Danish National Cohort Study of Effectiveness and Safety of SARS-Cov-2 Vaccines (ENFORCE)

Protocol Instruction Manual

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Contents

List of Abbreviations

AE	Adverse Event
AU	Aarhus Universitetshospital
CHIP	Centre for Health and Infectious Diseases Research
CPR	Det Centrale Personregister
CPT	Cell Preparation tube with Sodium Citrate
CRF	Case report Form
CV	Curriculum vitae
DSC	Data and Statistical Centre
eCRF	Electronic Case Report Form
EDTA	Ethylenediaminetetraacetic acid
ENFORCE	Danish National Cohort Study of Effectiveness and Safety of SARS-Cov-2 Vaccines
GCP	Good Clinical Practice
PIM	Protocol Instructions Manual
PAXgene	Blood RNA Tube
PBMCs	Peripheral blood mononuclear cells
REDCap	Electronic Data Capture System
SAE	Serious Adverse Event
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SSI	Statens Serum Institut
SST	Danish Health Authority (Sundhedsstyrelsen)

1 SITE-SPECIFIC COMMUNICATION AND PROCEDURES

1.1 Communication

Communication with investigators and staff regarding the ENFORCE study will take place electronically, via e-mail and telephone and conference calls (Zoom/Teams). This will allow rapid and uniform communication to ensure all participating investigators stay informed about the conduct and progress of the study.

1.2 Contact Information

Sponsor and Principal Investigator:

Sponsor/ Data and Statistical Centre (DSC):	**Name** **Title** **Department** **Address:** **Telephone:** **E-mail:**	Jens Lundgren Professor Centre for Health and Infectious Diseases Research (CHIP) Department of Infectious Diseases Blegdamsvej 9, 2100 Copenhagen Ø, Denmark +45 35 45 57 57 Enforce.rigshospitalet@regionh.dk
Principal Investigator/Coordination Center	**Name** **Title** **Department** **Address:** **Telephone:** **E-mail:**	Lars Østergaard Professor i infektionssygdomme og ledende overlæge Infektionsklinikken Aarhus Universitetshospital, Palle Juul-Jensens Boulevard 45, 8200 Aarhus N +4578452800 inf-forskning@rm.dk

List of Regional Coordinators:

Region	Regional Coordinator
Region Nordjylland	Henrik Nielsen, professor og ledende overlæge, Aalborg Universitetshospital
Region Syddanmark	Isik Johansen, professor og overlæge, Odense Universitetshospital
Region Sjælland	Lothar Wiese, ph.d. og overlæge, Sjællands Universitetshospital Roskilde
Region Midtjylland	Nina Breinholt Stærke, læge og forskningsleder
Region Hovedstaden	Thomas Benfield, professor og overlæge, Hvidovre Hospital

2 Participant Recruitment and Inclusion

2.1 Recruitment Strategies

The following recruitment strategies are approved by the Ethics Committee:

Citizens who are offered the invitation for vaccination via their e-boks and citizens who already have booked time for vaccination can afterwards be informed of the study per e-mail or letter (normal mail or e-boks). Study staff will be present at vaccination centres to inform citizens of the study.
An invitation through ENFORCE study information, which is disseminated by other channels (flyers, posters, websites, etc.) e.g. to health care staff which are notified via their employer about vaccination.

Patients followed at hospitals or General Practitioners that are offered vaccination can be invited to take part in the study by email or letter from their doctor/department. If a telephone contact is planned with the patient, information about the study may be given per telephone.

People considering participating are informed to contact the trial project staff before the vaccination and schedule a visit via www.enforce.dkwhere further information on the study can be found.

Participants should still book the vaccination according to instructions given in the invitation from SSI or by their employer.

2.2 Inclusion and Consent

In order to investigate the effect of the vaccine against COVID-19, participants are informed that blood samples will be drawn six (6) times within two (2) years. Currently a total of 10,000 participants, 2,500 per vaccine, are expected to participate.

Participants must answer questions about health and medicine and fill out four symptoms forms regarding possible side-effects from the vaccine.

To be enrolled into the trial the subject must meet the following inclusion criteria:

- Written informed consent must be obtained before any trial related procedures are performed
- Male or female eligible for SARS-CoV-2 immunization (as defined by SST in the national vaccination plan)
- The subject must be willing and able to comply with trial protocol (re-visits and biological samples)

2.3 Sub-studies

Within the ENFORCE main study there are sub-studies under separate consent:

- sub-study 1 (cellular immunity), which aims to include 250 patients (100 healthy and 150 high-risk individuals) from each vaccine group. At all 6 regular study visits extra samples are drawn - Live cells (PBMCs) and PAX tubes (for transcriptomic analysis). Not all sites participate in this sub-study as it requires a specific size spinner and that all samples are drawn early in the day (before 12 noon) to secure arrival at AUH or processing the same day.

- sub-study 2 (safety), which is a generic safety study that can be activated upon decision by the ENFORCE Scientific Steering Committee provided that the Danish Health Authority and/or Medicines Agency agrees that an “issue of concern” has arisen for one or more vaccines. There is no predefined number of subjects for the sub-study. Extra samples will be collected at one or more extra study visits - typically at day 7 after (first and second) vaccination.

2.4 3rd vaccination

For the 3rd vaccination, all participants in ENFORCE will receive an invitation for two additional visits: Visit X and visit Xc, for capturing this data.
The additional visits require: an additional consent to be signed, 1 or 2 extra visits and additional collection of blood 12 ml per visit (+27 ml per visit for participants in substudy 1). Data collected are the same as for the regular study visits and both visits can replace regular study visits, if within the window period.

For participants that receives the invitation to participate after they have received the 3rd vaccination:

- book a visit Xc within 28 days (+8) days of the vaccination.
- go to form X and tick ”*Studiebesøg ikke gennemført*” and tick “*Dato for 3.vaccination er udenfor besøgsvindue*”.

Remember that If visit X or Xc are within 30 days before a planned follow up visit (visit 4,5 or 6) **visit X or Xc can replace the visit 4,5 or 6:**

- Go to the future form 4, 5 or 6 and tick “*Studiebesøg ikke gennemført*”. Write reason: ”*Studiebesøg X eller Xc ligger 30 dage før det planlagte besøg*”.

3 Regulatory Requirements and Trial Documentation

3.1 Investigator Site File

Sites has received an Investigator Site File binder: Please ensure that the file contain the below trial related documentation:

- ENFORCE Protocol v2.0
- ENFORCE Site Information Form
- ENFORCE Signature Delegation and Training Log
- ENFORCE Source Data Location Agreement V2 (describing where source data is found at the site)
- ENFORCE Protocol Instructions Manual (PIM) 2021SEP05
- ENFORCE Training materials (slides)
- ENFORCE Recruitment Materials (poster, letter, letter-text)
- ENFORCE Laboratory manual (main study)
- ENFORCE Sub-study #1 (cellular immunity) Laboratory manual
- ENFORCE Monitoring Plan
- ENFORCE Subject Source Data Document V2 2021APR22
- ENFORCE Subject Identification log_V2
- ENFORCE Participant information_Informed Consent(s)_GDPR (one per Region)
- ENFORCE Worksheets (PDFs of all eCRF)
- ENFORCE Symptoms form 1AB and 2AB (for participants)

- ENFORCE Specimen Identification log
- ENFORCE Specimen Shipping log
- ENFORCE Sub-study #1 (cellular immunity) Specimen Identification Log
- ENFORCE Sub-study # 1 (cellular immunity) Specimen Shipping Log

Upon activation of ENFORCE Sub-study 2 for a specific safety-issue sites will receive:

- *ENFORCE Sub-study 2 Specimen Identification Log*
- *ENFORCE Sub-study 2 Specimen Shipping Log*
- *ENFORCE Sub-study 2 Lab-manual if required*

(see Annex 2 for details of the activated substudy 2 for the AstraZeneca participants and the documents to be kept in the Investigator Site File).

Additional documents

- ENFORCE Protocol V5.0
- ENFORCE Participant information and Informed Consent_V1.0 (tillægssamtykke)
- ENFORCE Worksheets (PDFs of visit X and Xc eCRF)
- ENFORCE Symptoms form 3A and 3B (for participants)
- ENFORCE Subject Source Data Document V3 2021OCT06
- ENFORCE Subject Identification Log Boost 2021OCT06

The Investigator Site File is a GCP requirement and it is the site's responsibility to add the requested documentation and keep the binder updated during the trial.

If a site uses worksheets (paper CRFs) to record study data, these must be kept and stored in a CRF binder. The Investigator Site File and the CRF binder should be stored in a safe location.

ALL DOCUMENTS MUST BE STORED AND KEPT

Trial related documents can also be found at the study website: ***https://chip.dk/Research/Studies/ENFORCE/Study-Documents***

3.2 Site Initiation procedures

Before initiation sites must complete and submit to Sponsor the requested regulatory documents:

- Curriculum vitae (CV) of Investigator and study staff – Dated and signed
- GCP certificate(s) for study staff
- ENFORCE Site Information Form
- ENFORCE Signature Delegation and Training Log
  (all staff participating in the study should be on this log)
- ENFORCE Source Data Location Agreement (describing where source data is found at the site)

All study staff at trial sites must be trained in GCP prior to study start. If staff have no previous GCP training they are recommended to follow the online GCP course https://gcp-enhed.dk/e-learning-webinar/e-learning/

Site initiation visits are not feasible due to the COVID pandemic; hence site staff will be trained online. All study staff listed on the delegation log are requested to be present at the online training. Trained staff may subsequently train other colleges that are to be involved in the study.

3.3 Source Data Verification

Subjects enrolled in the ENFORCE study have not necessarily had a contact to the hospital system and therefore may not have a medical record where sites can record the source data.

The protocol specifies a minimum requirement for the following data to be source data-verifiable in source documentation other than the worksheet/eCRF:

- Date of birth
- Confirmation of participation in the trial
- Confirmation of subject eligibility (in/exclusion criteria)
- Date of each trial visit and telephone contact
- Subject discontinuation from the trial, including reason.

The following items may be direct entries in the e-CRF:

- Concomitant diseases and medication
- Relevant medical history
- Any AE and SAE

It is up to the site where the source data will be documented as long as it can be verified during monitoring. Sites may use the ENFORCE Subject Source Data Document that has been developed to facilitate the process of documenting source data. Please store this form in the ENFORCE Investigator Trial File.

