ReaScan® TBE IgM

REF 114106 IVD CE

Instructions for use

Reagena

INTENDED PURPOSE

ReaScan TBE IgM is a rapid test for qualitative detection of tick-borne encephalitis (TBE, FSME) virus specific IgM antibodies in human serum and cerebrospinal fluid (CSF). The test result is read from the Test cassette with the ReaScan+ reader (REF 105100) (Reagena Oy, Toivala, Finland). The result is used as an aid in the diagnosis of TBE. The test is intended for in vitro diagnostic laboratory use by healthcare professionals. Device is not intended for self-testing or near-patient testing. Device is not automated.

INTRODUCTION

Tick-borne encephalitis (TBE) virus belongs to flaviviruses and it may cause an infection of the central nervous system (CNS). TBE virus, which is found in most European countries, Russia and Northern Asia, can be transmitted to humans by the bite of infected *Ixodes ricinus* and *Ixodes persulcatus* ticks.

Symptoms of TBE virus infection usually appear in a two-phase course. After an incubation period of 1-2 weeks, flu-like symptoms are developed in the viremic phase of the illness and then a brief symptom-free period occurs. The second phase of the disease may involve the CNS with symptoms of meningitis, meningoencephalitis, meningoencephalomyelitis or meningo-encephaloradiculitis. On average, the severity of the disease increases with patient's age and the case-fatality is 0,5–2% in Europe, although considerably higher mortality has been reported for Siberian and Far Eastern TBE virus subtype.

The laboratory diagnosis of TBE is based on the detection of TBE virus specific IgM and IgG antibodies in serum and CSF.

TEST PRINCIPLE

The IgM antibodies in the sample are captured onto the test line by anti-human IgM antibodies. TBE specific IgM antibodies are detected by recombinant TBE virus antigen-colloidal gold complex. The intensity of the formed test line is proportional to the amount of TBE virus IgM antibody in the sample and is converted into numerical value by the ReaScan+ reader. The result is either Negative, Equivocal (EQV) or Positive according to the lot-specific cut-off values.

KIT COMPONENTS

10 ReaScan TBE IgM Test cassettes

10 ReaScan TBE IgM Dilution buffer vials

10 ReaScan TBE IgM Conjugate vials

Method card for the ReaScan+ reader

Instructions for use

MATERIALS REQUIRED BUT NOT PROVIDED

ReaScan+ reader (REF 105100)

Pipettes for volumes of 5-100 µl

Timer

STORAGE AND HANDLING CONDITIONS

Store the test kits at 5…25 °C. Store the test cassettes unopened in the foil pouches until use. The product is stable until expiry date indicated on the kit label. Perform the test at 15…25 °C.

In-use stability:

Sample dilution stored at room temperature is stable for 4 hours. Diluted serum sample, or CSF sample mixed with conjugate is stable for 15 minutes. Test cassette should be used within 15 minutes after opening the foil pouch.

WARNINGS AND PRECAUTIONS

All human samples must be treated as potentially infectious. Use protective gloves. Follow biosafety precautions at each step and discard waste according to national safety guidelines.

Do not mix components of different kit lots.

Do not use expired kits.

Do not use the test cassette if the wrapper foil is damaged.

Do not write on or close to the 2D-code of the test cassette. The 2D-code is used by ReaScan+ reader for the identification of the type and lot of the test cassette.

Test components are for single use only.

If there is any undissolved conjugate left in the conjugate vial after you have transferred the sample-conjugate mixture into the test cassette, the result may not be reliable.

The volume of the sample-conjugate mixture transferred into the test cassette should be 80 µl. Too small or too large volume may give an erroneous result.

Detection of TBE IgM antibodies may be interfered by very high titer of TBE IgG antibodies in the sample.

Any serious incident that has occurred in relation to the ReaScan TBE IgM shall be reported to the manufacturer (Reagena Oy) and the competent authority of the Member State in which the user is established.

SPECIMEN

Use serum or CSF as a sample. It is recommended to use fresh samples (not frozen).

Samples can be stored for few days in refrigerator (2…8 °C) or for years in freezer (below -20 °C). Frozen samples may cause false positive results when aggregated. If the sample is aggregated or otherwise poor in quality, please centrifuge it for 5 min with 3000 x g before use.

REASCAN+ READER SETUP

ReaScan+ reader setup must be performed when using ReaScan TBE IgM test for the first time with your ReaScan+ reader or if you are using a new test kit lot.

1. Switch on the ReaScan+ reader and log in using your user ID and password.
2. Press 'Start new test' icon and press 'New Method' on the next window.
3. Insert the Method card (barcode facing up) into the drawer, close the drawer and press 'Read Card'.
4. Wait until the ReaScan+ reader has finished loading the test parameters (screen shows green color when ready).

Remove the Method card from the drawer after successful setup.

TEST PROCEDURE

Before using the ReaScan TBE IgM rapid test and the ReaScan+ reader, it is important to read the instructions for use carefully.

Please note that the test procedure is different for serum versus CSF.

