Rapid assay for the semi-quantitative detection of *Cryptococcus sp.* antigen in serum, plasma, whole blood and CSF.

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1. INTENDED USE
Bio-Rad RDT CryptoPS Assay is a single use rapid immunoassay for the semi-quantitative detection of Cryptococcus sp. antigen in serum, plasma, venous and capillary whole blood, and cerebrospinal fluid (CSF). Bio-Rad RDT CryptoPS Assay is a screening test intended for use as an aid in the diagnosis of cryptococcal infection, especially cryptococcal meningitis.

2. SUMMARY AND EXPLANATION OF THE TEST
Cryptococcosis is an opportunistic infection caused by both species of Cryptococcus neoformans and Cryptococcus gattii complex. Primary cryptococcal pneumonia following yeast inhalation from environment is mostly asymptomatic. Severity of the infectious is mostly associated to the tropism of the yeast to Central Nervous System. The infection can disseminate to extrapulmonary sites and progress to fatal cryptococcal meningitis after crossing blood-brain barrier. Immunocompromised patients are at greatest risk of developing severe disease. Cryptococcal meningitis is the world second deadliest opportunistic disease (after tuberculosis), amongst patients infected by HIV and severely immunosuppressed.

3. PRINCIPLE OF THE PROCEDURE
Bio-Rad RDT CryptoPS Assay is a lateral flow immunoassay for semi-quantitative detection of Cryptococcus sp. antigen in serum, plasma, venous and capillary whole blood or CSF sample. Lyophilized Conjugate composed of Cryptococcus sp.-specific antibodies conjugated with gold particles is immobilized on the membrane at conjugate pad level. Sample and Diluent are dispensed to the SAMPLE (S) well which generates migration of the sample together with conjugate. If present, Cryptococcus sp. antigen from the specimen will form antigen-antibody complex with specific antibodies from the Conjugate and will be captured by antibodies specific to Cryptococcus sp. antigen coated on the membrane solid phase at Test Lines level (T1 and T2) of the reaction zone. This capture will produce one or two purple colored bands at Test Line level depending upon antigen concentration in specimen (T1 test line appears at a concentration as low as approximately 25 ng/ml depending on the strain of Cryptococcus sp, while T2 test line appears at higher concentration estimated at 2.5 μg/ml. These concentration values are only indicative, and the assay cannot provide true antigen concentration in the specimen).

In the absence of Cryptococcus sp. antigen, no purple bands will be produced at Test Line level. Whether reactive or non-reactive, the sample continues to migrate along the membrane and produces a purple band at Control Line (C) level. This procedural control confirms that sufficient specimen volume was dispensed and indicates a proper procedural process.

4. REAGENTS

4.1 Description

<table>
<thead>
<tr>
<th>Label</th>
<th>Nature of reagents</th>
<th>Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Device</td>
<td>Individually foil pouchled test device with desiccant. Device containing a strip with antibodies specific to Cryptococcus sp. antigen coated Nitrocellulose membrane at Test Line levels (T1 and T2), goat anti-mouse IgG at Control Line (C) level and Cryptococcus sp.-specific antibodies conjugated with gold particles at conjugate pad level.</td>
<td>20 x 1 Ready-to-use</td>
</tr>
<tr>
<td>DIL 1</td>
<td>Diluent Dropper containing saline solution with EDTA and bovine serum albumin (BSA) with preservative (Sodium Azide &lt; 0.1%)</td>
<td>1 x 4 ml Ready-to-use</td>
</tr>
<tr>
<td>DIL 2</td>
<td>Diluent (filtration protocol) Vial containing saline solution with EDTA and bovine serum albumin (BSA) with preservative (Sodium Azide &lt; 0.1%)</td>
<td>1 x 4 ml Ready-to-use</td>
</tr>
<tr>
<td>CONTROL +</td>
<td>Positive Control Vial containing saline solution with inactivated ATCC 34877 strain with preservative (Sodium Azide &lt; 0.1%)</td>
<td>1 x 0.5 ml Ready-to-use</td>
</tr>
<tr>
<td>Microsafe Pipette</td>
<td>Microsafe plastic tube of 20 μl (for capillary blood specimens)</td>
<td>20 x 1</td>
</tr>
</tbody>
</table>

4.2. Storage and Handling Requirements
Bio-Rad RDT CryptoPS Assay should be stored at 2°C to 25°C, until expiration date stated on the kit.

Do not freeze. Do not open device pouch until performing a test. Diluent is stable in its original bottle until expiration date after first use.
When stored as indicated, test devices are stable until the expiration date printed on the pouch. Diluent should be stored at 2 to 25°C in its original bottle.
5. WARNINGS AND PRECAUTIONS
For in vitro diagnostic use only. For healthcare professional use only.

