TFS-Info-02b_Information on applications for biosamples

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1. Introduction

This information sheet describes the available biosamples, the corresponding application procedure and the review process for biosample applications.

NAKO's strategy for utilizing the limited supply of biosamples is to ensure their efficient transformation into perpetually accessible and analysable data. The finite nature of the biosamples necessitates more stringent requirements for the submission of applications.

2. Available biosamples

As part of the NAKO health study, the following biosamples were and are being collected in the 18 study centres:

Baseline examination (2014-2019):	• Blood sample • DNA from Buffy Coat • Urine sample • Saliva sample • Nasal swab • Stool sample
First re-examination (2019-2024):	• Blood sample • Urine sample
Second re-examination (from 2024):	• Blood sample • Urine sample • Stool sample

One-third of the biosamples are preserved in the biosample repositories of the respective study centres, while the remaining two-thirds are stored in the central biorepository at the HMGU in Munich.

Information on the available biosamples is continuously updated through a database maintained by the central biorepository. For detailed specifications regarding the collected samples—including manufacturer, product number, treatment, processing, pipetting procedure, and storage—please refer to **Appendix 1**.

The NAKO biosamples are distinguished by their high quality and standardization, ensured through specialized Standard Operating Procedures (SOPs) and a meticulously maintained cold chain from the local study centres to the decentralized biosample storage facilities and the central biorepository.

2.1. Sample preparation and analysis

The aim is to optimise the use of biosamples for molecular phenotyping based on established and innovative methods, covering the entire spectrum of analytical capacities from a clinical, biomedical, environmental and occupational perspective. In particular, the extensive utilization of omics technologies is planned, encompassing genomics, epigenomics, transcriptomics, metabolomics, proteomics, adductomics, microbiomics, and viromics. The following samples have been reserved for specific purposes and underwent appropriate pre-treatment prior to storage: Buffy coat samples and DNA samples, extracted from the buffy coat samples should be used for genomic analyses; stabilised stool samples for transcriptomics or genomics; RNA samples for transcriptomics. See **Appendix 2**.
The sample analysis conducted by an applicant must adhere either to an existing NAKO protocol or to a protocol submitted by the applicant as an annex to the application when applying for the use of biosamples.

3. Application

The data and biosamples from the German National Cohort (NAKO) are accessible for scientific research through the NAKO Transfer Unit. Use and access applications (as well as NAKO-internal notifications of use) can be submitted through the web portal TransferHub https://transfer.nako.de/; applicants can view the data dictionary including the descriptions of the study variables and biosamples.

Applicants from outside the EU or those intending to collaborate with laboratories outside the EU must undertake special formal procedures to ensure compliance with the GDPR and should always contact the NAKO Transfer Office in advance.

General information on the application procedure can be found in the info sheet TFS-Info-03_Information_on_use_and_access_procedure. The specific requirements for applying for biosamples are outlined below.

3.1. Biosamples tab

If biosamples are required as part of a use and access application, they must be selected in the TransferHub under the biosamples tab. Only samples listed here can be verified and delivered.

In the biosamples tab, applicants can select the storage location from which the desired samples will be retrieved. As a general rule, this will be the central biorepository.

Detailed information on completing the biosample tab can be found in **Appendix 3.**

3.2. Person(s) responsible for biosamples

A person must be appointed who is responsible for the biosamples as part of the use and access project. This person must be entered as the biosample recipient in the biosample tab of the TransferHub, as a use/access agreement must be established with their institution. This use/access agreement is a prerequisite for the dispatch of the requested biosamples. If the biosamples are to be sent to several institutions, one person responsible for the biosamples must be named for each institution.

3.3. Biosample Panel

A dedicated biosample committee has been established to review biosample use and access applications. Upon request, it advises the Use and Access Committee (UAC) on biosample applications or provides an assessment.

Tasks:

- Review and prioritisation of biosample applications in accordance with the criteria specified in the Use and Access Policy
- Recommendation on the necessity of central or decentral aliquotation of the samples and, if necessary, return of the biosample residues in accordance with the Use and Access Policy.

Permanent members:

- Representative of the executive/transfer office for coordination
- Head of the central biorepository
- Representatives of the expert groups OMICs as well as Biomaterials and Laboratory Analyses and the working group Biosamples

Optional members

- Representative of the OMICS data infrastructure
- Member of the board of directors responsible for biobanking
- Representative of the Use and Access Committee
- Representative of the respective expert group(s) whose content is the subject of the respective application
- Representative for the central biosamples

For details on the review procedure, see chapter 4.

