UPPSALA UNIVERSITET

Zoonosis Science Center

# Immune responses in TBEV infection and vaccination

Åke Lundkvist

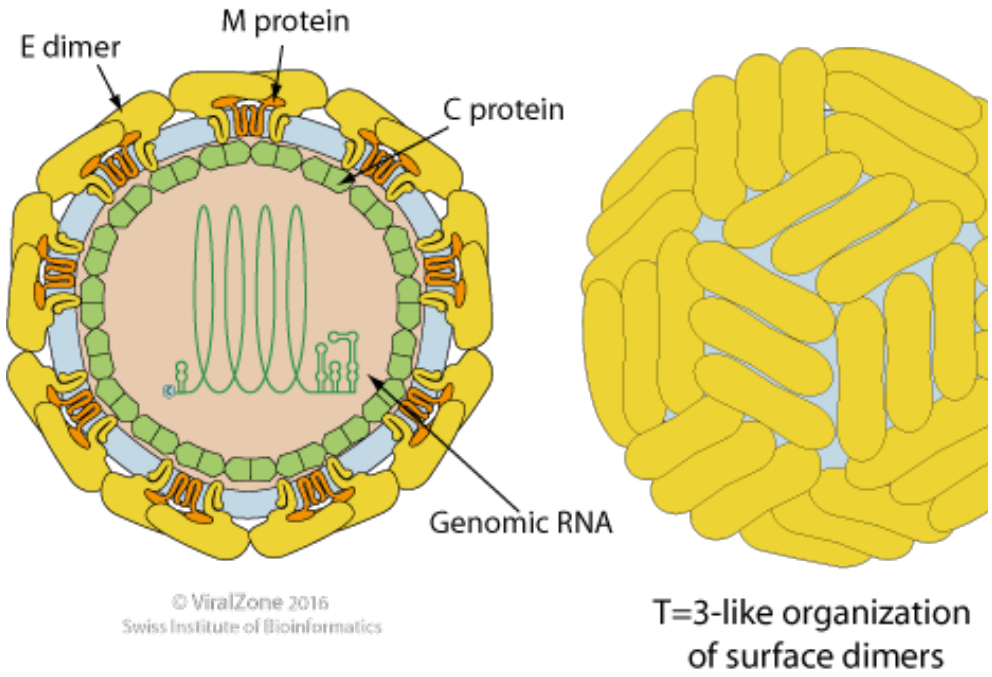
AKADEMISKA SJUKHUSET

UPPSALA UNIVERSITET

Zoonosis Science Center

# The virus

- Tick-Borne Encephalitis virus (TBEV) is an enveloped RNA virus of the family Flaviviridae. Size approx. 50 nm, single stranded +RNA 10 kb.



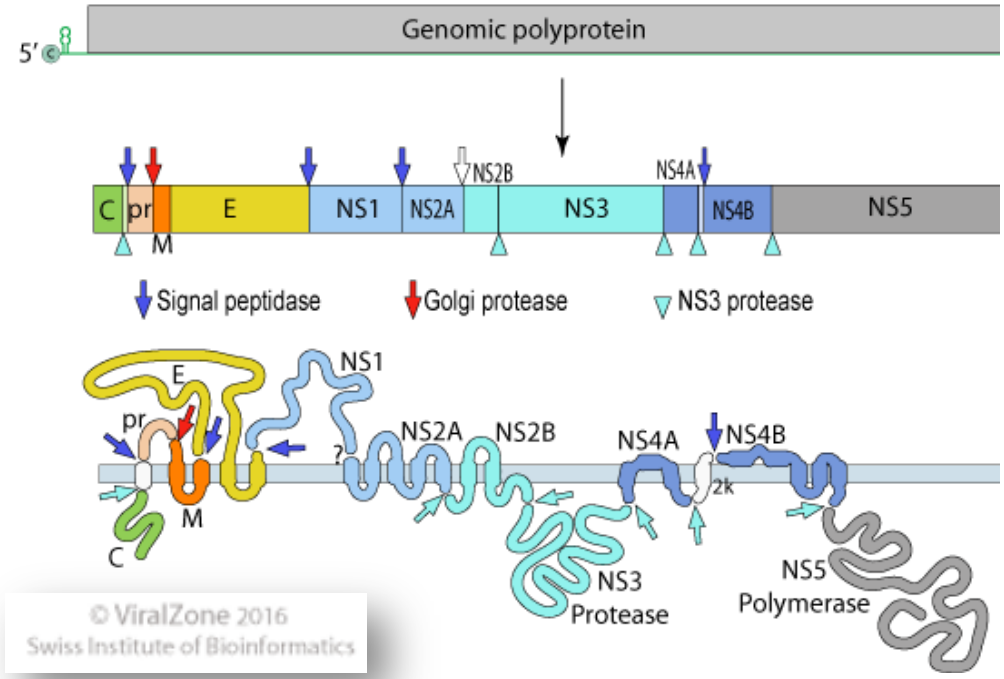

AKADEMISKA SJUKHUSET

UPPSALA UNIVERSITET

Zoonosis Science Center

# The genome

- The RNA serves as both the genome and the viral messenger RNA. The whole genome is translated into a polyprotein.
- C – capsid protein
- M – membrane protein
- E – envelope protein
- NS – Non-structural proteins



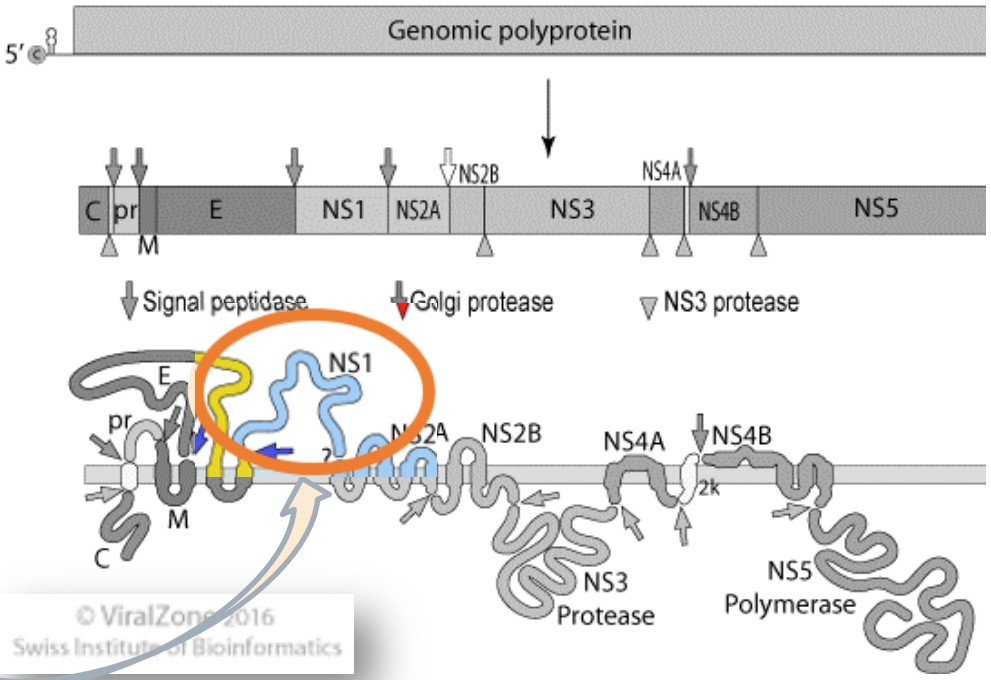

AKADEMISKA SJUKHUSET

# Non-structural proteins

- The virion RNA serves as both the genome and the viral messenger RNA. The whole genome is translated into a polyprotein.
- C – capsid protein
- M – membrane protein
- E – envelope protein
- NS – Non-structural proteins
- NS1– nonstructural glycoprotein

"enigmatic protein"

(Intracellular dimer/secreted hexamer)



