BIOSYNEX® CryptoPS

Ref. 1120001

Rapid test for the semi-quantitative detection and titration of Cryptococcus sp. capsular antigens in serum, plasma, whole blood and CSF

INTRODUCTION

BIOSYNEX® CryptoPS is a rapid, single use, immunochromatographic test for the semi-quantitative detection and titration of Cryptococcus sp. capsular antigens in serum, plasma, whole blood and CSF.
BIOSYNEX® CryptoPS plays a part in the diagnosis of cryptococcal infections, particularly in the case of meningitis. This test is intended for *in vitro* diagnosis use exclusively by health professionals.

SUMMARY

Cryptococcosis is an opportunist, cosmopolitan illness due to a yeast of the Cryptococcus species, the most common of which in human pathology is *Cryptococcus neoformans*.
High-risk patients are immunodepressed (mainly people infected with HIV and those who have recently undergone grafts).
Most often infection is through inhalation of spores.
The most common clinical expression of the illness is meningoencephalitis. There are about 1 million new cases each year throughout the world, including 625,000 deaths linked to cryptococcal meningitis (source: CDC, Center for the Disease Control and Prevention).
The large majority of cases (>70%) occur in sub-Saharan Africa, followed by Asia.
Cryptococcosis is the second cause of mortality in immunodepressed patients (HIV, grafts).

OBJECTIVES

1. Use of BIOSYNEX® CryptoPS with a view to the diagnosis of infection with Cryptococcus sp. in symptomatic patients
2. Identification of patients presenting a high concentration of capsular antigens in the blood, (serum, plasma, whole blood) by the visualisation of the T2 line for poor prognosis, which can be confirmed by titration.
3. Use of BIOSYNEX® Crypto PS with a view to the diagnosis of infection with Cryptococcus sp in asymptomatic patients presenting a CD4 level of less than 100 cells/µl.

TEST PRINCIPLE

BIOSYNEX® CryptoPS is a lateral flow immunochromatographic test for the semi-quantitative determination and titration of the Cryptococcus sp. antigen in serum, plasma,whole blood and CSF.
The test uses specific polysaccharide capsular antibodies of Cryptococcus sp. which are fixed in the test lines (T1 and T2). During the test, the antigen present in the patient sample reacts with the anti-polysaccharide antibodies conjugated with gold particles, present in a non-visible part of the test. The complex thus formed migrates and interacts with the antibodies fixed in the test lines.
The presence of a coloured line in the test line or lines (T1 or T1 & T2) indicates a positive result whilst the absence of a test line indicates a negative result. The line T2 will appear if there is a high concentration of capsular antigens.
A coloured line will appear in the control line (C). It serves as a procedural control, confirming good migration.

EQUIPMENT PROVIDED

- Test cassettes, individually packaged in a pouch with a dessicant
- Capillary tubes (20 µl)
- Dropper bottle of diluent
- Bottle of diluent for the titration protocol
- Positive control bottle
- A barcode card making it possible to identify the test batch to be used if the BIOSYNEX Reader is used for the reading.
- Directions for use

EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Receptacles for the collection of samples
- Laboratory pipette
- Haemolysis microtubes or tubes for dilutions (titration protocol)
- Disposable gloves
- Timer
- Vortex

STORAGE AND STABILITY

Store in the original packaging at room or refrigerated temperature (2-25°C). This test is stable until the expiry date printed on the packaging. The test must remain in its sealed pouch until it is used.

PRECAUTIONS

– For professional *in vitro* diagnostic use only.
– Single use only. Do not re-use.
– The directions for use should be read and followed carefully.
– Do not freeze the components of the kit.
– Do not use the components after the expiry date (refer to the packaging).
– Do not use the test if its pouch is damaged.
– Do not eat, drink or smoke in the area where the sample and kits are handled.
– All samples must be handled as potentially infectious material.
– Observe the usual precautions established for biological risks and adhere to the directives in force tor disposal of the samples.
– Wear protective clothing: lab coats, disposable gloves and eye protection
– Damp and high temperatures may affect results in a harmful way
– Do not use more than the required quantity of liquid.
– Do not tip the samples into the reaction area.
– Do not touch the device reaction area to prevent contamination.
– The test must remain in its sealed pouch until it is used.
– Always store the test between 2-25°C.
– Do not mix and interchange the reagents for the various batches.
– Prevent all cross-contamination of samples by adhering to good laboratory practices.

