EUROPE BIOBANK WEEK

SEPTEMBER 13-16, 2016
VIENNA - AUSTRIA

Marie-Noelle Ungeheuer: "WHO Human African Trypanosomiasis (HAT) specimen bank : a valuable tool for HAT diagnosis and research"

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European, Middle Eastern & African Society for Biopreservation & Biobanking

WHO Human African Trypanosomiasis (HAT) specimen bank : a valuable tool for HAT diagnosis and research

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2016 European Biobank Week – Wien, Österreich

FOR RESEARCH, FOR HEALTH,
FOR OUR FUTURE

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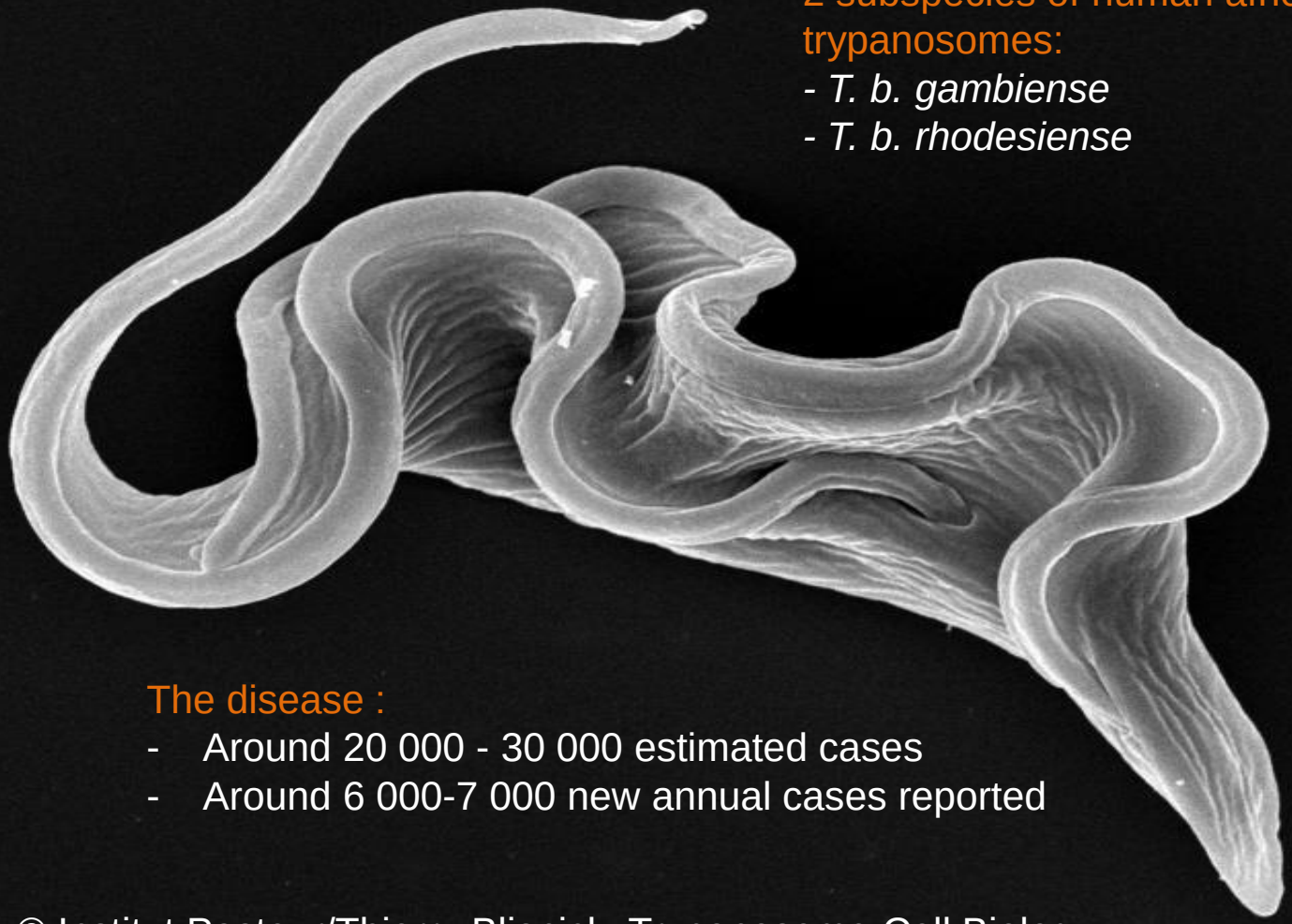
Summary

PART 1

Introduction

1.1 The parasite and the disease
1.2 HAT specimen bank
1.3 Status of the participating Centers
1.4. Diagnostic methods used for the biobank

1.1 Parasite and Disease



2 subspecies of human african trypanosomes:

- *T. b. gambiense*
- *T. b. rhodesiense*

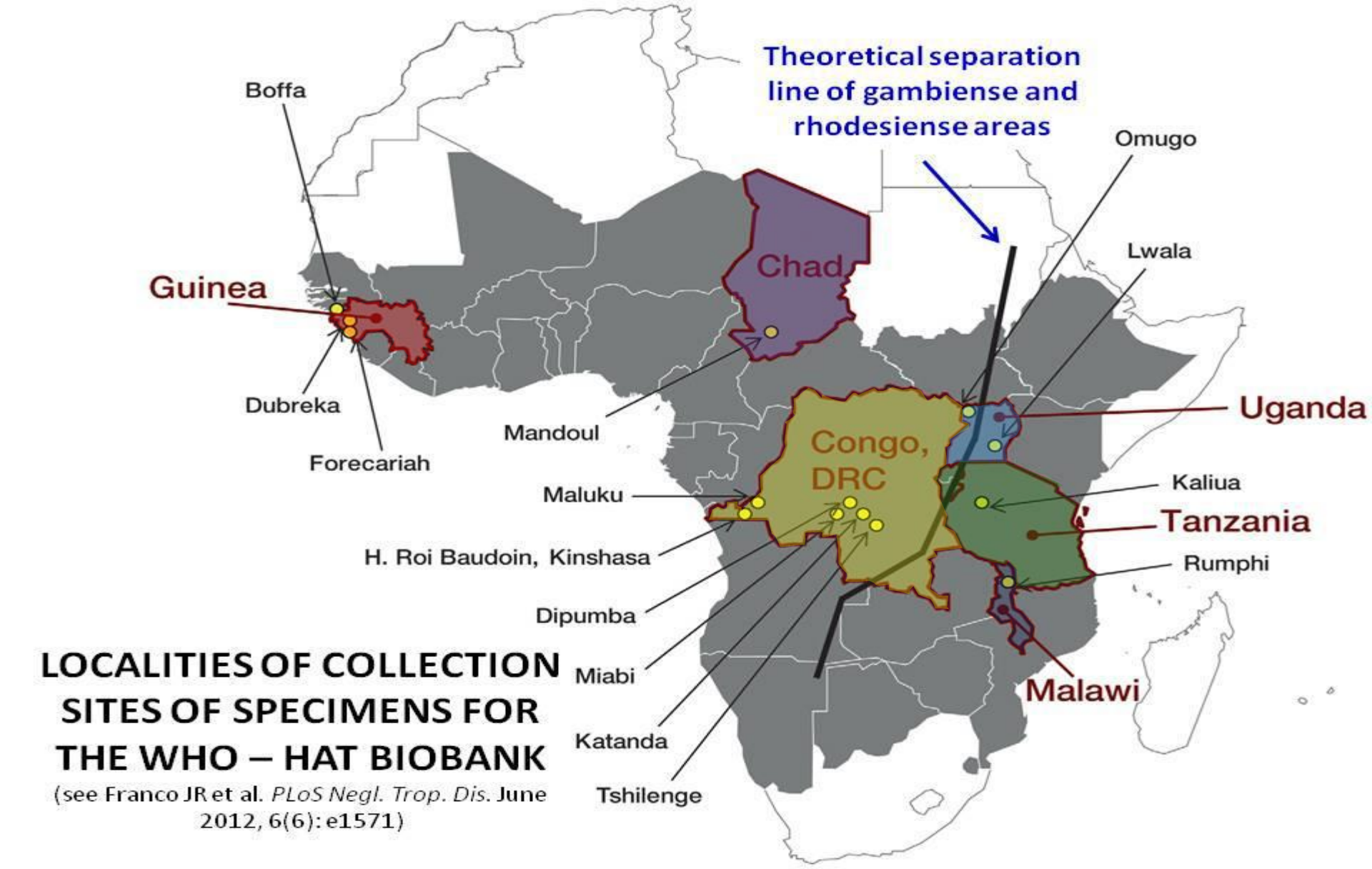
The disease :

