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The activities of the TBEV National Reference Center

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Rabies and TBEV National Reference Centres

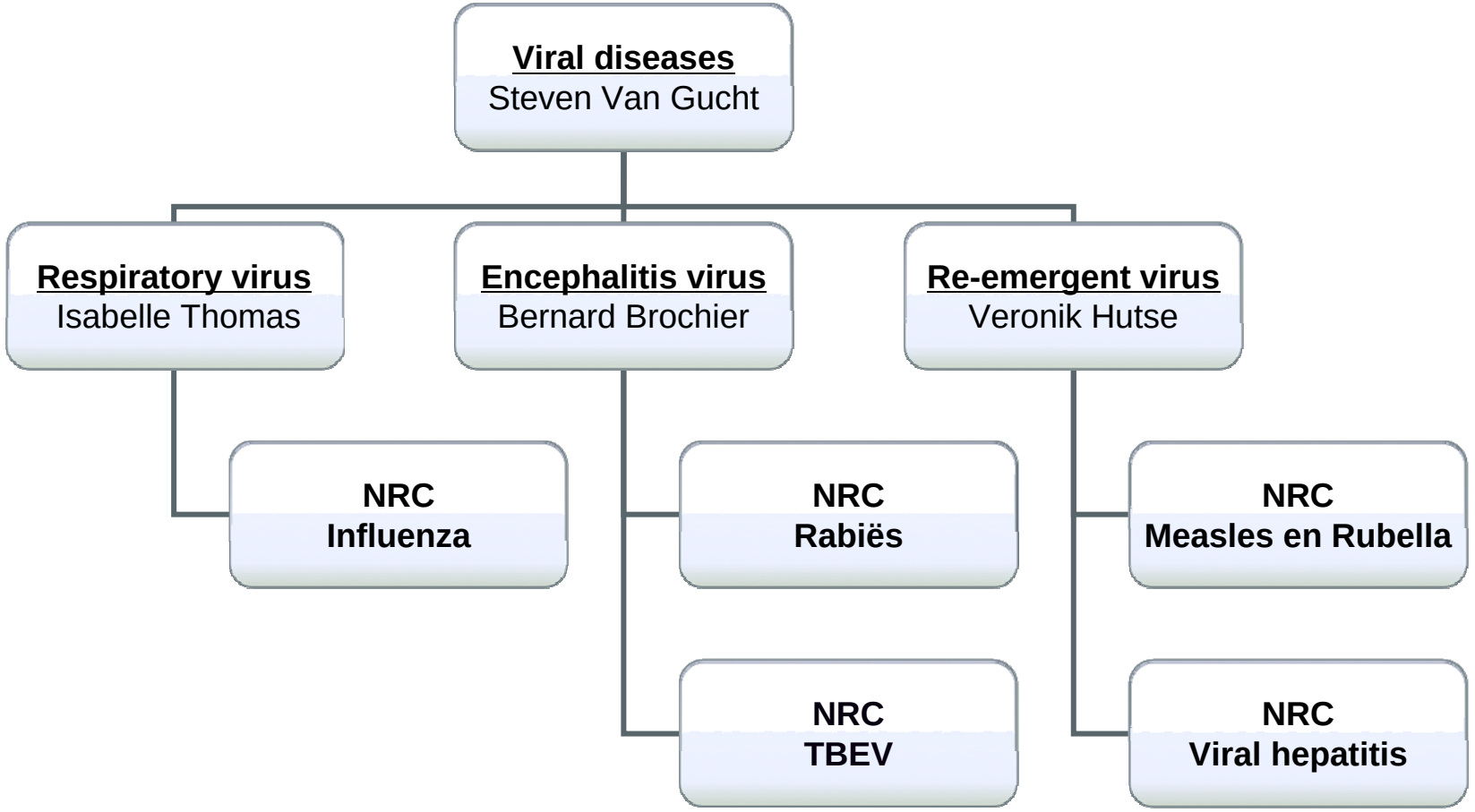
.be

NRC : TBEV National Reference Centre

- ✓ TBEV diagnostic methods used in the laboratory (IgM and IgG Elisa, seroneutralisation test and qRT-PCR)
- ✓ NRC activities : Human diagnosis and surveillance
- ✓ NRC activities : Seroprevalence in sentinel animal species
- ✓ Perspectives

