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BIORESOURCE PAPER

# Biological Resource Center for *Toxoplasma*

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The Biological Resource Center (BRC) for *Toxoplasma gondii* is a certified structure (NF S96-900 standard) that manages the storage of *Toxoplasma gondii* strains (protozoan parasite) and strain derivatives from human or animal toxoplasmosis to make them available to the scientific community.

**Keywords:** *Toxoplasma gondii*; strains; parasite; biological resources center

## (1) Bioresource Overview

### Project description

The Biological Resource Center (BRC) for *Toxoplasma gondii* is a certified structure (NF S96-900 standard) that manages the storage of *Toxoplasma gondii* strains (protozoan parasite) and strain derivatives from human or animal toxoplasmosis to make them available to the scientific community.

The BRC *Toxoplasma* works through a network of correspondents who send isolates to the parasitology departments of Reims and Limoges University hospitals (France). The managers are Pr Isabelle VILLENA (Reims) and Pr Marie-Laure DARDE (Limoges). The biological resources are stored in cryobanks located in both sites.

*Toxoplasma gondii* strains which integrate the BRC *Toxoplasma* collection come from:

- The French ToxoBs network
- National and international research collaborations
- Regular depositors

Development of BRC *Toxoplasma* has been also consolidated by the creation of a National Reference Center of Toxoplasmosis coordinated by Pr I.VILLENA, with an associated laboratory dedicated to *Toxoplasma* strains in Limoges (France).

Each site possesses its own specificity: cell culture multiplication, storage of DNA extracts from catalog's strains and distribution are managed by Reims's team, microsatellite genotyping and epidemiological data by Limoges's team.

The objectives of the BRC *Toxoplasma* are:

- The heritage conservation of strains and strains derivatives.
- The support for research projects thanks to the characterization of strains.
- Diversification of the geographical and host species origin of strains by collecting isolates from research teams at a national and international level.

The biobank stores around 1500 strains from different hosts (humans or animals) and from different countries around the world. Strains of human origin are accompanied by clinical data (congenital toxoplasmosis, immunodeficient or immunocompetent patient, severity of clinical form).

The online catalog currently offers 37 strains: reference strains (RH, Me49, PRU, VEG) and 33 strains selected for their belonging to the 16 haplogroups currently described for *Toxoplasma gondii* including 6 highly divergent strains from the Amazonian forest.

For 21 of them, the complete genome is available via ToxoDB and genomic analysis has been published in Lorenzi et al. [5]. The strain geographical origins are diversified: Europe, North and South America, Africa, Caribbean islands.

*Toxoplasma gondii* strains are available as tachyzoites suspensions obtained in cell culture ( $8 \times 10^6$ /cryovials) and as cysts in mouse's brain suspensions.

*Toxoplasma gondii* strains and their derivatives (DNA) are available to the research community or industrialists. The order is made via the website of the BRC where a catalog of well-characterized strains is available: [www.toxocrb.com](http://www.toxocrb.com).

The BRC *Toxoplasma* belongs to the following networks: Biobanques (INSERM), BBMRI-ERIC, FBRCMi and Club 3C-R.

### Classification (1)

Microorganism  
Parasite, protozoan

### Species

*Toxoplasma gondii*

### Classification (2)

Biological samples and associated data.

### Keywords

*Toxoplasma gondii*, strains, parasite, biological resource center.

### Context

#### Spatial coverage

France, Reims: 49° 15' 46" North/4° 02' 05" East  
France, Limoges: 45° 51' 00" North/1° 15' 00" East

#### Temporal coverage

Since 2002.

#### Temporal coverage for accessibility

Since 2010.

## (2) Methods

### Steps

To obtain *Toxoplasma gondii* strains, Swiss mice are inoculated with biological samples like amniotic fluid, placenta, blood or broncho-alveolar liquid for strains from human origin, or muscles or brains for strains from animal origin [2, 4, 7]. Then parasites are isolated from mouse's brain or ascitic fluid and genotyped by 15 microsatellite markers [1]. The isolation protocol has been approved and accepted by the relevant national and regional ethics committees.

According to acceptance criteria set by the BRC (number of cysts, genotyping results and clinical data), isolates are added to the collection.

Bradyzoites are purified by a Percoll gradient in order to initiate cell culture on VERO cells for tachyzoite multiplication.

Strains are stored in liquid nitrogen ( $-196^{\circ}\text{C}$ ) in 1 mL cryovials [6] (**Figure 1**) and DNA extracts are stored at  $-80^{\circ}\text{C}$ . Each cryovial contains a defined number of parasites (suspensions of tachyzoites or cysts).