5.1 Health and Safety
- This kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards.
- Wear protective clothing, including lab coat, eye/face protection and disposable gloves (synthetic, non-latex gloves are recommended) while handling kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- Handle patient samples and any material that comes directly in contact with them as potentially able to transmit infectious diseases.
- Biological spills: Human source material spills should be treated as potentially infectious.
- Spills not containing acid should be immediately decontaminated, including the spill area, materials and any contaminated surfaces or equipment, with an appropriate chemical disinfectant that is effective for the potential biohazards relative to the samples involved (commonly a 1:10 dilution of household bleach, 70-80% Ethanol or Isopropanol, an iodophor [such as 0.5% Wescodyne™ Plus, EPA Registration #4959-16-52], or a phenolic, etc.), and wiped dry. Spills containing acid should be appropriately absorbed (wiped up) or neutralized, the area flushed with water and wiped dry; materials used to absorb the spill may require biohazardous waste disposal. Then the area should be decontaminated with one of the chemical disinfectants.
- NOTE: DO NOT PLACE SOLUTIONS CONTAINING BLEACH INTO THE AUTOCLAVE.
- Patient samples and contaminated material should be discarded after decontamination.
- For hazard and precaution recommendation related to some chemical components in this test kit, please refer to the pictogram(s) mentioned on the labels and the information supplied at the end of the Instructions for Use. The safety Data Sheet is available on www.bio-rad.com.

5.2 Precautions related to the procedure
- For single use only.
- Completely read Product Insert before using the assay
- Strictly follow the assay procedure as not doing so may result in inaccurate result.
- Using the test with sample types other than those specifically approved for use with this device may result in inaccurate result.
- Do not use any part of the test beyond the expiration date.
- Always follow recommended storage conditions. If stored refrigerated, ensure the pouch is brought to room temperature before testing (18-30°C)
- Cold Diluent or moisture condensation on the membrane can lead to invalid test result.
- Do not open the pouch until performing the assay.
- Do not use test device if the pouch is damaged.
- Do not use test device if desiccant is missing from the pouch.
- Test should be performed at 18°C – 30°C.
- Do not mix reagent from different lot number of kits.

6. SPECIMENS
Serum, plasma or venous blood (EDTA or Heparin anticoagulants), capillary blood (without anticoagulant) and CSF are the recommended sample types.
Observe the following recommendations for handling, processing and storage of blood samples:
- Collect all blood samples observing routine precaution for venipuncture.
- For venous whole blood, samples should be stored at 2-8°C if test performed within 2 days. Do not freeze whole blood samples.
- For capillary whole blood, sample should be tested immediately.
- For serum, allow samples to clot.
- Keep tubes stoppered at all times.
- After centrifugation, separate the serum from the clot or red cells in a tightly stoppered storage tube.
- Serum, plasma and CSF specimens can be stored at 18-30°C if test is performed within 8 hours or at +2-8°C if test is performed within 3 days. If test is not completed within 3 days, or for shipment, freeze the samples at -20°C or colder.
- Bring the sample to room temperature (18-30°C) prior testing.
- Avoid repeated freeze/thaw of the specimen. Previously frozen specimens should be completely thawed and thoroughly mixed (vortex) prior to testing.
- Do not heat the samples.

7. PROCEDURE

7.1 Material required

7.1.1 Materials provided
- Individually foil pouched devices with desiccant (20), Dropper of Diluent (1 x 4 ml), Vial of Diluent (1 x 4 ml), Positive Control (1 x 0.5 ml) and Microsafe pipettes (20) per kit.
- Refer to § 4.1 Description.

7.1.2 Materials required but not provided
- Disposable gloves.
- Goggles or safety glasses.
- Automatic Pipettor(s) capable of delivering 20 µl of sample.
- Disposable tips.
- Clock, watch or other timing device
- Sodium hypochlorite (bleach) and sodium bicarbonate.
- Biohazard disposal container.

### 7.2 Reagents Preparation
All reagents are ready-to-use.

### 7.3 Assay Procedure

#### 7.3.1 Semi-quantitative Procedure

1. Remove Bio-Rad RDT CryptoPS device from its pouch and place it on a flat surface (it is not necessary to remove the desiccant from the pouch).  
   **NOTE:** if desiccant is missing from the pouch, DO NOT USE. Discard test device and use a new test device. Label the test device with patient ID or identification number.

2. Using a laboratory pipette or Microsafe pipette for capillary blood, dispense 20 µl of serum/plasma/whole blood/CSF to the center of the SAMPLE (S) well of the device.

   For Positive Control, dispense one drop using dropper.

3. Immediately following addition of sample, use dropper bottle to add 3 drops of Diluent to the center of the SAMPLE (S) well.
   **CAUTION:** avoid adding air bubbles into the well. Avoid splashes into the Test window.