If the site has its own system for documenting source data it is not necessary to use the ENFORCE Subject Source Data. Alternatively, if a subject already has a medical journal, a note about the patient's participation in the study can be a possibility.

ENFORCE

Subject enrollment and identification Log

Sitenumber: Site Name: Principal Investigator:

Patients who have signed the informed consent and are enrolled into the study

PID Number (REDCap Number)	CPR Number	Date of enrolment	Patient Name	Contact Information (phone) (Only if patient accepts)	End of trial
					☐ Completed ☐ Lost to follow-up ☐ Withdrawn ☐ Other
					☐ Completed ☐ Lost to follow-up ☐ Withdrawn ☐ Other
					☐ Completed ☐ Lost to follow-up ☐ Withdrawn ☐ Other
					☐ Completed ☐ Lost to follow-up

4 Preparation of Study visits

4.1 Print Forms and Logs

Before the study visits you must print and prepare the following forms and logs:

1 per participant:

- ENFORCE Participant information and Informed Consent (Protocol V4)
- ENFORCE Subject Source Data Document V2
- ENFORCE Symptoms Form (only if participant cannot fill out online) (1A and 1B for visit 1 and 2A and 2B for visit 2)
- ENFORCE Worksheets/prints of eCRFs (only if you cannot do online data entry during study visits)

1 per day:

- ENFORCE Subject Identification Log_V2
- ENFORCE Specimen Identification Log
- ENFORCE Specimen Shipping Log

If your site is participating in Sub-Study # 1 (Cellular Immunity) please also print:

- ENFORCE Sub-Study #1 (cellular immunity) Specimen Identification Log
  ENFORCE Sub-Study #2 (cellular immunity) Specimen Shipping Log

If sub-study 2 (safety) is activated specific documents are provided and must be printed.

If your site have participants that receives the 3rd vaccination, please also print:

- ENFORCE Participant information and Informed Consent_v1.0 (tillægssamtykke)
- ENFORCE Subject Source Data Document V3
- ENFORCE Symptoms Form 3A and 3B (only if participant cannot fill out online)
- ENFORCE Worksheets/prints of X and Xc eCRFs (only if you cannot do online data entry during study visits)

4.2 Barcode Scanner

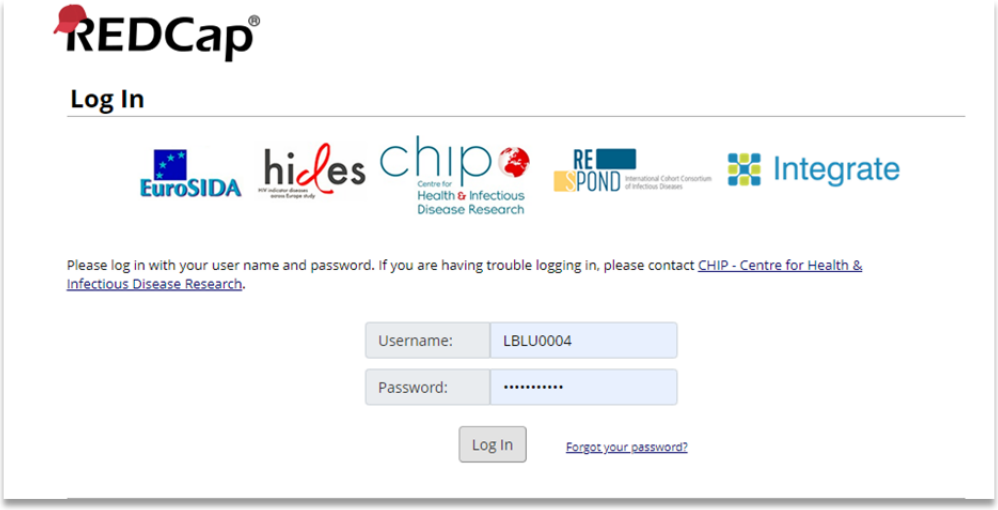
Sites are required to have a barcode scanner. To minimize errors, the use of the scanner ***is strongly recommended to*** be used to register:

- Subjects' CPR-number from Sundhedskort
- Specimen labels (see ENFORCE Laboratory manual for instructions)

4.3 Access to Electronic Data Capture System - REDCap

To request access to REDCap:

- Please send an email to **enforce.rigshospitalet@regionh.dk** The email must contain Name, Email-address and the name of the institution/ workplace for the site staff needing access.
- Within 3 days, an email will be sent to site staff, containing username and a link to a code



After receiving the login details for REDCap you need to:

- Go to https://redcap.regionh.dk/redcap
- Log-on with your username and password
- The first time you log on you will be asked to change the password and set up a password recovery question.
- REDCap uses a two (2) factor identification code, so besides entering username and code, you will receive a verification code by email each time you log in to REDCap, which also must be entered.

Please note that the login details are personal, and if more staff at your site needs access, please require additional individual logins by following the above procedure.

See also Chapter 8 for further REDCap instructions (in Danish)

4.4 Laboratory materials

Sites will receive the following laboratory materials for the specimen collection procedures:

- roll of labels with consecutive numbered barcodes
- EDTA plasma collection tubes
- Serum collection tubes
- Vacuette Transport Box
- Isothermal Carrier Bags that hold the Vacuette Transport Box

Main study specimens must be shipped to SSI daily. NOTE: Study visits can be performed Sunday-Thursday, as SSI can only receive specimens Monday-Friday. For more information on the specimen handling procedure, please see the ENFORCE Laboratory Manual.

Sub-study 1 (cellular immunity)

If your site is participating in Sub-Study #1 (cellular immunity) will also receive

- Sub-Study 1 labels
- PAXgene tubes
- CPT citrate tubes

Sub-study 1 specimens must be shipped to AU daily. Sub-study 1 samples MUST be taken in the morning – before noon – to ensure transport to AUH for processing on the same day. The day of specimens collection must be agreed with AUH

For more information on the specimen handling procedure for sub-study 1, please see the ENFORCE sub-study 1 (cellular immunity) Laboratory Manual.

Sub-study 2 (safety)
If the ENFORCE Sub-study 2 is activated a specific Lab-manual will be developed and shared as required, together with sub-study 2 labels and materials.

5 Study visits

5.1 Study visit overview schedule

Visit number	Schedule	Acceptable deviation (+/-) from date
1	Day of first vaccination	14 days to 30 minutes before first vaccination
2	Day of second vaccination [*If subject will only receive one vaccine shot, no Visit 2 will be performed]	5 days to 30 minutes before second vaccination
3	3 months after first vaccination [*If the day of Visit 2 was 3 months (+/- 14 days) after 1st vaccine, no Visit 3 will be performed]]	+/- 14 days
4	6 months after first vaccination	+/- 14 days
5	12 months after first vaccination	+/- 14 days
6	24 months after first vaccination (End of trial)	+/- 14 days

5.2 Study visits for 3rd vaccination

Visit number	Schedule	Acceptable deviation (+/-) from date
X	Day of third vaccination	14 days to 30 minutes before the third vaccination
XC	28 Days after third vaccination	-/+ 8 days

NOTE: Study visits can be performed Sunday-Thursday, as SSI can only receive specimens Monday-Friday

Sub-study 1 samples MUST be taken in the morning – before noon – to ensure transport to AUH for processing on the same day. The day of specimen collection must be agreed with AUH

5.3 Study visit procedures

Visit 1: Enrolment Visit (from 14 days before until 30 minutes before first vaccination)	**Study Personnel must:** **1.** Provide information and answer questions about the ENFORCE study **2.** Obtain the written informed consent from the participant before any other trial procedures are performed; all participants are invited to take part in the generic sub-study 2 (safety) **3.** Ensure compliance with inclusion and exclusion criteria **4.** Complete ENFORCE Subject Identification Log ***5.*** **Scan** CPR number into e-CRF **6.** Record date and place of planned vaccination **7.** Record relevant medical history and concomitant medication. **8.** Collect whole blood in 1 x 6 mL EDTA tube (for plasma isolation) and 1 x 6 mL "dry glass" (for serum isolation) **(For details on blood sample collection please see laboratory manual)** **9.** **Scan** specimen labels into eCRF form (or stick label onto paper worksheet and scan later) **10.** Stick a label onto the Specimens Identification Log and a label on the Specimens Shipping Log **11.** Give instructions to participant on how to complete the Symptoms Form 1A 7 days and 1B 14 days after the vaccination. Explain that the participant will receive a letter in E-boks with a non-clickable link to the form to fill it in online. Provide participants with 2 copies of paper form if requested. **12.** Schedule the next visit with the study participant or instruct the participant to book visits though the website: www.enforce.dk **13.** Ensure shipment of blood specimens and ENFORCE Specimens Shipping log to SSI (**please see laboratory manual)** *ENFORCE Sub-study 1 (cellular immunity) - for any details please see the sub-study 1 laboratory manual:* ***1.*** *Inform and offer entry into the sub-study 1 under separate consent* ***2.*** *Obtain written informed consent for the sub-study 1 before any other trial procedures are performed (part of the Participant Information document)* ***3.*** *Collect live cells (PBMCs) and PAX tubes* ***4.*** *Scan specimen labels into eCRF form (or stick label onto paper worksheet and scan later)* ***5.*** *Stick a label onto the CELLULAR IMMUNITY Specimens Identification Log and a label on the CELLULAR IMMUNITY Specimens Shipping Log* ***6.*** *Ensure shipment to AUH*

Visit 1b (7 days (+/- 2 days) after first vaccination)	Generic Sub-study 2 For participants who has consented to Sub-study No. 2 collect blood samples upon activation of sub-study 2
Visit 1c	Activated Sub-Study 2 - AZ For participants who received Astra Zeneca collect blood samples (see annex 2 for procedure)

Visit 2 (from 5 days before until 30 minutes before second vaccination) * *If subject will only receive one vaccine shot, no Visit 2 will be performed*	**Study Personnel must:** ***1.*** **<u>Scan</u>** the CPR number into e-CRF **2.** Record any signs and symptoms reported from participants (all grades) **3.** Record concomitant medication. **4.** Collect whole blood in 1 x 6 mL EDTA tube (for plasma isolation) and 1 x 6 mL "dry glass" (for serum isolation) **(For details on blood sample collection please see laboratory manual)** **5.** **<u>Scan</u>** specimen labels into eCRF form (or stick label onto paper form) **6.** Stick a label onto the Specimens Identification Log and a label on the Specimens Shipping Log **7.** Check electronic/collect paper symptom form 1A filled out by the subject 7 days after the first vaccination, and for, 1B 14 days after. **8.** Give instructions to participant on how to complete the symptoms form 2A 7 days after the second vaccination and 2B 14 days after. Explain that the participant will receive a letter in e-boks with a non-clickable link to the form to fill it in online. Provide participants with 2 copies of the paper form if requested **9.** Schedule the next visit with the study participant or instruct the participant to book visits though the website: www.enforce.dk **10.** Ensure shipment of blood specimens and ENFORCE Specimen Shipping Logs to SSI *ENFORCE Sub-study 1 (cellular immunity) - for any details please see the sub-study 1 laboratory manual:* ***1.*** *Collect live cells (PBMCs) and PAX tubes* ***2.*** *Scan specimen labels into eCRF form (or stick label onto paper worksheet and scan later)* ***3.*** *Stick a label onto the Sub-study 1 (cellular immunity) Specimens Identification Log and a label on the Sub-study 1 (cellular immunity) Specimens Shipping Log* ***4.*** *Ensure shipment to AUH*

Visit 3 (3 mts after first vaccination	**Study Personnel must:** **1.** Record any signs and symptoms reported from participants (all grades) **2.** Record concomitant medication.

