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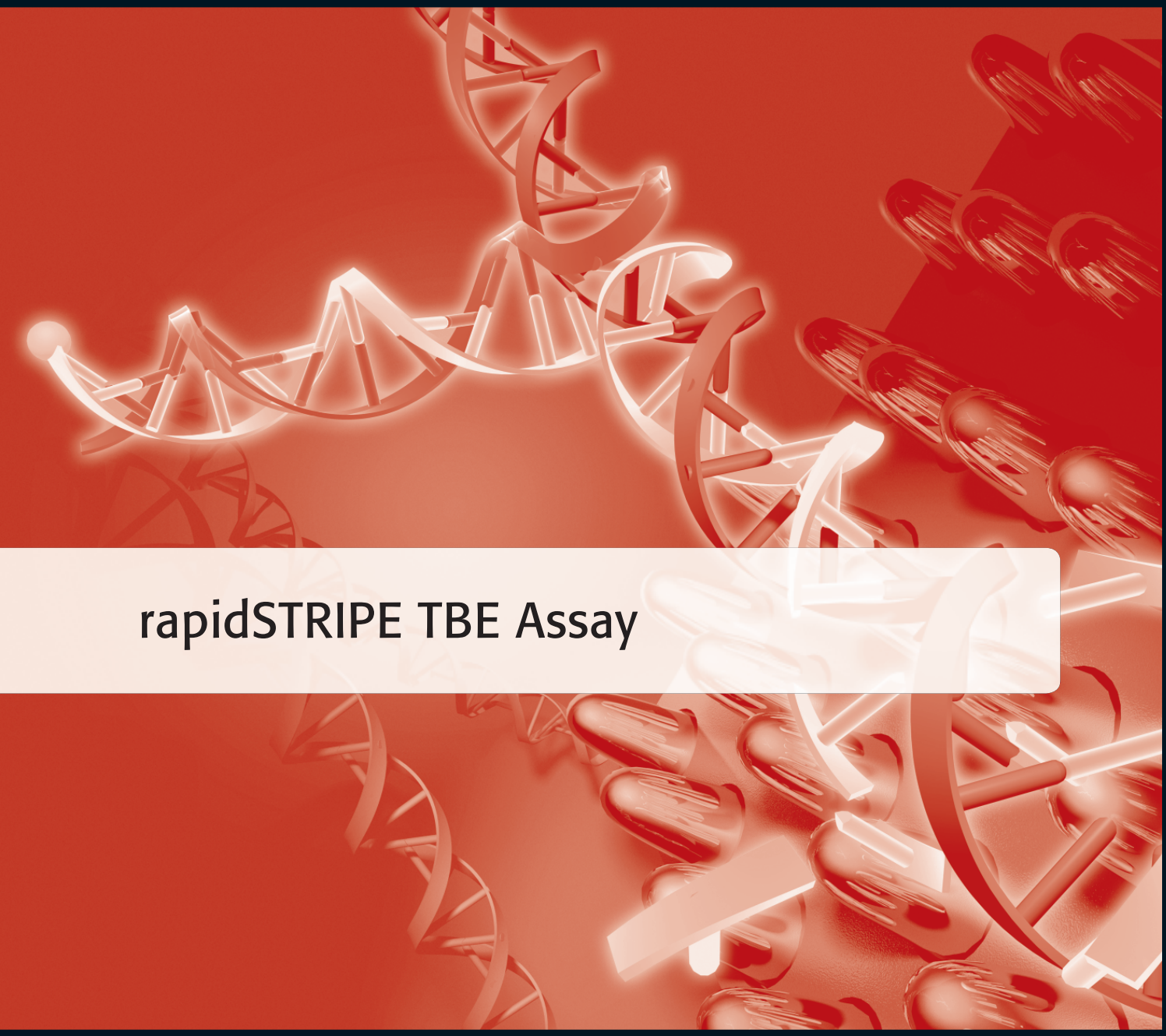
Manual



rapidSTRIPE TBE Assay

Order No.:

845-IS-1003010	10 reactions
845-IS-1003025	25 reactions
845-IS-1003050	50 reactions

Publication No.: HB_IS-1003_e_120618

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Content

1 Introduction

The arising warm climate in middle Europe will lead to a dramatic increasing of ticks. Referring to this fact, also the risk of a tick bite will be increased. Ticks are carrier of infectious pathogens and induce different diseases (zoonoses).