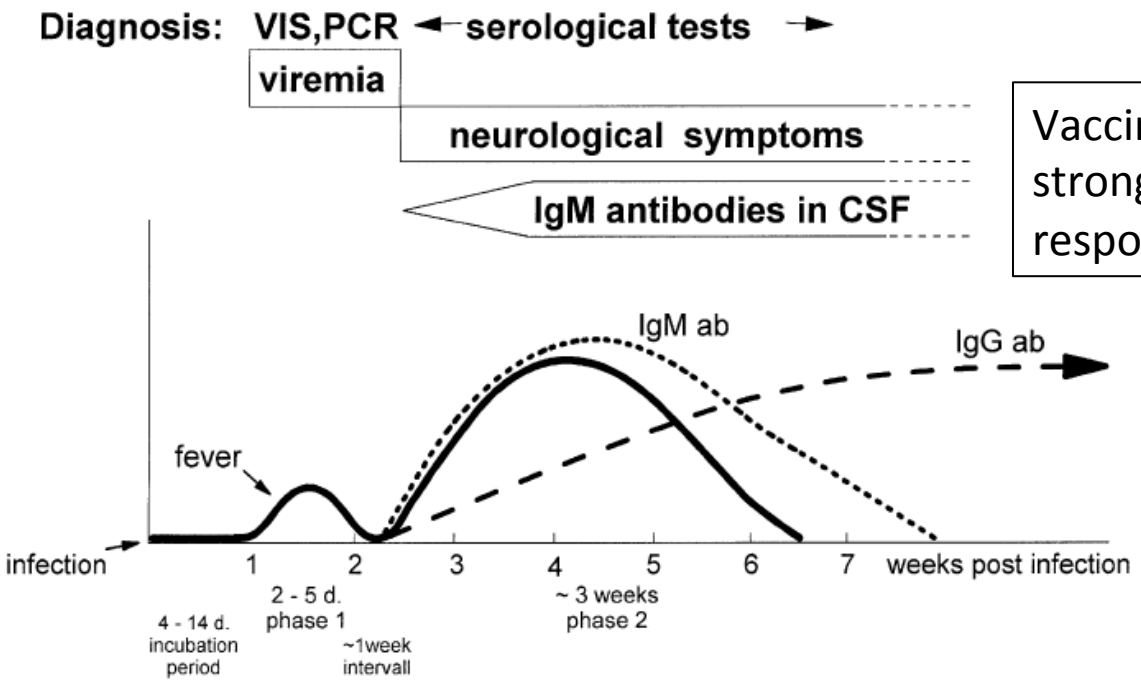
UPPSALA UNIVERSITET

Zoonosis Science Center

# TBE diagnostics – serology needed

## Antibody detection using EIA/ELISA

*H. Holzmann / Vaccine 21 (2003) S1/36–S1/40*



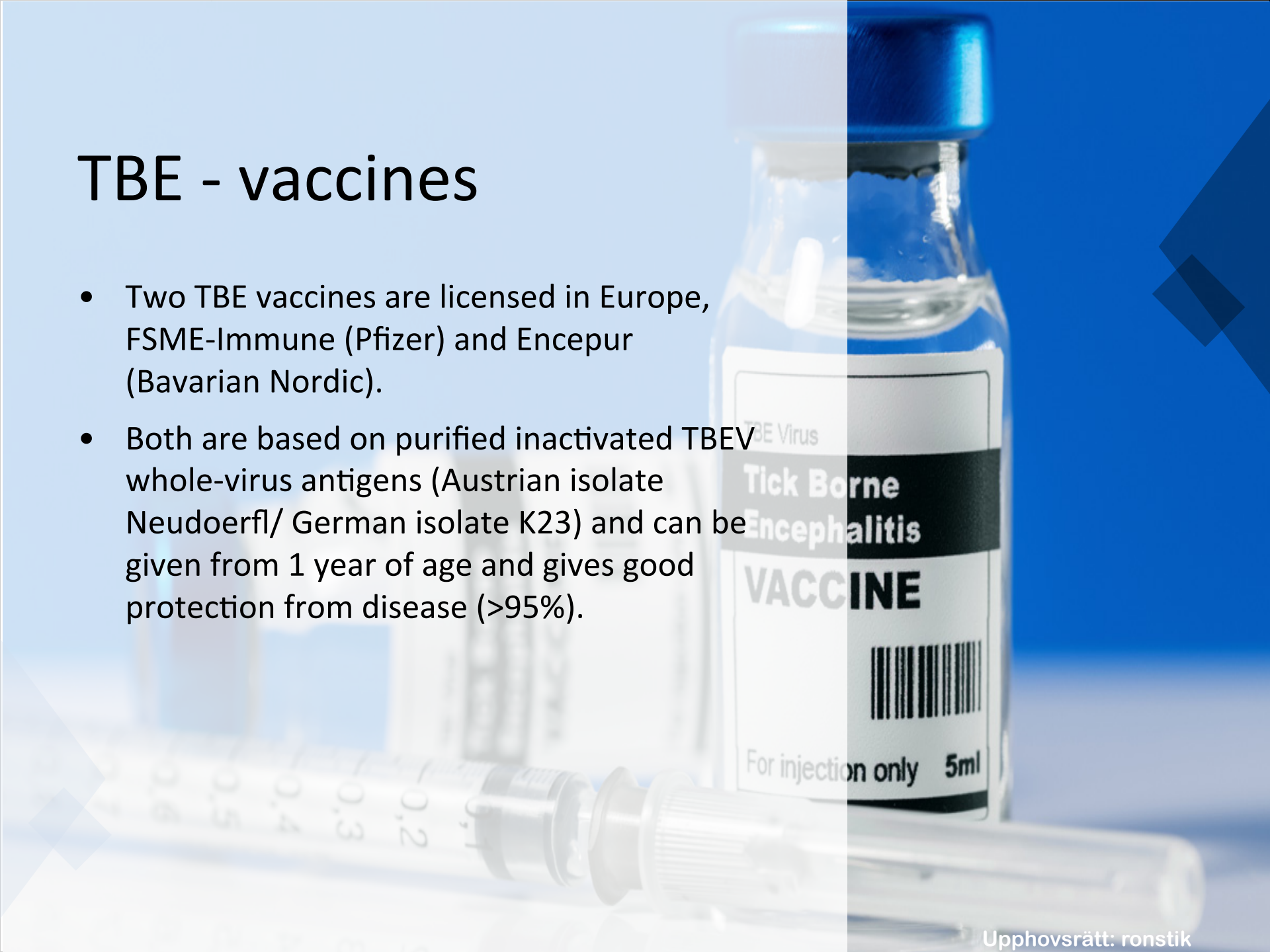

Vaccination gives strong IgG antibody responses



AKADEMISKA SJUKHUSET

# TBE - vaccines

- Two TBE vaccines are licensed in Europe, FSME-Immune (Pfizer) and Encepur (Bavarian Nordic).
- Both are based on purified inactivated TBEV whole-virus antigens (Austrian isolate Neudoerfl/ German isolate K23) and can be given from 1 year of age and gives good protection from disease (>95%).



UPPSALA UNIVERSITET

Zoonosis Science Center

# Vaccines

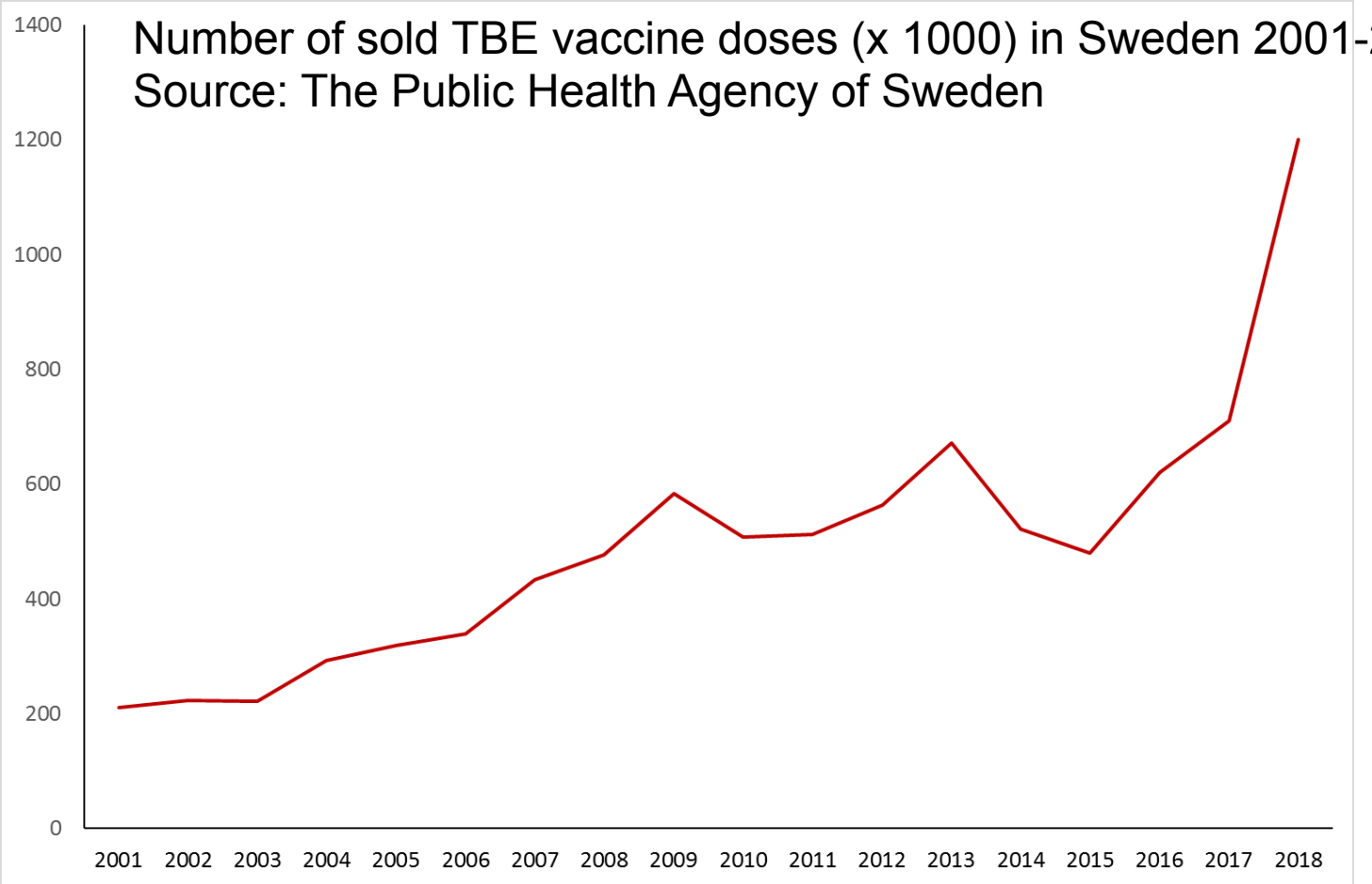
- Vaccination against TBE is effective to prevent TBE, but vaccine failures / vaccine breakthrough (VBT) occur.
- Andersson C, Vene S, Insulander M, Lindquist L, Lundkvist Å, Günther G. **Vaccine failures after active immunisation against tick-borne encephalitis**. Vaccine 28 (2010) 2827–2831
- Investigations of VBT is laborious and often include repeated antibody detection of blood and cerebrospinal fluid samples + neutralization test (NT) BSL 3.
- Simplify and improve diagnostics of VBT is a priority. TBEV SMIA has been tested.