+/- 14 days) * *If the day of Visit 2 was 3 months (+/- 14 days) after 1st vaccine, no Visit 3 will be performed*	**3.** Collect whole blood in 1 x 6 mL EDTA tube (for plasma isolation) and 1 x 6 mL "dry glass" (for serum isolation) **(For details on blood sample collection please see laboratory manual)** **4.** Scan specimen labels into eCRF form (or stick label onto paper form) **5.** Stick a label onto the Specimens Identification Log and a label on the Specimens Shipping Log **6.** Collect symptom form filled out by subjects at home **7.** Check electronic/collect paper symptom form filled out by the subject 7 days after the first vaccination **8.** Ensure shipment of blood specimens and ENFORCE Specimen Shipping Logs to SSI *ENFORCE Sub-study 1 (cellular immunity) - for any details please see the sub-study laboratory manual:* *1. Collect live cells (PBMCs) and PAX tubes* *2. Scan specimen labels into eCRF form (or stick label onto paper worksheet and scan later)* *3. Stick a label onto the Sub-study 1 (cellular immunity) Specimens Identification Log and a label on the Sub-study 1 (cellular immunity) Specimens Shipping Log* *4. Ensure shipment to AUH*

Visit 4 (6 mts after first vaccination +/-14 days)	**Study Personnel must:** **1.** IF NO VISIT 3 WAS PERFORMED: Record any signs and symptoms reported from participants (all grades) **2.** IF NO VISIT 3 WAS PERFORMED: Record concomitant medication. **3.** Scan CPR number into e-CRF (or write on worksheet) **4.** Collect whole blood in 1 x 6 mL EDTA tube (for plasma isolation) and 1 x 6 mL "dry glass" (for serum isolation) **(For details on blood sample collection please see laboratory manual)** **5.** Scan specimen labels into eCRF form (or stick label onto paper form) **6.** Stick a label onto the Specimens Identification Log and a label on the Specimens Shipping Log **7.** Ensure shipment of blood specimens and ENFORCE Specimen Shipping Logs to SSI *ENFORCE Sub-study 1 (cellular immunity) - for any details please see the sub-study 1 laboratory manual:* *1. Collect live cells (PBMCs) and PAX tubes* *2. Scan specimen labels into eCRF form (or stick label onto paper worksheet and scan later)* *3. Stick a label onto the Sub-study 1 (cellular immunity) Specimens Identification Log and a label on the Sub-study 1 (cellular immunity) Specimens Shipping Log* *4. Ensure shipment to AUH*

Visit 5 (12 mts after first	**Study Personnel must:** **1.** Scan CPR number into e-CRF (or write the CPR number on the worksheet)

vaccination +/-14 days)	**2.** Collect whole blood in 1 x 6 mL EDTA tube (for plasma isolation) and 1 x 6 mL "dry glass" (for serum isolation) (**For details on blood sample collection please see laboratory manual)** **3.** Ensure shipment of blood-specimens and ENFORCE Specimen Shipping Logs to SSI *ENFORCE Sub-study 1 (cellular immunity) - for any details please see the sub-study 1 laboratory manual:* *1. Collect live cells (PBMCs) and PAX tubes* *2. Scan specimen labels into eCRF form (or stick label onto paper worksheet and scan later)* *3. Stick a label onto the Sub-study 1 (cellular immunity) Specimens Identification Log and a label on the Sub-study 1 (cellular immunity) Specimens Shipping Log* *4. Ensure shipment to AUH*

Visit 6 End of trial (24 mts after first vaccination +/-14 days)	**Study Personnel must:** **1.** Scan CPR number into e-CRF (or write on worksheet) **2.** Collect whole blood in 1 x 9 mL EDTA tube (for plasma isolation) and 1 x 9 mL "dry glass" (for serum isolation) (**For details on blood sample collection please see laboratory manual)** **3.** Scan specimen labels into eCRF form (or stick label onto paper form) **4.** Stick a label onto the Specimens Identification Log and a label on the Specimens Shipping Log **5.** Ensure shipment of blood specimens and ENFORCE Specimens Shipping Logs to SSI **6.** Fill out end-of trial eCRF *ENFORCE Sub-study 1 (cellular immunity) - for any details please see the sub-study 1 laboratory manual:* *1. Collect live cells (PBMCs) and PAX tubes* *2. Scan specimen labels into eCRF form (or stick label onto paper worksheet and scan later)* *3. Stick a label onto the Sub-study 1 (cellular immunity) Specimens Identification Log and a label on the Sub-study 1 (cellular immunity) Specimens Shipping Log* *4. Ensure shipment to AUH*

5.4 Study Procedures for third vaccination

Visit X * (pre-3rd vaccination) (-14 days and up to 30 minutes	**Study Personnel must** 1. Sign additional consent 2. **Scan CPR** number into e-CRF (or write on worksheet) 3. Record date of planned vaccination 4. Collect whole blood in 1 x 6 mL EDTA tube (for plasma isolation) and 1 x 6 mL "dry glass" (for serum isolation) (For details on blood sample collection please see laboratory manual)

before 3rd vaccination)	5. **Scan specimen labels** into eCRF form (or stick label onto paper form) 6. Stick a label onto the Specimens Identification Log and a label on the Specimens Shipping Log 7. Information is given about the symptom form in e-box (3A and 3B), which must be filled in at home on the 7th and 14th day, respectively, after the 3rd vaccination 8. For participants who has consented to sub-study 1, blood samples are collected for this 9. Schedule the date for the next study visit 28 days after the 3rd session (- / + 8 days) *ENFORCE Sub-study 1 (cellular immunity) - for any details please see the sub-study laboratory manual:* *1. Collect live cells (PBMCs) and PAX tubes* *2. Scan specimen labels into eCRF form (or stick label onto paper worksheet and scan later)* *3. Stick a label onto the Sub-study 1 (cellular immunity) Specimens Identification Log and a label on the Sub-study 1 (cellular immunity) Specimens Shipping Log* *4. Ensure shipment to AUH*
Visit XC 28 days after 3. vaccination (-/+ 8 days)	**Study personnel must** 1. Study participants who have not had a visit must sign additional informed consent at this visit. 2. Collect whole blood in 1 x 6 mL EDTA tube (for plasma isolation) and 1 x 6 mL “dry glass” (for serum isolation) (For details on blood sample collection please see laboratory manual) 3. Scan specimen labels into eCRF form (or stick label onto paper form) 4. Stick a label onto the Specimens Identification Log and a label on the Specimens Shipping Log 5. For participants who has consented to sub-study 1, blood samples are collected 6. Record any signs and symptoms reported from participants (all grades) 7. Obtain symptom form day 7 and day 14 completed by the study participant *ENFORCE Sub-study 1 (cellular immunity) - for any details please see the sub-study laboratory manual:* ***1. 1. Collect live cells (PBMCs) and PAX tubes*** *2. 2. Scan specimen labels into eCRF form (or stick label onto paper worksheet and scan later)*

	3. 3. Stick a label onto the Sub-study 1 (cellular immunity) Specimens Identification Log and a label on the Sub-study 1 (cellular immunity) Specimens Shipping Log *4. Ensure shipment to AUH*

*Participants who have already received a 3rd vaccine will be called in by the site and a visit Xc will be scheduled.
For participants that receives the invitation to participate after they have received the 3rd vaccination:

- book a visit Xc within 28 days (+8) days of the vaccination.
- go to form X and tick ”Studiebesøg ikke gennemført” and tick “*Dato for 3.vaccination er udenfor besøgsvindue*”.

Remember that If visit X or Xc are within 30 days before a planned follow up visit (visit 4,5 or 6) **visit X or Xc can replace the visit 4,5 or 6:**

- Go to the future form 4, 5 or 6 and tick “*Studiebesøg ikke gennemført*”. Write reason: ”*Studiebesøg X eller Xc ligger 30 dage før det planlagte besøg*”.

6 Regulatory Documents (paper)

6.1 Informed Consent

The participant information document includes information about the study for potential participants, and four informed consent forms for subject signature:

- ENFORCE study (main study)
- Storage of samples for future research
- Sub-study 1 (cellular immunity)
- Sub-study 2 (safety) (participation offered to all participants as a generic add-on)
- ENFORCE Tillæg nr. 1 - Ekstra besøg I forbindelse med booster vaccination

6.2 Subject Identification Log

All subjects included into the study must be added to the Site-specific Subject identification log. Subject data to be entered on this log is:

- CPR number
- Date of enrollment
- Subject name
- Subject contact information

6.3 Subject Source Data Document

Sites must document source data for each subject in the study. Sites may use the ENFORCE Subject Source Data Document that has been developed to facilitate this process (see Section 3.4).