- Around 20 000 - 30 000 estimated cases
- Around 6 000-7 000 new annual cases reported

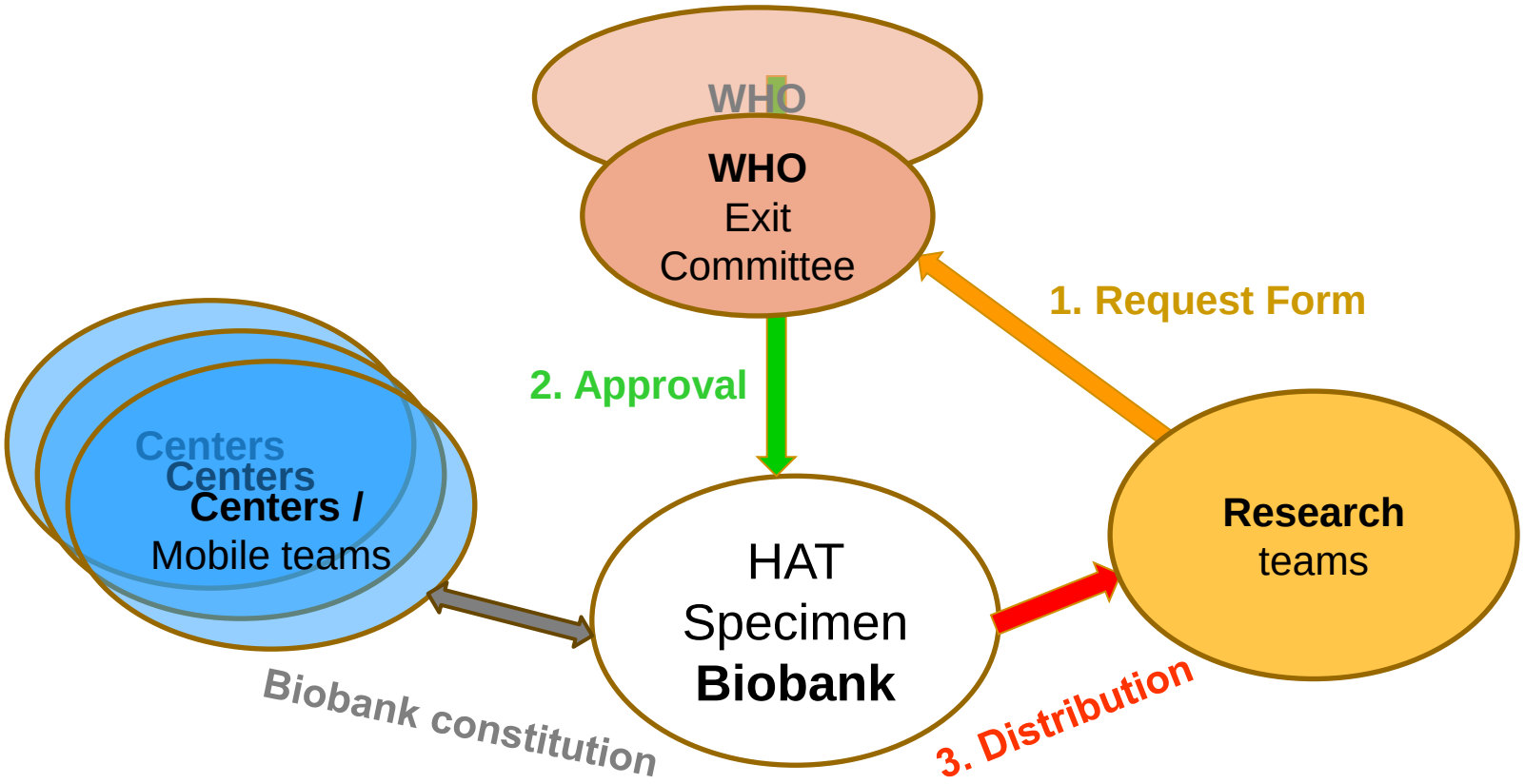
1.2 The WHO HAT specimen bank : WHO initiative

- Historical WHO-CNTD initiative in 2006
- WHO established a Human African trypanosomiasis (HAT) biobank in 2008 with financial support of FIND and SANOFI
- Cooperative framework between the WHO, the african centres and the ICAReB platform of Institut Pasteur
- **Goals : field-friendly, cost-effective, early stage and species-specific diagnostic tests**

1. 2 The WHO HAT Specimen Bank : Centers



1.2 The WHO HAT Specimen Bank : functioning and governance



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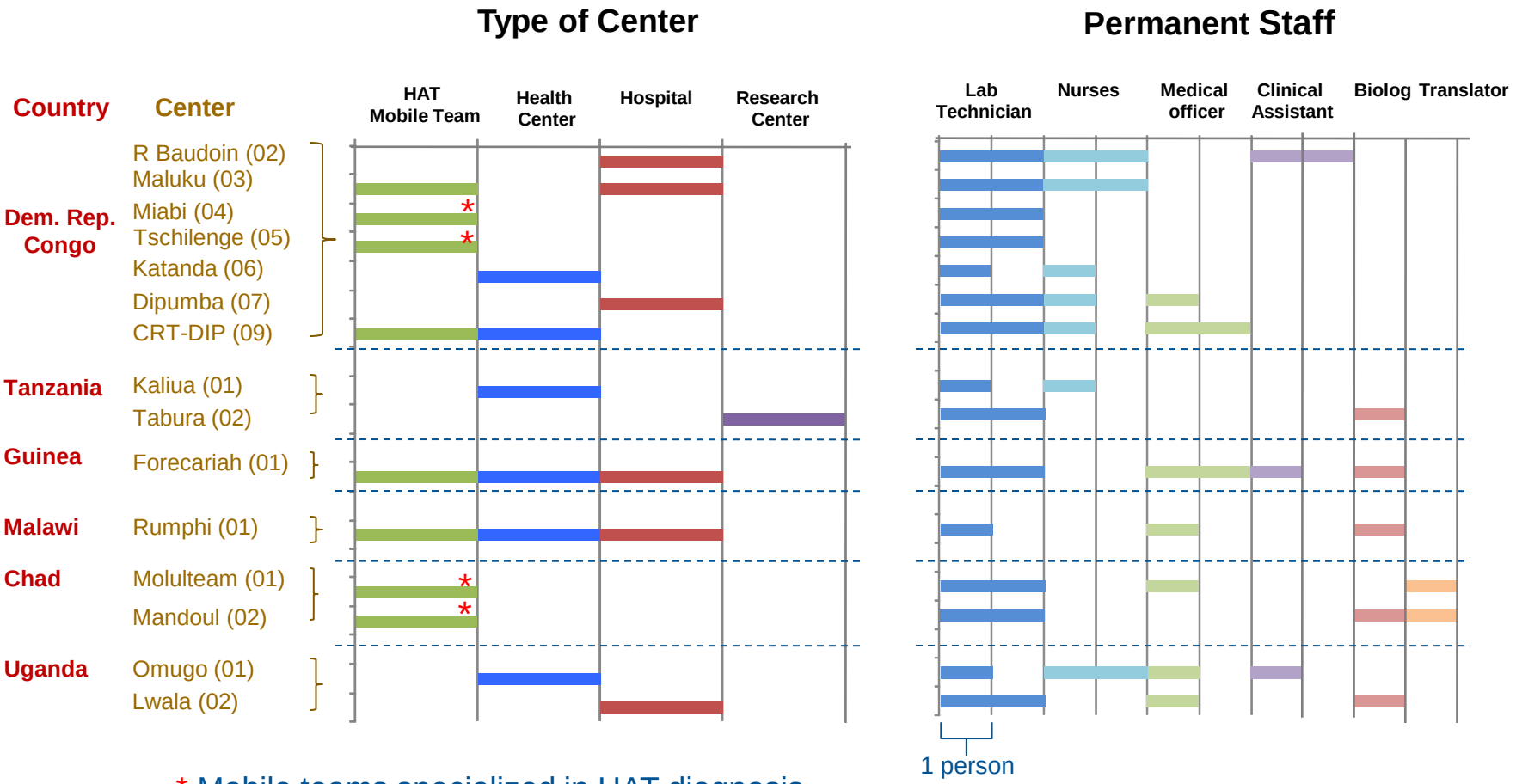
1.3 Status of the Centers