AKADEMISKA SJUKHUSET

UPPSALA UNIVERSITET

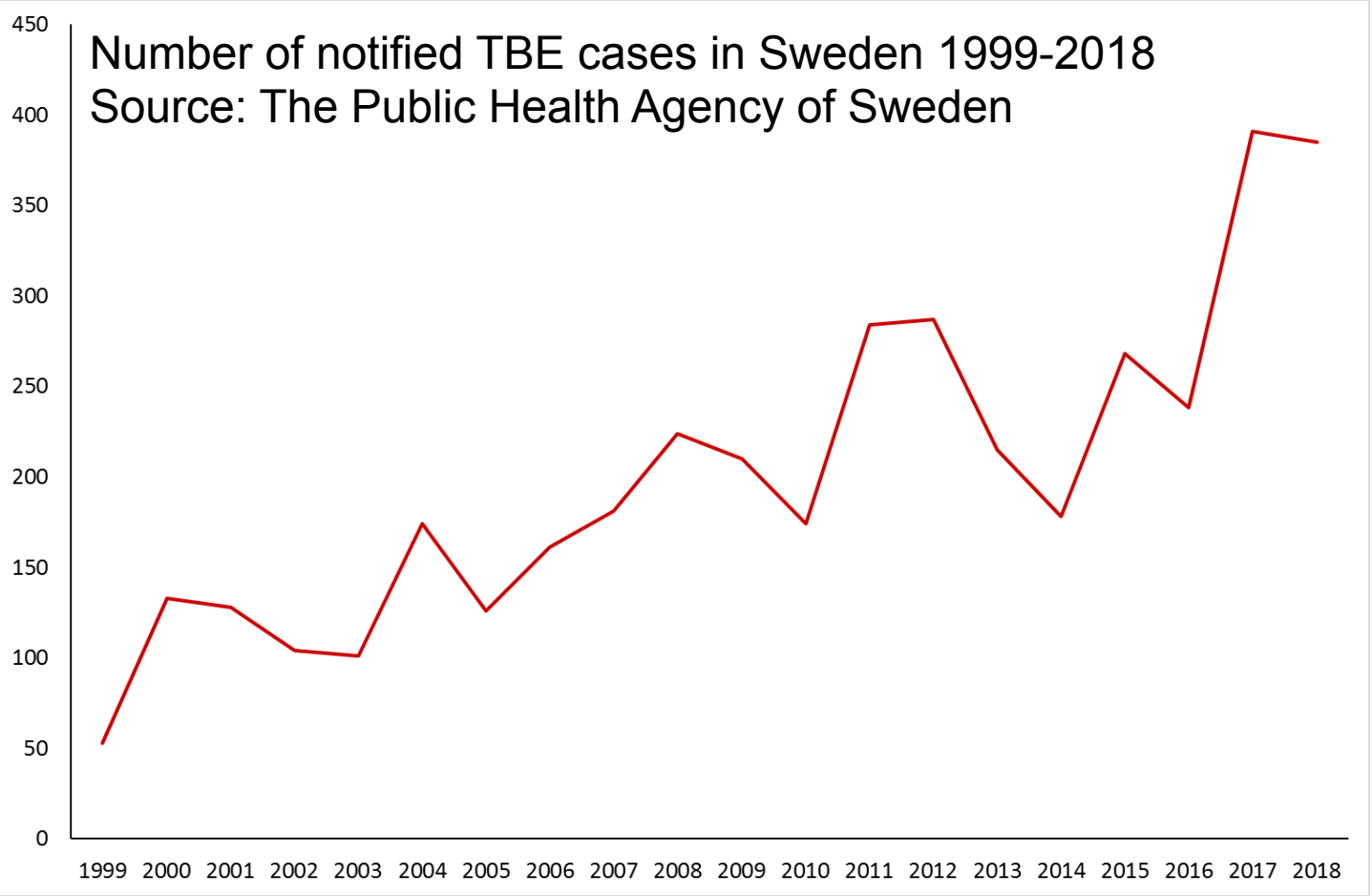
Zoonosis Science Center

AKADEMISKA SJUKHUSET

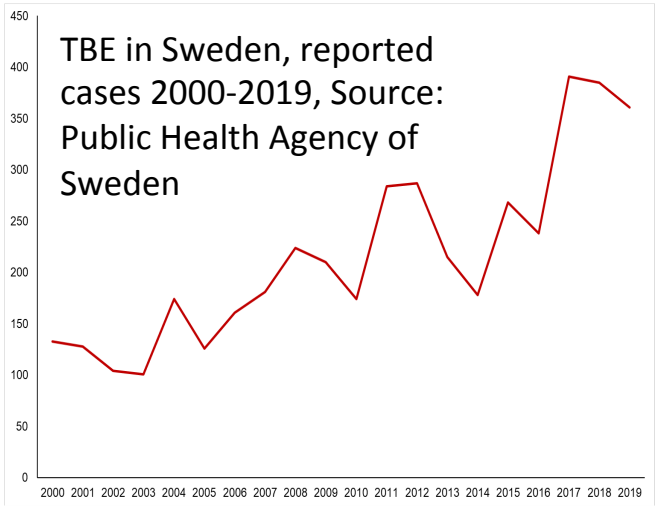


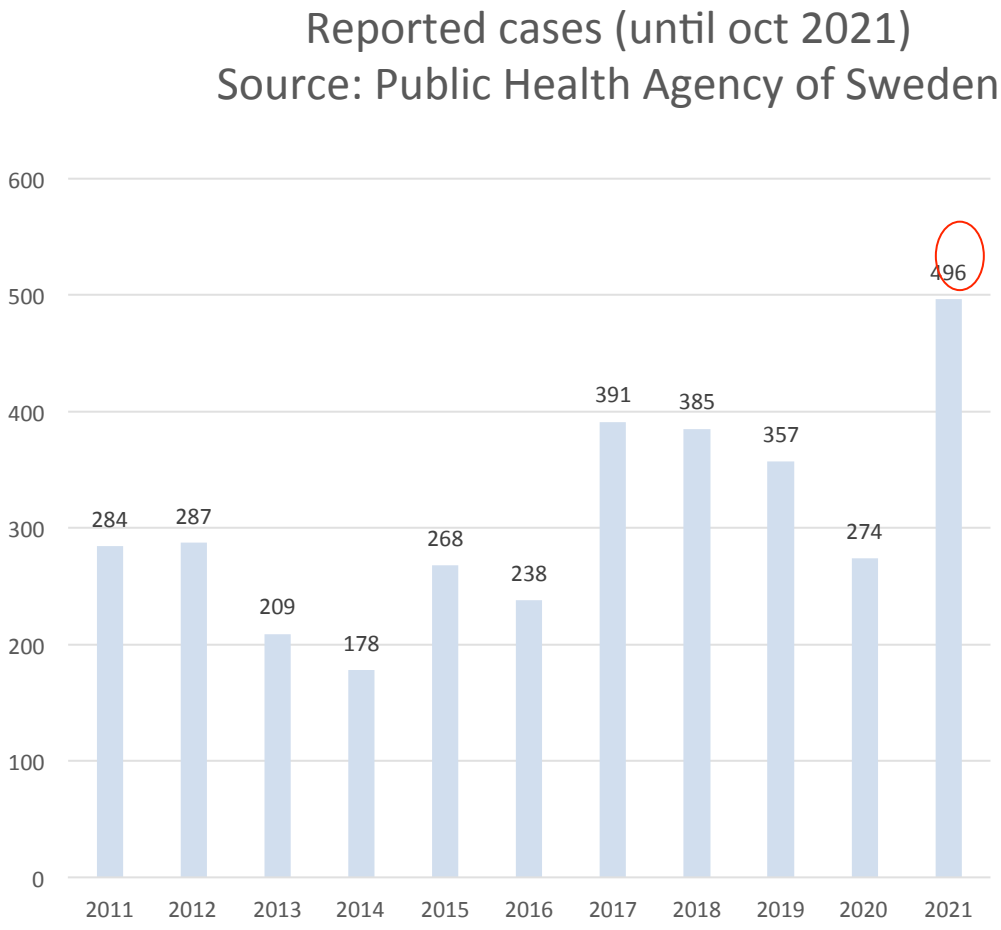
UPPSALA UNIVERSITET

Zoonosis Science Center



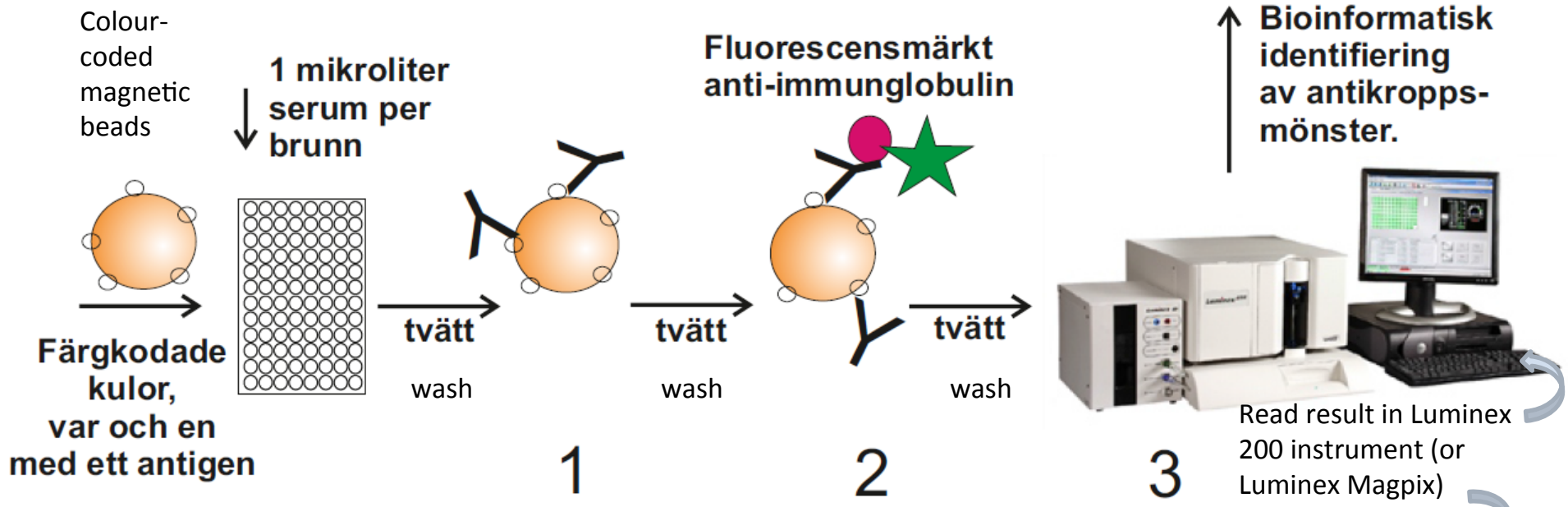

AKADEMISKA SJUKHUSET


TBE in Sweden, reported cases 2000-2019, Source: Public Health Agency of Sweden
450
400
350
300
250
200
150
100
50
0
2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019


Reported cases (until oct 2021)
Source: Public Health Agency of Sweden
600
500
400
300
200
100
0
284
287
209
178
268
238
391
385
357
274
496
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021

UPPSALA UNIVERSITET

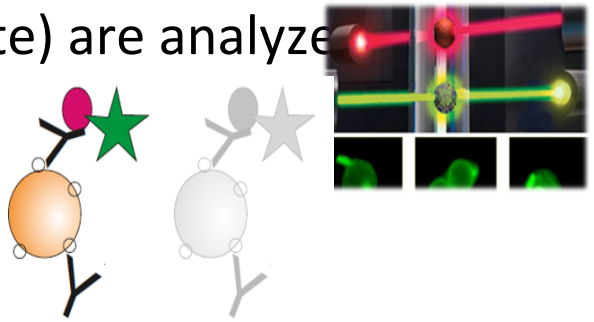
# Antibody detection using Suspension Multiplex Immuno Assay (SMIA)




AKADEMISKA SJUKHUSET

# TBEV Suspension Multiplex Immuno Assay (TBEV-SMIA)

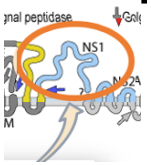
- The **TBEV-SMIA** method was developed from the more comprehensive Flavivirus suspension multiplex immunoassay, FSMIA (Rönnberg, Blomberg, Lundkvist et al, Med Microbiol Immunol (2017).
- **TBEV Whole Virus (WV)** and **Non-structural protein 1 (NS1)** antigens bound to differentially color-marked magnetic beads in a 96-well microtiter plate.
- Antibodies in serum samples binds to the antigen on the beads.
- Signals (via detection antibodies and conjugate) are analyze using laser beams in a Luminex/Magpix instru (Median Fluorescence Intensity) is calculated



UPPSALA UNIVERSITET

Zoonosis Science Center

# TBEV SMIA


- NS1 antigen is not present in existing vaccine preparations. 

