APPENDIX 2 (STUDY GROUP SELECTION AND RECRUITMENT)

2.2.1a) HREC (Human Research Ethics Committee) certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Kistiah et al

CLEARANCE CERTIFICATE	PROTOCOL NUMBER M070637
PROJECT	Studies of Bartonella and Toxoplasma gondii Species in Human Populations in South Africa
INVESTIGATORS	Miss K Kistiah et al
DEPARTMENT	Virology & Communicable Dis.
DATE CONSIDERED	07.06.29
DECISION OF THE COMMITTEE*	APPROVED UNCONDITIONALLY

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 07.08.06 CHAIRPERSON .....
(Professors PE Cleaton-Jones, A Dhai, M Vorster, C Feldman, A Woodiwiss)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Dr J Frean

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

**__please note__: the same set of samples for the human portion of this study were used for a Toxoplasmosis study that was being carried out by a colleague during the same period. Permission and ethics were received for both studies.*

2.2.1b) Informed consent forms for HIV positive human volunteers

Title of Project:

Studies of *Bartonella* and *Toxoplasma gondii* species in human populations in South Africa

Patient information sheet

Hello. My name is _____(*name of interviewer*)_____ and I would like to invite you to participate in a study. Your participation in this study is completely voluntary. If you decide not to participate, it will not affect your treatment in any way and you may also change your mind at any time. As we discuss the information, please feel free to ask me any questions.

I am studying infections called 'toxoplasmosis' and 'bartonellosis', caused by germs most commonly transmitted from humans animals and some foods. These infections can sometimes cause illness in humans. I would like to test your blood for signs of these infections, and ask your permission to take, at the same time that blood is taken from a vein in your arm for other tests routinely done in this clinic, two extra 5ml (2 teaspoons) tube of blood. This blood will be used to test for the presence of these infections and will not be used for any other purpose. Your blood will be disposed of safely once we have carried out these tests. Only the people involved in this study will have access to your blood and to these results. Your doctor will be given the results of my test, and will tell you about them when you return to the clinic for your normal appointment.

Thank you for your time. Once you have asked me any questions, you can decide in your own time whether you want to give the blood sample or not.

If you would like any more information on the study, you can contact any of the researchers that are here, or Miss K. Kistiah (011) 555 0332 or Miss A.N. Trataris (011) 555 0331

NICD

NATIONAL HEALTH LABORATORY SERVICE

Title of Project:

Studies of *Bartonella* and *Toxoplasma gondii* species in human populations in South Africa

Informed consent form:

I have understood the information (verbal and/or printed) about this study, and agree to donate a sample of blood to be tested only for toxoplasmosis and bartonellosis , and I understand that any remaining blood will be destroyed and not used for any other purpose.

I understand that I have been invited to participate in this study, and I understand that my agreeing to participate is fully voluntary and I can withdraw at any time.

Subjects name:

Signature

Date:

Consent record for blood tests for toxoplasmosis and bartonellosis.

Age: Gender:	**Date:**
Information provided and written consent witnessed by (print):	**Sign:**
Blood sample taken by (print):	**Sign:**
Study sample number:	

2.2.1c) Informed consent forms for healthy volunteers

Title of Project:

Studies of *Bartonella* and *Toxoplasma gondii* species in human populations in South Africa

Patient information sheet

Hello. My name is Anastasia Trataris and I would like to invite you to participate in a study. Your participation is completely voluntary and you may change your mind at any time. Please feel free to ask me any questions.

My colleague, Kesenthri Kistiah, and I are studying the seroprevalence of two zoonotic infections, toxoplasmosis and bartonellosis, caused by *Toxoplasma gondii* and *Bartonella* spp. respectively. These are infections people contract from contact with animals particularly cats and sometimes dogs. Although infection is often asymptomatic, disease does occur in humans. We would like to request that you volunteer two 4 ml tubes of blood (1x EDTA blood and 1x plain clotted blood) that will be drawn by a registered pathologist for us to use as controls for our study. This blood will be used to test for the presence of these infections and will not be used for any other purpose. Your blood will be disposed of safely once we have carried out these tests. Only the people involved in this study will have access to your blood and to these results. Should you want the results of these tests, feel free to contact me and I will be happy to provide you with the report, however, it does not mean that you are ill and require treatment, we are mostly looking for evidence of past or current exposure.

Thank you for your time. Once you have asked me any questions, you can decide in your own time whether you want to give the blood sample or not.

If you would like any more information on the study or would like to volunteer some blood for this study, please contact Miss A.N. Trataris (011) 555 0331 or Prof. J.A. Frean (011) 555 0308.

NICD

NATIONAL HEALTH LABORATORY SERVICE

Title of Project:

Studies of *Bartonella* and *Toxoplasma gondii* species in human populations in South Africa

Informed consent form:

I have understood the information (verbal and/or printed) about this study, and agree to donate a sample of blood to be tested only for toxoplasmosis and bartonellosis , and I understand that any remaining blood will be destroyed and not used for any other purpose.

