Eng.J.Med 330:670, 1994.
4. Hossein-Nia M, et al. Cardiac troponin I release in heart transplantation. Ann. Thorac.Surg. 61: 227, 1996.
5. Alpert JS, et al. Myocardial Infarction Redefined, Joint European Society of Cardiology American College of Cardiology: J. Am. Coll. Cardio., 36(3):959, 2000.

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Valid until: 27 February 2028

Vienna, 10 March 2025

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CEO

Mag. Dr. Werner Paar
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Registration No.: Q-27302/0

Date of initial issue: 28 February 2022

Valid until: 27 February 2028

Vienna, 10 March 2025

Quality Austria Certification GmbH,
AT-1010 Vienna, Zelinkagasse 10/3

Mag. Christoph Mondl
CEO

Mag. Dr. Werner Paar
CEO

Ing. Christoph Baumgartner, MSc, MBA
Authorised representative,
management Customer Service Center

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# 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

# Spore Strips (Steam Sterilization Monitor Strips)

**DD032**

Steam Sterilization Monitor Strips are used for evaluating sterilization process. These indicators which are specified by the U.S. military specification MIL-S- 36586 are GMP requirements of U.S. FDA.

## Directions

Place indicators in the areas of the pack or load least accessible to steam. Places such as the geometrical center, and the upper and lower regions of both front and rear of the load to be sterilized are considered suitable areas for placement of these indicators. A standard procedure should be established for the routine evaluation of each sterilizer. On completion of the sterilization cycle, remove the indicators from the test loads and deliver them to the laboratory for testing. All sterility tests should be performed in a clean dust free transfer area, preferably under positive air pressure, using rigid aseptic technique throughout the test procedure.

Using sterile scissors, cut open one end of the envelope. Thereafter remove the indicator with sterile tweezers and aseptically transfer it to a tube of sterile Soyabean Casein Digest Medium w/ Yeast Extract and Ferric pyrophosphate (M207) or Soyabean Casein Digest Medium (M011). Incubate the tubes for seven days at 55 - 60°C. Observe the tubes daily. If turbidity develops, failure of the sterilization process is indicated.

Precautions

The spore strips or broth cultures of *Bacillus stearothermophilus* must be autoclaved at 121°C for at least 30 minutes prior to discarding.

Each spore strip is individually packaged in a steam-permeable envelope.

## Principle And Interpretation

*Bacillus stearothermophilus* is a thermophilic bacteria which can grow at 65°C and above. The spores are highly heat resistant and are used to monitor autoclave performance (1).

Sterilisation is the freeing of an article from all living organisms including viable spores(1). Sterilization quality control can only be achieved through the use of calibrated biological indicators (endospores). These indicators consist of *Bacillus stearothermophilus* spores impregnated on chromatography paper strips, individually placed into envelopes. Number of spores present per strip : $10^6$. These organisms are difficult to destroy because they are more resistant to heat than other vegetative bacteria and viruses. Therefore, if they are destroyed during sterilization,it is assumed that all other life forms are also destroyed. This test is considered the most sensitive check of the autoclaves efficiency.

Precautions :

The spore strips or broth cultures of *Bacillus stearothermophilus* must be autoclaved at 121°C for at least 30 minutes prior to discarding.

Each spore strip is individually packaged in a steam-permeable envelope.

## Quality Control

**Appearance**
Filter paper strip impregnated with spores of standard culture of B.stearothermophilus

**Number of spores**
1000000 spores/strip

**Cultural response**
Sterility checking of the autoclave was carried out using Spore strip. After autoclaving, strip was inoculated in 100ml of st. Soyabean Casein Digest Medium(M011) and incubated at 55°C upto 7 days. An unexposed spore strip was also inoculated separately in 100ml M011

| Growth | Unexposed Spore Strip | Exposed Spore Strip | Positive control | Negative control |
|---|---|---|---|---|
| *Growth in M011* | Luxuriant | No growth | Luxuriant | No growth |

## Storage and Shelf Life

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

## Reference

1.Mackie and McCartney, 1996, Practical Medical Microbiology, 14th ed., Vol. 2, Collee J. G., Fraser A. G., Marmion B, P., Simmons A (Eds.), Churchill Livingstone, Edinburgh.