The security of the resources is ensured by the permanent temperature monitoring of the storage enclosures: freezers are temperature monitored by a software and the liquid nitrogen level in tanks is regularly monitored by the BRC staff. Moreover, the collection is duplicate in Reims and Limoges.



**Figure 1:** *Toxoplasma gondii* strains are stored in liquid nitrogen.

The following data are recorded on BRCLims software [3] and are available for each strain: genotype (microsatellite typing, and for some of them haplogroup membership), mouse virulence, host and tissue origin, date of isolation, geographical origin and disease.

To order a strain, a request must be submitted at the BRC *Toxoplasma* scientific council (crb.toxobs@unilim.fr) by filling an order form available on the website. After approval by the scientific council, a Material Transfer Agreement is signed and the resources are sent at controlled temperature by a qualified carrier.

The BRC valuation is ensured by the publications that must quote the bank when research is carried out with BRC *Toxoplasma* strains or derivatives.

To deposit a strain in the BRC *Toxoplasma*, a request must be submitted by filling a deposit agreement form available on the website. After approval by the direction of the BRC, the biological resources are sent at controlled temperature by a qualified carrier.

#### Stabilization/preservation

Strains are stored in liquid nitrogen.  
DNA is stored at  $-80^{\circ}\text{C}$ .

#### Type of long-term preservation

Liquid nitrogen

#### Storage temperature

$-80^{\circ}\text{C}$ ;  $-196^{\circ}\text{C}$

#### Shipping temperature from patient/source to preservation or research use

NA

#### Shipping temperature from storage to research use

Dry Ice ( $-80^{\circ}\text{C}$ )

#### Quality assurance measures

Parasite revival in cell culture or in mouse after defrosting.  
Storage location verification.

#### Source of associated data

National Reference Center for Toxoplasmosis  
Original research works

#### Ethics Statement

Animals were housed and fed according to the animal care facilities guidelines, and procedures were approved by ethic committees on animal use.

Number of authorization for Reims' site delivered by the ethic committee for animal experimentation: APAFIS#11913-2017102316076105 v2.

Number of authorization for Limoges' site delivered by the ethic committee for animal experimentation: APAFIS #15267-2018052909366202 v2.

#### Constraints

Animals were housed and fed according to the animal care facilities guidelines, and procedures were approved by ethic committees on animal use.

### (3) Bioresource description

#### Object name

*Toxoplasma gondii*

#### Bioresource name

Biological Resources Center of *Toxoplasma*  
Bioresource acronym: BRC *Toxoplasma*

#### Bioresource location

BRC *Toxoplasma*  
Pôle de Biologie Médicale et Pathologie du CHU de Reims  
Laboratoire de Parasitologie  
rue Général Koenig  
FR 51092 Reims Cedex FRANCE

BRC *Toxoplasma*

CHU DUPUYTREN - CBRS  
Laboratoire de Parasitologie  
2 avenue Martin Luther King  
FR 87042 Limoges FRANCE

#### Bioresource contact

crb-reims@wanadoo.fr

#### Bioresource URL

www.toxocrb.com

#### Identifier used

(if available: DOI, etc.)

#### Bioresource type

Tachyzoites or bradyzoites

#### Type of sampling

Each cryovial contains  $8 \times 10^6$  tachyzoites in 1 ml of cryopreserving media, or a precise number of cysts in 1.5 ml, or DNA extract from  $8 \times 10^6$  tachyzoites in 1 ml tubes.

#### Anatomical site

NA

#### Disease status of patients/source

- Human strains:
  - Congenital toxoplasmosis
  - Toxoplasmosis in immunodeficient patients (AIDS or transplant)
  - Acquired toxoplasmosis in immunocompetent patients
- Animal strains
  - Chronic infection
  - Abortion

#### Clinical characteristics of patients/source

NA

#### Size of the bioresource

The biobank stores around 1500 strains from different hosts (humans or animals): 1000 human strains and 500 from different animal species.

BRC *Toxoplasma* includes 30–60 new strains per year.

**Vital state of patients/source**

NA

**Clinical diagnosis of patients/source**

NA

**Pathology diagnosis**

NA

**Control samples**

Cysts and tachyzoites number per cryovial.  
Mycoplasma detection.  
Microsatellite genotyping before and after cell culture.

**Biospecimen type**

Microorganism class B and DNA

**Release date**

Since 2010

**Access criteria**

The catalogue of BRC *Toxoplasma* is built on several criteria validated by the scientific council. General conditions are available on the website. All requests are recorded.

**(4) Reuse potential**

NA

**Competing Interests**

The authors have no competing interests to declare.

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