



UK Health
Security
Agency

UK Health Security Agency

National Infection Service

UKHSA BRISTOL:

**Rapid SARS-CoV-2 Sequencing
Service: Referral Instructions**

Updated 08.10.21

1.Introduction

This document provides details on how to send samples for whole genome nucleotide sequencing at UKHSA Bristol. UKHSA Bristol offers a rapid sequencing service for Pillar 1 National Health Service (NHS) samples in England that have been identified as SARS-CoV-2 PCR positive. All NHS Trusts within the South regions 1-3 should send positive samples with Ct values ≤ 30 in at least one of multiple targets or where no Ct value is generated without delay to UKHSA Bristol using UKHSA funded or existing transport services for sequencing. Samples for genotyping/reflex testing must be sent to regional testing sites.

Sequencing is essential for identifying and managing the emergence of variants of concern. The results will be sent via the Second-Generation Surveillance System (SGSS) and through to government for key decision making around Variants of Concern and the easing of lockdown restrictions

Results will be forwarded to submitting laboratories via NPEX, local Trust systems, eLab electronic reporting system and to local Health Protection Teams via SGSS to facilitate rapid enhanced Public Health actions. These arrangements will be kept under review as further rapid sequencing assays are implemented in regional UKHSA and NHS laboratories.

Testing at UKHSA Bristol is open to receive samples 7-days per week. There is no requirement to batch samples and laboratories are requested to send samples daily so that results are timely and have greatest benefit to the public health response. This will also ensure a more streamlined workload into the UKHSA Bristol laboratory.

Trusts will NOT be charged for any samples referred to UKHSA Bristol sequencing for SARS-CoV-2.

Some sample collection buffers are not suitable for sequencing pathway. This is because the chemical lysis buffer is detrimental to the integrity of RNA. Failed sequencing runs offer poor public health value for money and delays public health responses. Specific instructions for unsuitable lysate buffers are detailed below.

2. Sample types for sequencing assay

Positive SARS-CoV-2 samples should be referred daily to UKHSA Bristol for sequencing testing. Please do not batch samples as this causes delay in identification of variants of concern and may impede the workflow of UKHSA Bristol by creating bottle necks.

Samples can be sent as:

- a. Specimens in lysis buffer (lysates) **(preferred)**
 - Please note that the preferred sample type for referral to UKHSA Bristol is Lysate. Specimens in lysis buffer, apart from the following sample buffers which are not suitable for the sequencing pathway:
 - **Samba / Liat buffer**, please send original sample in VTM or re-swab within 48 hours and send directly to UKHSA Bristol
- b. Primary clinical sample
- c. Pre-extracted RNA
 - Consideration needs to be given within each NHS network to explore how RNA can be provided rather than original sample material or sample lysates. The lead NHS or UKHSA regional genotyping/reflex testing sites and partner sending laboratories should agree where the extraction and onward referral of RNA will take place for each region. If sending laboratories are unable to perform extraction of RNA locally, then the preferred option is to refer the lysate or original specimen to the UKHSA Bristol for sequencing. This must take place in a timely manner so that RNA is referred for sequencing without unnecessary delay. If possible. A minimum volume of 25µl is required for sequencing only.

2.1 Primary samples

Primary samples should only be sent where lysate at the primary test site or regional genotyping/reflex testing site is not possible. Primary samples, defined as the swab in

a Viral Transport Medium (VTM), respiratory tract specimens such as sputum or BAL or heat-treated samples, can be sent via DX (UKHSA Bristol, DX 6120200, Bristol 90 BS) or courier (City Sprint):

- a. Minimum volume primary sample: **200µl** (preferably minimum 300µl)
- b. If there is no access to original sample material or extract for referral, re-swabbing is recommended

When sending samples, include the SARS-CoV-2 PCR Ct value if available. Only samples with Ct values ≤ 30 in at least one of multiple targets should be referred for sequencing. Positive samples detected using methods without Ct (qualitative tests) can be submitted but may fail to produce a reportable result in sequencing assays where the viral load is very low (equivalent to <1000 copies/ml).