4. Read test result 10 minutes after addition of Diluent.
   **CAUTION:** DO NOT read results after 15 minutes.
   **NOTE:** Discard used pipette tips, test device and any other material into biohazard container.

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![Image of Bio-Rad RDT CryptoPS device and reagents](image-url)
7.3.2 Titration Procedure
- Prepare 10 micro-tubes and number them from 1 to 10.
- From Vial of Diluent, dispense 225 μl of Diluent into micro-tube 1 and 120 μl in the 2 to 10 micro-tubes.
- Dispense 25 μl of sample into micro-tube 1 to obtain a 1:10 dilution. Mix well (vortex).
- Transfer 120 μl of the micro-tube 1 to micro-tube 2 and well mix to obtain a 1:20 dilution. Continue this process of dilution for the following micro-tubes. The result is 10 dilutions ranging from 1:10 dilution (micro-tube 1) to 1:5120 dilution (micro-tube 10).
- Take 10 pouches containing a cassette and prepare them according to §7.3.1 Semi-quantitative Procedure.
- Dispense 100 μl of the micro-tube 1 (dilution 1:10) onto the first cassette. Dispense 100 μl of the micro-tube 2 into the 2nd cassette. Continue this procedure until the micro-tube 10 with the 10th cassette.
- Read the result at 10 minutes of migration. Do not interpret any test strip appearing 15 minutes after sample dispense.
- Discard used pipette tips, test device and any other material into biohazard container.

7.4 Quality Controls
Built-in Control Feature
Internal procedural control is included in the test. A colored band appearing Control Line (C) level ensures that sufficient specimen volume has been loaded and that the correct procedure has been followed.

Good Laboratory Practices recommend the use of positive and negative controls to check the correct test operating. A positive control that will monitor the entire assay is provided in the kit.

Positive controls should be tested once for each new test kit opened and as otherwise required by your laboratory’s standard quality control procedures.

7.5 Test Validation Criteria
A purple band should always appear at Control Line (C) level, whether or not a band appears at Test lines (T1 and/or T2). If there is no distinct purple band visible at Control Line (C) then the test is INVALID.

An INVALID test cannot be interpreted and must be discarded. It is recommended to repeat INVALID test be repeated with a new device.

7.6 Interpretation of the Results
All visible bands, even faint band or weak, must be considered as reactive.

7.6.1 Semi-quantitative Procedure

<table>
<thead>
<tr>
<th></th>
<th>Absence of all Test Lines (T1 and T2) and presence of Control Line (C) band</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Presence of purple band at Test Line T1 level and presence of Control Line (C) band</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Presence of purple band at Test Line T1 AND T2 level and presence of Control Line (C) band</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strong Positive</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Absence of Control Line (C) band</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invalid</td>
<td></td>
</tr>
</tbody>
</table>

7.6.2 Titration Procedure
For reading, refer to the previous §7.6.1 paragraph (Visual reading / semi-quantitative procedure). The titer of the specimen is expressed as the reciprocal of the highest dilution showing a reactive Test line T1.
8. TEST LIMITATIONS

- Bio-Rad RDT CryptoPS Assay must ONLY be used with venous or capillary whole blood, serum, plasma or CSF. Using other types of samples or testing of venipuncture whole blood samples collected using a tube containing an anticoagulant other than heparin or EDTA may not yield accurate results.
- Bio-Rad RDT CryptoPS Assay must be used in accordance with the instructions in this product insert to obtain accurate results.
- Read results in a well-lit area.
- A reactive result using Bio-Rad RDT CryptoPS Assay suggests the presence of Cryptococcus sp. antigen in the sample.
- Bio-Rad RDT CryptoPS Assay is intended as an aid in the diagnosis of infection with Cryptococcus sp.
- Bio-Rad RDT CryptoPS Assay is a semi-quantitative test and intensity of T1 or T2 test lines cannot predict the titer of antigen in sample.
- A Negative result does not preclude the possibility of exposure to Cryptococcus sp. antigen.

9. PERFORMANCES CHARACTERISTICS

9.1 Precision Measurement
A panel of four members made of purified Cryptococcus sp. antigen at different concentrations (100 and 5 µg/ml with T1 and T2 bands, 25 ng/ml with only T1 band and 0.5 ng/ml without T1 or T2 band) was used for determining the precision of the assay.

9.1.1 Repeatability
Precision panel (N = 4) was tested in replicates of 10 on the same day on one lot of Bio-Rad RDT CryptoPS Assay to give a 100% agreement between the results.

9.1.2 Intermediate Precision
Run and Day precision
Precision panel (N = 4) was tested on one lot of Bio-Rad RDT CryptoPS Assay, in replicates of 2 per run, with 1 run per day and for 5 days to give a 100% agreement between the results.