7 Data Collection

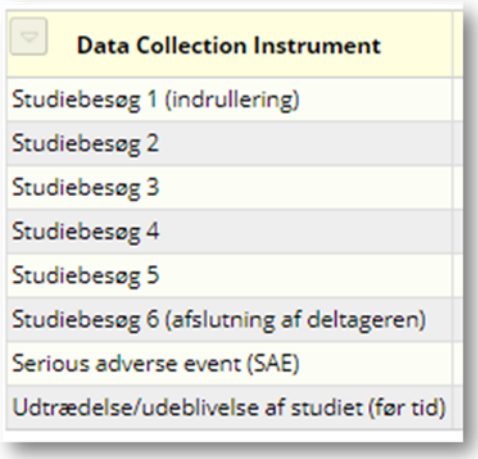
7.1 Data Collection Overview

Visit number	**1*** **(prior to 1st vaccination)**	***1b (substudy 2 – safety)***	***2** (prior to 2nd vaccination)***	***2b (substudy 2 saftey)***	***3*** 3 months after first vaccination***	**4**	**5**	**6**	**X*** **(prior to 3rd vaccination)**	***XC (28 days after 3rd vaccination)***
PARTICIPANT DATA - eCRF										
Informed consent	X	X							X	(X - if visit X has not been performed)
Medical history	X									
Medications	X		X		X	X****				
Vaccination date & brand	X		X		X				X	X
SPECIMEN COLLECTION										
Whole Blood samples and *Sub-study blood samples*	X	X	X	X	X	X	X	X	X	X
ASSESMENT (SYMPTOMS, AE, SAE) - eCRF										
Provision of symptoms form A + B (electronic or paper) to participant	X		X						X	
Review of electronic/ collection of paper symptoms form A + B			X		X	X****				X
AE assessment			X		X	X****				X
SAE (assessment of relation to vaccination by investigator)	Reported within 7 days after notification									X

*Visit must be before vaccination.
** If subject will only receive one vaccine shot, no Visit 2 will be performed
*** If the day of Visit 2 was 3 months (+/- 14 days) after 1st vaccine, no Visit 3 will be performed
**** Only If no Visit 3 was performed these data are collected at Visit 4

7.2 Data Collection Forms
The following data collection forms are used in the study:

- Visit 1
- *Visit 1B (only if substudy 2 is activated)*
- *Visit 1C (Astra Zeneca Safety visit – see annex 2)*
- Visit 2
- *Visit 2B (only if substudy 2 is activated)*
- Visit 3
- Visit 4
- Visit 5
- Visit 6
- Serious Adverse Event (SAE) form
- Withdrawal (before the end of study)
- Repeat visit 1 (for participants whose 1st vaccination was postponed after visit 1)
- Transfer form (participant move to another region)
- Symptoms form 1AB & 2AB (only for in between visits)
- Visit X
- Visit XC
- Symptoms form 3A & 3B



Data Collection Instrument
Studiebesøg 1 (indrullering)
Studiebesøg 2
Studiebesøg 3
Studiebesøg 4
Studiebesøg 5
Studiebesøg 6 (afslutning af deltageren)
Serious adverse event (SAE)
Udtrædelse/udeblivelse af studiet (før tid)

The forms are available electronically under ENFORCE in REDCap.

Sites may choose to collect data during study visits on paper CRFs/worksheets and subsequently enter the data into the eCRFs in REDCap. It is important to enter data into REDCap as soon as possible after the study visits and **no later than 48 hours after the visit**.

(Paper) worksheets should be stored and kept in Investigator Site File or a CRF Binder.

7.3 eCRFs Study Visits 1-6
At visit 1 - the first study visit or enrolment - the following data must be recorded in the eCRF:

- Informed Consent
- CPR-number (scan 'Sundhedskort')
- Inclusion criteria/Eligibility

- Vaccination priority group
- Scheduled date of 1st vaccination
- Medical history within last 12 months
- Concomitant medication within last 24 hours
- Information about Symptoms Form
- Blood samples (scan specimen labels)
-

At visit 1b – see annex 2
At visit 1c – see annex 2

At visit 2 - the following data must be recorded in the eCRF:

- Confirm 1st vaccination (date, brand/type)
- Scheduled date of 2nd vaccination
- Concomitant medication within last 24 hours
- Participants' Symptoms Form (1A and 1B)
- Grade 1 and 2 adverse events as present on the specific day of visit 2
- Grade 3 and 4 adverse events observed since last visit
- SAE
- Blood samples (scan specimen labels)

At visit 3- the following data must be recorded in the eCRF:

- Confirm 2nd vaccination (date, brand/type)
- Concomitant medication within last 24 hours
- Participants' Symptoms Form (2A and 2B)
- Grade 1 and 2 adverse events as present on the specific day of visit 3
- Grade 3 and 4 adverse events observed since last visit
- SAEs
- Blood samples (scan specimen labels)

At visit 4-6- the following data must be recorded in the eCRF:

- Blood samples (scan specimen labels)

For participants where no Visit 3 was performed the following data are collected at Visit 4

- Participants' Symptoms Form (2A and 2B)
- Grade 1 and 2 adverse events as present on the specific day of visit 3
- Grade 3 and 4 adverse events observed since last visit
- SAEs

At visit X – the following data must be recorded in the eCRF

- Sign additional Informed Consent
- Planned 3rd vaccination date
- Scheduled date for XC visit
- Information about Symptoms Form (3A and 3B)
- Blood samples (scan specimen labels)

At visit XC – the following data must be recorded in the eCRF

- If no visit X: Sign additional Informed Consent
- Confirm 3rd vaccination date
- Participants' Symptoms Form (3A and 3B)
- Grade 1 and 2 adverse events as present on the specific day of visit 2
- Grade 3 and 4 adverse events observed since last visit
- SAE

- Blood samples (scan specimen labels)

7.4 Symptoms Form (for subjects)

Local and systemic reactions to vaccination, shall be ascertained through the reactions reported on the four symptoms forms:

- 1A for days 1-7 completed on day 7 after first vaccination,
- 1B for days 8-14 completed on day 14 after first vaccination,
- 2A for days 1-7 completed on day 7 after second vaccination,
- 2B for days 8-14 completed on day 14 after second vaccination
- 3A for days 1-7 completed on day 7 after third vaccination,
- 3B for days 8-14 completed on day 14 after third vaccination

At visit 1, 2 and visit X, study staff must instruct participants in how to complete the symptoms forms:

Dag 7 efter vaccinationen
sæt kryds ved den graduering, der bedst beskriver *den højeste grad af symptomet* du har oplevet i løbet af de 7 dage siden du blev vaccineret.

	Ingen	**Mild**	**Moderat**	**Svær**
Lokale symptomer (det sted på kroppen hvor du blev stukket)				
Rødme				
Hævelse				
Ømhed				
Andre symptomer/ reaktioner				

All subjects will automatically receive a letter in E-boks with a link to the Participant Symptoms Form in electronic version (sender is Region Hovedstaden). Should the subject prefer to fill out the Symptoms Form on paper please hand out 2 copies at study visit 1 and at study visit 2.

For the electronic version subjects will receive the mail with link to form A in e-boks 1-2 days after the visit is registered in REDCap. The mail with link to form B arrives in e-boks 8-10 days after the visit. To avoid delays **it is important to enter visit data electronically within 48 hours.**

Please give the participants instruction on how to access the form electronically. The letter in e-boks contains a link – which is *not* clickable, but must be copied into a browser. These are the instructions:

1. **Modtaget mail i e-boks: markere og kopiere link**

Region Hovedstaden

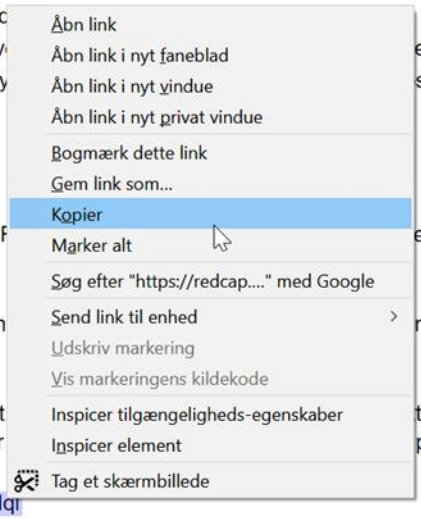
Kontaktoplysninger:
Projektnavn: ENFORCE
E-mailadresse: enforce.rigshospitalet@regionh.c
Hvis du kontakter os med email må du ikke skriv eks.
CPR-nummer eller oplysninger om helbred og sy s om
at ringe dig op.

Kære Deltager

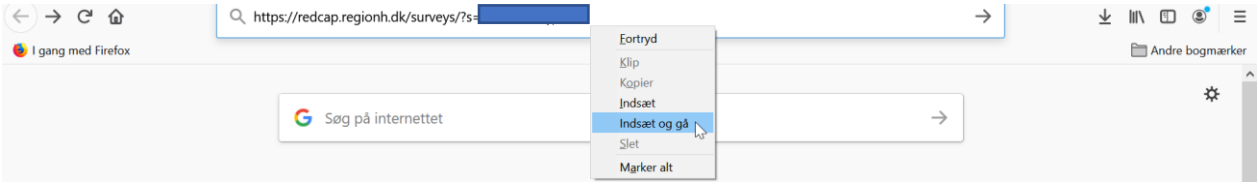
Tak fordi du vil deltage i det nationale studie ENF e
bivirkninger af de nye covid-19 vacciner.

Vi vil bede dig udfyld et kort skema om symptom nisk og
skal udfyldes på dag 7 efter vaccinationen.

Herunder er et link til skemaet. Det åbnes ved at te det
ind i en internet browser (f.eks. Internet Explorer på
linket - kun kopiere det:Â
https://redcap.regionh.dk/surveys/?s=STDuwd7Iqr
Dette link er kun til dig og må ikke sendes til andre.



2. Indsæt kopieret link i browseren, og gå



3. **Udfyld ”Symtomskema A” på dag 7 efter vaccinationen og ”Symptomskema B” på dag 14 - og klik på ”Submit” knappen, når du er færdig**

When the subject has filled out and submitted the electronic symptoms form, the data will automatically be uploaded to the eCRF.

If the subject has filled out paper forms, these should be handed to the study staff at the following study visit. (If a participant has forgotten to bring the forms, they can take a picture afterwards of the filled form and send via email/txt message to study personnel or fill in via the link received in e-boks.) The study staff must enter the data from the paper forms into REDCap.

If a participant hands in the paper form in-between the scheduled visits, there is a specific eCRF in RedCAP where this data can be typed in (Symptoms Form in between visits- only for staff).

There will be no other source documentation for these data than the electronic/paper form.

7.5 Adverse Events

At visit 2 and visit 3 study staff interview participants to capture AEs since last visit. For participants where no Visit 3 was performed AE data are collected at Visit 4.
For the grading of AE's the DAIDS toxicity table: (https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables) will be used. If event is not identified, the generic AE grading table below will be used. The grading table is included in the Protocol and in the eCRF for visit 2 and visit 3.