TEST PROCEDURE FOR SERUM SAMPLES

1. Please, confirm that the correct Method and Lot have been installed into the ReaScan+ reader.
2. Pipette **5 µl** of serum into the Dilution buffer vial and close the vial. Mix carefully by turning the vial upside down 10-20 times or by vortexing it for a few seconds.

Changes in the instructions for use are indicated with vertical lines.

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3. Open the test cassette's foil pouch. Place the test cassette on a flat surface (table). *Note! If you write down the patient information on the cassette, do not write on or close to the 2D-code of the test cassette.*
4. Transfer **100 µl** of diluted sample into the Conjugate vial. Mix the solution carefully until the conjugate is completely dissolved. You may do this by vortexing or by back-and-forth pipetting.

   Note! If you plan to incubate the sample inside the ReaScan+ reader (Incubation mode), please perform steps 1-3 of "HOW TO READ THE RESULT" before dispensing the mixture into the cassette.
5. Transfer **80 µl** of the conjugate-sample mixture into the test cassette's sample well.
6. Start the timer for 20-minute countdown **OR** insert the test cassette into the drawer of the ReaScan+ reader (Incubation mode).
7. Read the result with the ReaScan+ reader after **20 minutes**. The reading time frame is 19-21 minutes. The result may be invalid if the test is read beyond this time frame.

TEST PROCEDURE FOR CSF SAMPLES

1. Please, confirm that the correct Method and Lot have been installed into the ReaScan+ reader.
2. Pipette **90 µl of Dilution buffer** and **10 µl of CSF** into the Conjugate vial. Mix carefully until the conjugate is completely dissolved. You may do this by vortexing or by back-and-forth pipetting.
3. Open the test cassette's foil pouch. Place the test cassette on a flat surface (table). *Note! If you write down the patient information on the cassette, do not write on or close to the 2D-code of the test cassette.*

   Note! If you plan to incubate the sample inside the ReaScan+ reader (Incubation mode), please perform steps 1-3 of "HOW TO READ THE RESULT" before dispensing the mixture into the cassette.
4. Transfer **80 µl** of the conjugate-sample mixture into the Test cassette's sample well.
5. Start the timer for 20-minute countdown **OR** insert the test cassette into the drawer of the ReaScan+ reader (Incubation mode).
6. Read the result with the ReaScan+ reader after **20 minutes**. The reading time frame is 19-21 minutes. The result may be invalid if the test is read beyond this time frame.

HOW TO READ THE RESULT

1. Switch on the ReaScan+ reader by pressing the power key and log in using your user ID and password.
2. Press 'Start new test', select TBE IgM Method and the correct Lot ID.
3. Enter the sample identification using the keyboard or the optional barcode reader and press Continue.
4. Insert the test cassette into the drawer so that the reading area is pointing upwards and the sample well towards the reader. Close the drawer by pushing it gently back into the reader.
5. Select either 'Read Now mode' or 'Incubation mode' and start the measurement. If 'Incubation mode' was selected the test cassette will be analyzed after 20-min incubation. **Note! Do not remove the test cassette from the reader during incubation or measurement.**
6. Read the result. The result is defined as Positive, Equivocal (EQV) or Negative according to the lot-specific cut-off values.

Press 'Next sample' if you need to measure additional test cassettes of the same lot.

INTERPRETATION OF THE TEST RESULT

A **negative** result indicates that the sample does not contain TBE virus IgM antibodies. If acute TBE virus infection is still suspected, repeat the analysis with a new sample drawn after approximately one week.

A **positive** result indicates the presence of TBE virus specific IgM antibodies in the sample.

An **EQV** result means that the result is neither positive nor negative. In this case, follow the instructions of your organization. Typically, take a new sample after approximately one week and perform a new test.

When interpreting the results, patient's clinical picture and TBE vaccination history needs to be taken into account.

The test result is **invalid** if the control line in the test cassette is not visible or if it is too weak.

In the case that the test line appears blurred, unusually broad or multiple diffuse, repeat the test with a new test cassette.

CONTROL PROCEDURE

For internal quality assurance it is recommended to analyse a sample confirmed as TBE IgM antibody positive with each new ReaScan TBE IgM lot. Follow the quality control procedure guidelines of your laboratory.

TEST PERFORMANCE

Analytical sensitivity

Positive percent agreement (PPA) of ReaScan TBE IgM against reference methods with serum, plasma and CSF samples (n=157) was 98.7%.

PPA	ReaScan TBE IgM result		
N	Positive	EQV	Negative
157	155	0	2
PPA: 98.7% (95% CI: 95.5-99.9)			

Analytical specificity

Negative percent agreement (NPA) of ReaScan TBE IgM against reference methods with serum, plasma and CSF samples (n=130) was 96.9% when the equivocal results were counted as positive and 97.7% when the equivocal results were counted as negative.