Revision : 1 / 2011

**HiMedia Laboratories Private Limited**
C-40, Road No.21Y, MIDC, Wagle Industrial Area,
Thane(W) - 400604 , Website : www.himedialabs.com,
Email : info@himedialabs.com

# *Certificate of Analysis, Quality and Conformity*

| | | |
|---|---|---|
| **Material Code :** DD032 | **Material Name :** Spore Strips (25 strips / pack) | **Lot No** : 0000634709 |
| **Report No.:** 40001453480 | **Date of Release & Report :** 2024-03-20 | **Expiry Date :** 2026-02 |

## Appearance

Filter paper strip impregnated with spores of standard culture of *B.stearothermophilus* ATCC 7953

## Number of spores

1000000 spores/strip

## Cultural response

Sterility checking of the autoclave was carried out using Spore strip. After autoclaving, strip was inoculated in 100ml of st. Soyabean Casein Digest Medium(M011) and incubated at 55°C upto 7 days. An unexposed spore strip was also inoculated separately in 100ml M011

| Organism | Unexposed Spore Strip | Exposed Spore Strip | Positive control | Negative control |
|---|---|---|---|---|
| **Cultural response** | | | | |
| *Growth in M011* | Luxuriant | No growth | Luxuriant | No growth |

. ATCC is a registered trade mark of the American Type Culture Collection
. NCTC and National Collection of Type Culture are registered trade mark of the Health Protection Agency

Control Media :
. For Bacteria : Soyabean Casein Digest Agar / Columbia Blood Agar base enriched with 5% v/v Sheep/Horse blood.
. For Yeast & Mold : Sabouraud Dextrose Agar.

. All ISO 11133 : 2014/Amd.1:2018( E ) control strains are included in the Quality parameter
. HiMedia Laboratories Pvt Ltd is Certified for ISO 9001:2015, ISO 13485:2016 and WHO GMP

. The Quality Assurance Parameters are as per the guidelines specified in CLSI (NCCLS) document M22-A3 wherever applicable.

Storage & Shelf Life
Store at 15 - 27°C. Use before expiry date on the label.

. ycle .Positive control tubes are inoculated with *B.stearothermophilus* standard culture .The spore strips or broth cultures of *B. stearothermophilus* must be autoclaved at 121°C for at least 30 minutes prior to discarding

## STATUS OF THE MATERIAL : APPROVED

This is to certify that this lot passes and it confirms to the above mentioned tests and specifications . The information given here is believed to be correct and accurate, however, both the information and products are offered without warranty for any particulars use, other than that specified in the current HiMedia manual or product sheets. The results reported were obtained at the time of release.

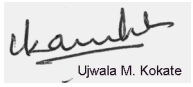
This document has been produced electronically and is valid

**HiMedia Laboratories Private Limited**
C-40, Road No.21Y, MIDC, Wagle Industrial Area,
Thane(W) - 400604 , Website : www.himedialabs.com,
Email : info@himedialabs.com

## *Certificate of Analysis, Quality and Conformity*

| | | |
|---|---|---|
| **Material Code :** DD032 | **Material Name :** Spore Strips (25 strips / pack) | **Lot No :** 0000634709 |
| **Report No.:** 40001453480 | **Date of Release & Report :** 2024-03-20 | **Expiry Date :** 2026-02 |


**Microbiologist/Sr.Executive Microbiologist**




**Asst./Dy/QC Manager**


**Dy/QA Manager**

**2024-03-20**

# 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

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EC REP

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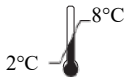
IVD ***In vitro* diagnostic medical device**

**CE Marking**




**Storage temperature**

**Do not use if package is damaged**

Disclaimer :

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.

# Soyabean Casein Digest Medium (Tryptone Soya Broth) M011

## Intended Use:

Recommended as a general purpose medium used for cultivation of a wide variety of microorganisms and recommended for sterility testing of moulds and lower bacteria.

## Composition**

| Ingredients | g / L |
|---|---|
| Tryptone | 17.000 |
| Soya peptone | 3.000 |
| Sodium chloride | 5.000 |
| Dextrose (Glucose) | 2.500 |
| Dipotassium hydrogen phosphate | 2.500 |
| Final pH ( at 25°C) | 7.3±0.2 |

**Formula adjusted, standardized to suit performance parameters

## Directions

Suspend 30.0 grams in 1000 ml purified/ distilled water. Heat if necessary to dissolve the medium completely. Mix well and dispense in tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

*Note: If any fibres are observed in the solution, it is recommended to filter the solution through a 0.22 micron filter to eliminate the possibility of presence of fibres.*

## Principle And Interpretation

Soyabean Casein Digest Medium is recommended by various pharmacopeias as a sterility testing and as a microbial limit testing medium (1,2,3). This medium is a highly nutritious medium used for cultivation of a wide variety of organisms (4). The combination of Tryptone and soya peptone makes the medium nutritious by providing nitrogenous, carbonaceous substances, amino acids and long chain peptides for the growth of microorganisms. Dextrose/glucose serve as the carbohydrate source and dibasic potassium phosphate buffer the medium. Sodium chloride maintains the osmotic balance of the medium.