- c. If using a PCR platform which does not provide a Ct value i.e. uses RLU or Cn values, please send all specimens which are reported as positive and the equivalent value **MUST** be included on the sample request form.

2.2 Extracted RNA

RNA extracted from clinical specimens including swabs, respiratory specimens, saliva, PM tissues etc. should be prepared as soon as possible after sample receipt to prevent degradation. Degraded RNA is likely to fail sequencing. Regional genomic testing hubs may offer to extract original samples, perform the reflex test and split the RNA sample for onward sequencing. This must be agreed at a regional level.

Samba / Liat users should refer to section 2.0 above.

Details for sending RNA:

- a. To prevent the degradation of RNA, please avoid extremes of temperatures, for example avoid repeat freeze thaw cycling (maximum of 2 cycles from primary test, through to shipping to sequencing laboratory).

- b. RNA samples should be shipped on dry ice using a courier (courier details below).
- c. Incorrect storage and handling conditions risk the integrity of RNA leading to potential sequencing failure. Use molecular grade RNase-free plasticware and do not handle samples or extracts without gloves.
- d. A minimum volume of 25µl is required for sequencing only. Extracts should be stored and transferred in molecular grade RNase-free plasticware
- e. RNA should be sent in individual tubes, labelled with laboratory specimen number as a minimum OR as plates if > 20 samples, accompanied by a clearly labelled plate map referencing the sending lab specimen number and plate well position.
- f. When sending samples for sequencing, include the Ct value if available. Ct's ≤ 30 in at least one of multiple targets should be referred for sequencing or genotyping/reflex testing. IF Ct not available because local platforms do not provide this, please indicate which platform has been used in primary screen and any alternative values e.g. RLU from Hologic **MUST** be included on the request form.

Please send primary material with the sequencing Request Form E53, see [appendix D](#)

It is important that the E53 is completed as fully as possible and **MUST** include sample collection date.

Place one copy of the **E53** form in the parcel (not in contact with the specimen container) indicating relevant safety information and sample types with the samples/RNA. Label the outside of the specimen parcel with identifier **P30 in red** to assist specimen reception at UKHSA Bristol identifying the urgent samples.

3.Data to send with samples

3.1 When sending samples in batches <20

Please send primary material with the sequencing request Form E53, see [Appendix D](#)

Complete as fully as possible :- Essential information should include:

- Patients full name
- reference/lab specimen number
- date of birth
- primary Sars-CoV-2 method and result
- NHS number
- Sample collection date
- Primary testing platform
- Ct or RLU

3.2 When sending batches of samples >20

Senders can use the excel template supplied for batches of 20 or more samples.

1. Batches of RNA eluates may be sent in a leak-proof sealed deep well plate, accompanied by a clearly labelled plate map referencing lab specimen number and plate well position.
2. The excel template detailed above must be completed instead of individual E53 forms if referring plates.
 - Do not use a line list for single/low sample numbers, use the E53 form
3. The excel template must be printed and a copy included with the samples/lysate or RNA plate samples. Samples received without this information will **NOT** be tested.

For general enquiries please contact 0117 414 6222

4. Packaging

Materials containing live virus need to be managed as ACDP Hazard Group 3 specimens.

For details on how to package samples please see link:

[Packaging and transport requirements for patient samples – UN3373 - GOV.UK \(www.gov.uk\)](http://www.gov.uk)

Please Place a UN3373 label on the outer packaging with postal address and annotation of **P30 in red**, on outside of specimen parcel to assist specimen reception.

5. Transport

There are several delivery options for transporting specimens.

Laboratories must refer positives directly from individual trusts.

UKHSA Bristol can receive specimens at Pathology Specimen reception 24/7.