Tick-borne meningoencephalitis or tick-borne encephalitis (TBE) is a tick-borne viral infection of the central nervous system affecting humans as well as most other mammals. It is transmitted by the bite of infected ticks.

TBE virus has two subtypes: (a) European subtype (tick vector: *Ixodes ricinus*) and (b) Far Eastern subtype (tick vector: *Ixodes persulcatus*).

The risk of a transfer of the pathogens is depending on how and when the tick is removed. A test on pathogens inside the tick is faster and safe in comparison to test the pathogen in human beings. If the PCR result is positive, the tick is carrier of the accordant pathogen. That means that the pathogen could be transferred during the progress of a tick bite. Frequently the diagnosis of the infection itself is not surely possible. Thus only the risk diagnostic via the tick analysis is able to determine an adequate therapy in case of an appearance of any symptoms.

2 Test description and principle

The rapidSTRIPE TBE Assay is a molecular diagnostic test system to determine *Tick-borne Encephalitis Virus* in ticks.

The rapidSTRIPE TBE Assay detects the nucleic acids of the pathogens directly inside the sample material (ticks). The test contains of 3 modules, which functions are optimized to each other.

2.1 Module nucleic acid purification

Module nucleic acid purification is used for the isolation of nucleic acids from the sample material (tick tissue). Thereby it is possible to extract only DNA (blackPREP Tick DNA Kit) or to process a simultaneously isolation of DNA and RNA from the tick tissue (blackPREP Tick DNA/RNA Kit).

The blackPREP Tick DNA Kit is used to isolate DNA form ticks and is applicable for the detection of bacteria and protozoa inside the tick tissue.

The blackPREP Tick DNA/RNA Kit is used for the simultaneous isolation of DNA and RNA directly from ticks. This is of especially interest, if next to the analysis of bacterial pathogens, also e.g. RNA viruses have to be determined (e.g. detection of TBE).

In both extraction kits, first the tick has to be mechanical homogenized using e.g. SpeedMill (Analytik Jena AG) or other commercial available homogenizers on the basis of beads.

After the mechanical homogenization, the lysis or denaturation of the sample is followed. Consecutively the released nucleic acids are specific bound onto a spin filter surface, washed and finally eluted. Now the nucleic acids are ready for any further down stream application.

2.2 Module PCR amplification / hybridization

Module PCR amplification / hybridization is used for the detection of the *TBEV* – specific RNA. The isolated RNA used for a virus specific RT (Reverse Transcription) reaction. The cDNA amplification is in the following combined with a hybridization reaction using a *TBEV* specific probe within the same well of the PCR plastic. This reaction formats allows thus a specific determination of the *TBEV* RNA and avoids false positive results because of a mispriming.

2.3 Module detection

Module detection is used to visualize the amplification – hybridization results by an user-friendly Lateral Flow Strip. To visualize the reaction the amplification mix will be transferred onto a Lateral Flow Strip. A positive PCR/hybridization result will be confirmed by a visible test line.

Attention

Results from ticks may not be adducted as exclusive basis for further therapies!

3 Performance assessment, spectrum of application and specificity

Sequences of primers and probe chosen for this assay allow to determine both, European strains (FSME) and Far Eastern strains (RSME) of TBE viruses. Furthermore *Louping ill virus* (also tick-borne virus enzephalitide) can be determined with rapidSTRIPE TBE Assay.

This assay has been validated with following TBEV strains:

- Tick-borne encephalitis virus strain K23
- Tick-borne encephalitis virus strain Sofjin
- Tick-borne encephalitis virus strain Neudörfl
- Louping ill virus

NCBI Sequence alignment for primers probe shows additionally following results:

- Tick-borne encephalitis virus strain Primorye
- Tick-borne encephalitis virus strain Glubinnoe
- Tick-borne encephalitis virus strain Oshima
- Tick-borne encephalitis virus strain Sofjin
- Tick-borne encephalitis virus strain Senzhang
- Tick-borne encephalitis virus strain 205, 178-79, MDJ-01, 886-84, K23, 263, EK-328
- Tick-borne encephalitis virus strain Vasilchenko
- Tick-borne encephalitis virus strain Neudörfl
- Louping ill virus
- Spanish sheep encephalitis virus
- Tick-borne encephalitis virus strain Zausaev
- Greek goat encephalitis virus
- Omsk hemorrhagic fever virus

4 Kit components, storage and stability

Important!