## Type of specimen

Pharmaceutical samples, Clinical samples - urine, pus, wound samples.

## Specimen Collection and Handling

For clinical samples, follow appropriate techniques for handling specimens as per established guidelines (5,6). For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per pharmaceutical guidelines (2). 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. Biochemical characterization is necessary to be performed on colonies from pure cultures for further identification.
2. This medium is general purpose medium and may not support the growth of fastidious organisms.

## 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

**Colour and Clarity of prepared medium**
Light yellow coloured clear solution without any precipitate.

**Reaction**
pH of 3.0% w/v aqueous solution at 25°C (after sterilization). pH : 7.3±0.2

**pH**
7.10-7.50

**Stability test**
Light yellow coloured clear solution without any precipitation or sedimentation at room temperature for 7 days

**Growth promoting properties**
Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating not more than 100 cfu (at 30-35°C for 18-24 hours for bacteria and 5days for fungal) Growth promotion is carried out as per USP/ EP/BP/JP/IP.

| Organism | Inoculum (CFU) | Growth | Incubation temperature | Incubation period |
|---|---|---|---|---|
| *Salmonella* Typhimurium ATCC 14028 (00031*) | 50 -100 | luxuriant | 30 -35 °C | 18 -24 hrs |
| *Salmonella* Abony NCTC 6017 (00029*) | 50 -100 | luxuriant | 30 -35 °C | 18 -24 hrs |
| ^*Pseudomonas paraeruginosa* ATCC 9027 (00026*) | 50 -100 | luxuriant | 30 -35 °C | 18 -24 hrs |
| *Staphylococcus aureus subsp. aureus* ATCC 6538 (00032*) | 50 -100 | luxuriant | 30 -35 °C | 18 -24 hrs |
| *Escherichia coli* ATCC 25922 (00013*) | 50 -100 | luxuriant | 30 -35 °C | 18 -24 hrs |
| *Escherichia coli* ATCC 8739 (00012*) | 50 -100 | luxuriant | 30 -35 °C | 18 -24 hrs |
| ** *Bacillus spizizenii* ATCC 6633 (00003*) | 50 -100 | luxuriant | 30 -35 °C | 18 -24 hrs |
| $ *Kokuria rhizophila* ATCC 9341 | 50 -100 | luxuriant | 30 -35 °C | 18 -24 hrs |
| *Pseudomonas aeruginosa* ATCC 27853 (00025*) | 50 -100 | luxuriant | 30 -35 °C | 18 -24 hrs |
| *Candida albicans* ATCC 10231 (00054*) | 50 -100 | luxuriant | 20 -25 °C | <=5 d |
| *Staphylococcus aureus subsp. aureus* ATCC 25923 (00034*) | 50 -100 | luxuriant | 30 -35 °C | 18 -24 hrs |
| **Sterility Testing- Growth promotion+Validation** | | | | |
| *Staphylococcus aureus subsp. aureus* ATCC 6538 (00032*) | 50 -100 | luxuriant | 20 -25 °C | <=3 d |
| # *Aspergillus brasiliensis* ATCC 16404 (00053*) | 50 -100 | luxuriant | 20 -25 °C | <=5 d |
| *Candida albicans* ATCC 2091 (00055*) | 50 -100 | luxuriant | 30 -35 °C | <=5 d |
| *Staphylococcus aureus subsp. aureus* ATCC 25923 (00034*) | 50 -100 | luxuriant | 20 -25 °C | <=3 d |
| *Escherichia coli* ATCC 25922 (00013*) | 50 -100 | luxuriant | 20 -25 °C | <=3 d |

| | | | | |
|---|---|---|---|---|
| ^*Pseudomonas paraeruginosa* ATCC 9027 (00026*) | 50 -100 | luxuriant | 20 -25 °C | <=3 d |
| ** *Bacillus spizizenii* ATCC 6633 (00003*) | 50 -100 | luxuriant | 20 -25 °C | <=3 d |
| *Salmonella* Typhimurium ATCC 14028 (00031*) | 50 -100 | luxuriant | 20 -25 °C | <=3 d |
| *Salmonella* Abony NCTC 6017 *(*00029*) | 50 -100 | luxuriant | 20 -25 °C | <=3 d |
| *Escherichia coli* ATCC 8739 (00012*) | 50 -100 | luxuriant | 20 -25 °C | <=3 d |
| *Pseudomonas aeruginosa* ATCC 27853 (00025*) | 50 -100 | luxuriant | 20 -25 °C | <=3 d |
| $ *Kokuria rhizophila* ATCC 9341 | 50 -100 | luxuriant | 20 -25 °C | <=3 d |

Key : (*) Corresponding WDCM numbers

^ Formerly known as *Pseudomonas aeruginosa*

**Formerly known as *Bacillus subtilis* subsp. *spizizenii*

# Formerly known as *Aspergillus niger*

$ Formerly known as *Micrococcus luteus*

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

## Reference

1.Indian Pharmacopoeia, 2022, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare Government of India.