- -> Vaccinees are not expected to develop any antibodies against NS1. 
- TBE exposed persons should develop antibodies to NS1. 

AKADEMISKA SJUKHUSET

UPPSALA UNIVERSITET

# Example newly infected (part of results)

| | IgG | | | | | | IgM | | | | | | NT | IgG | IgM | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | A75 | | | A14 | | | A75 | | | A14 | | | | Siemens | Siemens | Reascan |
| | TBEV wv | Urea | AI | TBEV NS1 | Urea | AI | TBEV wv | Urea | AI | TBEV NS1 | Urea | AI | | | | |
| 1 | 3208 | 148 | 0,0 | 659 | 50 | 0,1 | 3894 | 825 | 0,2 | 817 | 130 | 0,2 | ND | 46 | 2.2 | 287 |
| 2 | 992 | 32 | 0,0 | 155 | 23 | | 4776 | 1062 | 0,2 | 755 | 136 | 0,2 | ND | 12 | 2.0 | 280 |
| 3 | 3384 | 1011 | 0,3 | 408 | 127 | 0,3 | 2557 | 355 | 0,1 | 513 | 78 | 0,2 | ND | 29 | 2.0 | 259 |
| 4 | 1742 | 80 | 0,0 | 238 | 45 | 0,2 | 2882 | 422 | 0,1 | 299 | 22 | 0,1 | ND | 47 | 1.6 | 278 |
| 5 | 1493 | 40 | 0,0 | 363 | 36 | 0,1 | 2992 | 385 | 0,1 | 286 | 33 | 0,1 | ND | 19 | 1.9 | 282 |
| 6 | 2613 | 97 | 0,0 | 773 | 47 | 0,1 | 3485 | 489 | 0,1 | 677 | 76 | 0,1 | ND | 52 | 1.6 | 81 |
| 7 | 1751 | 260 | 0,1 | 0 | 24 | | 1232 | 106 | 0,1 | 162 | 10 | | ND | 21 | 1.2 | 120 |
| 8 | 4443 | 331 | 0,1 | 754 | 56 | 0,1 | 5009 | 1227 | 0,2 | 357 | 83 | 0,2 | ND | 89 | 2.0 | 241 |
| 9 | 3470 | 359 | 0,1 | 269 | 55 | 0,2 | 3186 | 759 | 0,2 | 317 | 23 | 0,1 | ND | 86 | 2.0 | 249 |
| 10 | 2618 | 180 | 0,1 | 615 | 118 | 0,2 | 2690 | 383 | 0,1 | 214 | 22 | 0,1 | ND | 81 | 1.2 | 291 |
| 11 | 2202 | 116 | 0,1 | 564 | 142 | 0,3 | 2641 | 390 | 0,1 | 384 | 77 | 0,2 | ND | 20 | 1.6 | 259 |
| 12 | 3077 | 309 | 0,1 | 838 | 148 | 0,2 | 4032 | 753 | 0,2 | 621 | 198 | 0,3 | ND | 57 | 2.2 | 267 |