* Mobile teams specialized in HAT diagnosis

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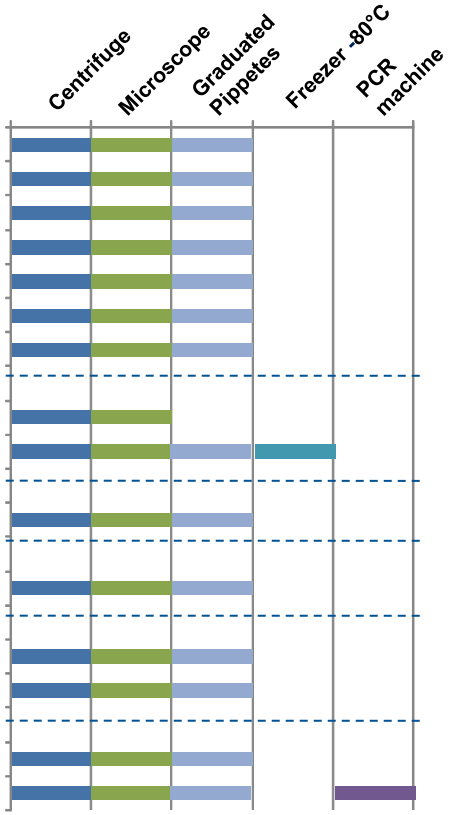
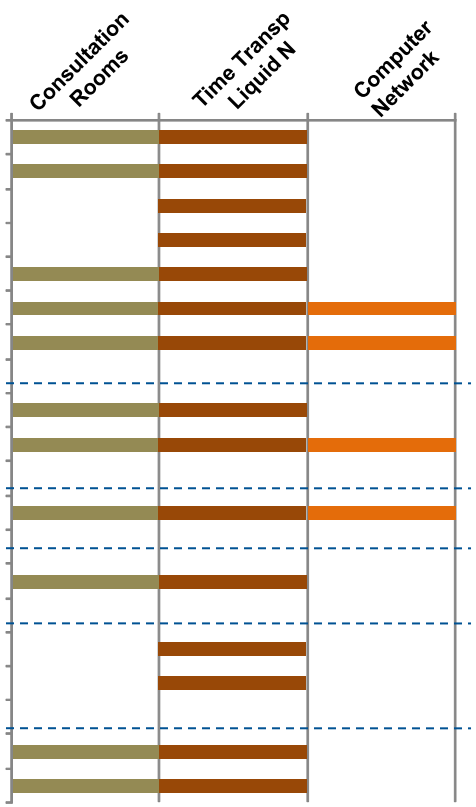
1.3 Status of the Centers

Country	Center
Rep. Dem. Congo	R Baudoin (02)
	Maluku (03)
	Miabi (04)
	Tschilenge (05)
	Katanda (06)
	Dipumba (07)
	CRT-DIP (09)
Tanzania	Kaliua (01)
	Tabora (02)
Guinea	Forecariah (01)
Malawi	Rumphi (01)
Chad	Mandoul 1 (02)
	Mandoul 2 (02)
Uganda	Omugo (01)
	Lwala

Available Equipment



Logistic Conditions



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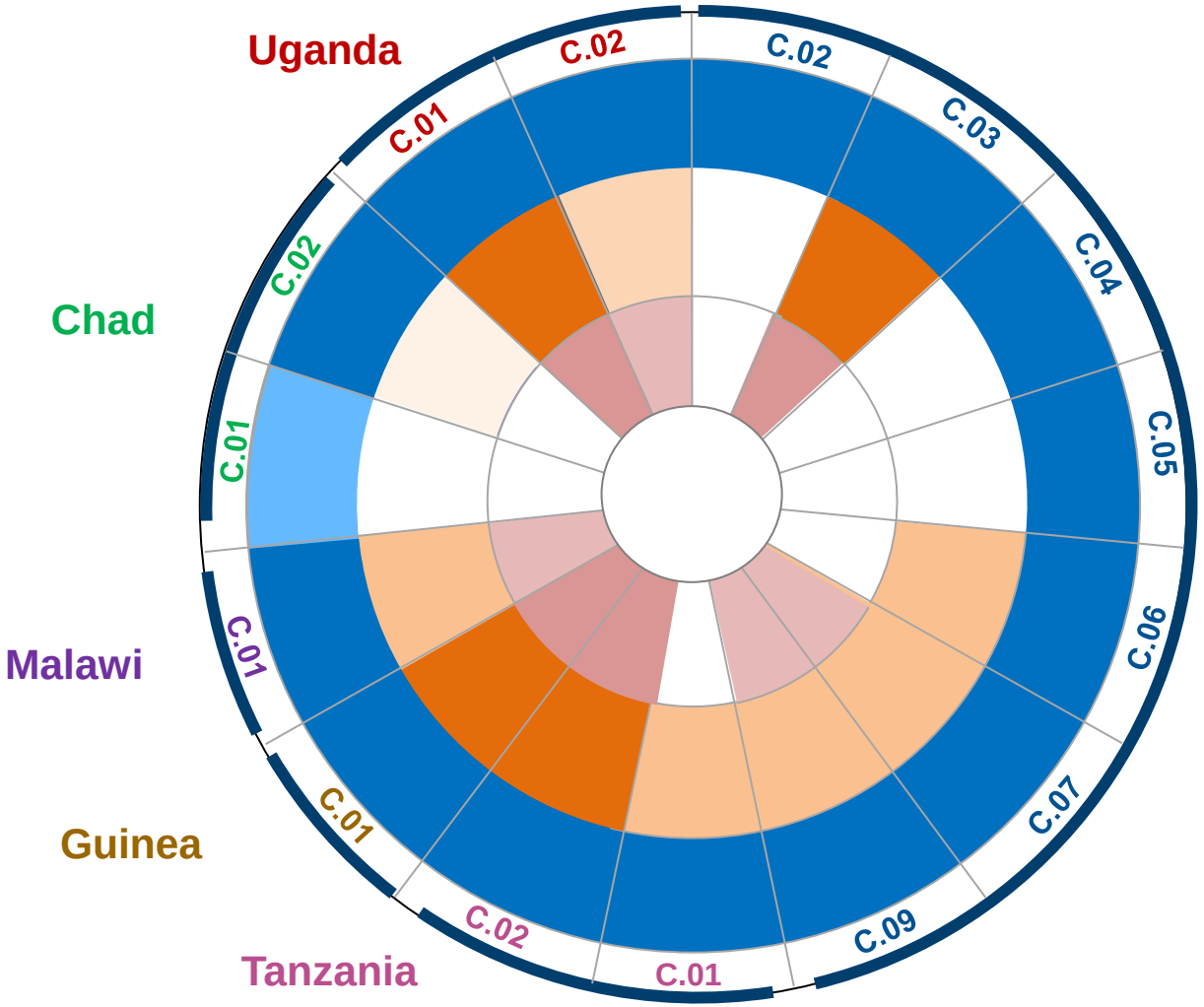
1.3 Status of the Centers

WHO Mobile medical team
http://www.who.int/trypanosomiasis_african/en/

1.3 Status of the Centers

Participation in Clinical Studies / Programs



HAT Programme

- >10 years
- >5 years

International Programme(s)

- >10 years
- >5 years
- 5 years
- 1 year

National Programme(s)