3.4. Special case: Use of decentrally stored biosamples

3.4.1. Notification of use

If biosamples stored decentrally at a study centre are to be applied for and the PI or head of the respective study centre is the main or co-applicant, the TransferHub provides a simplified application procedure through a notification of use. Notifications of use are only checked and, if necessary, authorised by the board of directors; the conclusion of a use/access agreement is not necessary due to the existing data processing agreement with the relevant study centre. Notifications of use for decentrally stored biosamples without the involvement of the PI or head of the study centre concerned are not possible. If samples stored decentrally are to be used by more than one study centre, a regular use and access application must be submitted. The procedure for notifications of use is described in detail in the info sheet TFS-Info-03_Information_on_use and access procedure .

The UAC and Biosample Panel are not involved in notifications of use.

When a notification of use is approved, the biorepository team is informed and creates a corresponding project in the laboratory information management system (LIMS) to document the sample collection. The documentation of the collection is carried out by the decentralised biobank.

If a notification of use is approved, the IT department of the Transfer Unit is also notified.

3.4.2. Use and access applications by external users for biosamples stored in study centres

External applicants, i.e. applicants who are not affiliated with the respective study centre, can only apply for biosamples that are stored at a study centre if a PI of the respective study centre acts as co-applicant in the application. In this case, a notification of use is not possible. External applicants must contact the respective study centre in advance and obtain confirmation that the desired samples may be requested. If samples from several study centres are to be applied for, the PIs of all these study centres must agree to the application and be included as co-applicants.

4. Application review

Detailed information on the application review can be found on the info sheet TFS-Info-03_Information_on_use and access_procedure. Only the additional review steps are described here. A schematic representation of the workflow can be found in **Appendix 4** .

4.1. Review by Use and Access Committee

After the formal review of an application by the Transfer Unit, the Use and Access Committee (UAC) reviews the submitted biosample use and access applications in accordance with the provisions of the Use and Access Policy. If the basic review is positive, the UAC informs the Transfer Office, which forwards the application to the Biosample Panel for a more detailed review. If UAC and Biosample Panel reviews lead to contradictory conclusions, the UAC will make reasonable attempts to resolve these contradictions. Ultimately, the UAC is responsible for the review.

Once the Biosample Panel has reported back the review results, the UAC finalises the review and sends its recommendation to the Transfer Office, which then proceeds according to the usual procedure.

4.2. Review by Biosample Panel

The Biosample Panel meets every four weeks or as required to discuss incoming biosample applications. If necessary, an application can also be reviewed in circulation. All applications involving biosamples from the central biorepository are reviewed and prioritised by this panel according to the following review criteria. Once the review has been completed, the results are sent to the Transfer Office.

4.3. Review criteria

Use and access applications that include biosamples are reviewed and prioritised on the basis of specific criteria for biosamples in accordance with the Use and Access Policy. These criteria go beyond those that apply to use and access applications that only concern study data. Notifications of use (for samples stored decentrally) are not subject to this prioritisation. The review is based on the following criteria:

- Availability of biosamples
- Checking the requirements for the data infrastructure/data volume (OMICS)
- Evaluation of competing applications
- Sample characteristics and sample size: Due to the large size of the NAKO study and its study design, applications that optimally use the breadth and depth of phenotyping in the following order are preferred:
    1. Projects that analyse as many participants as possible using one methodology, e.g. all participants at one point in time (baseline survey);
    2. Projects that analyse data and samples from all participants in the L2 or MRI subgroup at one point in time;
    3. Projects that carry out combined measurements of baseline and follow-up examinations in selected subgroups;
    4. Projects that want to use participants for large case-control studies and have great added value for the NAKO;
    5. Projects that want to analyse large control samples from NAKO for studies with external cases and offer great added value for NAKO.
- Measurements in selected sub-samples should be scientifically justified.
- Pilot studies for the development of new standardised procedures are only accepted if there is a particular legitimate interest.
- Optimal use of aliquots. In principle, as little sample material as possible should be used:

    - Applications for samples with one aliquot per biosample type are preferred over projects with two or three aliquots in order to minimise material usage.
    - Applications in which the volume is fully utilised by as many analyses as possible are given priority.
- The Biosample Panel will make a recommendation as to where biosamples should be aliquoted and what to do with any remaining samples.
- Sample quality: If applicable, justification of the quality specifications of the requested samples. Requests for samples of the highest quality (e.g. strictest compliance with SOPs and no thaw-freeze cycle) are scrutinised most closely.
- Quality-assured and well-documented methods
    - Justification, quality and documentation of the planned method. Planned methods of analysis must be well documented. If possible, protocols recognised by the NAKO and listed in the application should be used. Otherwise, the planned protocols must be submitted with the application for review.
    - A high degree of standardisation has priority, e.g. in the OMICs analysis. Approaches that can later be repeated or extended to larger sample sizes are favoured.
- Scientific priorities and value for NAKO: The review includes an assessment of the consistency or conflict of a proposal with the scientific priorities.
- Check of the required data infrastructure at the central data management of the NAKO (by the Biosample Panel).

4.4. Review results

If the review of an application results in the UAC recommending a revision, an automatic request for resubmission will be sent to the applicant. The application is released for revision and forwarded to the UAC for re-examination after resubmission. If the revision concerns biosample aspects of the application, the Biosample Panel is involved again.

If the review leads to a recommendation for approval or rejection, the procedure is carried out as specified for NAKO use and access applications. See info sheet TFS-Info-03_Information_on_use and access_procedure

5. Application release

5.1. Reservation of biosamples

1. Once an application has been reviewed by UAC and Biosample Panel, the biosamples are internally assigned to a project in LIMS. These assignments are non-binding and reversible. The time of the application review is recorded.
2. After approval by the NAKO board of directors, the samples are placed on a waiting list. At this point, the allocation becomes an exclusive reservation for the applicants.
3. After signing the use/access agreement between NAKO e.V. and (co-)users, the biosamples for the requested project are released for retrieval.

5.2. Aliquotation in central biorepository

The Biosample Panel will make a recommendation as to where biosamples should be aliquoted and what to do with any remaining samples.
If applicants use less than the total quantity per aliquot, there are generally two options:

(1) an aliquotation at the biorepository. Aliquotation can be offered centrally for all samples, except for stool samples and nasal swab samples;

or

(2) Aliquotation at the recipient.

Applicants can specify the requirement and the preferred option for aliquoting in the biosamples tab. As part of its review, the Biosample Panel then decides which type of aliquoting should be used (centralised in the biorepository or decentralised by the applicants).

In general, the following issues will be considered:

- Aliquoting makes sense if the requested volume is within certain threshold values. The decision on threshold values is made by the expert group Biomaterials and Laboratory Analyses in consultation with the biorepository team.
- In general, it is preferable to send complete aliquots to a recipient, even if not all the material has been requested, in order to avoid an additional thawing/freezing cycle.
- Remaining, unused material must be returned to the storage by the recipient. The recipient is responsible for compliance with SOPs that meet the NAKO standards.
- The aliquoting of DNA extracted from buffy coat samples will always be done centrally according to the required DNA concentration, regardless of the volume. Exceptions may be granted if special DNA isolation procedures are absolutely necessary.

The costs for centralised or decentralised aliquoting, including return transport and the necessary tubes and racks, are borne by the user of the biosamples. The specifications for tubes and racks are provided by the central biorepository.

6. Transfer of biosamples

(Info sheet TFS-Info-05 (in progress))

6.1. Release of biosamples

- The Trust Centre and biorepository must be notified of the board of directors` approval of an application. At this point, the applicants are informed of an estimated time for the sample compilation. The times may vary depending on the storage location and call-offs to be carried out in parallel.
- Release requirements.
  - After signing the agreement between NAKO e.V. and (co-)users, the samples can be shipped to the recipient.
  - Biosamples with valid informed consent can be retrieved from storage. However, consent can also be withdrawn after the samples have been sent to an applicant. In such cases, there is no impact on the processes started, including the sample analyses. If the samples are withdrawn during the sample analysis, the associated data is removed from CentraXX and no further connections with the ID-LIMS are possible.

7. Costs

The following costs must be covered by the applicant's institution for centrally stored NAKO biosamples:

- Racks, tubes, packaging material, dry ice, transport and, if applicable, the same costs for returning residual samples.