2.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams & Wilkins, Baltimore, M.d.

3.The United States Pharmacopoeia-National Formulatory (USP-NF), 2022.

4.Forbes B. A., Sahm D. F. and Weissfeld A. S., 1998, Bailey & Scotts Diagnostic Microbiology, 10th Ed., Mosby, Inc. St. Louis, Mo.

5.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

6.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.

Revision : 05/2024

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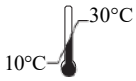
Plot No.C-40, Road No.21Y,
MIDC, Wagle Industrial Area,
Thane (W) -400604, MS, India

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




**Storage temperature**

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CE **CE Marking**

**Do not use if package is damaged**

Disclaimer :

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.

# Tinsdale Agar Base

M314

## Intended Use:

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

## Composition**

| Ingredients | g / L |
|---|---|
| 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 H2S 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% CO2 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).

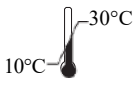
## Reference

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.

Revision :06/2024

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

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




**Storage temperature**

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

CE **CE Marking**

**Do not use if package is damaged**

Disclaimer :

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.

## 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)

*** Not for Medicinal Use**

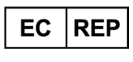

In vitro diagnostic medical device

CE Marking


Storage temperature

Do not use if package is damaged

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




CE Partner 4U ,Esdoornlaan 13, 3951
DB Maarn The Netherlands,
www.cepartner 4u.eu

Revision : 02 / 2022

**Mast Group Ltd.**
Mast House, Derby Road, Bootle
Liverpool, Merseyside, L20 1EA
United Kingdom
Tel: + 44 (0) 151 472 1444
Fax: + 44 (0) 151 944 1332
email: sales@mast-group.com
Web: www.mast-group.com

**Mast Diagnostica GmbH**
Feldstrasse 20
DE-23858 Reinfeld
Germany
Tel: + 49 (0) 4533 2007 0
Fax: + 49 (0) 4533 2007 68
email: mast@mast-diagnostica.de
Web: www.mast-group.com

**Mast Diagnostic**
12 rue Jean-Jacques Mention
CS91106, 80011 Amiens, CEDEX 1
France
Tél: + 33 (0) 3 22 80 80 67
Fax: + 33 (0) 3 22 80 99 22
email: info@mast-diagnostic.fr
Web: www.mast-group.com

# 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

**Mast Group Ltd.**
Mast House, Derby Road, Bootle
Liverpool, Merseyside, L20 1EA
United Kingdom
Tel: + 44 (0) 151 472 1444
Fax: + 44 (0) 151 944 1332
email: sales@mast-group.com
Web: www.mast-group.com

**Mast Diagnostica GmbH**
Feldstrasse 20
DE-23858 Reinfeld
Germany
Tel: + 49 (0) 4533 2007 0
Fax: + 49 (0) 4533 2007 68
email: mast@mast-diagnostica.de
Web: www.mast-group.com

**Mast Diagnostic**
12 rue Jean-Jacques Mention
CS91106, 80011 Amiens, CEDEX 1
France
Tél: + 33 (0) 3 22 80 80 67
Fax: + 33 (0) 3 22 80 99 22
email: info@mast-diagnostic.fr
Web: www.mast-group.com

Mast Group

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2

3

4a

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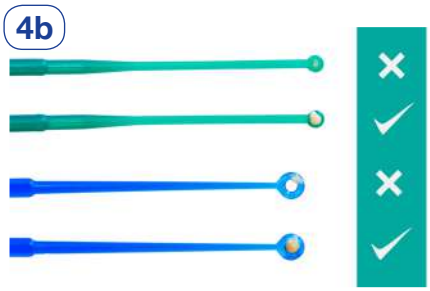