- >10 years
- >5 years
- 5 years

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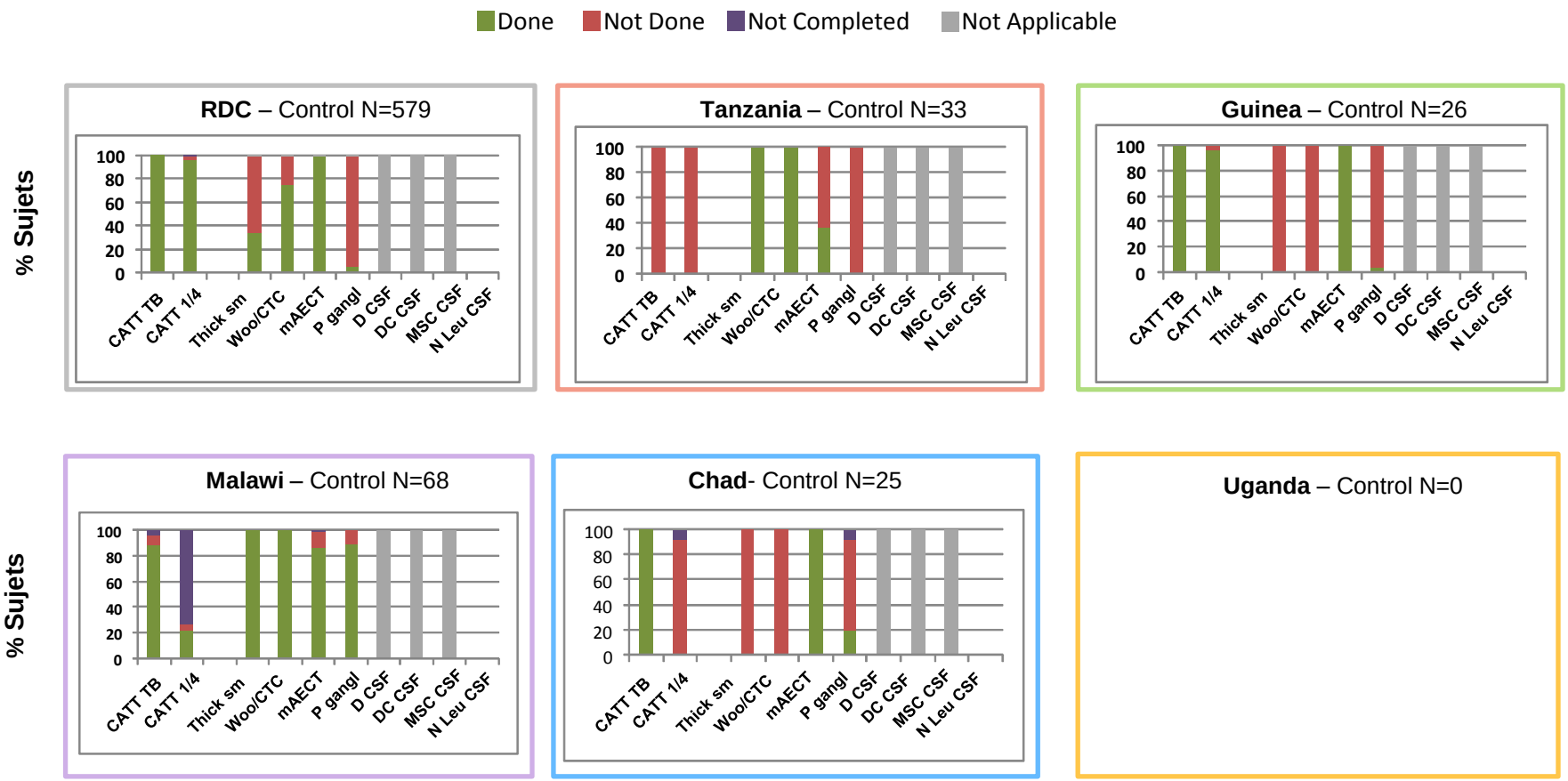
1.4 Diagnostic tests

Control Groups



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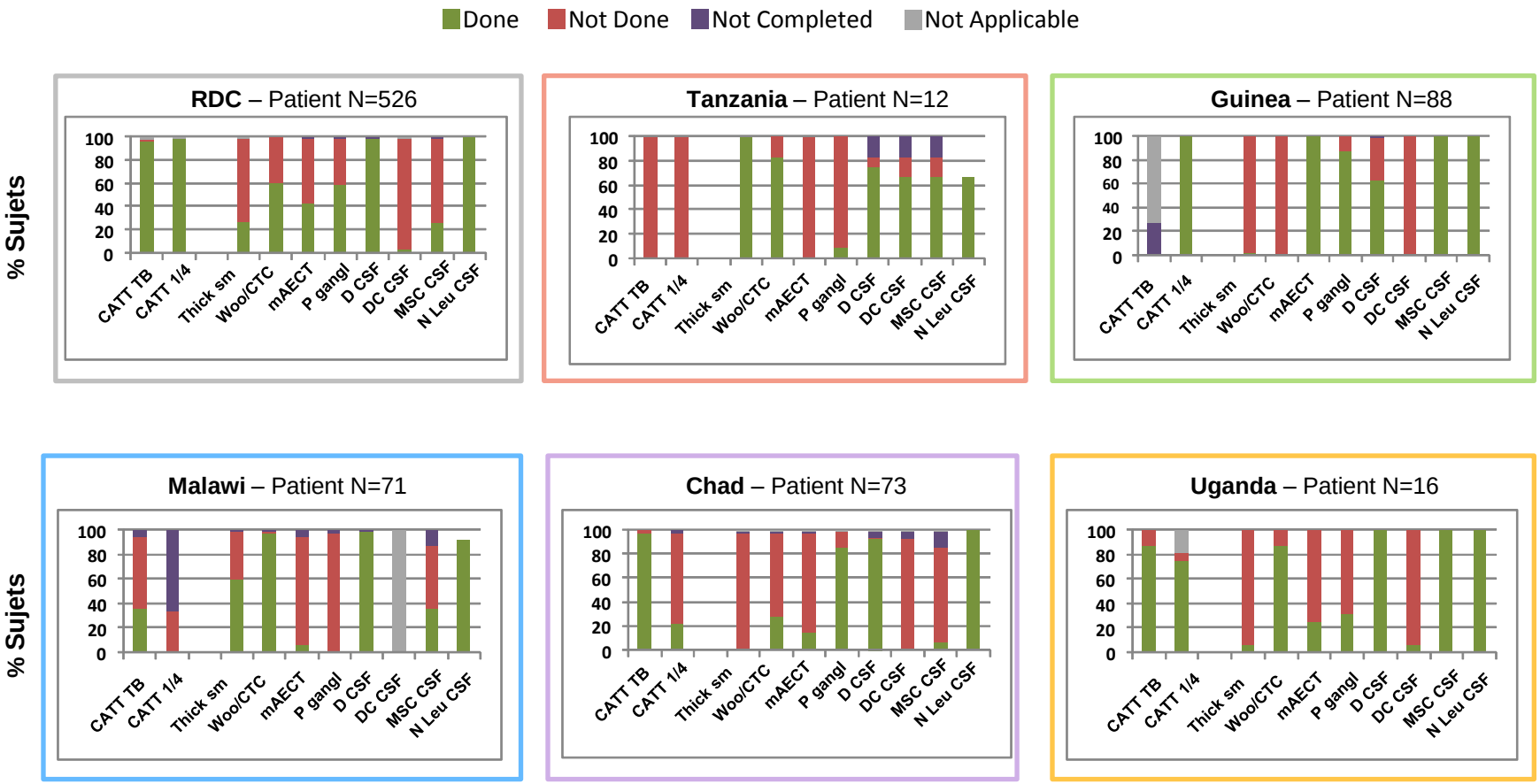
1.4 Diagnostic tests

Patients Groups



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PART 2

The WHO HAT Specimen bank

2.1 Processes of the biobank
2.2 Study subjects
2.3 Processing / Reception
2.4 Non compliances and Resolution
2.5 Processing / Aliquoting
2.6 Processing / Distribution