If you are sending specimens by courier at the weekend ideally these should be sent to arrive at UKHSA Bristol between 08:00-16:00 when the Central Specimen Reception is open. UKHSA Bristol would prefer to receive RNA samples on dry ice during the day between 08:00-16:00. However, should a delivery be delayed there will be a facility to receive these on the UKHSA Bristol site 24/7.

5.1 If transporting RNA

RNA must be transported by using the City Sprint courier who provides a 7 day/week **NATIONAL** service for collection of SARS-CoV-2 RNA samples requiring transfer on dry ice as detailed below:

- Each booker **MUST** request the ad hoc dry ice courier service by email.
- The service is a Day 1 and Day 2 service and NOT same day.
- Day 1 - The booking form template [Appendix A](#) must be completed in full and submitted by email to UKHSAdryice@citysprint.co.uk by 12:00hrs each day 7 days/week.
- All RNA samples transported will require compliant transport within a suitable Bio-Therm box with dry ice. The box size required must be stated on the request form and will be provided and delivered by City Sprint on Day 1.
- Table 1: Guide to City Sprint Dry Ice Units - box size to request

Unit	Code	Height	Width	Depth	With dry ice
Bio 10	MX-48	19	25	20	2
Bio 15	MX-72	25	25	25	3
Bio 30	MX-115	32	32	32	12
Bio 45	MX-120	30	44	38	20

- There is no longer the need to telephone City Sprint to order a courier hence users should be aware that all communication will be directly by email.
- Busy locations requiring daily collections can book in block in perpetuity until further notice by email request.
- Day 2 – City Sprint courier collects from location and delivers to UKHSA Bristol.
- Compliance with UN3373 P650 packaging and labelling are required in all instances, with postal address and annotation of **P30 red label** on outside of the specimen parcel.
- The City Sprint customer services is managed by the Strategic Support Team and may be contacted on 01173 041374 in the event of problems.

5.2 If transporting primary specimens, specimens in lysis buffer (lysates) or heat-treated specimens

5.2.1 DX

Primary specimens, specimens in lysis buffer and heat-treated specimens do NOT require dry ice for transport. If laboratories have a DX account, they should use this for delivery of samples to UKHSA Bristol.

DX ROUTINE Monday to Friday collection for next day delivery.

UKHSA does not provide DX boxes, laboratories will need to secure these through their usual procurement system.

- **P30 (in red)**, UKHSA Bristol, DX 6120200, Bristol 90 BS
- Compliance with UN3373 P650 packaging and labelling are required in all instances, with postal address and annotation of **P30 red** label on outside of the specimen parcel.

DX SPECIAL Saturday and Sunday collection for next day delivery

DX are now operating a bespoke dedicated service for UKHSA Bristol on Saturday and Sunday. The weekend service has been established to ONLY transport sequencing samples from NHS Trust sites to UKHSA Bristol and therefore differs from the normal Monday – Friday service.

How to send your samples to UKHSA Bristol on the weekend

1. Package your samples in Category B compliant UN3373 P650 packaging and labelling

2. Affix the address details for UKHSA Bristol to the outside of the box **P30 (in red)**, UKHSA Bristol, DX 6120200, Bristol 90 BS
3. Attach the UKHSA Bespoke Weekend Service DX label, as per **Appendix B**, on the outside of the box
4. Place all boxes into a green sack for collection and apply a UKHSA Bespoke Weekend Service DX label to the sack as well as per **Appendix B**
5. DX can send laboratories a supply of these labels and green sacks, please email health@dxdelivery.com with your label/green sack requirements.
6. Place the green sack at your nominated collection point
7. DX need all laboratories to confirm the nominated collection point for their hospital for the Bespoke Weekend Service: (a) Main Reception, (b) Pathology Reception. Please email this information asap to health@thedx.co.uk

DX will attend every site and collect from your nominated collection point after 4pm on Saturday and Sunday, delivering into UKHSA Bristol on Sunday and Monday. You do NOT need to contact DX should there be no samples to collect; the courier should be notified of such on arrival.