Until further notice, transport will be carried out via TNT Express (or FedEx Express in future), using the TNT customer number of the biosample recipient. Prices can be found on the homepage of TNT (TNT - Shipping costs).

The costs for aliquoting must be agreed upon with the biorepository (contact person: Julia Six-Merker; julia.sixmerker@helmholtz-munich.de).

The costs for the use of decentralised samples must be clarified with the decentralised biosample storage facility.

Appendix 1: Specifications of the samples collected during the baseline and first re-examination (manufacturer, pre-analysis and storage conditions)

Baseline				
Collection	**Manufacturer/ product number**	**Handling/processing in the study centre**	**Pipetting procedure**	**Storage**
Blood samples				
2x 10 ml serum tube with clotting activator without gel	BD Life Science, Franklin Lakes, USA / 367896	4 x inverted, clotting time 30-45 minutes, centrifugation 10 minutes, 15°C, 2500xg	Pipetting robot (Hamilton Robotics, Reno, USA), plates with 96 wells (Azenta Life Sciences, Chelmsford, USA) 250 µl and 500 µl tubes)	• Central biorepository (2/3 of all collected samples): ○ 500µl at -80°C ○ 250µl tubes at -180°C • Local back-up storage (1/3 of all collected samples) ○ -80°C/-180°C
3x 10 ml $_2$EDTA tube (1.8mg/ml)	BD Life Science, Franklin Lakes, USA / 367525	4 x inverted, 30 seconds - max. 5 minutes blood mixer, interim storage until shipping by room temperature	Pipetting robot, plates with 96 wells, separated into: • Plasma (250 µl)	• Central biorepository (2/3 of all collected samples) ○ -180°C • Local back-up storage (1/3 of all collected samples) ○ -80°C/-180°C
			• Erythrocytes (250 µl tubes)	• Central biorepository (2/3 of all collected samples) ○ -180°C • Local back-up storage (1/3 of all collected samples) ○ -80°C/-180°C
			• Extended buffy coat (for later DNA isolation) (500 µl tubes)	• Central biorepository (all collected samples) ○ 2x500µl -80°C, one used for DNA isolation ○ 4x500µl -180°C ○
1x 250/400 µl gDNA	Revvity, Waltham, MA, USA / CMG-714 R	Fully automated DNA isolation with the chemagic DNA BC/Saliva Kit H96 on the chemagic Prime 8	• DNA isolated from 1x buffy coat	• Central biorepository ○ 1 x 250/400 µl -80°C
1x 175 µl, 60 ng/µl gDNA	Revvity, Waltham, MA, USA	Fully automated DNA normalisation on the chemagic Prime 8	• Normalisation of 1x gDNA to 60 ng/µl	• Central biorepository ○ 1 x 175 µl -80°C
2.5 ml RNA stabilised blood with PAXgene not yet available	BD Life Science, Franklin Lakes, USA / 762165	10 x inverted, 1-2 hours stored at room temperature, 24 hours -max. 72 hours by -20°C, then put into -80°C		• Central biorepository (all collected samples) -80°C (semi-automated storage system) Prior to storage, the RNA samples were volume reduced in central biorepository. The workflow is as follows: thaw, transfer to 50 ml flacons, centrifuge, remove supernatant, collect in resuspension buffer and vortex, transfer to Azenta tubes
Urine samples				

100 ml collecting cup	BD Life Science, Franklin Lakes, USA / Urine collection cup 364941, Urine tube / 365000	10 ml transferred into urine vacutainer, centrifugation 10 minutes, 15 °C, 2500xg	Pipetting robot, plates with 96 wells (250 µl and 500 µl tubes)	• Central biorepository (2/3 of all collected samples) ○ 500µl at -180°C ○ 250µl at -180°C • Local back-up storage (1/3 of all collected samples) ○ -80°C/-180°C
Saliva samples				
Paraffin chewing gum	GC Germany, Bad Homburg, Germany,	Participant chews gum for one minute, then saliva is dispensed; if necessary, the chewing time is extended by another minute	Pipetted by hand, plates with 96 wells (500 µl tubes)	• Central biorepository (all collected samples) ○ -80°C
Medicine cup with lid	Sarstedt, Nümbrecht, Germany / 75.1337.500 + 76.1340.560			
Nasal swabs				
Sterile nylon flock swabs	Copan Flock Technologies, Brescia, Italy	Two swabs are transferred into one 2 ml cryotube pre-filled with RNA-later	-	• Central biorepository (all collected samples) ○ -80°C
Stool samples				
At the same day or one day before the visit in the study centre, at home with stool catcher for sample collection	Süsse Labortechnik, Gudensberg, Germany / S1000	Stabilised stool sample is sent to the central university laboratory Greifswald, where it is aliquoted in 500 µl tubes	-	• Central biorepository (all collected samples) ○ -80°C
Two stool collecting tubes were filled: • one is without additives	Biosepar, Mühldorf am Inn, Germany			
• one tube was prefilled with 3.5ml RNA-later (RNA stabilising additive with K2EDTA)	Quiagen, Hilden, Germany			
The samples are packed in a protective bag "Süsse Schutzbeutel" in a box "Süsse Post Box	Labortechnik, Gudensberg, Germany / H64490 and H11227			