I understand that I have been invited to participate in this study, and I understand that my agreeing to participate is fully voluntary and I can withdraw at any time.

Subjects name: _____
Please print

Signature: _____

Date: _____

Consent record for blood tests for toxoplasmosis and bartonellosis.

Age:	**Gender:**				**Recent contact with domestic/wild animals:**				
	male		**female**		**yes**		**No**		**Type:**
Information provided and written consent witnessed by (print):					**Sign:**				
Blood sample taken by (print): Dr. J.A. Frean					**Sign:**				
Study sample number:									

2.2.1d) Animal ethics approval from the NHLS (National Health Laboratory Service)

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NATIONAL HEALTH LABORATORY SERVICE

ANIMAL ETHICS CLEARANCE CERTIFICATE.

CLEARANCE CERTIFICATE NUMBER:

2007	112

APPLICANT: MS A. TRATARIS

DEPARTMENT/COMPANY: NHLS

PROJECT TITLE: STUDIES OF BARTONELLA SPECIES IN ANIMALS POPULATION IN SOUTH AFRICA

SPECIES	NUMBER	TYPE OF APPLICATION
CATS, DOGS, WILD CAUGHT RODENTS	384 OF EACH SPECIES	EXPERIMENTAL

i) Approval is hereby given for the experiment/routine procedure described in the above application.

The use of these animals is subject to the National Code 1990 Guidelines as used by the NHLS AEC. If an application for a routine procedure then the recommended guide lines or SOP must be followed. It is limited to the procedure specified in the application form and to:

SIGNED---------- DATE: 14.06.2007
(Chairperson: Animal Ethics Committee)

ii) I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23(1) (C) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

SIGNED---------- DATE: 14.06.2007
(Registered Veterinarian)

2.2.1e) Animal ethics screening committee (AESC), Wits University.

AESC 3

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2008/56/03

APPLICANT: Ms AN Trataris

SCHOOL: School of Pathology
DEPARTMENT:
LOCATION:

PROJECT TITLE: **Studies of Bartonella species in human and animal population in South Africa**

Number and Species

384 Cats, 384 dogs, 384 various rodents

Approval was given for the use of animals for the project described above at an AESC meeting held on 25.11.2008. This approval remains valid until 25.11.2010

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and to the following additional conditions:

Signed: _____ Date: 28/11/2008
(Chairperson, AESC)

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: _____ Date: 28/11/2008
(Registered Veterinarian)

cc: Supervisor:
Director: CAS

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2.2.2) Five percent rabbit blood supplemented media for culture of Bartonella spp.

Components

39 g	**Columbia agar base (Sigma Aldrich)**
1000 ml	**Distilled sterile water (Laboratory produced)**
50 ml	**Sterile, defibrinated (heparin) rabbit blood**

Method

- Thirty-nine grams (39 g) of the Columbia agar base [a] was weighed out and added to 1000 ml distilled water in a 2000 ml glass schott bottle.
- The mixture was rapidly boiled for 10 to 15 min until the agar has fully dissolved.
- Media was autoclaved at 121 ℃ for 30 min/liter of media then placed into a shaking water bath set at 50 ℃. The media was allowed to cool to 50 ℃ and the volume was adjusted to 1000 ml with warmed, sterile water.
- Once the media reached 50 ℃, 50 ml of heparinised rabbit blood [b] per 1000 ml of media was added, swirling the mixture to ensure complete mixing.
- The media was poured into sterile plastic petri-dishes and were allowed to solidify [c, d].
- The media was placed into plastic bags to prevent drying out and placed into a 35 ℃ incubator for a week to examine for any contamination. Examination of the plates also occurred in sterile environment to prevent contamination. If the plate was free of contamination, the media was repacked into the plastic bags, sealed, dated and stored at 4 ℃ until use. The media was used within 2 months of production to ensure the media freshness.

***NOTE:** [a.] 25 g heart infusion broth powder (Becton Dickenson) and 15 g Difco* granulated agar (Becton Dickenson) together replace the 39 g of Columbia agar base.

[b.] 50 ml of horse blood replaces the 50 ml of rabbit blood.

[c.] When pouring blood or water into the media, or pouring media into the petri dishes, rims of the bottles/ containers/ tubes are heat sterilized over a flame. All pouring is done sterilely by working in a laminar flow hood or on a surface wiped down with 10 % (v/v) formalin followed by 70 % (v/v) ethanol. Separate laboratory coats are reserved for sterile pouring and are used ONLY for this purpose in order to minimize contamination during pouring or handling.

[d.] Bijou bottles or tissue culture flasks may be used in place of Petri dishes. Bijou bottles need to be tilted at 90° in a metal rack during the setting period to produce a slope.