# Example vaccinated (part of results)

| | IgG | | | | | | IgM | | | | | | NT | IgG | IgM | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | A75 | | | A14 | | | A75 | | | A14 | | | | Siemens | Siemens | Reascan |
| | TBEV wv | Urea | AI | TBEV NS1 | Urea | AI | TBEV wv | Urea | AI | TBEV NS1 | Urea | AI | | | | |
| **707-0** | 212 | 42 | | 0 | 14 | | 5 | 0 | | 1 | 0 | | <5 | ND | ND | ND |
| **707-120** | 692 | 82 | 0,1 | 39 | 14 | | 28 | 0 | | 6 | 0 | | <5 | ND | ND | ND |
| **707-390** | 4209 | 3258 | 0,8 | 111 | 123 | | 24 | 0 | | 0 | 0 | | >20 | ND | ND | ND |
| 708-0 | 160 | 61 | | 12 | 13 | | 93 | 30 | | 40 | 0 | | <5 | ND | ND | ND |
| 708-120 | 201 | 35 | | 16 | 6 | | 86 | 16 | | 34 | 0 | | <5 | ND | ND | ND |
| 708-390 | 0 | 0 | | 10 | 30 | | 59 | 21 | | 0 | 0 | | <5 | ND | ND | ND |
| **709-0** | 223 | 46 | | 0 | 7 | | 0 | 0 | | 4 | 0 | | <5 | ND | ND | ND |
| **709-120** | 804 | 111 | 0,1 | 44 | 24 | | 73 | 7 | | 0 | 0 | | <5 | ND | ND | ND |
| **709-390** | 1153 | 643 | 0,6 | 4 | 35 | | 2 | 0 | | 0 | 0 | | 20 | ND | ND | ND |
| 710-0 | 204 | 44 | | 0 | 17 | | 0 | 0 | | 0 | 0 | | <5 | ND | ND | ND |
| 710-120 | 225 | 37 | | 0 | 0 | | 0 | 0 | | 0 | 0 | | <5 | ND | ND | ND |
| 710-390 | 2256 | 878 | 0,4 | 0 | 0 | | 0 | 0 | | 0 | 0 | | >20 | ND | ND | ND |

AKADEMISKA SJUKHUSET

UPPSALA UNIVERSITET

# Example newly infected (part of results)

| | IgG | | | | | | IgM | | | | | | NT | IgG | IgM | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | A75 | | | A14 | | | A75 | | | A14 | | | | Siemens | Siemens | Reascan |
| | TBEV wv | Urea | AI | TBEV NS1 | Urea | AI | TBEV wv | Urea | AI | TBEV NS1 | Urea | AI | | | | |
| 1 | 3208 | 148 | 0,0 | 659 | 50 | 0,1 | 3894 | 825 | 0,2 | 817 | 130 | 0,2 | ND | 46 | 2.2 | 287 |
| 2 | 992 | 32 | 0,0 | 155 | 23 | | 4776 | 1062 | 0,2 | 755 | 136 | 0,2 | ND | 12 | 2.0 | 280 |
| 3 | 3384 | 1011 | 0,3 | 408 | 127 | 0,3 | 2557 | 355 | 0,1 | 513 | 78 | 0,2 | ND | 29 | 2.0 | 259 |
| 4 | 1742 | 80 | 0,0 | 238 | 45 | 0,2 | 2882 | 422 | 0,1 | 299 | 22 | 0,1 | ND | 47 | 1.6 | 278 |
| 5 | 1493 | 40 | 0,0 | 363 | 36 | 0,1 | 2992 | 385 | 0,1 | 286 | 33 | 0,1 | ND | 19 | 1.9 | 282 |
| 6 | 2613 | 97 | 0,0 | 773 | 47 | 0,1 | 3485 | 489 | 0,1 | 677 | 76 | 0,1 | ND | 52 | 1.6 | 81 |
| 7 | 1751 | 260 | 0,1 | 0 | 24 | | 1232 | 106 | 0,1 | 162 | 10 | | ND | 21 | 1.2 | 120 |
| 8 | 4443 | 331 | 0,1 | 754 | 56 | 0,1 | 5009 | 1227 | 0,2 | 357 | 83 | 0,2 | ND | 89 | 2.0 | 241 |
| 9 | 3470 | 359 | 0,1 | 269 | 55 | 0,2 | 3186 | 759 | 0,2 | 317 | 23 | 0,1 | ND | 86 | 2.0 | 249 |
| 10 | 2618 | 180 | 0,1 | 615 | 118 | 0,2 | 2690 | 383 | 0,1 | 214 | 22 | 0,1 | ND | 81 | 1.2 | 291 |
| 11 | 2202 | 116 | 0,1 | 564 | 142 | 0,3 | 2641 | 390 | 0,1 | 384 | 77 | 0,2 | ND | 20 | 1.6 | 259 |
| 12 | 3077 | 309 | 0,1 | 838 | 148 | 0,2 | 4032 | 753 | 0,2 | 621 | 198 | 0,3 | ND | 57 | 2.2 | 267 |

# Example vaccinated (part of results)

| | IgG | | | | | | IgM | | | | | | NT | IgG | IgM | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | A75 | | | A14 | | | A75 | | | A14 | | | | Siemens | Siemens | Reascan |
| | TBEV wv | Urea | AI | TBEV NS1 | Urea | AI | TBEV wv | Urea | AI | TBEV NS1 | Urea | AI | | | | |
| **707-0** | 212 | 42 | | 0 | 14 | | 5 | 0 | | 1 | 0 | | <5 | ND | ND | ND |
| **707-120** | 692 | 82 | 0,1 | 39 | 14 | | 28 | 0 | | 6 | 0 | | <5 | ND | ND | ND |
| **707-390** | 4209 | 3258 | 0,8 | 111 | 123 | | 24 | 0 | | 0 | 0 | | >20 | ND | ND | ND |
| 708-0 | 160 | 61 | | 12 | 13 | | 93 | 30 | | 40 | 0 | | <5 | ND | ND | ND |
| 708-120 | 201 | 35 | | 16 | 6 | | 86 | 16 | | 34 | 0 | | <5 | ND | ND | ND |
| 708-390 | 0 | 0 | | 10 | 30 | | 59 | 21 | | 0 | 0 | | <5 | ND | ND | ND |
| **709-0** | 223 | 46 | | 0 | 7 | | 0 | 0 | | 4 | 0 | | <5 | ND | ND | ND |
| **709-120** | 804 | 111 | 0,1 | 44 | 24 | | 73 | 7 | | 0 | 0 | | <5 | ND | ND | ND |
| **709-390** | 1153 | 643 | 0,6 | 4 | 35 | | 2 | 0 | | 0 | 0 | | 20 | ND | ND | ND |
| 710-0 | 204 | 44 | | 0 | 17 | | 0 | 0 | | 0 | 0 | | <5 | ND | ND | ND |
| 710-120 | 225 | 37 | | 0 | 0 | | 0 | 0 | | 0 | 0 | | <5 | ND | ND | ND |
| 710-390 | 2256 | 878 | 0,4 | 0 | 0 | | 0 | 0 | | 0 | 0 | | >20 | ND | ND | ND |