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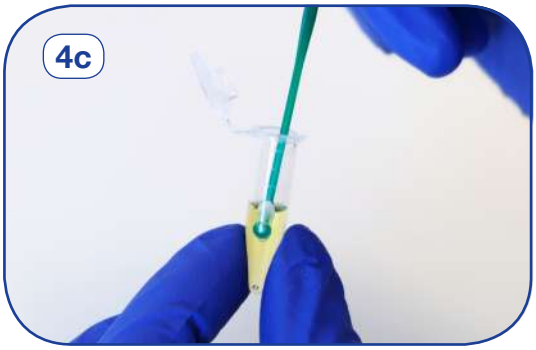

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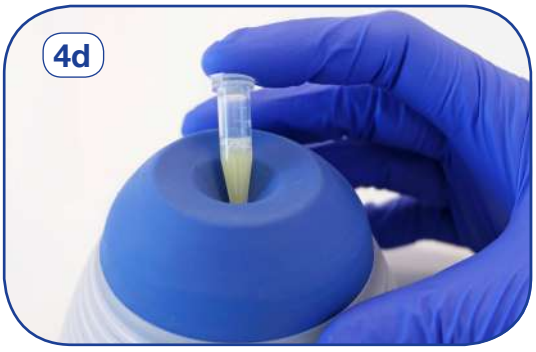

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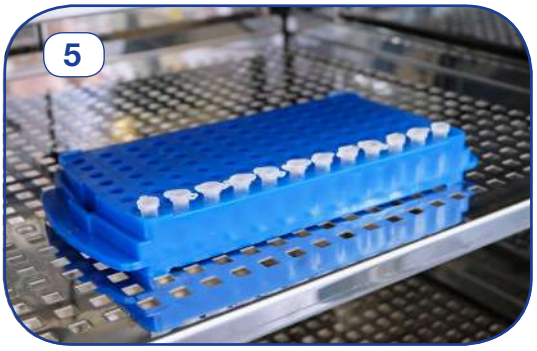


6

**Mast Group Ltd.**
Mast House, Derby Road,
Bootle, Merseyside, L20 1EA
United Kingdom
Tel: + 44 (0) 151 472 1444
Fax: + 44 (0) 151 944 1332
email: sales@mast-group.com
Web: www.mast-group.com

EC REP **Mast Diagnostica GmbH**
Feldstrasse 20
DE-23858 Reinfeld
Germany
Tel: + 49 (0) 4533 2007 0
Fax: + 49 (0) 4533 2007 68
email: mast@mast-diagnostica.de
Web: www.mast-group.com

**Mast Diagnostic**
12 rue Jean-Jacques Mention
CS91106, 80011 Amiens, CEDEX 1
France
Tél: + 33 (0) 3 22 80 80 67
Fax: + 33 (0) 3 22 80 99 22
email: info@mast-diagnostic.fr
Web: www.mast-group.com

# **MAST**DISCS® *Combi*
# Ceftazidime ESβL ID Disc Set

## D64C

## Intended use
For the detection of extended spectrum beta-lactamases (ESβLs) in Enterobacterales.

FOR IN VITRO DIAGNOSTIC USE ONLY

## Contents and Formulation*
3 x paired sets of cartridges per pack, each cartridge containing approximately 50 discs:

| | |
|---|---|
| CAZ30 | Ceftazidime 30 µg discs (x3) |
| CAZCV | Ceftazidime 30 µg + clavulanic acid 10 µg discs (x3) |

## 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.

## 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, Mueller-Hinton agar, swabs, forceps, callipers etc., as well as an incubator capable of maintaining 35 ± 2°C.

## Procedure
1. Using a pure, fresh culture of the test organism, prepare a suspension equivalent in density to a 0.5 McFarland standard.
2. Using a sterile swab, spread the suspension uniformly across the surface of a single Mueller Hinton Agar plate in accordance with the Clinical and Laboratory Standards Institute (CLSI) procedure.
3. Using a MAST® DISCMASTER Dispenser, or alternatively a sterile needle or forceps, place one of each type of disc onto the plate of inoculated medium, ensuring sufficient space between the discs to allow formation of clearly defined zones of inhibition.
4. Incubate at 35 ± 2°C for 17±1 hours.
5. Measure and record the diameter of any zones of inhibition, to the nearest whole millimetre. Discs showing no zone of inhibition should be recorded as 6 mm.

## Interpretation of results
Compare the zone of inhibition for the ceftazidime disc to that of the ceftazidime plus clavulanic acid combination disc. An increase in zone diameter of ≥5 mm in the presence of clavulanic acid indicates the presence of ESβL in the test organism.

