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Study of the immune cell function before and after freezing

Submission date 17/07/2025	**Recruitment status** No longer recruiting	[X] Prospectively registered [_] Protocol [_] Statistical analysis plan [_] Results [_] Individual participant data [X] Record updated in last year
Registration date 22/07/2025	**Overall study status** Completed	
Last Edited 31/10/2025	**Condition category** Other	

Plain English summary of protocol

Background and study aims
This study is looking at how freezing and thawing blood cells may affect immune cell functions. Immune cells are cells in our body that help fight infections. The study focuses on two types of immune cells: Natural Killer (NK) cells, which help kill infected cells; and Monocytes, which help "eat up" bacteria and other harmful things in the body. In future research, scientists want to study how stress affects our immune system. They will therefore analyze several parameters in the blood. But sometimes, blood samples can't be tested right away and need to be frozen. This is why this study is conducted: to check if freezing the cells changes their function too much compared to “fresh cells”, and if letting them "rest" overnight after thawing helps them to recover.

Who can participate?
Healthy female or male volunteers aged 18 to 60 years inclusive at the time of the blood draw.

What does the study involve?
Participants will voluntarily give a blood sample (about 26 mL, or roughly five teaspoons) that will be analyzed in this study. There will be one single visit during which the volunteers will first discuss the study with the study personnel and sign an Informed Consent Form (document explaining the study and that is signed by the participant to confirm the willingness to participate in the study). Then, some demographic information from the participants will be collected and a blood sample will be drawn. The blood will be processed to separate plasma (the fluid remaining after removing the blood cells) and immune cells. Some cells will be tested immediately, while the others will be frozen and tested later. Researchers will compare the fresh versus frozen cells to see if they perform in a similar way. They’ll also measure certain immune-related molecules (called cytokines) in the blood plasma.

What are the possible benefits and risks of participating?
No direct benefits are expected for participants. The goal is to improve future studies and research methods.
The only procedure is a blood draw, which is low risk. Minor issues like bruising or swelling may happen, and very rarely, infection or long-term marks might occur.

Where is the study run from?
This study is being led by Yakult Honsha European Research Center for Microbiology VOF, with help from Harmony Clinical Research (CRO) in Flanders, Belgium.

When is the study starting and how long is it expected to run for?
July 2025 to October 2025

Who is funding the study?
Yakult Honsha, Japan

Who is the main contact?
Mr Masatoshi Morikawa, masatoshi.morikawa@yher.be

Contact information

Type(s)
Public, Scientific, Principal investigator

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Additional identifiers

Clinical Trials Information System (CTIS)
Nil known

ClinicalTrials.gov (NCT)
Nil known

Protocol serial number
Protocol ID: YHER25-PBC_study protocol_v3.0_02Jul2025

Study information

Scientific Title
Study for the impact of PBMC cryopreservation on the immune function assays

Acronym
YHER25-PBC

Study objectives

Human peripheral blood mononuclear cells (PBMCs) are widely used in clinical studies as they can assess systemic immune function in a relatively minimally invasive manner. For an upcoming interventional study examining the link between psychological stress and upper respiratory tract infections (URTIs), PBMC-based assessments will be essential to elucidate the underlying mechanisms (Naito et al., 2025). However, immediate PBMC processing may not be feasible on all blood collection days. This study aims to evaluate the impact of cryopreservation of the PBMC cells on the outcome of the analysis of the immune function, particularly Natural Killer (NK) cell cytotoxicity and monocyte phagocytic activity. This pilot study is therefore designed to: (1) assess the effects of cryopreservation on NK cell cytotoxicity and monocyte phagocytic activity, (2) determine whether overnight recovery post-thaw improves functional readouts, and (3) validate methodological feasibility for future large-scale observational and interventional studies.

Ethics approval required
Ethics approval required

Ethics approval(s)
approved 14/07/2025, Committee for Medical Ethics UZA-UA (Drie Eikenstraat 655, Edegem, 2650, Belgium; +32 3 821 38 97; ethisch.comite@uza.be), ref: 7759

Study design
Single-arm blood collection study

Primary study design
Observational

Study type(s)
Other

Health condition(s) or problem(s) studied
To evaluate the impact of cryopreservation on immune functions

Interventions
Immune functions, focusing on cell-mediated responses such as NK cell cytotoxicity and monocyte phagocytic activity, were assessed under fresh, thawed, and recovered PBMC conditions.

This study involves a single-visit, non-interventional blood collection from healthy adult volunteers. After obtaining written informed consent, a total of 26 mL of venous blood is drawn from each participant. The blood samples are then processed to isolate peripheral blood mononuclear cells (PBMCs) and plasma.
PBMCs are subjected to cryopreservation and subsequent immune functional assays, including:
- Natural Killer (NK) cell cytotoxicity assay using K562 cancer cell line
- Monocyte phagocytic activity assay using fluorescent bacteria
These functional assays are performed under three conditions: fresh, post-thaw (immediately after thawing), and post-thaw with recovery in culture. Plasma samples are stored for cytokine measurement using ELISA.
There is no follow-up for participants beyond the single blood collection visit. The total duration per participant is approximately 30 minutes.

Intervention Type

Other

Primary outcome(s)
1. NK cell cytotoxicity, assessed by a flow cytometry-based assay using K562 target cells under fresh, post-thaw, and post-thaw with recovery conditions at one timepoint
2. Monocyte phagocytic activity, assessed by uptake of fluorescent bacteria via flow cytometry at one timepoint

Key secondary outcome(s))
1. Concentrations of IFN-γ and IL-12 measured using ELISA in cryopreserved plasma samples collected at the time of blood draw, in terms of assay feasibility, reproducibility, and sensitivity at one timepoint

Completion date
29/10/2025

Eligibility

Key inclusion criteria
1. A female or male participant aged 18 to 60 years inclusive at the time of the blood draw
2. Willing to participate in blood collection
3. A participant in good health, as confirmed by the participant (self-declaration)

Participant type(s)
Healthy volunteer

Healthy volunteers allowed
No

Age group
Adult

Lower age limit
18 years

Upper age limit
60 years

Sex
All

Total final enrolment
12

Key exclusion criteria
1. Difficulty providing blood samples
2. A history or evidence of immunodeficiency
3. Use of any prescription medication or NSAIDs within 14 days before blood draw, except for contraceptives
4. Fever at the time of the blood draw (body temperature >38°C)

5. Recent vaccination (within the past 6 months)
6. Pregnancy

Date of first enrolment
28/07/2025

Date of final enrolment
29/10/2025

Locations

Countries of recruitment
Belgium

Study participating centre
Bio-incubator
Technologiepark 94 bus 3
Ghent-Zwijnaarde
Belgium
9052

Sponsor information

Organisation
Yakult Honsha European Research Center for Microbiology VOF

Funder(s)

Funder type
Industry

Funder Name
Yakult Honsha

Alternative Name(s)
Yakult

Funding Body Type
Private sector organisation

Funding Body Subtype
For-profit companies (industry)

Location
Japan

Results and Publications

Individual participant data (IPD) sharing plan
No, individual participant data (IPD) will not be shared. This study involves a small sample size and sensitive immunological data from healthy volunteers. Due to privacy concerns and the nature of the internal research conducted by the sponsor, there is no plan to make individual-level data publicly available.

IPD sharing plan summary
Not expected to be made available