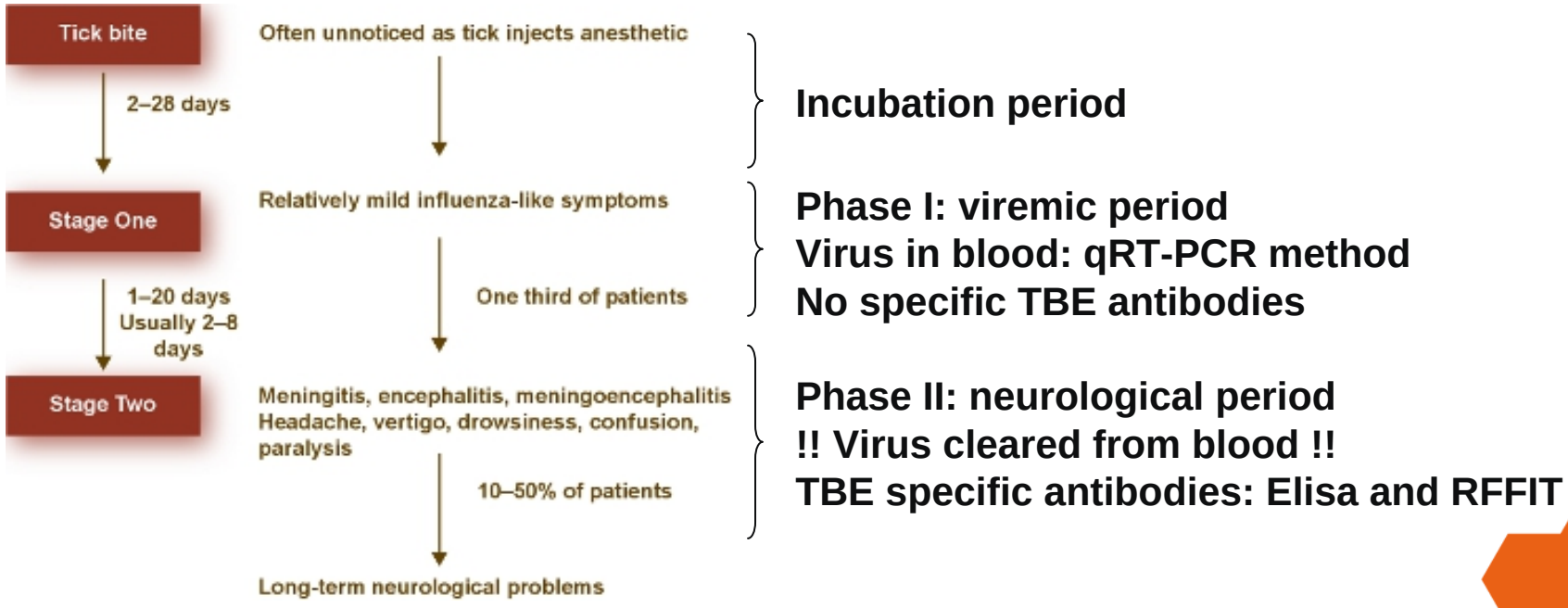
NRC : TBEV National Reference Center

WIV-ISP



TBEV : Diagnosis

- Usually used : Elisa test to detect specific IgM and IgG antibodies in serum and/or cerebrospinal fluid (CSF)
- Confirmation test by seroneutralisation test
- TBEV qPCR to detect viral RNA



NRC : TBEV diagnostic methods

Elisa test : Detection of IgM (human) and IgG (all species) anti-TBEV
- Commercial test (Progen)
- Samples: serum and CSF

Use: screening ⟶ Used for all human and animal samples

Interpretation:

IgM = recent infection
IgG = older infection
or vaccination

VIEU^{i} / ml	anti-TBE-IgG antibodies
< 63	negative
63 - 126	borderline
> 126	positive

Confirmed by a seroneutralisation test

NRC : TBEV diagnostic methods

Elisa test specificity : Cross reactions with other Flavivirus

	ELISA IgM		ELISA IgG	
	VIEU/ml	Interpretation	VIEU/ml	Interpretation
IgM Dengue positive	<40	Negative	45	Negative
IgM Dengue positive	<40	Negative	55	Negative
IgM Dengue positive	54	Negative	<40	Negative
IgG Dengue positive	<40	Negative	**> 600**	**Positive**
IgG Dengue positive	<40	Negative	**290**	**Positive**
IgG Dengue positive	42	Negative	**390**	**Positive**
IgG Yellow fever positive	<40	Negative	**75**	**Borderline**
IgG Yellow fever positive	<40	Negative	<40	Negative
IgG West Nile positive	<40	Negative	**155**	**Positive**
IgG West Nile positive	57	Negative	**130**	**Positive**
IgG West Nile positive	45	Negative	**105**	**Borderline**
IgG West Nile positive	<40	Negative	**115**	**Borderline**