SAMPLE COLLECTION

- For **capillary blood:** Use the capillary tube supplied and collect a 20 µl volume blood sample. The sample should be used immediately.
- For venous blood, **serum, plasma and CSF**: collect aseptically, in accordance with recommended procedures. **Blood or plasma collected in EDTA or heparin** can be used. The sample may be used immediately.

STORAGE OF SAMPLES

Do not leave the samples at room temperature for long periods.

- **Whole blood collected by finger puncture** must be used immediately.
- **Whole blood collected by venous puncture** must be stored at 2-8°C if the test takes place in the 2 days following the collection. Do not freeze samples of whole blood.
- Samples of **serum, plasma and CSF** may be stored for 8 hours at 15-30°C or at 2-8°C for up to 3 days. In the case of longer storage the samples must be stored at -20°C.

Return the samples to room temperature before performing the test. The frozen samples must be completely thawed or be mixed well before being tested. Avoid repeated cycles of freezing/thawing of samples.

TEST PROCEDURES

Preparation

Before performing the test make sure that all the test components have been brought to room temperature.

Semi-quantitative procedure

1. Open the pouch and take out the cassette; then put it on a flat, horizontal surface.
2. Put 20 µl of the sample (serum, CSF, plasma or whole blood) in the sample well (S) of the cassette.
3. Open the dropper bottle containing the diluent. Pour 3 drops of diluent into the sample well by applying slight pressure on the sides of the bottle. Avoid adding air bubbles to the cassette sample well and tipping the liquid into the result reading window.
4. Start the timer. As the test progresses a reddish colour migration front appears and migrates along the membrane.
5. Read the result after 10 minutes' migration. Very positive results will be seen more quickly.
Do not interpret any test band appearing 15 minutes after the sample is deposited in the cassette.
6. After reading, dispose of the test components and the sample in accordance with the established procedure for disposal of biological waste.

Titration protocol

1. Prepare 10 tubes and number them 1 to 10.
2. Place 225 µl of diluent (use the bottle of diluent intended for the titration protocol) in Tube 1 and 120 µl in Tubes 2 to 10.
3. Place 25 µl of sample in Tube 1 so as to obtain a dilution of 1/10th. Mix well (vortex).
4. Transfer 120 µl from Tube 1 to Tube 2 and mix well so as to obtain a dilution of 1/20th.
Continue this dilution procedure for the following tubes.
In this way 10 dilutions from 1/10th (Tube 1) to 1/5120th (Tube 10) are obtained.
5. Take 10 pouches containing a cassette and prepare them as in Point 1 of the semi-quantitative procedure
6. Place 100 µl of Tube 1 (1/10th dilution) in the 1st cassette.
Place 100 µl of Tube 2 in the 2nd cassette.

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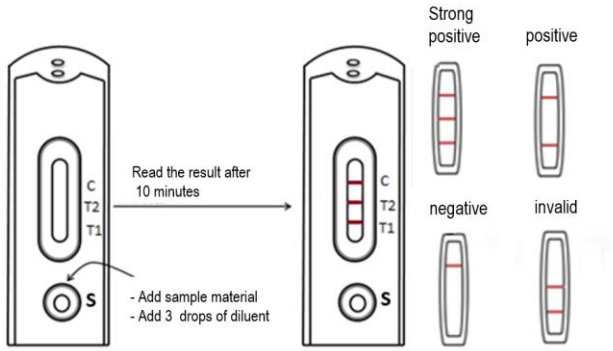
Continue this procedure up to Tube 10 with the 10th cassette.
7. Read the result after 10 minutes' migration.
Do not interpret any test band appearing 15 minutes after the sample is deposited in the cassette.
8. After reading, dispose of the test components and the sample in accordance with the established procedure for disposal of biological waste.

Procedure for the Positive Control
1. In the sample well (S) of the test cassette place one drop of the positive control.
2. Add 3 drops of diluent.
3. Read the result at 10 minutes.
Note: The positive control must be positive. If the control does not function as expected, do not interpret the test results. Repeat the test or contact your supplier.