Slim" and brought to the study centre at the time of the examination				
Follow-up examination				
Collection	Manufacturer/ product number	Handling/processing in the study centre	Pipetting procedure	Storage
Blood samples				
1x 10 ml serum tube with clotting activator without gel	BD Life Science, Franklin Lakes, USA / 367896	4 x inverted, clotting time 30-45 minutes, centrifugation 10 minutes, 15°C, 2500xg	Pipetting robot (Hamilton Robotics, Reno, USA), plates with 96 wells (Azenta Life Sciences, Chelmsford, USA) 250 µl tubes)	• Central biorepository (2/3 of all collected samples): ○ 1x250µl tube at -80°C* ○ 7x250µl tubes at -180°C • Local back-up storage (1/3 of all collected samples) ○ -80°C/-180°C
2x 10 ml K_2EDTA tube (1,8mg/ml)	BD Life Science, Franklin Lakes, USA / 367525	4 x inverted, 30 seconds - max. 5 minutes blood mixer, interim storage until shipping by room temperature	Pipetting robot, plates with 96 wells, separated into: • Plasma (250 µl tubes)	• Central biorepository (2/3 of all collected samples) ○ 250 µl at-180°C • Local back-up storage (1/3 of all collected samples) ○ -80°C/-180°C
			• Extended buffy coat (for later DNA isolation) (500 µl tubes)	• Central biorepository (2/3 of all collected samples) ○ 1x500µl -80°C** ○ 1x500µl -180°C • Local back-up storage (1/3 of all collected samples) ○ -80°C/-180°C
Urine samples				
100 ml collecting cup	BD Life Science, Franklin Lakes, USA / Urine collection cup 364941, Urine tube / 365000	10 ml transferred into urine vacutainer, centrifugation 10 minutes, 15 °C, 2500xg	Pipetting robot, plates with 96 wells (250 µl)	• Central biorepository (2/3 of all collected samples) ○ 250µl at -180°C • Local back-up storage (1/3 of all collected samples) ○ -80°C/-180°C
Second Follow-Up				
Collection	Manufacturer/ product number	Handling/processing in the study centre	Pipetting procedure	Storage
Blood samples				
1x 10 ml serum tube with clotting activator without gel	BD Life Science, Franklin Lakes, USA / 367896	4 x inverted, clotting time 30-45 minutes, centrifugation 10 minutes, 15°C, 2500xg	Pipetting robot (Hamilton Robotics, Reno, USA), plates with 96 wells (Azenta Life Sciences, Chelmsford, USA) 250 µl and 500 µl tubes)	• Central biorepository (2/3 of all collected samples): ○ 500µl at -80°C ○ 250µl tubes at -180°C • Local back-up storage (1/3 of all collected samples) ○ -80°C/-180°C