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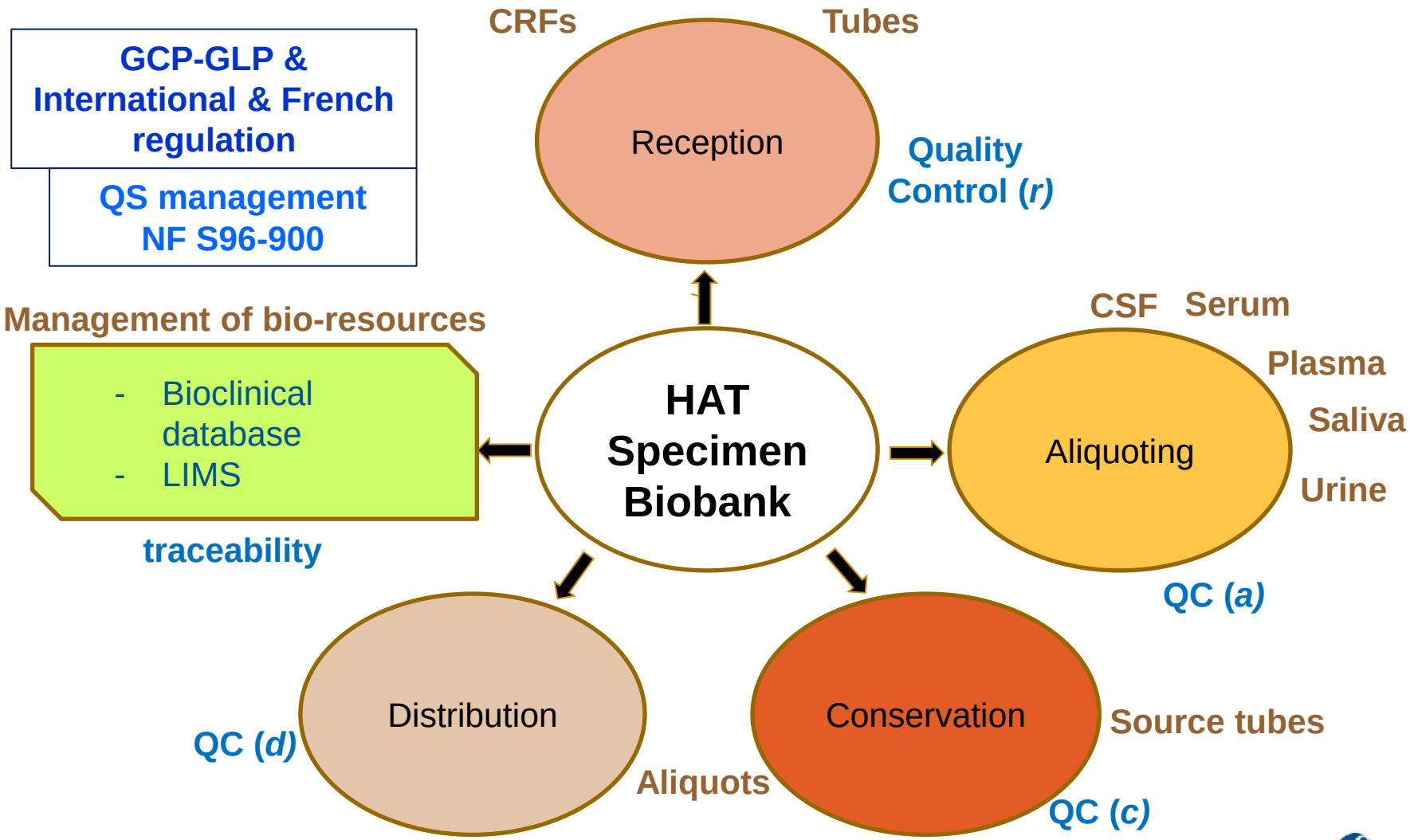
2.1 Processes of the biobank



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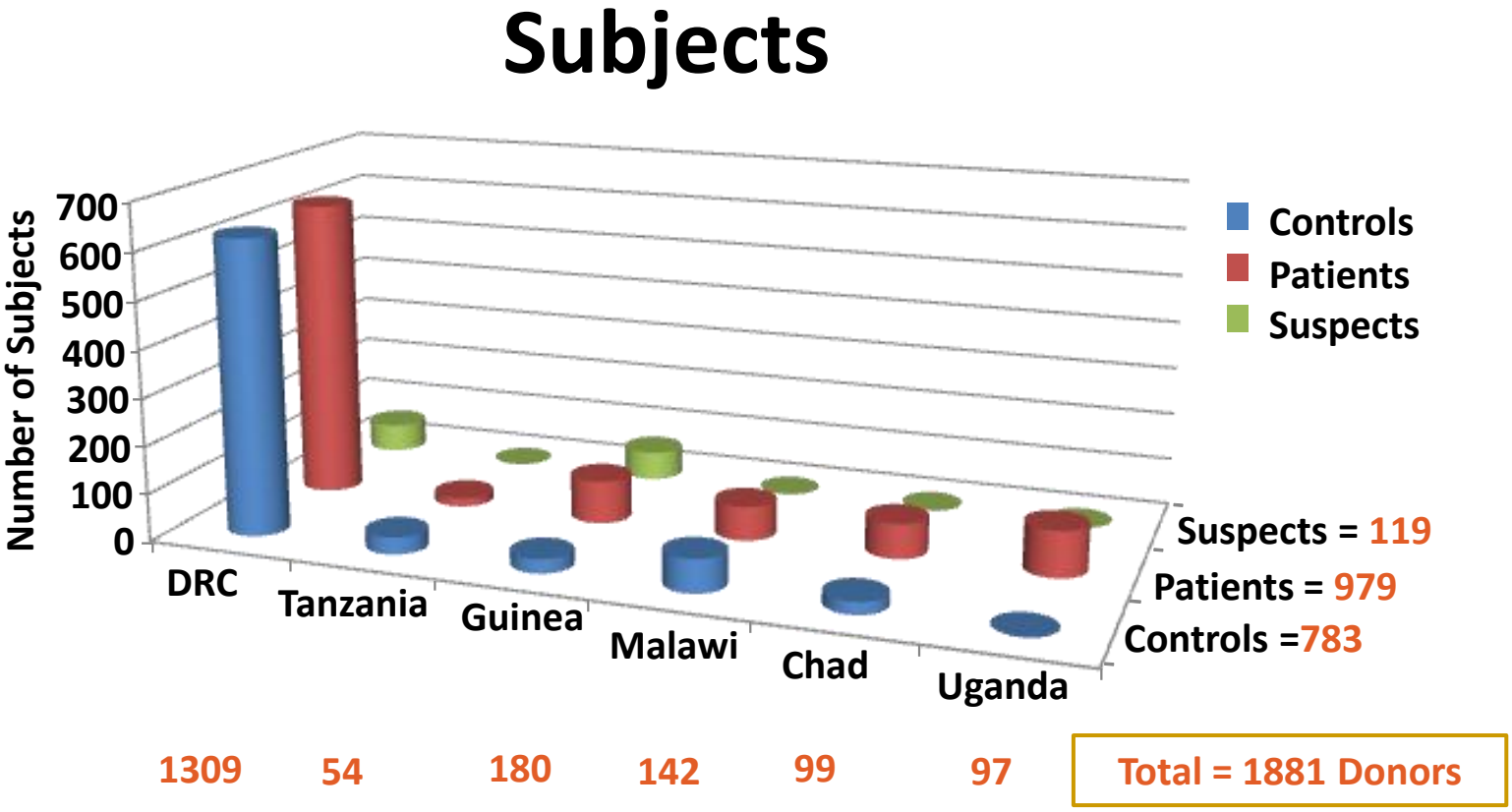
2.2 Study subjects