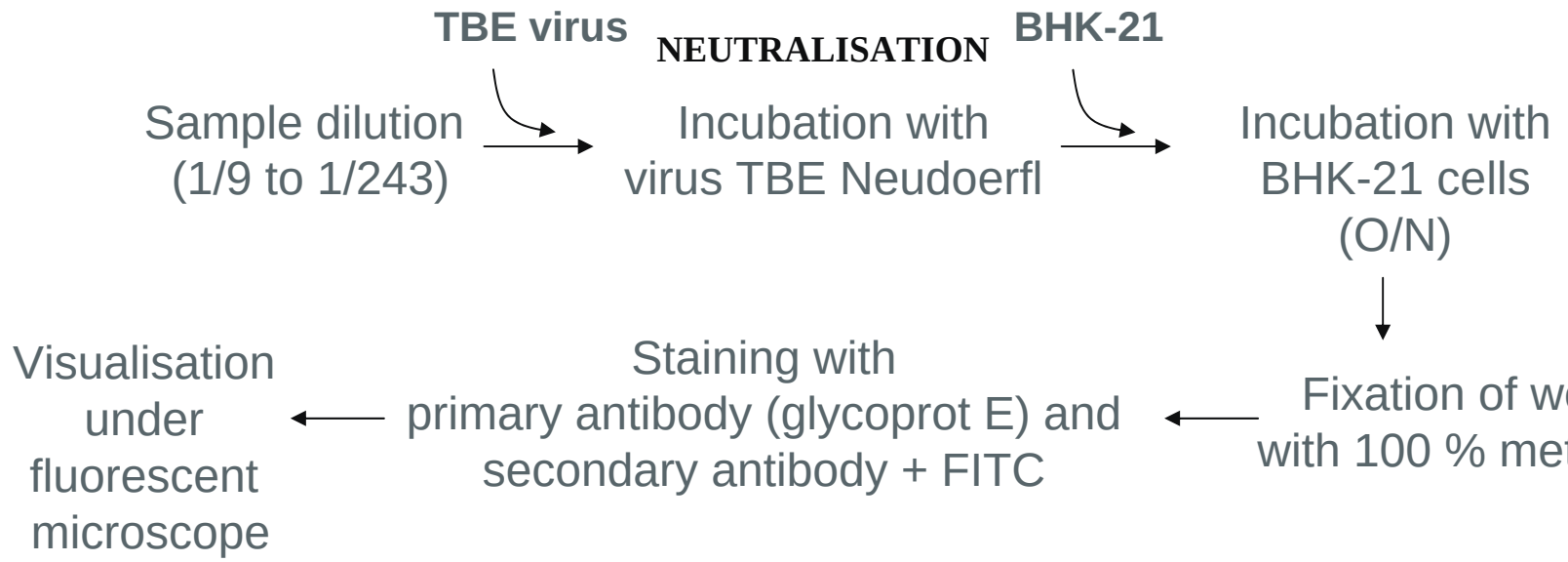
NRC : TBEV diagnostic methods

Seroneutralisation test or RFFIT

Rapid Focus Fluorescent Inhibition Test

- « In house » developed test (well known technique in Rabies NRC)
- Samples : Serum and CSF

Principle: !! This method allows distinction between different flaviviruses !!



NRC : TBEV diagnostic methods

RFFIT : Rapid Focus Fluorescent Inhibition Test (serum and CSF)

Use: Confirmation of all positive and borderline Elisa results

Interpretation:

13 microscopic fields are examined and the number of fluorescent positive fields are counted

DIL_{50} = the dilution at which 50% of the virus is neutralized

DIL_{50}	**Interpretation**
< 10	Negative
>10	Positive

NRC : TBEV diagnostic methods

qRT-PCR : Reverse transcription real-time PCR

- TBEV specific detection : Schwaiger *et al*, 2003
- Samples : serum, CSF and brain

Principle:

RNA extraction → Reverse transcription → Real-time PCR (Taqman probe)

Interpretation:

Sample with ct < 38 : positive
Sample with ct > 38 : negative
Sample not detected : negative

NRC activities : Human TBEV diagnosis

Network with belgian hospitals

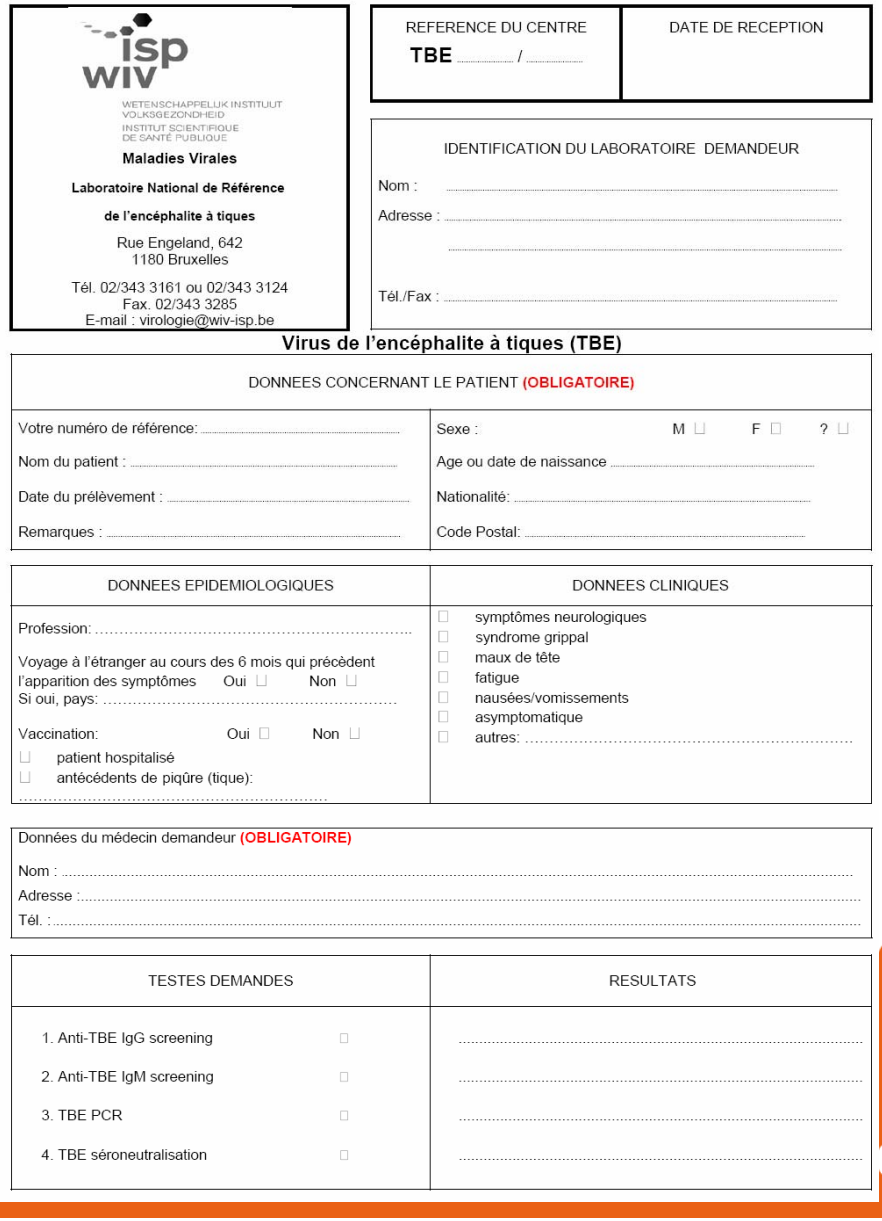
Analysis request form

Patient data →

Epidemiology and clinical data →

Doctor data →

Test requested and results →



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1180 Bruxelles
Tél. 02/343 3161 ou 02/343 3124
Fax. 02/343 3285
E-mail : virologie@wiv-isp.be

REFERENCE DU CENTRE	DATE DE RECEPTION
TBE ..... /	

IDENTIFICATION DU LABORATOIRE DEMANDEUR

Nom : .....
Adresse : .....
.....
Tél./Fax : .....