Between-Operator Precision
Precision panel (N = 4) was tested on one lot of Bio-Rad RDT CryptoPS Assay by two different operators, in replicates of 2 during the same day to give a 100% agreement between the results.

Between-Lot Precision
Precision panel (N = 4) was tested on 3 lots of Bio-Rad RDT CryptoPS Assay, in replicates of 3 during the same day to give a 100% agreement between the results.

9.2 Diagnostic Performance

9.2.1 Diagnostic Specificity
A total of 58 specimens including 33 whole venous blood and 25 serum, all diagnosed Cryptococcus sp. antigen negative with a CE-marked rapid assay, were tested with the Bio-Rad RDT CryptoPS Assay. All specimens were also found non-reactive with the RDT CryptoPS Assay to give a 100% (58/58) relative specificity with a confidence interval at 95% of [93.84–100.0].

9.2.2 Diagnostic Sensitivity
A total of 56 specimens including 35 whole venous blood and 21 serum, all diagnosed Cryptococcus sp. antigen positive with a CE-marked rapid assay, were tested with the Bio-Rad RDT CryptoPS Assay. Fifty-five (55) specimens were found reactive with the RDT CryptoPS Assay and one was found non-reactive to give a 98.2% (55/56) relative sensitivity with a confidence interval at 95% of [90.45–99.96].

9.3 Analytical Specificity

9.3.1. Cross reactivity Study
Eight potentially cross-reacting samples representing 3 different diseases (histoplasmosis, coccidioidomycosis and aspergillosis) were tested with the Bio-Rad RDT CryptoPS Assay to give a 100% (8/8) relative specificity.

9.3.2. Interference Study
Bio-Rad RDT CryptoPS Assay gave accurate results when Cryptococcus sp. antigen negative or positive samples were tested with abnormal concentrations of two endogenous blood components (up to 0.12 g/L bilirubin and up to 25 g/L human albumin). It has been observed a yellow color and antigen signal decrease with 0.3 g/L bilirubin.
9.4 Analytical Sensitivity

9.4.1. Analytical Sensitivity with recombinant antigen
Strains of *Cryptococcus neoformans* of four different serotypes (A, B, C and D) were cultivated for 48H at 37°C and number of colonies estimated by turbidimetry (Mac Farland technique). Strains suspension were then serially diluted and tested with the Bio-Rad RDT CryptoPS Assay until absence of detection. The limit of detection (LOD) in cfu/ml of Bio-Rad RDT CryptoPS Assay for *Cryptococcus neoformans* has been estimated as:

- **T1 band**:  \(2.1 \times 10^6 \) cfu/ml (serotype A);  \(0.22 \times 10^6 \) cfu/ml (serotype B);  \(52 \times 10^6 \) cfu/ml (serotype C);  \(0.9 \times 10^6 \) cfu/ml (serotype D)
- **T2 band**:  \(35 \times 10^6 \) cfu/ml (serotype A);  \(10 \times 10^6 \) cfu/ml (serotype B);  \(17 \times 10^6 \) cfu/ml (serotype D)

9.4.2. Analytical Sensitivity with purified antigen solution
Purified *Cryptococcus* sp. antigen was quantified and then serially diluted and tested with three different lots of Bio-Rad RDT CryptoPS Assay and in three replicates until absence of detection. The LOD in ng or µg/ml of the Bio-Rad RDT CryptoPS Assay for *Cryptococcus* antigen has been estimated as:

- **T1 band**: 25 ng/ml
- **T2 band**: 2.5 µg/ml

9.5 Hook Effect
A hook effect was studied by testing by serial dilution a high concentration (100 µg/ml) purified *Cryptococcus* sp. antigen. All results demonstrated a downward trend of band intensity with increased dilution and that high dose hook effect interference was not present with the Bio-Rad RDT CryptoPS Assay.

10. QUALITY CONTROL OF THE MANUFACTURER
All manufactured reagents are prepared according to manufacturer’s Quality System, starting from reception of raw material to commercialization of the final product. Each lot is submitted to quality control assessments and is released to the market only after conforming to pre-defined acceptance criteria. The records related to production and controls of each single lot are kept within manufacturer.
11. BIBLIOGRAPHY REFERENCES


** BIOSYNEX S.A.  
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Fax : +33 (0) 1 47 41 91 33  

**SYMBOLES**

- **Consult Instructions for Use**  
- **LOT**  
- **Batch code**

- **In Vitro Diagnostic Medical Device**  
- **Manufacturer**

- **Temperature limitation : 2 – 25°C**  
- **Distributor**

- **Contains sufficient for <n> tests**  
- **Catalogue number**

- **Use by**  
- **Do not reuse**

**Bio-Rad**