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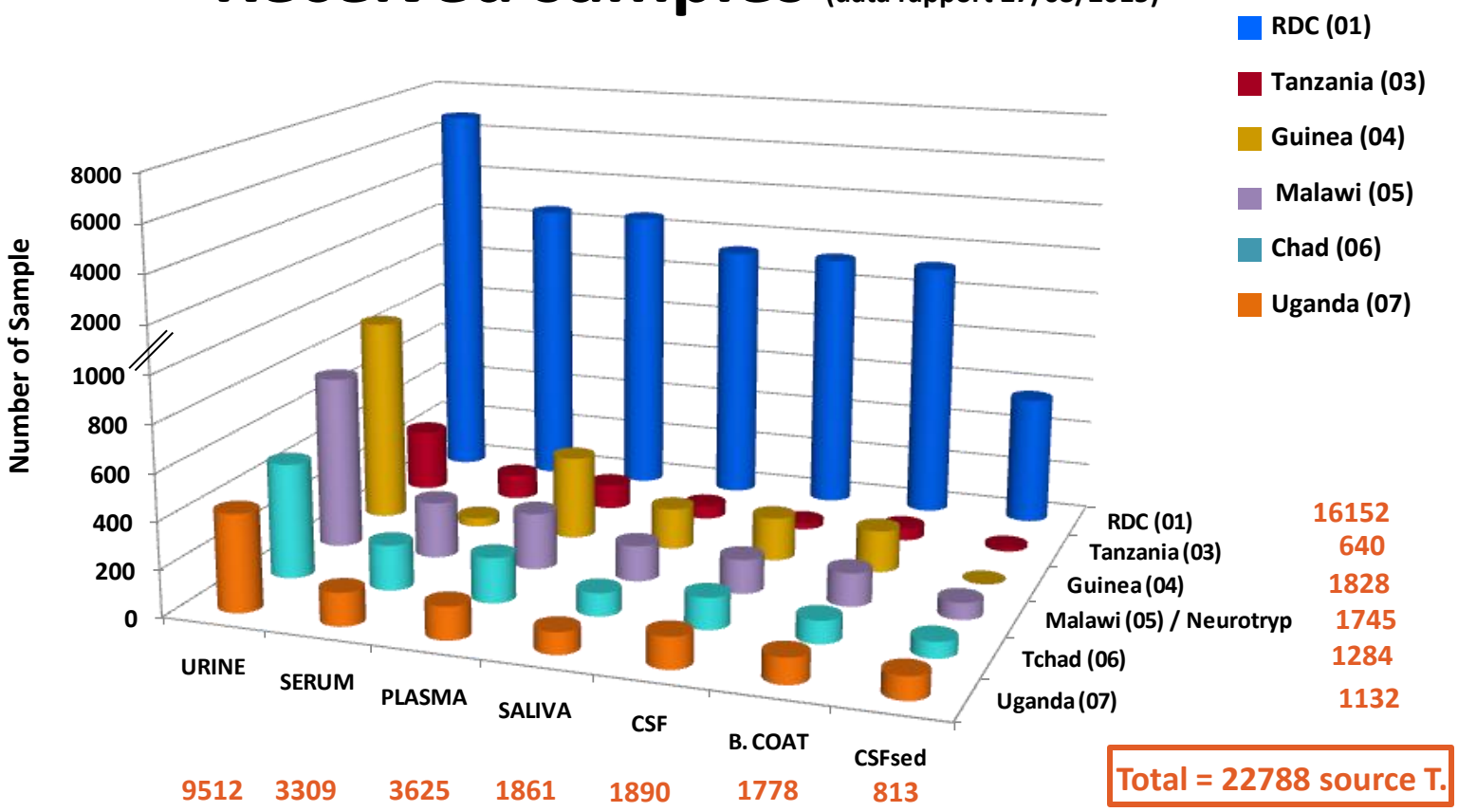
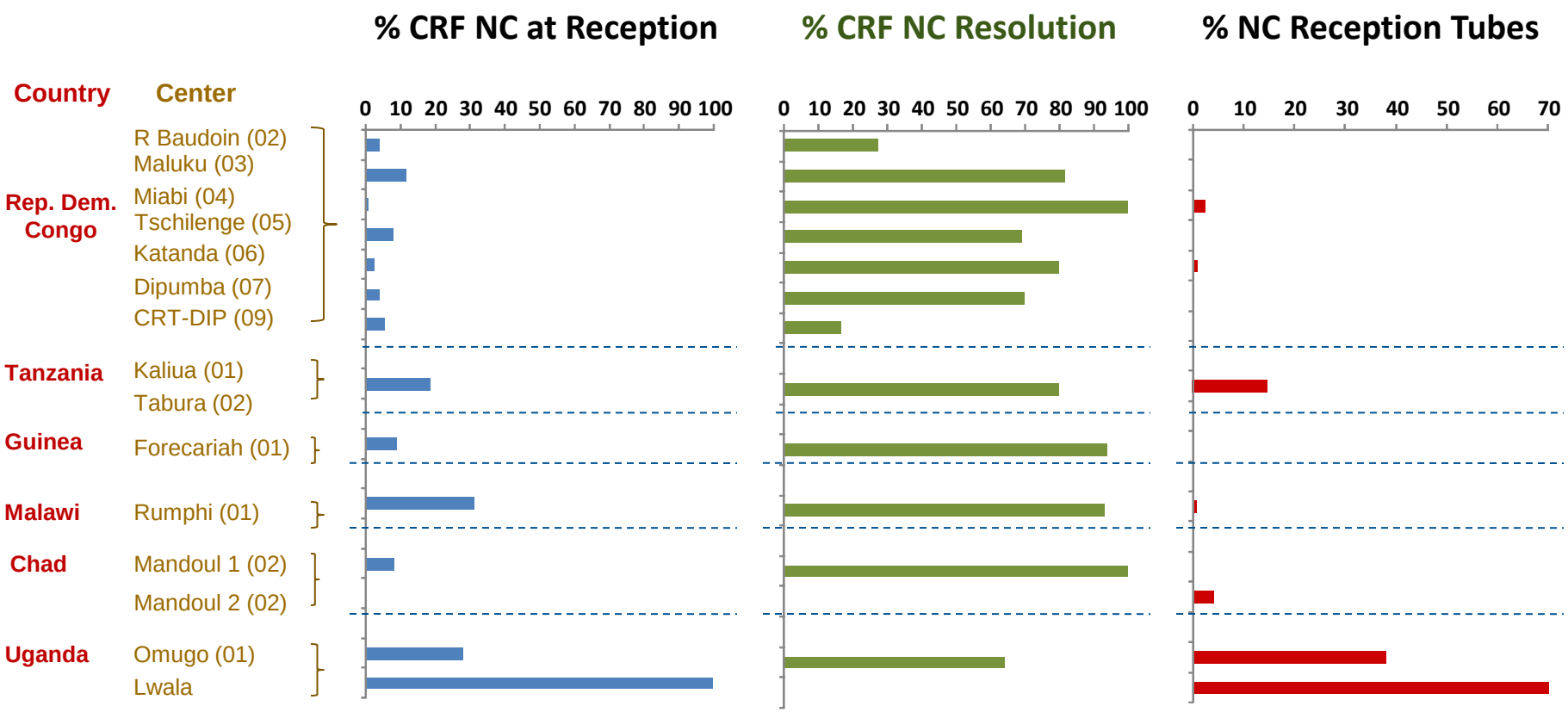
2.3 Processing / Reception

Received samples (data rapport 27/03/2015)