## Quality control
Check for signs of deterioration. Quality control must be performed with at least one organism to demonstrate a positive reaction and at least one organism to demonstrate a negative reaction. Zones of inhibition obtained using the combination disc plus clavulanic acid and corresponding ceftazidime only disc against ESβL-negative control organism *E. coli* ATCC® 25922, should be equal or show no greater difference in diameter than ±2 mm. Any greater difference implies malfunction or deterioration. 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 |
|---|---|
| *Escherichia coli* NCTC 13351 | Positive |
| *Escherichia coli* NCTC 13353 | Positive |
| *Escherichia coli* ATCC® 25922 | Negative |

## Limitations
D64C is not suitable for testing *Pseudomonas* spp. or *Acinetobacter* spp. D64C should always be used in conjunction with **MAST**DISCS® *Combi* Cefotaxime ESβL ID disc set (D62C); a positive result using one or both tests indicates the presence of an ESβL in the test organism. To avoid potentially erroneous results do not mix cartridges from different batches of D64C and ensure both discs in the set are tested on the same plate.

## References
Bibliography available on request.

IFU170 GB 03/21 V8

*Formulation may change to meet performance criteria

**Instruction for use**
**A solid-phase enzyme immunoassay kit**
**for the quantitative determination of**
**free thyroxin in human serum or plasma**

# fT4 EIA

Catalogue number REF **K214**

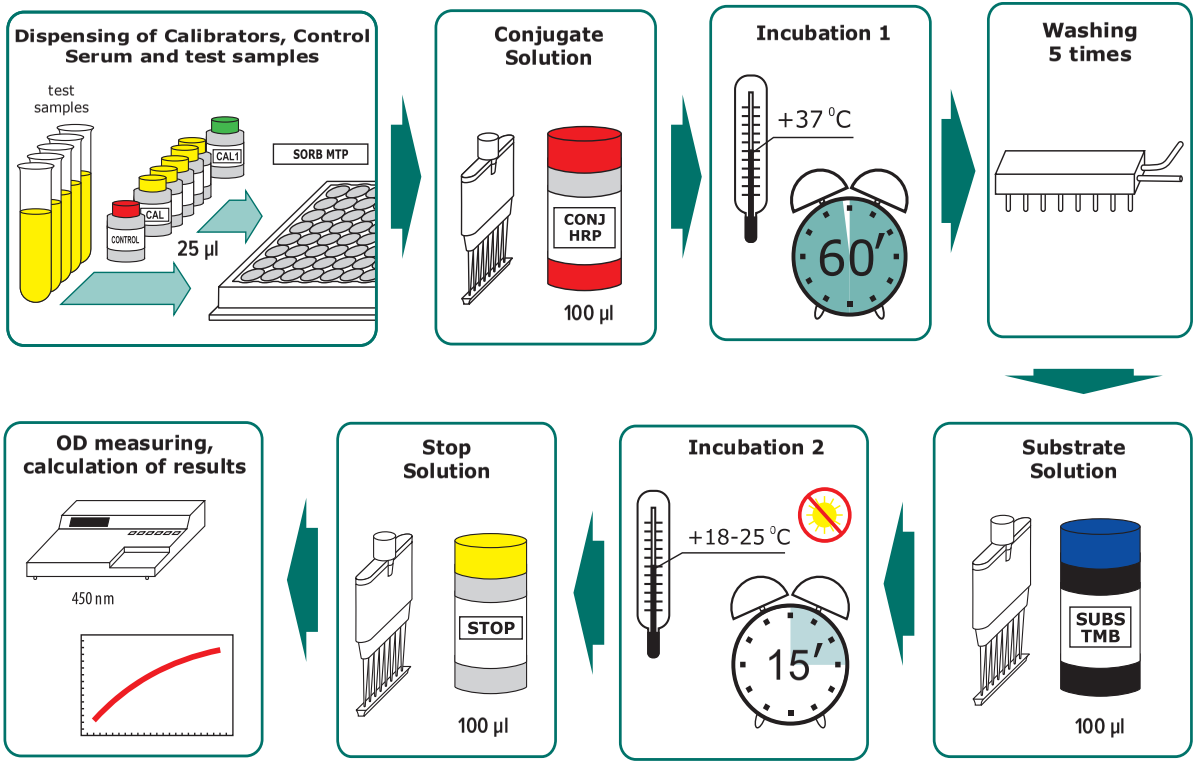
Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

XEMA LLC
Akademika Yefremova St. 23
03179, Kyiv, Ukraine
tel.:+38 044 422-62-16
tel.:+38 044 294-69-78
E-mail: qa@xema.com.ua
www.xema.in.ua

EC REP Authorized Representative in EU:
Polmed.de Beata Rozwadowska
Fichtenstr. 12A, 90763 Fuerth, Germany
tel.:+ 49 911 931 639 67
E-mail: info@polmed.de
www.polmed.de

## ASSAY PROCEDURE




K214

**CONTENT**

# Instruction for use
# A solid-phase enzyme immunoassay kit for the quantitative determination of free thyroxin in human serum or plasma
# fT4 EIA

## 1. INTENDED USE

The fT4 EIA kit is an enzyme immunoassay, intended for the quantitative determination of free thyroxin in human serum or plasma.