Please note: this service is only for sequencing samples to UKHSA Bristol, all other DX deliveries will need to be sent Monday-Friday.

All referring laboratories are responsible for ensuring the safety and security of all DX boxes left at their nominated collection point pending collection by the weekend courier.

5.2.2 Courier

If DX is not available for any reason or if referring laboratories do NOT have a DX account, primary specimens, specimens in lysis buffer and heat-treated specimens can be couriered by using City Sprint as detailed below:

- City Sprint: Telephone: 0117 304 1374
- Quote account reference CS013545, password UKHSADAB
- Request a **same day vehicle** and specify the **service does NOT need dry ice**
- You will need to provide contact details and addresses for package collection.
- Compliance with UN3373 P650 packaging and labelling are required in all instances, with postal address and annotation of **P30 red** label on outside of the specimen parcel.
- All samples should be sent to UKHSA Bristol, Pathology, Southmead Hospital,

Bristol, Westbury on Trym, BS10 5NB

6. Reporting

Sequencing results will be sent electronically to the requesting laboratory via UKHSA eLab system or via NPEX if requested via that route.

Examples of sequencing reports issued by UKHSA Bristol are provided. ([Appendix C](#))

Sequences are submitted by UKHSA to CLIMB as soon as they are complete and are available 12-24 hours after receiving the eLab report/NPEX.

Please find the following link to Standardised Variant Definitions. This is a repository containing the up-to-date lineage definitions for variants of concern (VOC) and variants of interest (VUI) as curated by UKHSA.

https://github.com/UKHSA-genomics/variant_definitions

For queries relating to eLab, please contact: eLAB.Helpdesk@phe.gov.uk. This operates Monday – Friday, 9am – 5pm.

The UKHSA-eLab system provides laboratories with a web-based pdf report delivery

- Users control their own user access
- There is no limit to the number of users allowed within the system
- Secure and confidential system - no chance of reports being lost in the post
- Faster receipt of reports (Reports are available 30 minutes after release)
- Access is available to historical copies of reports for at least 6 months
- No software to install and minimal PC requirements

7. Enquiries

Contact telephone number for enquiries is:

Tel: 0117 414 6222

Appendix A City Sprint Dry Ice email booking template to UKHSA Bristol



**UK Health Security Agency - Dry Ice Packaging
(send to UKHSAdryice@citysprint.co.uk)**

Request Date (collection date will be the day after if requested before 12:00)		Bookers Name	
Sample Collection Address		Lab Address for Delivery	
Collection Department		Delivery Department	UKHSA Bristol
Full Collection Address Including Postcode.		Full Delivery Address Including Postcode.	UKHSA Bristol, Pathology, Southmead Hospital, North Bristol NHS Trust, Westbury on Trym, Bristol, BS10 5NB
Contact Name		Contact Name	UKHSA Bristol Admin team
Phone Number		Phone Number	0117 4146222

Required Packaging size

<p>Biotherm 10 (MX48) internal dimensions are 25cm x 20cm x 19cm</p> <p>Biotherm 15 (MX72) internal dimensions are 25cm x 25cm x 25cm</p> <p>Biotherm 30 (MX115) internal dimensions are 32cm x 32cm x 32cm</p> <p>Biotherm 45(MX120) internal dimensions are 44cm x 38cm x 30cm</p>	<p>[Please insert your packaging requirement here – All size boxes are supplied with Dry Ice]</p>
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Appendix B Label for DX weekend delivery to UKHSA Bristol



DELIVERED EXACTLY™

WEEKEND DELIVERY TO:

PHE Bristol

DX 6120200

Bristol 90 BS

E: health@dxdelivery.com

T: 0333 241 5100



DXDMKT/PHE/01JUN21

Appendix C Example Sequencing Report



Public Health
England

PHE Colindale

61 Colindale Avenue, London NW9 5HT

Switchboard: 020 8200 4400

Website: <https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services>

CONSULTANT MICROBIOLOGIST
HEALTH PROTECTION AGENCY HPA
CENTRE FOR INFECTIONS
61 COLINDALE AVENUE
LONDON

NW9 5HT

Sender's ref. No. M,21.000123456
PHE ref. No. ZZ 2223 1593
Date received 24.05.2021