AKADEMISKA SJUKHUSET

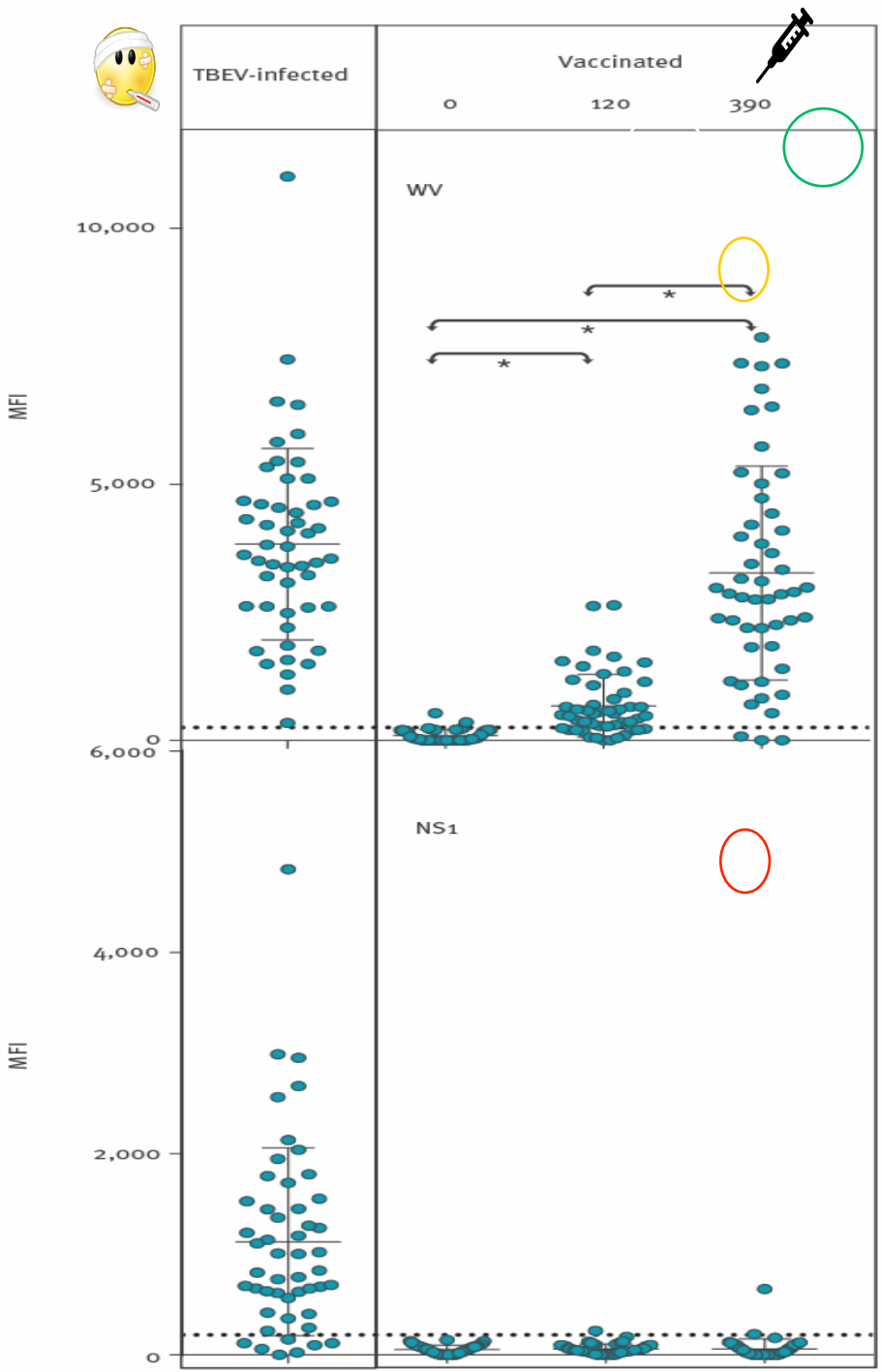



Fig. TBEV SMIA IgG reactivity whole virus vs NS1 antigens in infected (n = 50) vs vaccinated individuals (n = 50) at 0, 120 and 390 days after first vaccination.

Comment: 3 positive. 2 low close to cut-off. Infection during study period can not be ruled out.

UPPSALA UNIVERSITET

Zoonosis Science Center

AKADEMISKA SJUKHUSET

# Aim

- To study if a TBEV Suspension Multiplex Immunoassay (TBEV SMIA), based on non-structural protein 1 (NS1) and whole virus (WV) antigens, can be used for efficient detection of **TBE vaccination failure cases**.
- Serum and cerebrospinal fluid (CSF) samples from 14 patients previously analyzed at the Public Health Agency of Sweden were tested.
- All 14 patients had a documented history of previous TBEV vaccination and were **all considered to be TBE vaccine failure cases**.

UPPSALA UNIVERSITET

# Results

| Patient # | Age at disease (years) | Sex | Number of vaccine doses | Time from last vaccine dose to disease | No of sampling points | Neutralisation titer | IgM antibodies to NS1 antigen in sera or csf | IgM antibodies to NS1 antigen in sera alone | IgM and IgG antibodies to wv antigen in sera |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 63 | Male | 4 | 2 years | 2 | 10 -> 160 | Positive | Negative | Positive |
| 2 | 67 | Male | 3 | 2 years | 1 | 640 | Positive | Positive | Positive |
| 3 | 39 | Male | 2 | 3 months | 1 | 80 | Positive | Positive | Positive |
| 4 | 21 | Male | 2 | 5 months | 1 | 40-80 | Positive | Positive | Positive |
| 5 | 6 | Male | 3 | 1 year | 1 | > 160 | Positive | Positive | Positive |
| 6 | 67 | Female | 3 | Unknown | 2 | 640 -> 1280 | Positive | Positive | Positive |
| 7 | 55 | Female | 2 | 4 months | 1 | 160 | Positive | Positive | Positive |
| 8 | 52 | Female | 5 | 3 years | 1 | 5 | Negative | Negative | Positive |
| 9 | 68 | Male | 4 | 5 months | 1 | 80 | Positive | Positive | Positive |
| 10 | 64 | Male | 3 | 1 year | 2 | 20 -> 80 | Positive | Positive | Positive |
| 11 | 74 | Male | 4 | 3 years | 1 | 160 | Positive | Positive | Positive |
| 12 | 62 | Male | 4 | 3 years | 1 | n.d. | Negative | Negative | Positive |
| 13 | 72 | Male | 3 | 1 year | 1 | 320 | Positive | Positive | Positive |
| 14 | 76 | Male | 3 | 2 years | 1 | 80-160 | Negative | Negative | Positive |
| | Mean = 56 | M/F ratio: 80/20 | | | | | 11/14 | 10/14 | 14/14 |

AKADEMISKA SJUKHUSET

UPPSALA UNIVERSITET

Zoonosis Science Center

AKADEMISKA SJUKHUSET

# Conclusion

- The detection of antibodies directed to TBEV WV vs. NS1 antigen is a most useful tool to **differentiate previous TBEV infections from vaccinations**.
- The detection of antibodies directed to TBEV NS1 antigen considerably simplify and improve the quality in investigations regarding **suspected TBEV infection in vaccinated patients**.
- In a material from Slovenia, 9/9 VBT patients had NS1 antibodies.

UPPSALA UNIVERSITET

Zoonosis Science Center

AKADEMISKA SJUKHUSET

# Seroprevalence TBE (blood donors) TBEV SMIA whole virus/NS1 antigens

| Region | Antal serumprover |
|---|---|
| Gotland | 300 |
| Gävle | 300 |
| Göteborg | 300 |
| Karlstad | 300 |
| Lund | 300 |
| Stockholm | 300 |
| Umeå | 300 |
| Uppsala (inkl Hofors...) | 300 |
| Växjö | 300 |
| Totalt | 2700 |