INTERPRETATION OF THE RESULTS
The test result may be read visually or with the help of the ***BIOSYNEX Reader.***

➢ Visual reading:

Semi-quantitative procedure

POSITIVE:
- The test line T1 appears at 25 ng/ml of capsular antigen.
- The test line T2 appears at 2.5 µg/ml of capsular antigen. If there is positivity in T2 the T1 line is also present.

Positive (T1): presence of 2 distinct coloured lines. A line appears in the control line C and a coloured line (even of low intensity) appears in the test line T1.
Strong positive (T1&T2)): presence of 3 distinct coloured lines. A line appears in the control line C and two coloured lines (even of low intensity) appear in the test lines T1 and T2.

NEGATIVE:
A coloured line appears in the control line (C). No line appears in the test lines T1 and T2.

INVALID:
. No visible coloured line in the control line C, even if bands appear in T1 and T2. The results of a test without a control line must be discarded. Review the procedure and repeat the test with a new cassette.
. If there is a line in T2 and in C, without a line in T1, do not interpret, and repeat the test with a new cassette.
If the problem persists contact your local distributor.

Titration protocol
For reading refer to the previous paragraph (visual reading/semi-quantitative procedure).

The interpretation is made taking only the test line T1 into account.
The titre of Cryptococcus antigens corresponds to the last dilution allowing a positive result to be obtained.

➢ Reading with the Biosynex Reader:

- The BIOSYNEX® CryptoPS test is compatible with the BIOSYNEX Reader, in combination with the BIOSYNEX® CryptoPS SD card.
  To read a result please refer to the instructions for use of the reader.
- The barcode making it possible to identify the test batch and the compatibility of the SD card is printed on a card supplied in the kit.

QUALITY CONTROL
- A coloured line appearing in the control line (C) ensures that migration has taken place.
- Good laboratory practices recommend the use of positive and negative controls *to* check the test functions correctly.

A positive control is supplied in the kit. This control must be tested at least when the kit is opened. It may be tested often according to the control procedures in place within the laboratory.

LIMITATIONS
1. As for any diagnostic test, the test result must be correlated with clinical results.
2. The test results should be interpreted taking into account the epidemiological, clinical and therapeutic contexts. Other reference techniques should be considered if necessary.

EXPECTED VALUES
- The test line T1 appears at 25 ng/ml of capsular antigens of Cryptococcus sp.
- The test line T2 appears at 2.5 µg/ml of capsular antigens of Cryptococcus sp.

PERFORMANCE
Internal evaluation
The BIOSYNEX® CryptoPS test has been compared with another rapid test. The results are as follows:

	Plasma	Whole blood
Relative sensitivity*	95.2% (20/21)	-
Relative specificity	100% (23/23)	100% (23/23)

* T1 line only

External evaluation
The BIOSYNEX® CryptoPS test has been compared to the CrAg LFA test from IMMY using 100 samples of whole blood from the serum bank (50 positive samples and 50 negative samples). Relative sensitivity of 96% (48/50) and relative specificity of 96% (48/50) were obtained. (Comparison of a novel semi-quantitative prototype and a commercial lateral flow assay for detection of cryptococcal antigen from thawed whole blood samples, Sriruttan C et al., ECCMID, 2016)

External evaluation
The BIOSYNEX® CryptoPS test has been compared to another rapid test. using 35 samples of CSF (2 positive samples and 33 negative samples). Relative sensitivity of 100% (2/2) and relative specificity of 100% (33/33) were obtained.

Cross reactivity
No detection of cross reactivity with Aspergillus sp.

REFERENCES

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3. WHO, December 2011 http://apps.who.int/iris/bitstream/10665/44786/1/9789241502979_eng.pdf
4. Centers for Disease Control and Prevention, http://www.cdc.gov/fungal/pdf/at-a-glance-508c.pdf
5. Centers for Disease Control and Prevention, http://www.cdc.gov/fungal/diseases/cryptococcosis-neoformans/
6. Guidelines for Management of Cryptococcosis, http://www.idsociety.org/uploadedFiles/IDSA/Guidelines-Patient_Care/PDF_Library/Cryptococcal.pdf

SYMBOLS

	Caution, see the Directions for use.	LOT	Batch No.
IVD	For *in vitro* diagnostic use only.		Manufacturer
2 25	Store between 2-25°C		Do not re-use.
Σ	Tests per kit	REF	Catalogue No.
	Expiry	DIL	Diluent

IFU_1120001_EN_V05201810R01

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