NPA	ReaScan TBE IgM result		
N	Positive	EQV	Negative
130	3	1	126
NPA: 96.9% (95% CI: 92.3-99.2) NPA (EQV counted as negative): 97.7% (95% CI 93.4-99.5)			

Repeatability and reproducibility

The repeatability and reproducibility testing was done on three different sites, on five days, and using five replicates per sample (3x5x5 design). One sample (EQV) showed a ReaScan+ reader value between the negative and positive cut-off values and one sample (POS) represented a sample near the medical decision point, i.e. near the positive cut-off value.

			Repeatability		Within-Lab Precision		Reproducibility	
Sample	Mean reader value	N	SD	CV%	SD	CV%	SD	CV%
EQV	6,19	75	1,04	16,8 %	1,09	17,6 %	1,19	19,2 %
POS	15,44	75	2,29	14,8 %	3,18	20,6 %	3,18	20,6 %

Diagnostic sensitivity

Based on a total of 172 serum samples from earlier diagnosed TBE patients, the sensitivity was 99.4% (95% confidence interval between 96.80% and 99.99%)*.

ReaScan TBE IgM showed sensitivity of 91,89% (95% confidence interval between 78,09% and 98,30%) with a panel of CSF samples from 37 patients with acute TBE.

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Diagnostic specificity

Based on 141 TBEV IgM negative samples a specificity of 97.9% (95% confidence interval between 93.91% and 99.56%) was found*.

ReaScan TBE IgM showed specificity of 100% (95% confidence interval between 89,11% and 100,00%) with a panel of CSF samples from 32 non-TBE patients.

INTERFERING SUBSTANCES, LIMITATIONS

High levels of rheumatoid factor (RF) may cause interference.

Possible interference of heterophilic antibodies was assessed by analyzing serum/plasma samples positive for human anti-mouse antibodies (HAMA, n=10), rheumatoid factor (RF, n=29), Epstein–Barr virus (EBV, n=23), cytomegalovirus (CMV, n = 27) or system lupus erythematosus (SLE, n=10).

Interference	n	ReaScan TBE IgM		
		NEG	EQV	POS
HAMA	10	10	0	0
RF	29	24	2	3
EBV	23	22	1	0
CMV	27	24	0	3
SLE	10	10	0	0

In the publication by Albinsson B et al, a total of 73 serum samples from cytomegalovirus (CMV), Epstein barr virus (EBV), herpes simplex virus (HSV) or Varizella Zoster virus (VZV) infected patients and 10 serum samples from *A. phagocytophilum* infected individuals were tested. Based on those 83 potentially interfering samples, a specificity of 96.4% (95% confidence interval between 89.80% and 99.25%) was found.

CSF samples of patients with neuroborreliosis have not shown interference. TBE virus IgG antibodies do not cause a false positive result.

CROSS-REACTIVITY

To evaluate the level of potential cross-reactivity of the ReaScan TBE IgM to other flavivirus infections, a total of 57 serum samples from Dengue virus (DENV), Japanese encephalitis virus (JEV), West Nile virus (WNV), and Zika virus (ZIKV) infected individuals were analyzed. In addition, 15 sera from JEV and Yellow fever virus (YFV) vaccinated individuals and 10 sera from individuals infected with Chikungunya virus (CHIKV) were analyzed. Of these 82 sera 81 showed a negative result giving a specificity of 98.8% (95% confidence interval between 93.39% and 99.97%).

WASTE DISPOSAL

Patient samples and used test cassettes should be handled as infectious waste. All materials must be disposed in accordance with local disposal regulations.

LITERATURE

Lindquist L and Vapalahti O. 2008. Tick-borne encephalitis. Lancet, May 31;371 (9627):1861-71.

Mansfield N et al. 2009. Tick-borne encephalitis virus – a review of an emerging zoonosis. J.Gen. Virol. 90: 1781–1794.

Parviainen M et al. 2011. Detection of acute hantavirus infections using novel instrument-readable rapid tests. Eur. Infect. Dis.; 5(1):35-7.

Bogovic P and Strle F. 2015. Tick-borne encephalitis: A review of epidemiology, clinical characteristics, and management. World J Clin Cases. May 16;3(5):430-41.

Lotrič-Furlan S et al. 2017. Tick-borne encephalitis in patients vaccinated against this disease. J Intern Med. Apr 25.

Laaksonen M et al. 2017. Crowdsourcing-based nationwide tick collection reveals the distribution of Ixodes ricinus and I. persulcatus and associated pathogens in Finland. Emerg Microbes Infect. May 10;6(5).

Beauté J et al. 2018. Tick-borne encephalitis in Europe, 2012 to 2016. Euro Surveill. Nov;23(45).

Ruzek D et al. 2019. Tick-borne encephalitis in Europe and Russia: Review of pathogenesis, clinical features, therapy, and vaccines. Antiviral Res. Apr;164:23-51.

**Albinsson B et al. 2020. Multi-laboratory evaluation of ReaScan TBE IgM rapid test, 2016 to 2017. Euro Surveill. 2020 Mar;25(12). doi: 10.2807/1560-7917.ES.2020.25.12.1900427.*

Bjonholm E et al. 2022. Tick-borne encephalitis in pregnant women: A mini narrative review. New Microbe and New Infect 2022; Aug 29;48:101017.

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