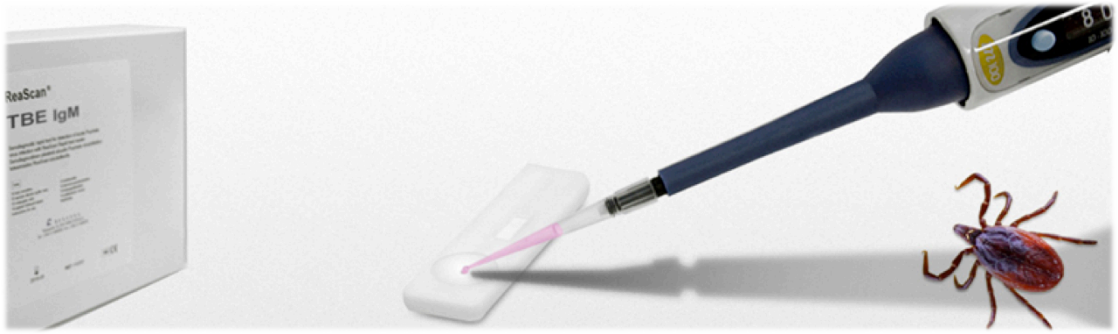
UPPSALA UNIVERSITET

Zoonosis Science Center

AKADEMISKA SJUKHUSET

# Reagena ReaScan® TBE IgM rapid test

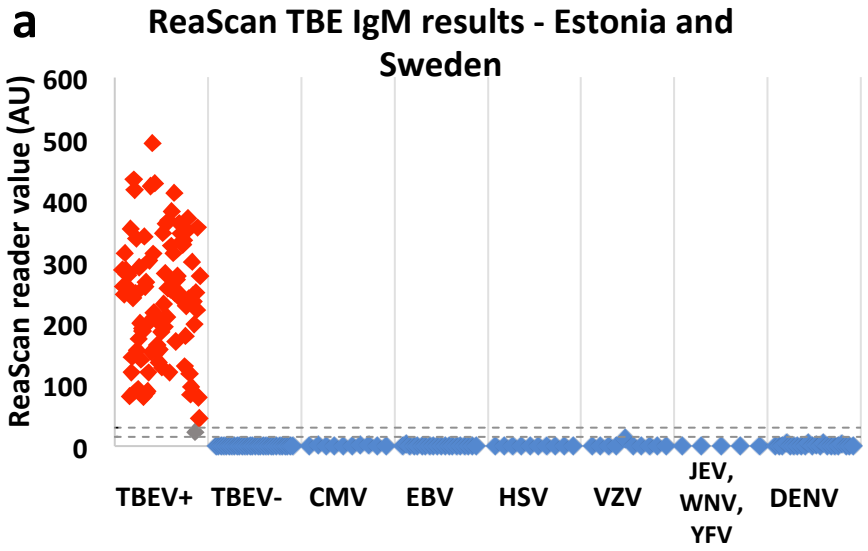
- The Reagena ReaScan® TBE IgM rapid test (ReaScan TBE) was evaluated for usage in a clinical laboratory setting in five diagnostic laboratories in Estonia, Finland, Slovenia, Sweden, and the Netherlands.

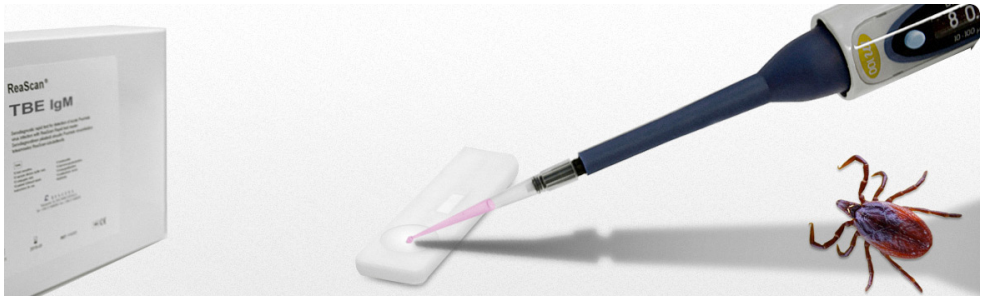


UPPSALA UNIVERSITET

Zoonosis Science Center

# Reagena ReaScan® TBE IgM rapid test




AKADEMISKA SJUKHUSET

# Distinction between serological responses following tick-borne encephalitis virus (TBEV) infection vs vaccination, Sweden 2017

**Bo Albinsson[1,2], Sirkka Vene[3,5], Lars Rombo[4,5], Jonas Blomberg[1,6], Åke Lundkvist[1], Bengt Rönnberg[1,2]**
1. Department of Medical Biochemistry and Microbiology, Zoonosis Science Center, Uppsala University, Uppsala, Sweden
2. Laboratory of Clinical Microbiology, Uppsala University Hospital, Uppsala, Sweden
3. The Public Health Agency of Sweden, Solna, Stockholm, Sweden
4. Department of Infectious diseases, Eskilstuna, Sweden
5. Clinical Research Centre, Sormland county council, Uppsala University, Uppsala, Sweden
6. Department of Medical Sciences, Uppsala University, Uppsala, Sweden

**Correspondence: Åke Lundkvist (ake.lundkvist@imbim.uu.se)**

Citation style for this article:
Albinsson Bo, Vene Sirkka, Rombo Lars, Blomberg Jonas, Lundkvist Åke, Rönnberg Bengt. Distinction between serological responses following tick-borne encephalitis virus (TBEV) infection vs vaccination, Sweden 2017. Euro Surveill. 2018;23(3):pii=17-00838. https://doi.org/10.2807/1560-7917.ES.2018.23.3.17-00838

Article submitted on 18 Dec 2017 / accepted on 18 Jan 2018 / published on 18 Jan 2018

**Tick-borne encephalitis virus (TBEV) is an important European vaccine-preventable pathogen. Discrimination of vaccine-induced antibodies from those elicited by infection is important. We studied anti-TBEV IgM/IgG responses, including avidity and neutralisation, by multiplex serology in 50 TBEV patients and 50 TBEV vaccinees. Infection induced antibodies reactive to both whole virus (WV) and non-structural protein 1 (NS1) in 48 clinical cases, whereas 47 TBEV vaccinees had WV, but not NS1 antibodies, enabling efficient discrimination of infection/vaccination.**

Sweden reported record-high numbers of tick-borne encephalitis (TBE), 391 cases, during 2017. TBE diagnosis is mainly performed by serology. Improved serology should distinguish TBE virus (TBEV) antibodies induced by infection from those induced by vaccination; it should control for cross-reactions and detect suboptimal vaccinations. The major surrogate indicator of protection, neutralising antibodies measured by neutralisation test (NT), requires a biosafety level 3 facility and is time-consuming. We aimed to address all these issues. In this study, we made use of the fact that TBEV NS1 antigen is not present in existing vaccine preparations; thus vaccinees are not expected to develop a serological response against NS1, so that anti-TBE antibodies induced by infection can be distinguished from those induced by vaccination.

## Proof of concept study of immune responses after infection or vaccination

### Serum samples

We analysed 50 serum samples drawn between 2011 and 2014 from patients in the region of Uppsala Akademiska hospital, Sweden, with clinical suspicion of acute TBEV infection. All had a serological profile consistent with current or recent TBEV infection, i.e. high levels of TBEV-reactive IgM and low or borderline levels of TBEV-reactive IgG in a commercial assay (Siemens Healthcare Diagnostics AG, Marburg, Germany), and were confirmed as TBEV IgM-positive by another commercial test (Reagena OY, Toivala, Finland) (data not shown).

We also analysed 150 serum samples from 50 healthy individuals who were vaccinated in Eskilstuna, Sweden, between 2012 and 2013 with TBEV vaccine (FSME-Immun, Pfizer, New York, United States (US)). Three serum samples per vaccinee were drawn: on day 0, the day of the first dose (n = 50), on day 120 after the first vaccination dose, i.e. a minimum of 30 days after at least two doses (n = 50), and on day 390 after the first vaccine dose, 30 days after at least three doses (n = 50). For all time points after the first dose, a difference of +/− 2 days was accepted.

### Suspension multiplex immunoassay reactivity in acute-phase TBE patients vs TBE vaccinees

TBEV whole virus (WV) antigen was purchased from Jena Bioscience, Jena, Germany (Cat. No. PR-BA112) and TBEV non-structural protein 1 (NS1) antigen from Native Antigen Company, Heyford Park, United Kingdom (Cat. No. TBEV-NS1–100). The TBEV-specific suspension multiplex immunoassay (SMIA) was performed as earlier described for the more comprehensive Flavivirus suspension multiplex immunoassay (FSMIA) [1]. Briefly, each antigen was coupled to carboxylated differentially colour-marked magnetic microspheres using carbodiimide. For IgG determination, serum diluted 1:50 was added to 96-well microtitre plates. Vortexed and



INFECTION ECOLOGY & EPIDEMIOLOGY
2019, VOL. 9, 1696132
https://doi.org/10.1080/20008686.2019.1696132

Taylor & Francis
Taylor & Francis Group

ARTICLE

OPEN ACCESS Check for updates

# Antibody responses to tick-borne encephalitis virus non-structural protein 1 and whole virus antigen–a new tool in the assessment of suspected vaccine failure patients

Bo Albinsson [A,B], Bengt Rönnberg [A,B], Sirkka Vene[A,C] and Åke Lundkvist [A]

[A]Department of Medical Biochemistry and Microbiology, Zoonosis Science Centre, Uppsala University, Uppsala, Sweden; [B]Laboratory of Clinical Microbiology, Uppsala University Hospital, Uppsala, Sweden; [C]Department of Microbiology, The Public Health Agency of Sweden, Solna, Sweden

**ABSTRACT**

We report a new tool for improved serological diagnostics in suspected tick-borne encephalitis (TBE) vaccine failure cases. Due to an increase in the incidence of disease as well as the number of vaccinees, specific and simplified diagnostic methods are needed. Antibody responses to TBE-virus (TBEV) non-structural protein 1 (NS1) are detectable post TBEV infection but not post vaccination. We have used samples from 14 previously confirmed Swedish TBEV vaccine failure patients to study antibody responses against NS1 and whole virus antigens, respectively. Our conclusion is that the detection of antibodies directed to TBEV NS1 antigen is a useful tool to considerably simplify and improve the quality in investigations regarding suspected TBEV infection in vaccinated patients.