2x 10 ml K_2EDTA tube (1,8mg/ml)	BD Life Science, Franklin Lakes, USA / 367525	4 x inverted, 30 seconds - max. 5 minutes blood mixer, interim storage until shipping by room temperature	Pipetting robot, plates with 96 wells, separated into: • Plasma (250 µl) • Extended buffy coat (for later DNA isolation) (500 µl tubes)	• Central biorepository (2/3 of all collected samples) o -180°C • Local back-up storage (1/3 of all collected samples) o -80°C/-180°C • Central biorepository (all collected samples) o 2x500µl -80°C, one used for DNA isolation o 4x500µl -180°C o
1x 10 ml lithium-heparin tube	BD Life Science, Franklin Lakes, USA / 367526	4 x inverted, 30 seconds - max. 5 minutes blood mixer, interim storage until shipping by room temperature	Pipetting robot, plates with 96 wells, separated into: • Plasma (250 µl)	• Central biorepository (2/3 of all collected samples) o -180°C • Local back-up storage (1/3 of all collected samples) o -80°C/-180°C
2.5 ml RNA stabilised blood with PAXgene not yet available	BD Life Science, Franklin Lakes, USA / 762165	10 x inverted, 1-2 hours stored at room temperature, 24 hours -max. 72 hours by -20°C, then put into -80°C		• Central biorepository (all collected samples) -80°C (semi-automated storage system) Prior to storage, the RNA samples were volume reduced in central biorepository. The workflow is as follows: thaw, transfer to 50 ml flacons, centrifuge, remove supernatant, collect in resuspension buffer and vortex, transfer to Azenta tubes
Urine samples				
100 ml collecting cup	BD Life Science, Franklin Lakes, USA / Urine collection cup 364941, Urine tube / 365000	10 ml transferred into urine vacutainer, centrifugation 10 minutes, 15 °C, 2500xg	Pipetting robot, plates with 96 wells (250 µl and 500 µl tubes)	• Central biorepository (2/3 of all collected samples) o 500µl at -180°C o 250µl at -180°C • Local back-up storage (1/3 of all collected samples) o -80°C/-180°C
Stool samples				
At the same day or one day before the visit in the study centre, at home with stool catcher for sample collection	Süsse Labortechnik, Gudensberg, Germany / S1000	Stabilised stool sample is sent to the central university laboratory Greifswald, where it is aliquoted in 500 µl tubes	-	• Central biorepository (all collected samples) o -80°C
Stool collecting tube was filled:	Biosepar, Mühldorf am Inn, Germany			

• one tube was prefilled with 3.5ml RNA-later (RNA stabilising additive with K2EDTA)	Quiagen, Hilden, Germany
The samples are packed in a protective bag "Süsse Schutzbeutel" in a box "Süsse Post Box Slim" and brought to the study centre at the time of the examination	Labortechnik, Gudensberg, Germany / H64490 and H11227

* samples stored at -80°C since August 2020

** samples stored at -180°C since August 2020

Appendix 2: Samples and their availability from the central biorepository of the baseline and follow-up examination (status: January 2024)

Sample type	Volume	Aliquots per subject (Total/in Biorep)	Study participants (Completeness, %)	Study participants at central storage (Completeness, %)	Retrievable	Pre-processing required	Preferred analysis
Baseline examination*							
Plasma	250 µl	48/ 32	97.1	94.9	Yes (-180°C storage temperature)	No	
Serum	250 µl	30/ 20	97.4	96.0	Yes (-180°C storage temperature)	No	
Serum	500 µl	1/ 1	96.3	94.3	Yes (-180°C storage temperature)	No	
Erythro-cytes	250 µl	6/ 4	96.0	94.6	Yes (-180°C storage temperature)	No	
Buffy Coat	500 µl	6/ 5	95.5	93.7	Yes (-80°C and -180°C storage temperature)	DNA isolation	DNA isolation → genomics
gDNA	400 (250) µl	1/ 1	88.3	88.3	Yes (-80°C storage temperature)		
gDNA 60 ng/µl Normalisation	175 µl	1/ 1			Yes (-80°C storage temperature)	Currently underway at the HMGU	Genotyping, Whole Genome Sequencing, any genomic analysis
Urine	250 µl	12/ 8	97.3	95.9	Yes (-180°C storage temperature)	No	
Urine	500 µl	4/ 4	96.4	94.6	Yes (-180°C storage temperature)	No	
Saliva	500 µl	2/ 2	93.5	92.5	Yes (-80°C storage temperature)	DNA isolation, to be done by user	DNA isolation → genomics (microbiome)
Nasal Swab	2 ml	1/ 1	93.5	91.5	Yes (-80°C storage temperature)	DNA isolation, to be done by user	DNA isolation → genomics (microbiome)
Native Stool	5 ml	1/ 1	78.3	75.3	Yes (-80°C storage temperature)	No	
Stabilised Stool	500 µl	4/ 4	84.2	78.3	Yes (-80°C storage temperature)	RNA isolation	RNA isolation -> transcriptomics
RNA (PAX genes) not available available	10 ml	1/ 1	9.9	7.9	No, will be volume reduced (500 µl) at the central Biorepository	-	-
RNA (500 µl) not yet available	500 µl	1/ 1	83.6	83.5	Yes, depending on the state of the RNA volume reduction (-80°C storage temperature)	RNA isolation, to be done by user	RNA isolation → transcriptomics