----- Adverse Events -----

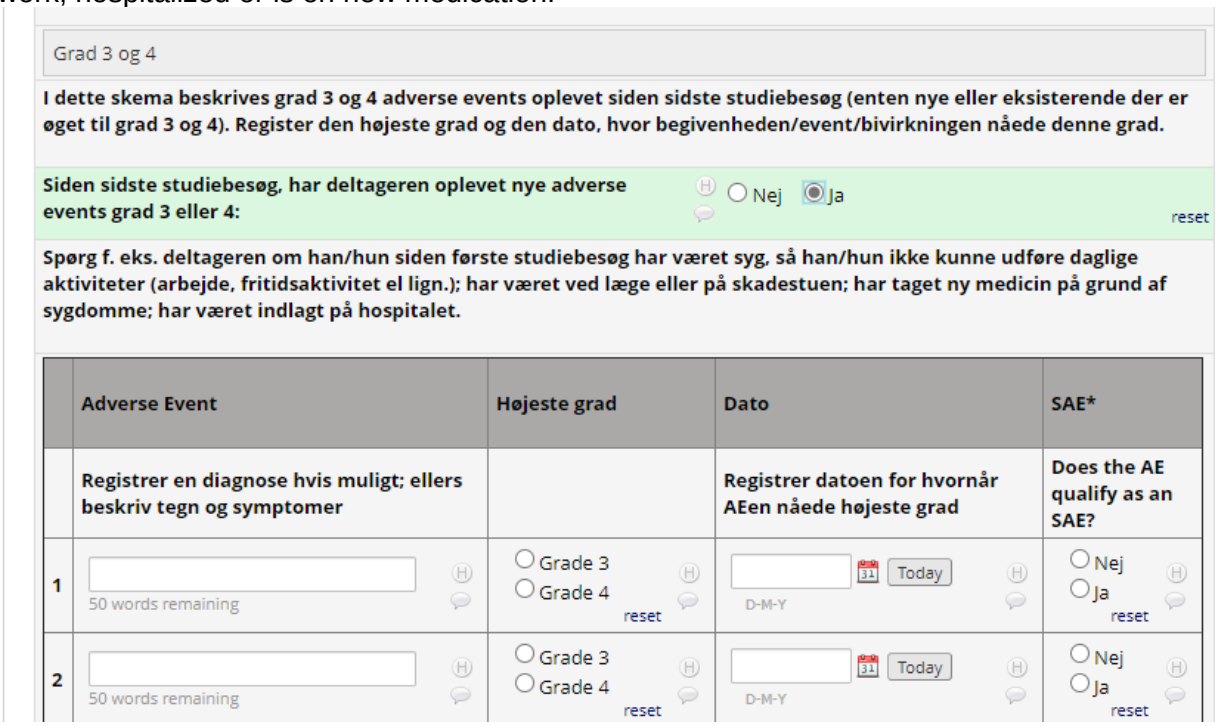
For the grading of AE's the DAIDS toxicity table: (https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables) will be used. If event is not identified, the generic AE grading table below will be used.

GENERIC AE GRADING SCALE

Grade 1	**Events causing no or minimal interference with usual social and functional activities, and NOT raising a concern, and NOT requiring a medical intervention/ therapy.**
Grade 2	**Events causing greater than minimal interference with usual social and functional activities; some assistance may be needed; no or minimal medical intervention/therapy required.**
Grade 3	**Events causing inability to perform usual social and functional activities; some assistance usually required; medical intervention/therapy required.**
Grade 4	**Events causing inability to perform basic self-care functions; medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death**
Grade 5	**Events resulting in death**

Grade 1 and 2 AE's are captured on the specific day of the visit (visit 2 and visit 3 (or 4)) by asking the subject if they have any clinical symptoms including mild ones.

Grade 3 and 4 AE's are captured at visit 2 and visit 3 by staff asking participants about any events happening since last visit, e.g., asking whether the subject has been ill, unable to work, hospitalized or is on new medication.



It is important to assess whether the event is an SAE – and if yes, the event must be reported in a separate SAE form within 24 hours of site being aware.

7.6 Serious Adverse Event (SAE)

The study uses the below definition of SAE's, which is included in the Protocol and in the eCRF:

*Definition - Serious Adverse Event (SAE) / Serious Adverse Reaction (SAR)
A serious adverse event/reaction is an experience that at any dose results in any of the following: **• Results in death (reporting of death will be done in a specific Death form)** **• Is life-threatening - this refers to an event in which the subject was at risk of death at the time of the event** **• Requires in-subject hospitalisation or prolongation of existing hospitalisation** **• Results in persistent or significant disability or incapacity** **• Is a congenital anomaly or birth defect** **• Is judged medical important (this refers to an event that may not be immediately life-threatening or result in death or hospitalisation, but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed above)**

SAEs are recorded and reported during the first 3-6 months a subject is enrolled in the study. Within 24h hours of being notified, site staff must report all SAEs in the SAE-form and include data on:

- Relation of SAE to previous AEs
- Date of SAE
- Diagnosis or description
- The site's study responsible/doctor must review the SAE report and assess causality, expectedness and relation to the vaccines.

The initial SAE report must contain as much information as possible. Any follow-up data must be detailed in a subsequent SAE Form in due time.

When an SAE form is filled out the system immediately sends an automated alert to the Sponsor's project email. Sponsor study staff reviews will review all incoming SAE reports on a daily basis for quality and completeness of data. Sponsor's Medical Officer reviews all SAE's and assesses causality, expectedness and relation to the vaccines.

7.7 Withdrawal (before the end of study)/exclusion

If a subject withdraws from the trial (before the end of study) or is excluded the withdrawal form must be filled out. Here you must record data on:

- Date of withdrawal
- Type of withdrawal – it can be one of the 3 below options.
- Reason for withdrawal/exclusion

☐ ønsker ingen studiebesøg (indsamlet data kan fortsat bruges i studiet og registerdata kan fortsat indsamles) ☐ udtræder af studiet fra dd. (indsamlet data kan fortsat bruges i studiet, men ingen ny registerdata indsamles) ☐ tilbagetrækker samtykke og får al data slettet

7.8 Data Queries

Reported study data will be submitted to automated and manual checks of quality and completeness. REDCap contains a data resolution workflow that will be used to send queries to sites regarding the reported data.

See next chapter for further instructions (in Danish).

8 ENFORCE REDCap Instructions (in Danish)

8.1 Adgang

For at få adgang til REDCap skal man sende en email til enforce.rigshospitalet@region.dk med følgende detaljer på den/dem der skal have adgang:

- Navn
- email adresse (OBS: vær opmærksom på at man skal have adgang til sin mail når man logger på REDCap)
- hvor man er fra (site, klinik, hospital)

Indenfor 3 dage burde man få en email med brugernavn og link til at sætte ens kode.

REDCap bruger 2-faktor identifikation, så udover at indtaste brugernavn og selvvalgt kode, får man tilsendt en email med en ny kode hver gang man skal logge ind i REDCap.

8.2 Opret ny deltager

1. Når du har logget ind, skal du under **My Projects** klikke ENFORCE (du skal måske skrolle lidt ned for at kunne se det.
2. Klik **Add / Edit record** i venstre side.
3. Klik **Add new record**.
4. Udfyld studiebesøg 1 formen. OBS: For at undgå tastefejl skal CPR-nummer altid scannes ind fra Sundhedskort.

VIGTIGT! CPR-nummer skal være UDEN bindestreg.

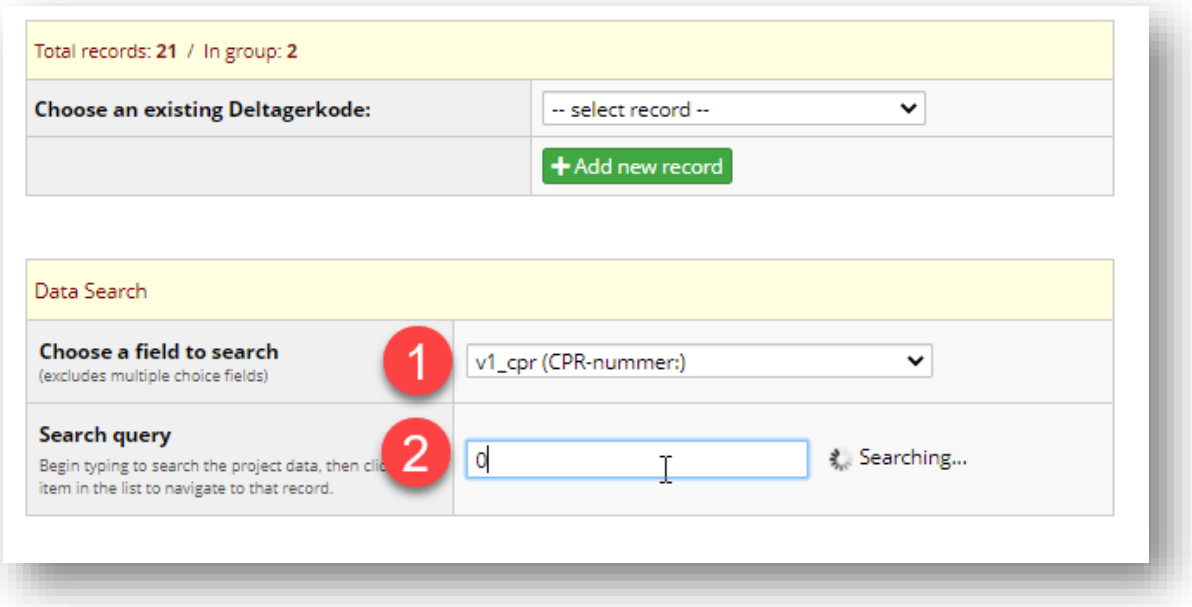
8.3 Find eksisterende deltager

Der er flere måder at finde en eksisterende deltager. I **ADD / EDIT RECORD** er det muligt at søge efter CPR nr eller deltagerkoden.

Søg efter CPR nr.:

1. I Add / Edit record, under Data search, vælg **v1_cpr (CPR-nummer:)**.

2. Skan sygesikring eller skriv CPR-nummeret i Search query feltet, der kommer resultater op efterhånden som du skriver.



3. Vælg deltager.

8.4 Record Status Dashboard

'Record status dashboard' giver en liste over alle deltagere per site med status ikoner for hver deltager, så man hurtigt kan få et overblik over status på alle deltagerne. Med 'custom dashboard' kan man filtrere på alle mulige ting f.eks. hvis man kun vil se deltager over 65 år.