**ARTICLE HISTORY**
Received 3 July 2019
Accepted 14 October 2019

**KEYWORDS**
Tick-borne encephalitis (TBE); vaccine failure; vaccine breakthrough; vaccine-preventable diseases; vaccines and immunization; antibody detection; serology; diagnostics

## Introduction

Long-term neurological sequelae and case fatalities can occur in tick-borne encephalitis (TBE) patients [1,2], but also mild cases are probably common and often remain undiagnosed. The disease typically follows a biphasic pattern with flu-like symptoms in the first phase and a second phase with symptoms ranging from meningitis to encephalitis. Transmission to humans occurs almost exclusively from tick bites, although viral transmission via milk products has been shown.

In the second phase of the disease, when neurological symptoms are present, laboratory diagnosis is highly dependent on the detection of TBE-virus (TBEV)-specific IgM and IgG in blood and/or cerebrospinal fluid (CSF) [3]. Viral RNA can normally be found in patient samples only during the early first phase of the disease. Immune-compromised patients with delayed antibody responses may have a prolonged viremic phase that, in rare cases, enables TBEV-RNA detection.

TBE is an important and growing public health problem in Europe; France reported a marked increase in TBE cases in 2016, and in Finland, the number of TBE cases has not only more than doubled during the last decade, but the virus has also spread to new geographical areas. The Netherlands, previously TBE-free, most recently reported its emergence. In Sweden, the number of notified cases is increasing and reached a record-high in 2017 (391 cases), with almost the same level in 2018 (385 cases) (Figure 1) [4]. Effective vaccines are available, but vaccine failures occur [5,6]. The number of sold vaccine doses has also increased during the same period and reached 1.2 million doses in 2018, which is double the amount sold the years before (Figure 1). The commercial or in-house serological tests that are commonly used are not designed to separate antibody responses induced by infection from those induced by vaccination, and interpretation of serological patterns is most challenging. This is even more the case if the patient has been vaccinated in close proximity to the onset of suspected TBE illness during the TBE season. As TBE vaccination is becoming more common, this diagnostic problem will increase even further. A diagnostic tool that can distinguish antibody responses induced by TBEV infection from those induced by vaccination is thus highly desirable.

Our recently published method for detection of non-structural protein 1 (NS1) and whole virus (WV) antibodies to TBEV using TBEV suspension multiplex immunoassay (SMIA) was proven to efficiently differentiate between antibodies induced by infection and vaccination. All but two (48/50) samples from TBEV-infected patients had antibodies to NS-1 antigen as compared to only three serum samples from the vaccinated group (3/50). In one

**CONTACT** Åke Lundkvist akelundkvist@imbim.uu.se Department of Medical Biochemistry and Microbiology, Zoonosis Science Centre, Uppsala University, Uppsala, Sweden

# Multi-laboratory evaluation of ReaScan TBE IgM rapid test, 2016 to 2017

Bo Albinsson[1,2], Anu E. Jääskeläinen[3,4], Kairi Värv[4,5], Mateja Jelovšek[6], Corine GeurtsvanKessel[6], Sirkka Vene[1], Josef D. Järhult[7], Chantal Reusken[6,10], Irina Goloviljova[4,5], Tatjana Avšič-Županc[8], Olli Vapalahti[3,4,9], Åke Lundkvist[1]

1. Department of Medical Biochemistry and Microbiology, Zoonosis Science Centre, Uppsala University, Uppsala, Sweden
2. Laboratory of Clinical Microbiology, Uppsala, Sweden
3. Department of Virology, University of Helsinki, Helsinki, Finland
4. Helsinki University Hospital Laboratory Services (HUSLAB), Department of Virology and Immunology, Helsinki, Finland
5. Department of Virology and Immunology, National Institute for Health Development, Tallinn, Estonia
6. WHO Collaborating Centre for Arbovirus and Viral Haemorrhagic Fever Reference and Research, Department of Virology, Erasmus University Medical Centre, Rotterdam, the Netherlands
7. Department of Medical Sciences, Zoonosis Science Centre, Uppsala University, Uppsala, Sweden
8. Institute for Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
9. Department of Veterinary Biosciences, University of Helsinki, Helsinki, Finland
10. Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, the Netherlands

**Correspondence: Åke Lundkvist (ake.lundkvist@imbim.uu.se)**

**Citation style for this article:**
Albinsson Bo , Jääskeläinen Anu E. , Värv Kairi , Jelovšek Mateja , GeurtsvanKessel Corine , Vene Sirkka , Järhult Josef D. , Reusken Chantal , Goloviljova Irina , Avšič-Županc Tatjana , Vapalahti Olli , Lundkvist Åke . Multi-laboratory evaluation of ReaScan TBE IgM rapid test, 2016 to 2017. Euro Surveill. 2020;25(12):pii=1900427. https://doi.org/10.2807/1560-7917.ES.2020.25.12.1900427

Article submitted on 28 Jun 2019 / accepted on 14 Feb 2020 / published on 26 Mar 2020

**Background:** Tick-borne encephalitis (TBE) is a potentially severe neurological disease caused by TBE virus (TBEV). In Europe and Asia, TBEV infection has become a growing public health concern and requires fast and specific detection. **Aim:** In this observational study, we evaluated a rapid TBE IgM test, ReaScan TBE, for usage in a clinical laboratory setting. **Methods:** Patient sera found negative or positive for TBEV by serological and/or molecular methods in diagnostic laboratories of five European countries endemic for TBEV (Estonia, Finland, Slovenia, the Netherlands and Sweden) were used to assess the sensitivity and specificity of the test. The patients' diagnoses were based on other commercial or quality assured in-house assays, i.e. each laboratory's conventional routine methods. For specificity analysis, serum samples from patients with infections known to cause problems in serology were employed. These samples tested positive for e.g. Epstein–Barr virus, cytomegalovirus and *Anaplasma phagocytophilum*, or for flaviviruses other than TBEV, i.e. dengue, Japanese encephalitis, West Nile and Zika viruses. Samples from individuals vaccinated against flaviviruses other than TBEV were also included. Altogether, 172 serum samples from patients with acute TBE and 306 TBE IgM negative samples were analysed. **Results:** Compared with each laboratory's conventional methods, the tested assay had similar sensitivity and specificity (99.4% and 97.7%, respectively). Samples containing potentially interfering antibodies did not cause specificity problems. **Conclusion:** Regarding diagnosis of acute TBEV infections, ReaScan TBE offers rapid and convenient complementary IgM detection. If used as a stand-alone, it can provide preliminary results in a laboratory or point of care setting.

## Introduction

Tick-borne encephalitis virus (TBEV) is the most important tick-transmitted virus causing human disease in Europe and Asia [1–4]. TBEV belongs to the genus *Flavivirus*, within the *Flaviviridae* family, and can be divided into three distinct subtypes: the European (TBEV-Eur, formerly known as Central European encephalitis virus), the Siberian (TBEV-Sib, formerly known as Siberian encephalitis virus), and the Far Eastern (TBEV-FE, formerly known as Russian Spring Summer encephalitis virus) subtypes [3]. Recently, two new subtypes of TBEV (Himalayan and Baikalian) have been characterised [5,6]. Several studies suggest that the case fatality rate for TBE caused by TBEV-Eur is 0–4% [1,7] by TBEV-Sib 2-3% [1,7] and by TBEV-FE 6–40% [1,3,7,8]. However, according to Ruzek et al., 2019 [9] the overall TBE mortality rate in Russia is approximately 2% (i.e. TBEV-Sib and TBEV-FE infections). Thus, data on fatality rates of TBEV are not comprehensive since, aside from the infecting subtype, other factors (such as healthcare system efficiency, population genetics or living conditions) may come into play. TBEV is maintained in ticks and in their wild vertebrate hosts in forested natural foci [10]. The main reservoir hosts are found among small mammals (e.g. rodents, insectivores), while larger animals (e.g. deer), despite being important feeding hosts for ticks, do not seem to play any considerable role in the maintenance of the virus within its foci [2,11].



And then came Covid-19…

UPPSALA UNIVERSITET

# Acknowledgements


- Bo Albinsson
- Bengt Rönnberg
- Sirkka Vene

- Thank you!


AKADEMISKA SJUKHUSET