The master mix has to be prepared freshly before each application. Storage of a ready-to-use master mix could lead to false positive results due to the formation of primer – probe – dimers.

Kit components and volumes or amounts are listed in the component table below. All components are ready to use and stable until expiry date mentioned on the kit packaging, if stored as specified in the following.

Module PCR amplification / hybridization

Component	Content per reactions			Storage
	10	25	50	
8 well strip 0.2 ml with lid	4	8	14	Packed within **Module detection**
TBEV positive control	7.5 µl	15 µl	25 µl	- 20 °C
Primer 1 F	35 µl	80 µl	150 µl	- 20 °C
Primer 2 F	25 µl	60 µl	100 µl	- 20 °C
Probe F	25 µl	60 µl	100 µl	- 20 °C
dNTP mix	15 µl	30 µl	60 µl	- 20 °C
RT-Enzyme	10 µl	25 µl	50 µl	- 20 °C work on ice
10x RT buffer	25 µl	50 µl	100 µl	- 20 °C
100 mM DDT	25 µl	50 µl	100 µl	- 20 °C
10x PCR Buffer	50 µl	100 µl	200 µl	- 20 °C
PCR-grade H_20	250 µl	500 µl	1000 µl	- 20 °C
innuTaq HOT DNA Polymerase	10 µl	15 µl	20 µl	- 20 °C work on ice

Module detection

Components	Content per reactions			Storage
	10	25	50	
Lateral Flow Strips	10	25	50	4 °C close airproof
Running buffer	2 ml	5 ml	10 ml	4 °C
Sample Tubes (2.0 ml)	10	25	50	Room temperature

5 Necessary laboratory equipment and additives

- SpeedMill (Analytik Jena AG) or other commercial available homogenizers on the basis of beads
- Standard PCR thermal cycler with heated lid and 0.2 ml wells (e.g. FlexCycler, Analytik Jena AG)
- Microcentrifuge
- Vortexer
- Variable pipettes for 10 µl, 100 µl and 1.000 µl (use separate pipettes for extraction, amplification and detection)
- Sterile pipette tips with protection against contamination (filter tips)

6 Remarks and safety precautions

All reagents in this kit only have to be used for the intention mentioned inside the user manual. The application may only be exercised by authorized personal.

During the operation, the described protocol has to be followed strictly. Furthermore the regularities to operate quality controls within medical laboratories have to be considered.

The reagents should be stored inside the original vessels at the mentioned temperatures. Single components of different charges and consumables may not be exchanged. The mentioned expiry dates have to be considered.

The material to be determined has to be categorized as potential infectious. The accordant precautions have to be noticed.

For the exposure to the kit reagents and the sample material, the accordant regulations to prevent accidents for the medical service have to be observed. Particularly the following precautions have to be considered:

- Don’t eat, drink or smoke!
- Always wear protective clothing and gloves!

The reagent vessels could be disposed with the normal laboratory waste.

7 Perfomance of the test

Important notes!

- Do not exchange the components of different kits or kit charges
- Open and close the vessels of single components always separately
- Change contaminated gloves immediately
- Spatial separation of the amplification and detection area
- Perform the procedure in the order of the following steps:

    1. Sample preparation / nucleic acid extraction
    2. Reverse transcription
    3. Amplification and hybridization
    4. Detection

- Do not open PCR plastics, which contain amplified samples in the area of sample preparation (NA isolation) or preparation of amplification
- Amplified samples and controls are potential sources of contamination
- Use separate pipettes with sterile filter tips for the preparation of the PCR reaction master mixes
- Open the reaction vessels carefully to avoid the generation of aerosols

7.1 Nucleic acid isolation

The isolation of the TBEV RNA has to be done using the blackPREP Tick DNA/RNA Kit. The protocols inside the accordant user manual have to be followed exactly. The simultaneous isolated DNA may be used for determination of further tick-borne diseases (caused by bacteria or protozoa).

Note: The operation of the test was optimized by using nucleic acids, which were isolated by the above mentioned extraction kit. Alternatively, also nucleic acids, which were isolated by other methods could be used.

7.2 Reverse transcription, PCR amplification and hybridization

Note

Only for application of Module PCR amplification / hybridization

Initially, the isolated RNA will be transcribed in a cDNA using a reverse transcriptase and a TBE-specific primer. Then the cDNA will be amplified and labeled in a PCR-hybridization reaction with TBE-specific primers and a probe.