Det er dog kun administratorer, der kan lave 'custom dashboards', så hvis du har behov for et eller flere 'custom dashboards', så skriv venligst en email til enforce.rigshospitalet@region.dk
og forklar behovet. Vær dog opmærksom på at ALLE vil kunne se 'custom dashboards'.

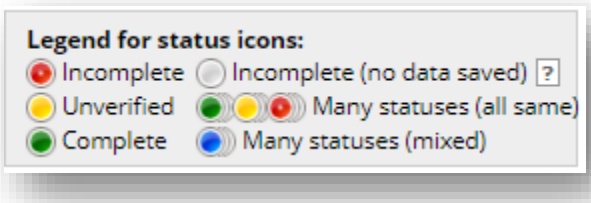
8.5 Status og gem

STATUS

I bunden af hver form er det muligt at sætte status for formen. Brug følgende:

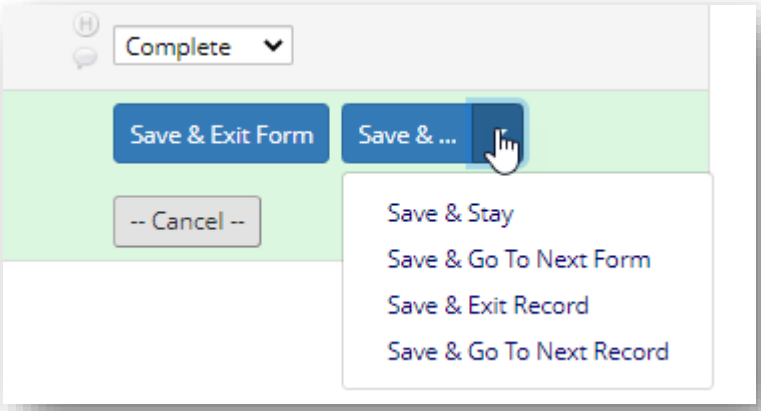
- Incomplete – brug denne hvis du er startet, men af en eller anden grund bliver nødt til at vende tilbage senere
- Complete – brug denne når du er helt færdig med at indtaste data i formen

OBS: Forms skal "completes" så hurtigt som muligt



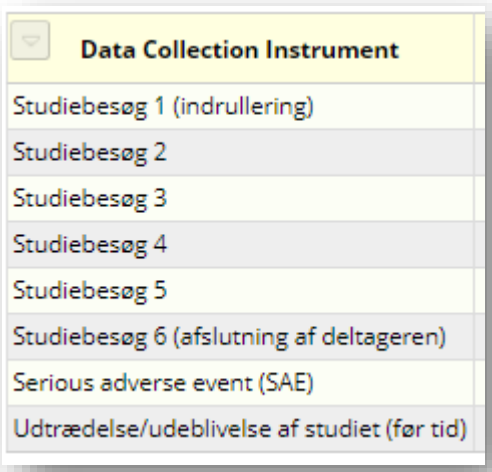
GEM
Der er mange muligheder når man skal gemme og det kan måske være lidt forvirrende. I de fleste tilfælde skal man kun bruge:

- Save & Exit Record – bruges når du er færdig med en form for en deltager og skal starte på en anden deltager
- Save & Exit Form – bruges når du er færdig med en form for en deltager og skal starte på en ny form for samme deltager
- Save & Stay – bruges når du bare gerne vil gemme det du har indtastet.



8.6 Oversigt over forms
Der er en form for hvert besøg og derudover er der også forms, hvis

- der skal rapporteres SAEer
- deltagere udtræder af studiet
- besøg 1 skal gentages hvis deltageren ikke fik sin første vaccine som planlagt
- transfer form, hvis en deltager ønsker at blive overflyttet til en anden region eller tage et enkelt studebesøg i en anden region.



8.7 Rapporter

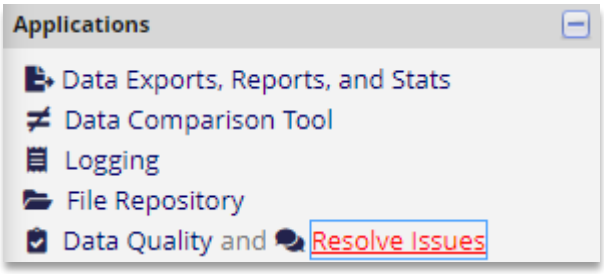
I venstre side under **Reports**, kan man se en liste med forskellige rapporter. Rapporterne lister deltagere ud fra forskellige kriterier f.eks. alle deltagere der har tilbagetrukket samtykke osv. Hvis du har brug for en rapport der ikke findes endnu, så skriv gerne til enforce.rigshospitalet@region.dk, så kan det være, at den kan blive oprettet.

8.8 Data queries

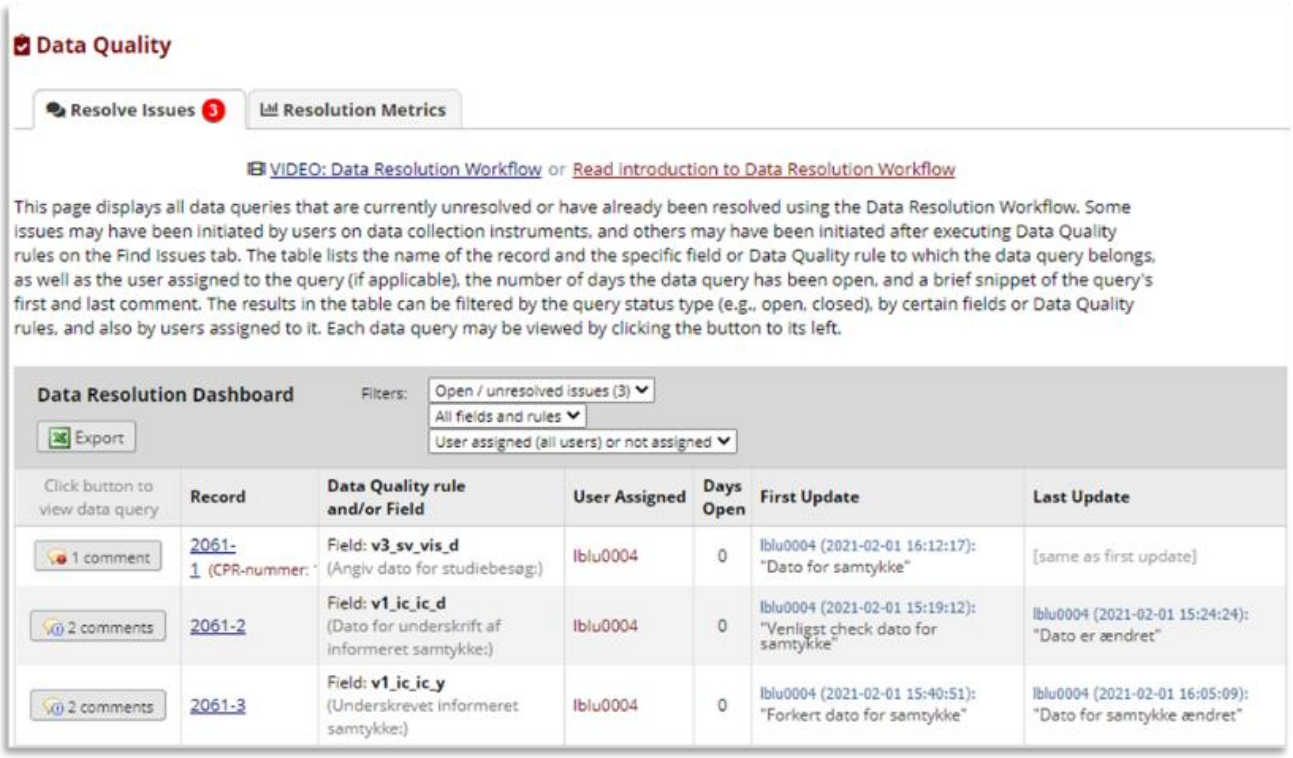
Data Queries er spørgsmål til site fra hvis/ når en dataværdi ikke er indtastet korrekt, dette kan f.eks. være en dato. Data Queries vil blive gennemgået løbende - oftere i starten af studiet og senere i studiet ca hver måned.

Som notifikation på at der en Query som skal løses, vil du modtage en e-mail om at der queries som skal løses i REDCap.

Data Queries vil blive løst via et workflow i REDCap, denne proces hedder ”**Data Resolution Workflow”**. Dette er en korrespondance imellem dig og den som har oprettet Querien. Query løsnings processen foregår ved at du logger ind på REDCap og vælger ”Resolve Issue”, i menuen til vestre.



Herefter kommer du til ’Data Quality/ Resolve Issue’ siden, hvor alle data queries er listede, både uløste og løste queries.

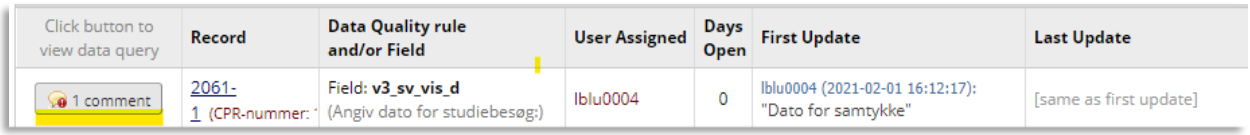


Som vist på billedet vil der under ” Click button to view data Query” være ”gule tale bobler” markeret med enten et rødt udråbstegn eller et blåt udråbstegn.