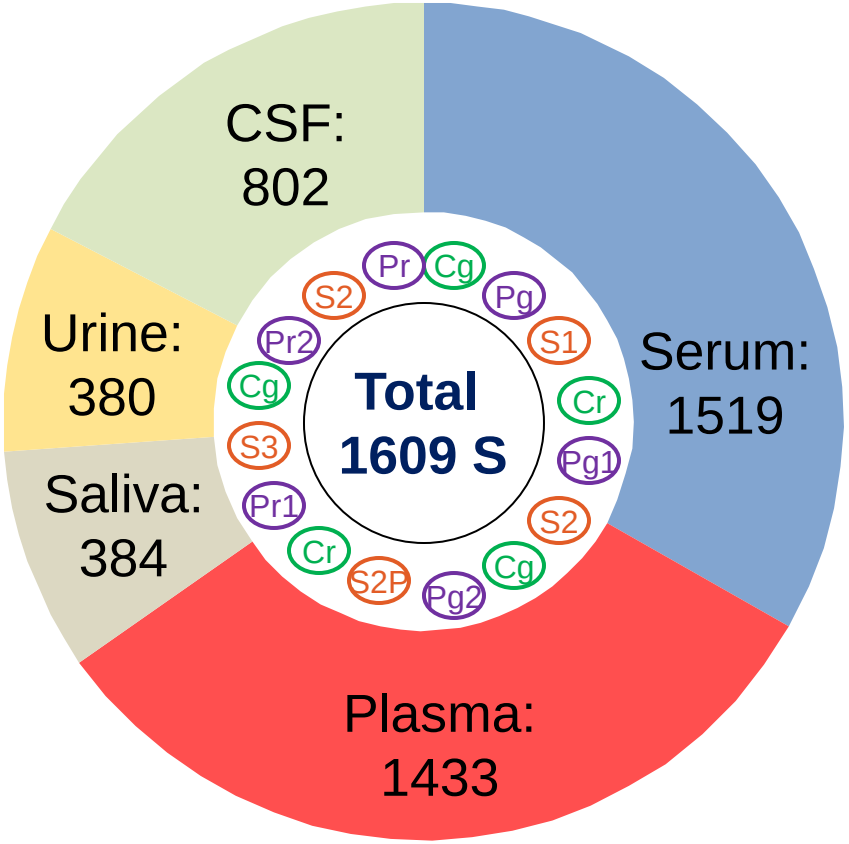


2.4 Non Compliances and Resolution



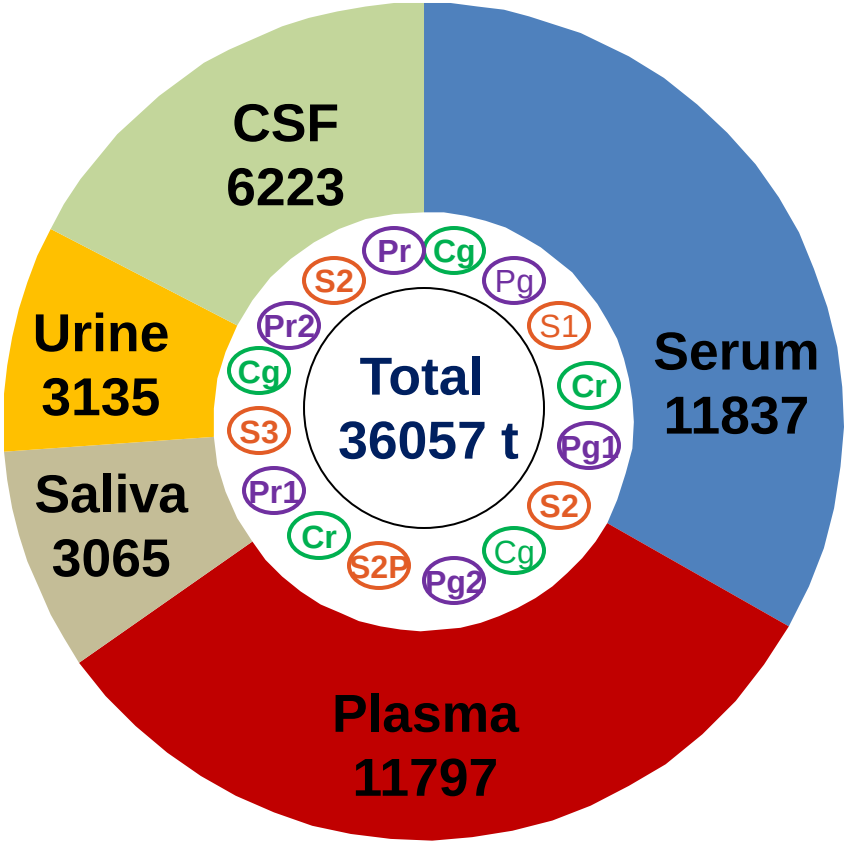
2.5 Processing / Aliquoting

N Subjects Aliquoted



(data rapport 27/03/2015)

N Aliquots produced



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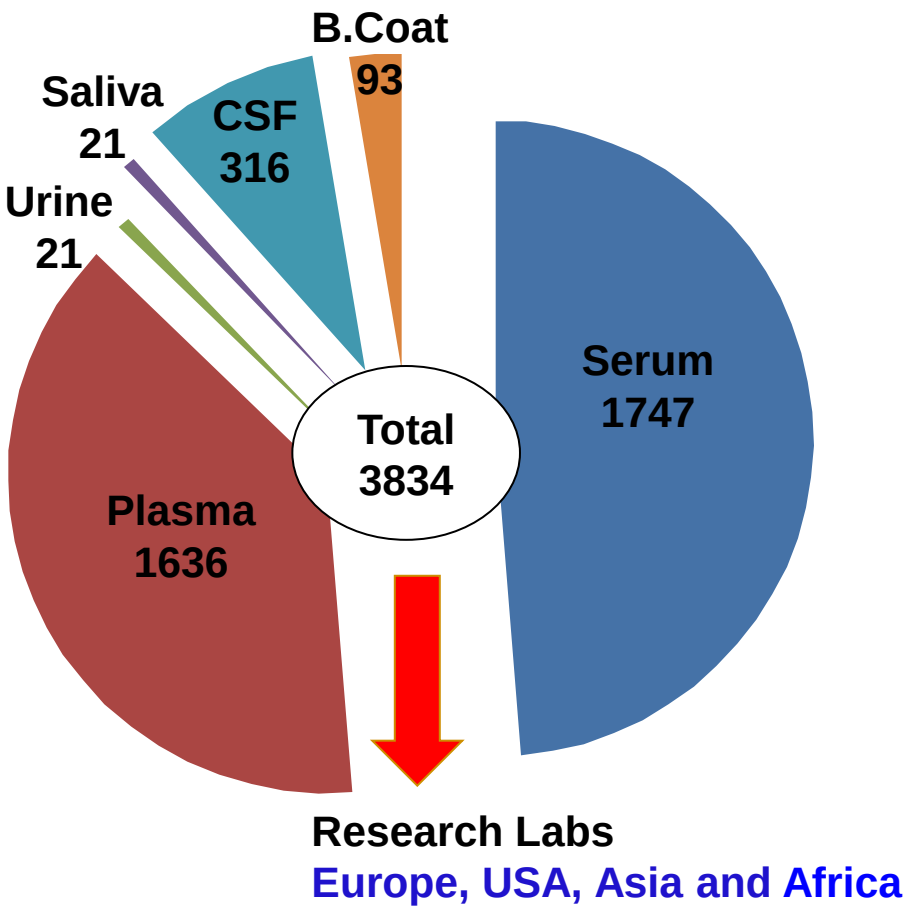
2.6 Processing / Distribution

Distribution of Samples

(data rapport 27/03/2015)



PART 3

New diagnostic tests set up

3.1 « Reference » diagnosis methods
3.2 Overall performances
3.3 New diagnosis methods set up
3.4 Performances optimization
3.5 Synthesis

3.1 Reference diagnosis Methods

Reference tests used for the WHO HAT biobank constitution :

Stage 1 : Blood

- INDIRECT (SERO) :
  - **CATT** : **C**ard **A**gglutination **T**est for **T**rypan. (pure or diluted to 1/4th)
    very sensitive method+++ / but numerous limitations

- DIRECT :
  * **Woo** (or Haematocrit Centrifuge technique or capillary centrifugation technique) / QBC
  * **mAECT** (mini-Anion Exchange Centrifugation Technique) (more ss)

3.1 Reference diagnosis Methods

Stage 2 : CSF examination

- LEUCOCYTE count > 5/ µL (but « grey zone » to 20 WC / µL)
- INDIRECT :
  - **IgM** : in the CS
- DIRECT :
  - **Trypa.** observed at the microscope after centrifugation single (SC), double (DC) or modified (MSC)

3.2 Performances of reference tests

Performances of the tests :

- CATT : excellent sensitivity
- Direct (parasitological tests) : excellent specificity

Parasitological tests	nb parasites detected /mL	sensitivity	specificity
Direct examination of blood	6 000 - 10 000		100%
thick blood smear	600 - 5 000	26 - 35%	100%
Woo (capillary centrif.)	500 - 600	56%	100%
QBC	15 - 300	77%	100%
mAECT	15 - 100	78%	100%

3.2 Performances of reference tests

Additional reference tests used by the research teams :

Stage 1 :

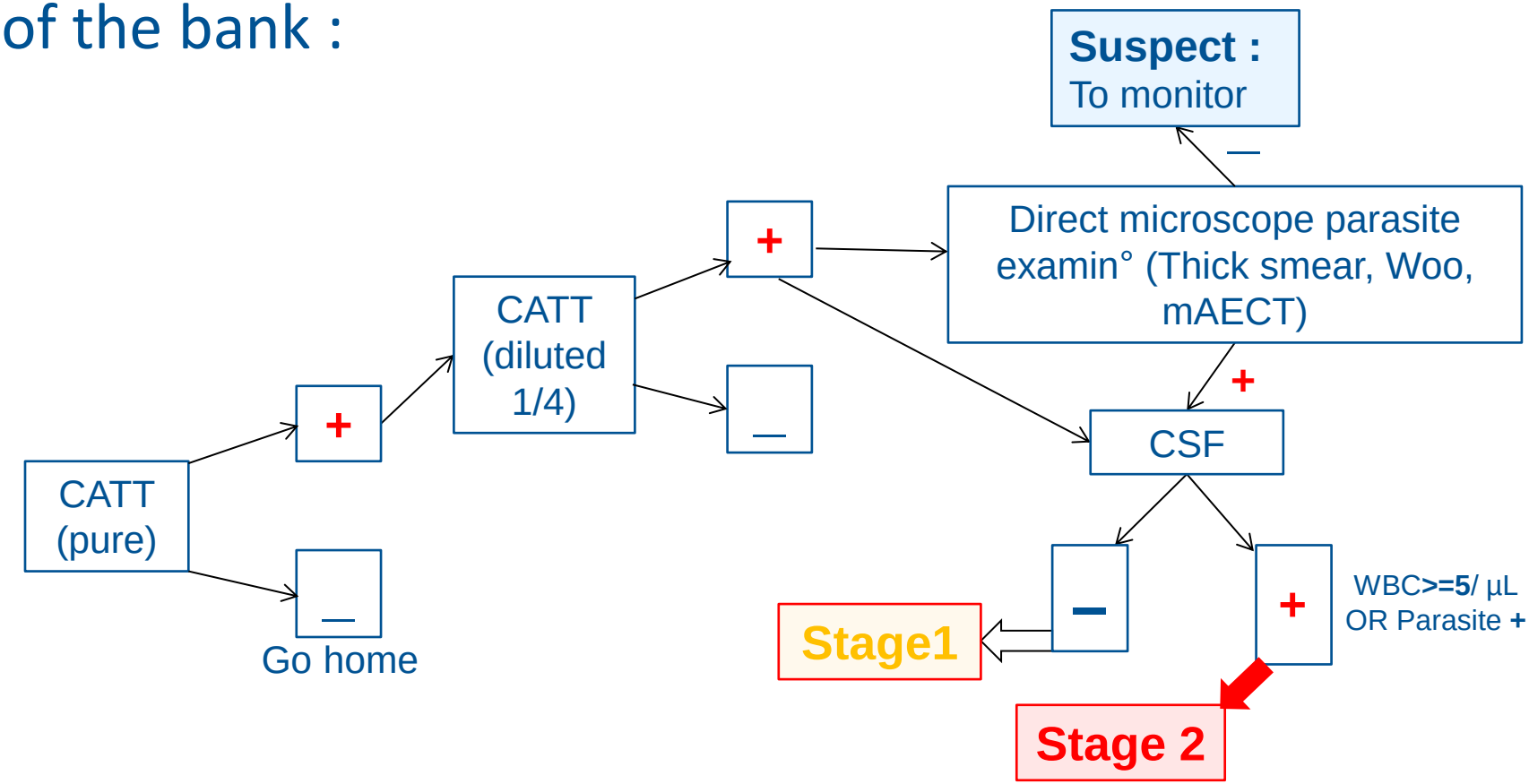
- **Trypanolysis** consists in lyse of trypanosome by the patient 's antibodies
  (Live parasite culture)