The field of application is clinical laboratory diagnostics.

## 2. GENERAL INFORMATION

Thyroid hormones thyroxin (T4) and 3,5,3'-triiodothyronine (T3) exert regulatory influences on growth, differentiation, cellular metabolism and development of skeletal and organ systems. T4 and T3 in blood are found both in free and bound form – mostly, they are bound to thyroxin binding globulin (TBG). Only free forms of T3 and T4 exert hormonal activity also their percentage is very low – 0.3% for T3 and 0.03% for T4.

The concentration of T4 is generally accepted as an index of thyroid function which provide enough information to differentiate between hyper-, hypo- and euthyroidism.

Elevation of total T4 is found in hyperthyroidism, in patients with tumours of pituitary gland, in subjects with elevated TBG level (pregnancy, acute or chronic active hepatitis, estrogen-secreting tumours or estrogen intake, hereditary elevation of TBG), in patients taking oral contraceptives, heroin, methadone, thyroid preparations, TSH, thyroliberin.

Low total T4 is found in hypothyroidism, in patients with panhypopituitarism, in subjects with low TBG level (acromegaly, nephritic syndrome, hypoproteinemia, chronic liver diseases, androgen-secreting tumours, hereditary reduction), in patients taking aminosalicylic and acetylsalicylic acids, cholestyramine, reserpine, potassium iodide, triiodothyronine.

## 3. TEST PRINCIPLE

Determination of free thyroxin is based on competition principle of the enzyme immunoassay. Microwells plate is coated with specific murine monoclonal antibodies to T4. fT4 conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage fT4 from the specimen competes with the conjugated fT4 for coating antibodies. As a result, a complex bounded to the solid phase and containing peroxidase is formed.
- during the second stage, the complexes formed due 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. Optical density in the microwell is inversely related to the quantity of the measured fT4 in the specimen of the serum (plasma).

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of fT4 in the calibration samples.

## 4. KIT COMPONENTS

| Code of component | Symbol | Name | Volume | Qty, pcs. | Description |
|---|---|---|---|---|---|
| P214Z | SORB MTP | **Microplate** | - | 1 | 96-well polystyrene strip microplate coated with murine monoclonal antibodies to T4; ready to use |
| C214Z | CAL 1 | **Calibrator C1** | 0.5 mL | 1 | Solution based on human plasma, free of fT4, with preservative, ready to use (yellow liquid) |
| C214Z | CAL 2-6 | **Calibrators** | 0.5 ml | 5 | Solutions based on human plasma, containing 5; 10; 25, 50 and 100 pmol/L of fT4, with preservative, ready to use (red liquids) |
| Q214Z | CONTROL | **Control Serum** | 0.5 ml | 1 | Solution based on human plasma, containing of known fT4 content, with preservative, ready to use (colourless liquid) |
| T214Z | CONJ HRP | **Conjugate Solution** | 12 ml | 1 | Solution of fT4 conjugated to the horseradish peroxidase; ready to use (red liquid) |
| R055Z | SUBS TMB | **Substrate Solution** | 12 ml | 1 | Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid) |
| S008Z | BUF WASH 26X | **26x Concentrate Washing Solution** | 22 ml | 1 | Buffer solution with detergent, 26x concentrate (colourless liquid) |
| R050Z | STOP | **Stop Solution** | 12 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 (1 pcs.) | | | | | |

## 5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

- microplate photometer with 450 nm wavelength;
- dry thermostat for +37°C±1°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 fT4 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 fT4 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:

- the remaining strips should be immediately resealed in the bag along with the silica gel, closed with the zip-lock, and stored at +2...+8°C within 2 months
- 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;
  *NOTE: Single freezing of Calibrators and Control Serum in aliquots is allowed.*
- 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. The remaining strips should be immediately resealed in the bag along with the silica gel and closed with the zip-lock to prevent moisture from affecting the plate's strips.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 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 | 1.8 | 3.6 | 5.4 | 7.2 | 9 | 10.8 | 12.6 | 14.4 | 16.2 | 18 | 19.8 | 22 |
| Volume of water, mL | 45 | 90 | 135 | 180 | 225 | 270 | 315 | 360 | 405 | 450 | 495 | 550 |

## 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 14 wells for Calibrators and Control Serum (2 wells for each calibrator (CAL 1-6) and 2 wells for control serum (Q)).