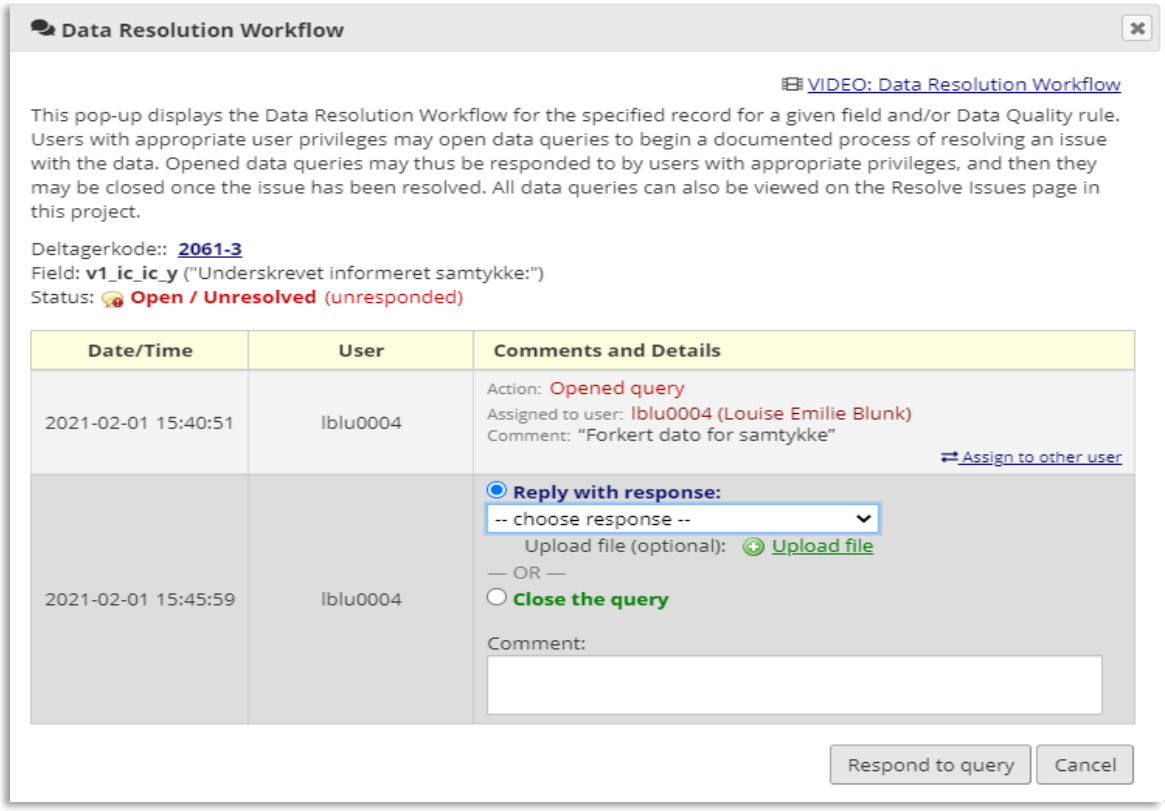
- Gul tale boble med rødt udråbstegn, indikerer at ”querien” er uløst og der er behov for at data bliver tjekket for korrekt indtastning.
- Gul tale boble med blåt udråbstegn indikerer at der er svaret på boblen og modtager skal tage stilling til om querien kan lukkes (her skal du ikke foretage dig noget)
- Under data Quality rule and/ or field: Kan du læse hvad querien er udløst af, ex manglende dato.
- User assigned: Data querien vil være ’assigned’ til en bestemt person, dvs. stilet til en bestemt person. Denne person vil være listet under ”User Assigned”. Hvis det er dit navn/ User-id som står under ”user Assigned” er det dig som skal besvare/rette querien.

For at korrigere en query skal du gøre følgende:

- Vælg under” Click button to view data query” Den gule tale boble med rødt udråbstegn:

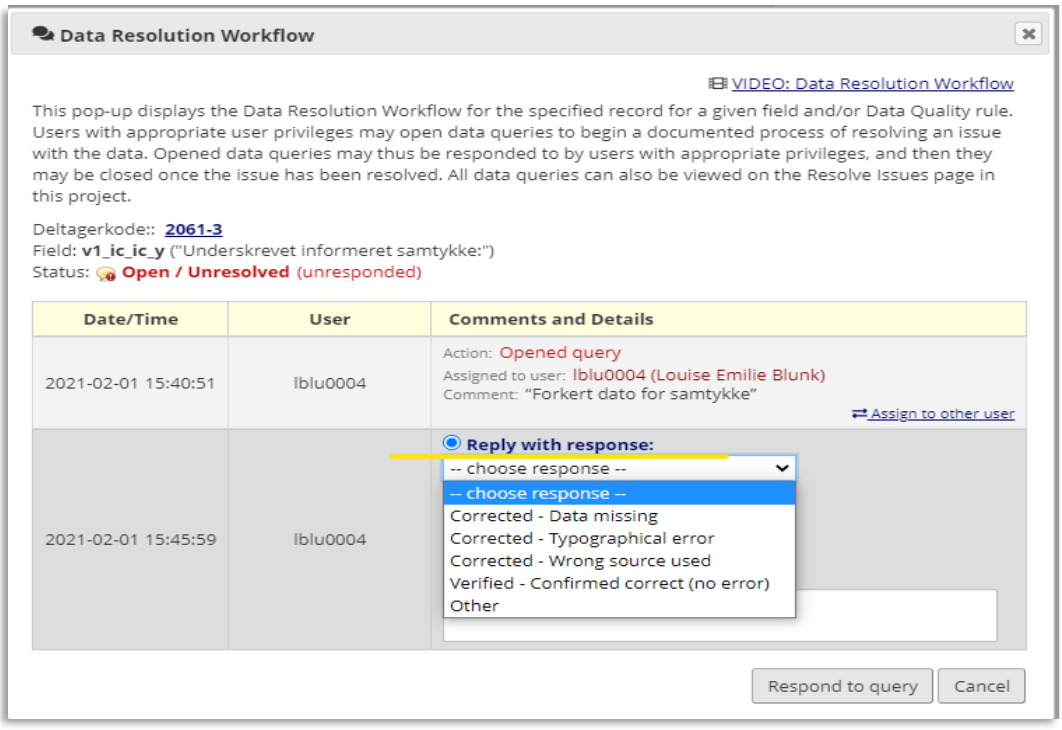


Herefter åbner Data resolution workflowet.

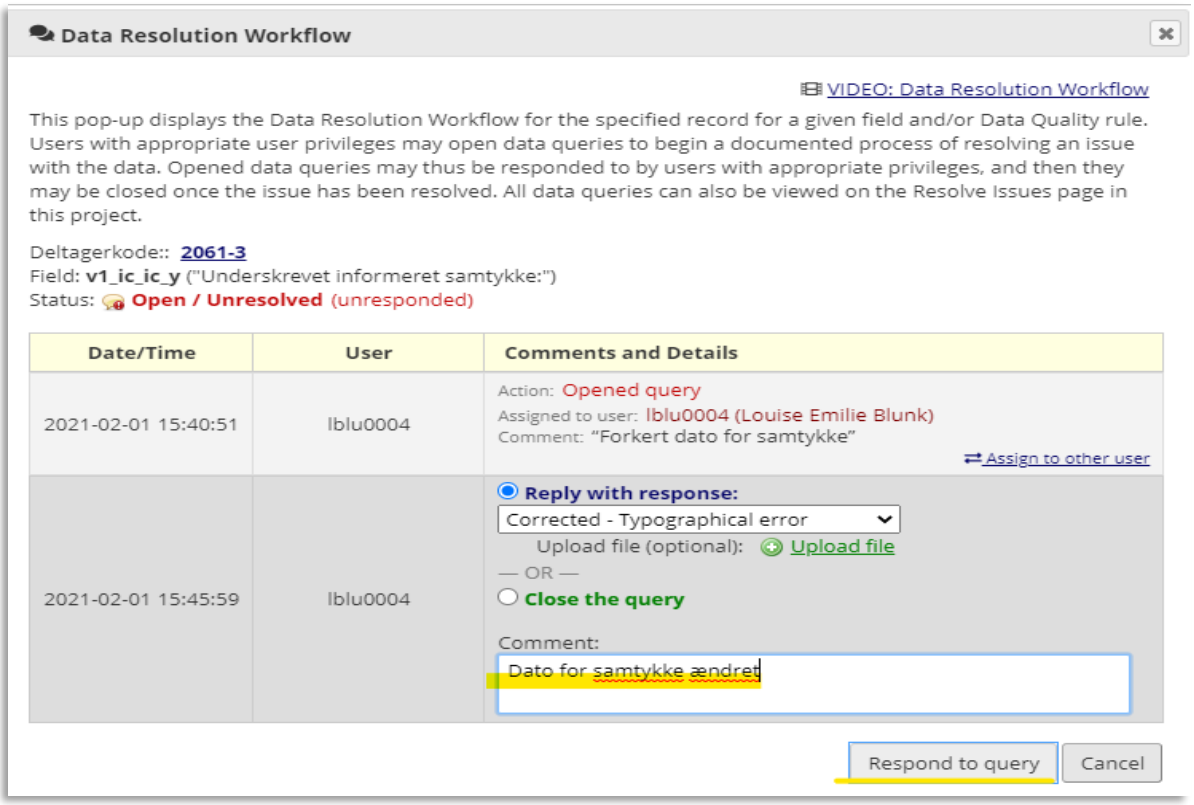


Som vist på de næste to slides og det er muligt at vælge et svar fra drop down menuen (Reply with response) og/eller indsætte en kommentar:

Vælg Reply with repsonse/ fra drop down menuen og find det svar som besvarer til querien.

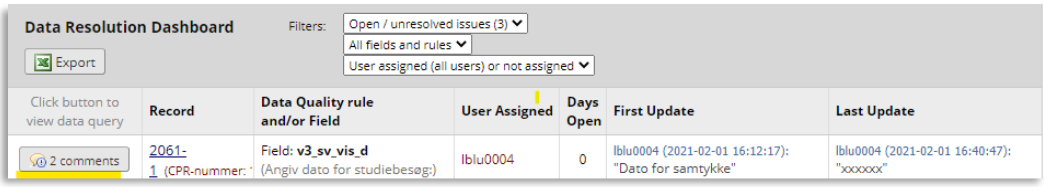


Du kan også vælge at indtaste en kommentar, i kommentarfeltet, som vist nedenfor.



Når du har kommenteret på Querien eller rettet data, vælger du " Repsond to Query", som markeret nedenfor:

- Når du har valgt "**Respond to Query**" kommer du tilbage til oversigten over Queries og den gule tale boble har nu fået har nu et blåt udråbstegn.



- Gå tilbage til **Resolve Issues** og gentag processen hvis du har flere uløste queries.

Oplever du problemer, så skriv gerne til enforce.rigshospitalet@region.dk.