Stage 2 :

- **IgM detection** in CSF
  (*too sophisticated)*

3.1 Reference diagnosis Methods

WHO algorithm guidelines used in the framework of the bank :



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3.1 The new tests

RDT (Rapid Diagnostic Test) – Dipstick :

Rapid Diagnostic Test for Sleeping Sickness

To the editor

N Engl J Med 368; march 14, 2013

P. Büscher (1), Ph.D., Q. Gilleman (2) , and V. Lejon (1)

(1) Institute of Tropical Medicine Antwerp, Belgium

(2) Coris BioCOncept, Gembloux, Belgium

3.3 The new tests

Lateral Flow Test

1. Proteomic selection of immunodiagnostic antigens for Human African trypanosomiasis and generation of a prototype Lateral Flow immunodiagnostic device

PLOS Neglected Tropical Diseases, 2013

L Sullivan[1], S J. Wall[2], M Carrington[3], M. A. Ferguson[1]
1 College of Life Sciences, University of Dundee, Dundee, United Kingdom,
2 BBInternational, Alchemy House, Dundee, United Kingdom,
3 Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom

2. Identification of sVSG117 as an immunodiagnostic antigen and evaluation of a dual-antigen lateral flow test for the diagnosis of human African trypanosomiasis.

PLoS NTD 2014

Sullivan L[1], Fleming J[1], Sastry L[1], Mehlert A[1], Wall SJ[2], Ferguson MA[1].

3.3 The new tests

Biomarker for S2 : Neopterin test

1. Cerebrospinal fluid neopterin as marker of the meningo-encephalitic stage of Trypanosoma brucei gambiense sleeping sickness

PloS One 2012

N Tiberti [1.], A Hainard [1.], V Lejon [2.], B Courtioux [3], E Matovu [4,], JC Enyaru [5], X Robin [1.], N Turck [1.], K Kristensson [6], D Mumba Ngoyi [7],G M. L. Vatunga [8], S Krishna [9], P Büscher [2], S Bisser [3], J Mathu Ndung'u [10], JC Sanchez [1.] *

1.University of Geneva, Geneva, Switzerland, 2. ITM Antwerp, Belgium, 3. INSERM UMR1094, Tropical Neuroepidemiology, Limoges, France, 4-5 Makerere University, Kampala, Uganda, 6. Karolinska Institutet, Stockholm, Sweden 7. INRB, Kinshasa, DRC, 8 Control of Trypanosome Institute, Luanda, Angola, 9. St George's University, London, UK, 10. FIND, Geneva, Switzerland 11. Neuroepidemiology Institute, University of Limoges, France.

2. Neopterin is a cerebrospinal fluid marker for treatment outcome evaluation in patients affected by Trypanosoma brucei gambiense sleeping sickness

N Tiberti [1.], V Lejon [2.], A Hainard [1.], B Courtioux [3], X Robin [1.], N Turck [1.], K Kristensson [6], E Matovu [5], JC Enyaru [5], D Mumba Ngoyi [7], S Krishna [9], S Bisser [3] J Mathu Ndung'u [10], P Büscher [2], JC Sanchez [1.]

3.3 The new tests

Definition of the new tests

Publication / Journal, year	Author	Organism of affiliation	New test method	Sample	Reference method
NEJM 2013	Ph. Büscher	Institute for Tropical Med., Antwerp - B	TDR : sero-strip /LiTat 1.3 (sVSG)	BLOOD	trypanolysis
			TDR : sero-K-SeT /LiTat 1.5 (sVSG)	BLOOD	trypanolysis
PLoS NTD 2013	L. Sullivan	Dundee univ., - UK	Lateral Flow immunodiag. / sVSG 117	Serum	
PLoS NTD 2014	L. Sullivan	Dundee univ., - UK	Lateral Flow immunodiag. / sVSG 117 & rISG 65	Serum	
PLoS One 2012	J.C. Sanchez	Geneva, univ., - Switz.	ELISA kits / Neopterin biomarker	CSF	IgM in CSF
PLoS NTD 2013	J.C. Sanchez	Geneva univ., - Switz.	ELISA kits / Neopterin biomarker	CSF	IgM in CSF
Parasitol. Int. 2016	S.M. Nzou	KEMRI, Kenya &	Luminex multiplex Tb **gambiense**	Serum	
Parasitol. Int. 2016	S.M. Nzou	Nagasaki univ., Japan	Luminex multiplex Tb **rhodesiense**	Serum	

3.4 Properties of the new tests

Caracteristics of the new tests

New test method	field friendly?	rapidity?	cost?	stage specific (%)?	species specific (%)?	overall sensit.	overall specif.
TDR : sero-strip /LiTat 1.3 (sVSG)	Yes +++ (TDR)	15 min	2,50 USD			97.5 %	98.0 %
TDR : sero-K-SeT /LiTat 1.5 (sVSG)	Yes +++ (TDR)	15 min	2,50 USD			93.7 %	99.0 %
LFT / sVSG 117	Yes + (LFT)	30 min	1 USD		Tb gambiense	88%	93%
LFT / sVSG 117 & rISG 65	Yes + (LFT)	30 mn	1 USD		Tb rhodesiense	58, 9 (83.9 %)	87,3 (85.3 %)
ELISA kits / Neopterin biomarker	+/-	4-5 hours		Neurotrypanos. (S2)		97.9 %	100%
ELISA kits / Neopterin biomarker	+/-	4-5 hours		post-ther outcome		92%	92%
Luminex multiplex Tb **gambiense**	No	?	?		Tb gambiense	low 50%	95%
Luminex multiplex Tb **rhodesiense**	No	?	?		Tb rhodesiense	low 25%	95%

PART 4

Conclusions and Perspectives

4. Conclusions - I

The results update :

- **1881** subjects enrolled in the specimen bank project
- 36 000 samples of various bio-resources
  of high intrinsic quality, and annotated

- **≈1/10**th so far distributed

- **Several New HAT diagnosis methods** developed :
  - RDT,
  - ELISA- or Luminex-based,
  - S2 stage biomarker

4. Conclusions - II

The experience in the field :

- **YES, THEY DID IT !**
- Centers located in remote regions endemic for neglected tropical diseases have been successfully implicated in this work

- The work was best implemented in nearly all centers
  (completion of bc data, cold chain, tests realization…)

- These centers have gained important experience

4. Perspectives

- **To accompany the development** of the current new methods towards **validation**

- **To engage now with the WHO in benefit sharing** with the affected populations *(information, affordability)*

- **open the bank to broader research topics**

THANK YOU !!!

ICAReB Platform –
Institut Pasteur - Paris

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Catherine OTTONE
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José Ramon FRANCO MINGUELL
Pere Perez SIMARRO
Gerardo PRIOTTO
Jean JANNIN

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WHO & FIND