7.2.1 Reverse transcription

1. Thaw 10x RT-Buffer, dNTP's, DTT und Primer 1 F from Module PCR amplification / hybridization, vortex, spin down and store the components on ice during the preparation. Spin down the RT-Enzyme and store it also on ice during the preparation.
2. Divide 10 µl of the RNA eluates to the wells of the accordant PCR plastic
3. Keep the RNA samples on ice till RT-master mix (→ table below) is prepared. The preparation of the master mix has to be done for the number of used samples

	RT master mix volume for 1 sample
10x RT Buffer	1.5 µl
Primer 1 F	1.0 µl
dNTP Mix	0.3 µl
RT-Enzyme	0.8 µl
DTT	1.4 µl
Final PCR volume	5 µl/reaction

4. Divide 5 µl of the RT-master mix to the accordant RNA samples.
5. Close the PCR plastic, put it into the thermal cycler and close the lid
6. Start cycler program for Reverse Transcription (see below).

Time and temperature protocol for reverse transcription:

Reverse transcription

Step	**Cycle**	**Profile**	**Temperature**	**Holding time**
1	1	Reverse transcription	50 °C	2.700 sec
2	1	Deactivation of RT-Enzyme	95 °C	300 sec

Standby: 18 °C **Time:** depending on thermal cycler

7.2.2 Amplification and hybridization

The performance of amplification and the hybridization of the PCR product could be done using a standard PCR thermal cycler (including a heated lid) and a sample protection system (SPS), which holds samples cooled during heating of the lid to set temperature (105 °C – 120 °C) to avoid primer – probe – dimers, unspecific annealing and premature elongation.

Initial steps

1. Divide the DNA eluates and controls to the accordant PCR plastic

	8 Well Strip 0,2 ml
Sample (extracted DNA)	2.5 µl
Positive (positive control)	2.5 µl
Negative (PCR-grade H_2O)	2.5 µl

2. The prepared plastic has to be stored on the cooling block until the amplification is started

Preparation of the PCR reaction mix:

! **Important!**
The master mix has to be prepared freshly before each application. Storage of a ready-to-use master mix could lead to false positive results due to the formation of primer – probe – dimers.

1. Thaw all reagents of Module PCR amplification / hybridization, which are needed for PCR and hybridization, vortex, spin down and store the components on ice during the preparation
2. The preparation of the master mix for one sample is described in the following table. The preparation of the master mix has to be done for the number of used samples (including positive and negative controls)

Mastermix	**standard PCR**
Plastic	8 well strip (0.2 ml)
10x PCR Buffer	2.5 µl
Primer 1 F	1.5 µl
Primer 2 F	1.5 µl
Sonde F	1.5 µl
dNTP Mix	0.5 µl
innuTaq Hot DNA Polymerase	0.25 µl
PCR-grade H_2O	14.75 µl
Final PCR volume	25 µl/ reaction

3. The master mix has to be added to the wells, which still contain the pre-pipetted DNA samples (positive and negative controls respectively)
4. Close the PCR plastic, put it into the thermal cycler and close the lid
5. Start the PCR time and temperature protocol

Time and temperature protocol for amplification and hybridization:

The PCR protocol contains two steps:

Step 1: Amplification and labelling of the *TBE* specific DNA fragment.

Step 2: Hybridization of the amplified DNA sequence using the *TBE* specific probe.

Amplification and hybridization

Step	Cycle	Profile	Temperature	Holding time
1	1	Initial denaturation	95 °C	120 sec
2	42	Denaturation	95 °C	30 sec
		Annealing	55 °C	30 sec
		Elongation	72 °C	60 sec
3	1	Denaturation	95 °C	300 sec
		Hybridization	45 °C	650 sec

Standby: 18 °C **Time:** depending on thermal cycler

7.3 Detection

7.3.1 Introduction

The determination of the combined amplification / hybridization reaction is done by visualization on a Lateral Flow Strip (fig. 1). The Lateral Flow Strip consists of the following areas:

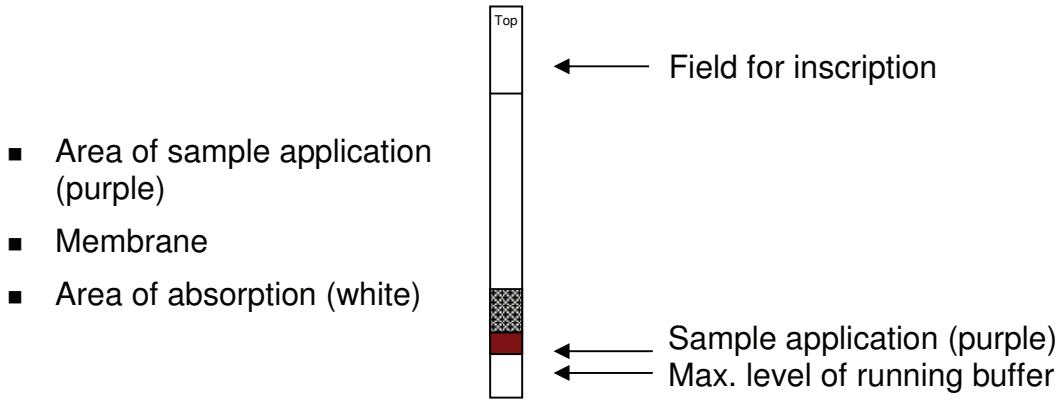

Fig. 1: Design of the Lateral Flow Strip

The whole Lateral Flow Strip, besides the lower part of the sample application area, is covered with a foil and can be touched on this foil. The foil above the absorption area can be used for any inscriptions. After the test is finished, the Lateral Flow Strips can be archived in a progress report.

7.3.2 Performance

1. Take the needed number of Lateral Flow Strips out of its package, inscribe it and place it ready

   Note: Only areas, which are covered with a foil can be touched and inscribed. Store the residual Lateral Flow Strips closed under adequate conditions.

2. Apply 10 µl of the PCR / hybridization reaction on the head of the sample application area (fig. 1, purple) at the border of the foil and incubate for at least 1 minute at room temperature.

   Note: Thereby the occurrence of a smear is normal.

3. Add **150 µl Running Buffer** to each single 2.0 ml Sample Tube
4. Place the Lateral Flow Strips with the membrane into the prepared 2.0 ml Sample Tubes and incubate until the area of sample application is discolored (approx. 20 min).

8 Analysis

The test is valid, if for each determined sample (positive control, negative control and sample) a colored control line is visible (fig. 2).

For each current test performance, the accordant positive and negative controls have to be correct. In case of the PCR negative control the test line has to be invisible (fig. 2B). If the test line of these samples is visible, the analysis for all tested samples has to be repeated.

1. Two red lines are visible: (Test and control line) — The sample is **positive** (fig. 2A).

Attention

Also a light colored test line has to be valued as positive. Compare with the negative control. If necessary repeat the whole test to confirm the result. The intensity of the control line has no influence on the result validation, because the control line is always more intensive in comparison to the test line.

Positive results can be visible before the incubation is finished.

2. Only one red line (level of the control line) **is visible:** — The sample is **negative** (fig. 2B).

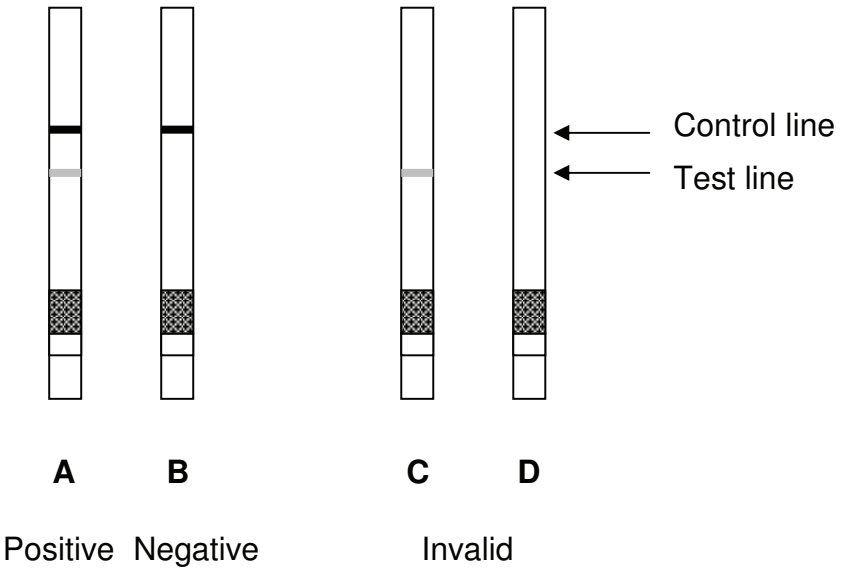

Fig. 2: Analysis of the reaction on the Lateral Flow Strip

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