Billing reference NO PO GIVEN
Outbreak/Investig. No
Ilog number
Project code ECOVP30

Hospital No. abc123456
Sex **M** Date of birth **12.02.1911** Age **110y**
NHS number **999 004 159 8** Patient postcode **LS1 6AE**
Name **EDITESTPATIENT, THIRTYONE**
Date of collection **20.05.2021 13:45**

Specimen Type Nose and throat swab

Final report

Sample : **sample for sequencing**

SARS-CoV-2 Sequencing B.1.617,PHEC-31X5A80
SARS-CoV-2 Sequencing Report VOC-21APR-02 CONFIRMED

SARS CoV-2 Genotyping RT-PCR SNP E484K Wild type
SARS CoV-2 Genotyping RT-PCR SNP K417N Wild type
SARS CoV-2 Genotyping RT-PCR SNP K417T Wild type
SARS CoV-2 Genotyping RT-PCR SNP P681R Mutant

P681R mutation DETECTED by SARS CoV-2 genotyping assay

Report comment:

This sample has a mutation/s that is/are compatible with a SARS-CoV-2 variant of concern/variant under investigation.

This sample has been tested by SARS-CoV-2 genotyping assays to identify amino acid changes at E484K, K417N, K417T and P681R.

Authorised by Please contact the number above


Date reported: 27.05.2021 17:33

Page 1/1

Date printed: 27.05.2021 17:34

Appendix D

PHE Bristol request form



COVID-19 Genotyping and Genome Sequencing - Bristol

Public Health England

PHE Bristol
Pathology, Southmead Hospital
Westbury on Tyldesley
Bristol, BS10 5NB

Phone 0117 414 6222
Open 8am – 8 pm
www.gov.uk/phe

PHE
Bristol
DX 6120200
Bristol 90 BS

Please write clearly in dark ink

SENDER'S INFORMATION

Report to be sent **FAO**

Contact Phone Ext

Purchase order number

Project code

HPZONE or local outbreak ID

Postcode

PATIENT/SOURCE INFORMATION

NHS number

Surname

Forename

Hospital number

Hospital name (if different from sender's name)

Sex male female

Date of birth Age

Patient's postcode

Patient's HPT

Ward/ clinic name

Ward type

ADDITIONAL TEST REQUEST

Genotyping not performed -Requested Genotyping performed -Not Required

SAMPLE INFORMATION

Your reference

Original Sample (please specify type)

Original sample (State volume buffer) in lysis buffer

Nucleic Acid Please tick if NA sample volume <50ul

(Include a plate map if sending extracts on plates)

Other (please specify)

Date of collection Time

Date sent to PHE

All samples submitted should be treated as though the patient is infected with a Hazard Group 3 Pathogen. All samples must be sent in accordance with Cat B transport guidance.

SARS-CoV2 PCR Assay used?

CT value?

Provide result of genotyping if already performed

No Ct generated by assay used.

Tick if known co-infection (please specify)

CURRENT PATIENT STATUS

Care Home At Home Hospitalised ICU ECMO Deceased Other (eg. Prison) (please specify)

Please tick the box if your clinical sample is post-mortem

REASON FOR SEQUENCING

Outbreak HCW Travel Study/project Vaccine Failure VOC/VUI query/contact Other

(Give details eg. Study name/location of outbreak/travel country and date of return)

Have previous samples from this patient/outbreak been sequenced? No Yes (State where)

CLINICAL/EPIDEMIOLOGICAL INFORMATION

Asymptomatic URTI ILI Pneumonia Other (please specify)

Onset Date

OTHER COMMENTS

All requests are subject to PHE standard terms and conditions.

Version effective from Oct - 2021

YW-2258.02