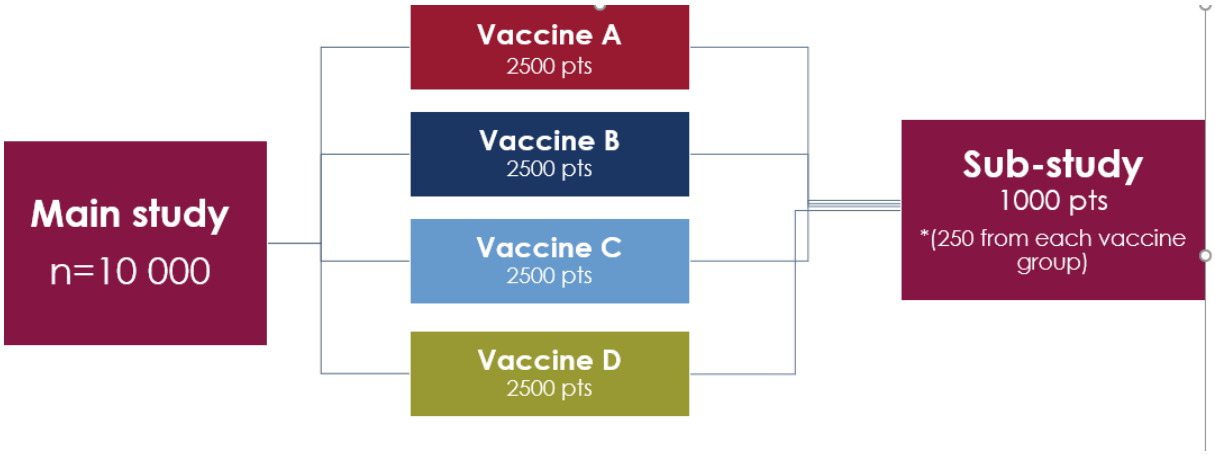
9 Laboratory and Sample Handling Instructions

Please find instructions in the Laboratory Manual:

- *ENFORCE Laboratory manual (main study)*
- *ENFORCE Sub-study 1 (cellular immunity) Laboratory manual*
- *If activated: *ENFORCE Sub-study 2 (safety) Laboratory manual*

Annex 1: ENFORCE Sub-Study 1 (cellular immunity)

Under separate participant informed consent, a sub-study 1 cohort will be established including 1000 participants (250 from each vaccine group) from the ENFORCE main study.



The sub-study aims to generate a well curated and detailed biobank of material to investigate the immunology and safety of the vaccine roll-out.

At time of first vaccination, 10% of participants enrolled in the ENFORCE national cohort study are also offered entry into this sub-study under separate consent. The informed consent form for the sub-study is contained in the ENFORCE Participant Information document.

The sub-study runs in parallel with the ENFORCE study and at the regular six (6) ENFORCE study visits the sub-study subjects will have additional blood extracted, and live cells will be stored in liquid nitrogen to assess cellular immunity.

Sample collection table – sub-study No. 1

Sample	Visit (Days)	Collection tube	Sample Volume
Blood for cellular nucleic acid	Baseline and Visit 2, 3, 4, 5 and 6	One PAXgene tube	2,5 mL
Blood for PBMCs	Baseline and Visit 2, 3, 4, 5 and 6	Three CPT citrate tubes	8 mL x 3

For further instructions please read the ENFORCE Sub-study 1 (cellular immunity) Laboratory Manual.

Annex 2: ENFORCE Sub-study 2 (Safety)

Generic Description of ENFORCE Sub-study 2 (safety)

All ENFORCE participants are invited to take part in the generically described sub-study 2 under a separate consent. The sub-study 2 will be activated upon decision by the ENFORCE Scientific Steering Committee provided that the Danish Health Authority and/or Medicines Agency agrees that an “issue of concern” has arisen for one or more vaccines.

An appropriate number of participants in ENFORCE vaccinated with other vaccines without such “issues” of concern” may also be invited to participate in the sub-study as “controls”. There is no predefined number of subjects for the sub-study.

With two new visits 1b and 2b 7 days (+/- 5 days) after first and second vaccination date the aim is to collect additional biological samples that can be used to address changes in inflammation, coagulation, epigenetic regulation, and cell phenotypes from before the first SARS-CoV-2 vaccine to 7 days after vaccination. The visit and sample collection maybe repeated as needed at later timepoints depending on the issue of concern.

The comparison of pre-and postvaccination samples will enable the identification of any changes in soluble markers, cell characteristics and activation, and cell transcription related to each vaccine type.
The laboratory assessments made in conjunction with implementing this sub-study will need to be adaptive depending on the issue(s) of concern. Proposed biochemistry samples for analysis at routine local biochemistry department includes leucocyte count and differential, thrombocyte count, CRP, and possible other biomarkers incl D-Dimer and markers of organ dysfunction. A maximum of 80 ml blood will be drawn at each visit, but no more than 240 ml will be drawn in total.

Samples will be collected typically at day 7 after (first and second) vaccination and only once, but maybe needed to be repeated depending on the issue of concern.

In principle, all ENFORCE participants can be invited to take part in the sub-study 2, but we will specifically try to recruit as many individuals as possible from the ENFORCE sub-study 1. Because for the sub-study 1 subjects, we have already collected blood cells and PAX mRNA gene stabilizing tubes (in addition to serum and plasma) prior to vaccination. Thus, collecting new samples 7 days after vaccination and booster will provide a unique opportunity to compare changes in soluble markers, cell characteristics and activation, and cell transcription from pre- to post-vaccination.

Activated 25 April 2021: ENFORCE Substudy 2 Safety - AstraZeneca patients - study procedure

Under the ENFORCE protocol v4 April 09, 2021 approved generic Substudy 2 related to specific safety issues, the Danish health Authorities have requested ENFORCE to implement an extra safety visit for all participants that have been vaccinated with Astra Zeneca.

Sites are requested to call in all participants as soon as possible starting from Sunday 25 April 2021. Please see specific emails concerning this activation.

Study documents related to this activated sub-study 2 (safety – AZ) have been sent by email to PIs and study coordinators, including:

- ENFORCE Substudy 2 Safety AstraZeneca procedure_2021APR20
- ENFORCE - Oplysningspligt_V2_2021MAR26
- Forsoegspersoners rettigheder i sundhedsvidenskabelige forskningsprojekter_NVK_2020MAR11
- Substudie 2 ekstra samtykke, 1 side kaldet ENFORCE_DK_IC_v4.0_Sub-2-2021APR09 – A - specific AstraZeneca
- CRF/Worksheet – Studiebesøg 1C (safety)
- ENFORCE Source Subject Document – Visit 1C (safety – AZ)
- ENFORCE_Substudy 2(Safety-AstraZeneca SSI) Specimens Identification Log_2021APR14
- ENFORCE_Substudy 2(Safety-AstraZeneca SSI) Specimens Shipping Log_2021APR14
- ENFORCE_Substudy 2(Safety-AstraZeneca AUH) Specimens Identification Log_2021APR19
- ENFORCE_Substudy 2(Safety-AstraZeneca AUH) Specimens Shipping Log_2021APR19

Study procedures:

Substudy 2 Safety Studiebesøg 1 C: (start 25 april 2021)	**Study Personnel must:** **1.** Invite all AstraZeneca participants from the ENFORCE study to participate in Substudy 2 (safety) (AstraZeneca who have received one or both vaccinations) **2.** Hand out the informed consent a. ENFORCE _DK_IC_v4.0_Sub-2_2021APR09 - A - specific AstraZeneca b. Oplysningspligt_v2_2021MAR26 c. Forsøgspersoners rettigheder I sundhedsvidenskabelige forskningsprojekter_NVK_2020MAR11 **3.** Provide information, and answer questions about the substudy 2 (safety) **4.** Obtain the written informed consent (ENFORCE _DK_IC_v4.0_Sub-2_2021APR09 - A - specific AstraZeneca) from the participant before any other trial procedures are performed

	5. Store this informed consent with the participants ENFORCE consent form that has previously been signed. ***6.*** Scan CPR number into e-CRF Substudie 2 (Safety) Studiebesøg 1C (or write on worksheet) **7.** Record date of informed consent **8.** Record date of study visit **9.** Collect whole blood in 1 x 6 mL EDTA tube (for plasma isolation) and 1 x 6 mL "dry glass" (for serum isolation) **10.** Scan orange specimen labels into eCRF form Substudie 2 (Safety) Studiebesøg 1C (or stick label onto paper worksheet and scan later) **11.** Stick orange specimen label onto the ENFORCE Substudy 2 (Safety-AstraZeneca SSI) Specimens Identification Log and a label on the ENFORCE Substudy 2 (Safety-AstraZeneca SSI) Specimens Shipping Log **12.** Inform the participant to contact you when they are invited to receive a new vaccine or instruct the participant to book visits though the website: www.enforce.dk **13.** Ensure shipment of blood specimens and ENFORCE Substudy 2 (Safety-AstraZeneca SSI) Specimens Shipping Log to SSI *Procedured for AstraZeneca participants that have provided consent to SUB-STUDY #1 (the ENFORCE CELLULAR IMMUNITY Sub-Study) (selected sites only)* ***1.*** *Collect live cells (PBMCs) 3 x 8 ml and PAX and 1 x 2,5 tubes* ***2.*** *Scan yellow specimen labels into eCRF* Substudie 2 (Safety) Studiebesøg 1C (or write on worksheet) *(or stick label onto paper worksheet and scan later)* ***3.*** *Stick a yellow label onto the ENFORCE_Substudy 2 (Safety-AstraZeneca AUH) Specimen Identification Log and a yellow label on the ENFORCE_Substudy 2_(Safety-AstraZeneca AUH)) Specimen Shipping Log* ***4.*** *Ensure shipment to AUH*

Blood samples:
For Substudy 2 visit 1C the same blood samples as in the main ENFORCE study are taken, and for participants already consented to the sub-study No. 1 (cellular immunity), the extra samples related to this are also taken.
The sample handling and transport flow to SSI and AUH is identical to all other ENFORCE study visits:

Alle AstraZeneca deltagere som samtykker til Substudie 2

Blodprøve	Besøg 1C	Prøveglas	Volume	Sendes til
Plasma	1 Substudy 2 besøg	Et EDTA Plasma rør	6 mL	SSI
Serum	1 Substudy 2 besøg	Et Serum rør	6 mL	SSI

Alle AstraZeneca deltagere som har sagt ja til Substudie 1 (cellular immunity) ved baseline og som nu samtykker til Sub-Studie 2 (safety)

Blodprøve	Besøg 1C	Prøveglas	Volume	Sendes til
PBMCs	1 Substudy 2 besøg	Tre CPT citrate rør	3 x 8 mL	AUH
Blod til cellular Nucleic acid	1 Substudy 2 besøg	Et PAXgene rør	1 x 2,5 mL	AUH