Virus de l'encéphalite à tiques (TBE)

DONNEES CONCERNANT LE PATIENT **(OBLIGATOIRE)**

Votre numéro de référence:	Sexe : M ☐ F ☐ ? ☐
Nom du patient :	Age ou date de naissance .....
Date du prélèvement :	Nationalité: .....
Remarques :	Code Postal: .....

DONNEES EPIDEMIOLOGIQUES	DONNEES CLINIQUES
Profession:	☐ symptômes neurologiques
Voyage à l'étranger au cours des 6 mois qui précèdent l'apparition des symptômes Oui ☐ Non ☐	☐ syndrome grippal
Si oui, pays:	☐ maux de tête
Vaccination: Oui ☐ Non ☐	☐ fatigue
☐ patient hospitalisé	☐ nausées/vomissements
☐ antécédents de piqûre (tique):	☐ asymptomatique
	☐ autres: .....

Données du médecin demandeur **(OBLIGATOIRE)**

Nom : .....
Adresse : .....
Tél. : .....

TESTES DEMANDES	RESULTATS
1. Anti-TBE IgG screening ☐	.....
2. Anti-TBE IgM screening ☐	.....
3. TBE PCR ☐	.....
4. TBE séroneutralisation ☐	.....

NRC activities: Human TBEV diagnosis

I. Samples received from belgian hospitals

- Control of vaccination, tick bite, patients with neurological symptoms and no confirmed diagnosis
- 2011 : 9 samples
  2012 : 72 samples → IgM and IgG Elisa
  RFFIT

1 positive imported case from Norway

Results: IgG positive (150 VIEU/ml)
IgM negative
RFFIT positive (DIL_{50} = 31)

Sequelae: Neurological disorders
Sight disorders
Visual migraines

Old infection

1 positive imported case from Austria

Results: IgG positive (> 600 VIEU/ml)
IgM positive (448 VIEU/ml)
RFFIT positive (DIL_{50} > 243)

Symptoms: 1°) Flu-like syndrome
2°) headaches (+++), myalgia

Recent infection

NRC activities: Human TBEV diagnosis

II. Flavivirus project

Aim: Development of a differential diagnosis plateform for undiagnosed encephalitis

Generic pan-**lyssavirus** qRT-PCR → 7 species of lyssavirus (Rabies)

Generic pan-**flavivirus** qRT-PCR → TBEV, WNV, YF, Dengue, Japanese encephalitis virus

2012 : 21 human samples (serum or CSF) → All negative for both genus

NRC activities: Seroprevalence in animals

→ Sentinel animal species
Ruminants (sheep - goat - cattle)
Wildlife (roe deer, wildboar,…)
Domestic animals (dog,…)

Up to now in Belgium:

- Screening of the canine population by the Coda-Cerva (Roelandt et al, 2011). Detection of one confirmed TBEV positive dog (n = 880). This dog came from West Flanders and travelled in German Mosel region (2002) and Alsace (2003)

- Screening of roe deers in Southern Belgium (Linden et al, 2012). Two confirmed TBEV positive roe deers (n = 498). We do not know the exact region where these roe deers were collected.

NRC activities: Seroprevalence in sentinel animals

In progress:

- **Cattle** from Wallonia (n = 608)

  → Collaboration with the Coda-Cerva
  Results: 12/450 (2,67%) positive in IgG - RFFIT

- **Roe deers** from Flanders (n = 78)

  → Collaboration with the Coda-Cerva
  Results: All roe deers were negative

- **Wildboars** from Flanders (n = 76)

  → Collaboration with the Agentschap voor Natuur en Bos (ANB)
  Results: 2 positive in IgG Elisa (RFFIT to do)

NRC : Perspectives

Surveillance and / or seroprevalence studies:

- in **forestry workers**
  Collaboration with the Agentschap voor Natuur en Bos (ANB)

- in **rodents** (TBEV reservoir: wood mouse, common vole, …)
  Collaboration with ? for TBE virus isolation

- in **domestic animal species** (ruminants)
  Collaboration with the Coda-Cerva

- In **wildlife** (roe deers, wild boars,…)
  Collaboration with the Coda-Cerva (for Flanders)
  Collaboration with the Réseau de Surveillance Sanitaire de la Faune Sauvage, Ulg (for Wallonia)?
  Collaboration with the Agentschap voor Natuur en Bos (ANB) ?

Acknowledgments

The scientific team of the division of Viral Diseases

Thank you for your attention !!

Questions ?