Follow-up examination**							
Plasma	250 µl	18/ 12	97.9	94.6	Yes (-180°C storage temperature)	No	
Serum	250 µl	12/ 8	97.2	94.7	Yes (-80°C samples since August 2022 and -180°C storage temperature)	No	
Buffy Coat	500 µl	2/ 2	96.8	88.8	Yes (-180°C storage temperature)	DNA isolation, extraction will take place at HMGU, stored at -80 C	DNA isolation → genomics
Urine	250 µl	6/ 4	98.7	96.1	Yes (-180°C storage temperature)	No	
Second Follow-up examination ***							
Buffy Coat	500 µl	3/ 2	12.6	7.3	Yes (-80°C storage temperature)	DNA isolation, extraction will take place at HMGU, stored at -80 C	DNA isolation → genomics
Plasma	250 µl	18/ 12	12.8	7.2	Yes (-180°C storage temperature)	No	
Heparin-Plasma	250 µl	10/ 6	12.6	7.1	Yes (-180°C storage temperature)	No	
RNA (PAX genes) not yet available	10 ml	1/ 1	12.1	4.1	Yes (-80°C storage temperature)	-	
RNA (500 µl) not yet available	500 µl	1/ 1	0	0	Yes, depending on the state of the RNA volume reduction (-80°C storage temperature)	RNA isolation, to be done by user	RNA isolation → transcriptomics
Serum	250 µl	12/ 8	12.6	7.2	Yes (-180°C storage temperature)	No	
Serum	500 µl	1/ 1	12.5	7.1	Yes (-180°C storage temperature)	No	
Urine	250 µl	6/ 4	12.8	7.4	Yes (-180°C storage temperature)	No	
Urine	500 µl	6/ 4	12.8	7.5	Yes (-180°C storage temperature)	No	

Numbers are subject to change when samples are released from stock or consent is withdrawn.

*Planned number of participants for baseline examination has been set to 205,000 except for stool samples. Collection of stool samples had not been introduced by the start of the study, but only in October 2016. In the case of the stool samples, the planned number of participants was set at 100,000.

**Planned number of participants for follow-up examination has been set to 135,000.

*** Planned number of participants for second follow-up examination has been set 87.000

Appendix 3: Biosamples Tab

Person responsible for biosamples

Note: *Which of the co-applicants should be responsible for the project? Person will be contacted by biorepositories. Person will be responsible for managing logistics with laboratories and ensuring that samples are utilised or destroyed after end of project.*

Description of planned analyses and analytes

Note: *1. Describe the planned analyses, or name standard protocols for analyses.*
2. If you plan a sample aliquot or return of residual samples, please indicate this here.
3. Processing biosamples using a non-published method, you must upload your own biosample protocol.

Analysis (Description of planned analyses and analytes)

Biosample type (please select)

- *Buffy Coat*
- *gDNA*
- *Erythrocytes*
- *Plasma*
- ~~*RNA blood*~~
- *Serum*
- *Nasal swab*
- *Saliva*
- *Urine*
- *Stabilised stool*
- *Native stool*

Required quantity (By 'required quantity' is meant the amount for the respective analysis and additionally also the required amount for processing (sample division / pipetting).)

- *xxx µl*
- *xxx µg*
- *xxx ng/µl*

Subpopulation

- *Data Freeze 100.000*
- *MRI study*
- *Soccer Health Cohort*
- *NAKO participants (200k)*

Examination wave

- *Baseline examination*

Storage location

- *No biosample type selected*

Shipping address (The address (person in the laboratory, street, postcode and city) of the laboratory should be entered here. For biosamples that are to be analysed in different laboratories, the order can be adjusted using the arrow keys. The person responsible for the biosamples is responsible for the logistics of the intermediate dispatch.)

Add analysis
This button can be used to add further biosample analyses to the application.

Justification of feasibility for analysis of biosamples
Note: *Justification/information on costs incurred, information on technical/financial feasibility*

Appendix 4: Workflow biosample application