10.2 Dispense **25 µL of Calibrators and Control Serum as well as 25 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 Sample 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 | SAMP2 | SAMP2 | SAMP10 | SAMP10 | | | | | | |
| B | CAL2 | CAL2 | SAMP3 | SAMP3 | SAMP11 | SAMP11 | | | | | | |
| C | CAL3 | CAL3 | SAMP4 | SAMP4 | SAMP12 | SAMP12 | | | | | | |
| D | CAL4 | CAL4 | SAMP5 | SAMP5 | | | | | | | | |
| E | CAL5 | CAL5 | SAMP6 | SAMP6 | | | | | | | | |
| F | CAL6 | CAL6 | SAMP7 | SAMP7 | | | | | | | | |
| G | Q | Q | SAMP8 | SAMP8 | | | | | | | | |
| H | SAMP1 | SAMP1 | SAMP9 | SAMP9 | | | | | | | | |

10.3 Add **100 µL of the Conjugate Solution** to all wells.

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 **60 minutes at +37°C**.

10.5 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 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 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 15 minutes**.

10.7 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.

10.8 Read the optical density (OD) of the wells at 450nm using a microplate photometer within 5 minutes of adding the Stop Solution.

10.9 Plot a calibration curve in semi-logarithmic coordinates: (x) is the decimal logarithm of the fT4 concentration in the calibrators pmol/L, (y) – OD versus fT4 concentration (OD 450 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. Adjust the concentration of CAL1 to an infinitesimally small value, for example, 0.001 pmol/L.

10.10 Determine the corresponding concentration of fT4 in tested samples from the calibration curve.

## 11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 1.2, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

## 12. EXPECTED VALUES

Therapeutical consequences should not be based on 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 fT4. Based on data obtained by XEMA, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

*NOTE: values of fT4 concentrations in the tested samples that are below the LoD (0.75 pmol/L) and also exceed the value of the upper calibrator (100 pmol/L) should be provided in the following form : «the fT4 concentration of tested sample X is «lower than 0.75 pmol/L» or «higher than 100 pmol/L»*.

| Sex, age | Units, pmol/L | |
|---|---|---|
| | Lower limit | Upper limit |
| Healthy donors | | |
| < 60 yrs | 10 | 25 |
| > 60 yrs | 10 | 21 |
| Pregnancy week | | |
| 1st trimester | 9 | 26 |
| 2nd trimester | 6 | 21 |
| 3rd trimester | 6 | 21 |

## 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, pmol/L | CV, % |
|---|---|---|
| 1 | 54.4 | 5.83 |
| 2 | 85.23 | 3.67 |

*Reproducibility (Inter assay reproducibility)* was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

| Sample | Concentration, pmol/L | CV, % |
|---|---|---|
| 1 | 54.36 | 1.15 |
| 2 | 85.73 | 3.23 |

*Reproducibility between lots* was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

| Sample | Concentration1, pmol/L | Concentration2, pmol/L | Concentration3, pmol/L | CV, % |
|---|---|---|---|---|
| 1 | 54.59 | 52.67 | 60.39 | 7.19 |
| 2 | 85.23 | 87.53 | 85.13 | 1.58 |

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 fT4 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 5-100 pmol/L ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest fT4 concentration in the serum or plasma sample that is detected by the fT4 EIA kit is no lower than 0.75 pmol/L.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for fT4 EIA kit is 5 pmol/L.

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 fT4 with other analytes is shown in the table:

| Analyte | Cross-reactivity, % |
|---|---|
| L-Thyroxin | 100 |
| D-Thyroxin | 94 |
| 3,3′,5′-Triiodo-L-Thyronine (Reverse T3) | 86 |
| 3,3′,5-Triiodo-L-Thyronine (T3) | 3.3 |
| 3,3′,5′-Triiodo-D-Thyronine | 1.8 |
| 3,3′,5′-Triiodothyropropionic acid | 0.6 |

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## 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 _____

| | |
|---|---|
|  | 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**

**+38 044 294-69-78**

**or write to:**

**qa@xema.com.ua**

XEMA LLC
Akademika Yefremova St. 23
03179, Kyiv, Ukraine
tel.:+38 044 422-62-16
tel.:+38 044 294-69-78
E-mail: qa@xema.com.ua
www.xema.in.ua