Histopathology User Guide

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AUTHORISED BY: Laura Wright	
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Histopathology Policy	
Histopathology User Guide	
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DATE	**AMENDED BY**	**DETAILS**
18/1/21	P Hinson/ L Wright	UKAS scope update after accreditation visit **V19**
19/02/22	Jackie Wilkinson	Removed signature table, no change to body of document. New version not created
03/08/2022	L Wright	Addition of antibodies added to scope after recent UKAS. Change of one of the contacts. **V20**
07/03/2023	L Wright	Addition of specimen details on pots and removal of Bouin's fluid. **Version 21 created.**
20/12/2023	L Wright	Update to ISO 15189 standards. How to contact on call BMS. Clarification on placentas. Update to contacts. Addition of risk to patients and PDL-1.Minor changes. Addition of WGS. **Version 22 created.**
04/11/2024	L Wright	Update to include digital pathology (2.16). Clarification on handwritten requests in section 2.6. ISS transport changed to EMED Global hospital replaced by HealthShare. Clarification on section 4.3.3 frozen sections. **Version 23 created.**

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1.0 PURPOSE OF PROCEDURE

The purpose of this procedure is to provide contact details and helpful information concerning specimen handling. This is the master Histopathology user guide (HIS.GEN.P.9, department also issues daughter policies, that contain user specific information :-

HIS.GEN.P.09a Histopathology User Guide for GPs

HIS.GEN.P.09b Histopathology User Guide for JPUH

HIS.GEN.P.09c Histopathology User Guide for HealthShare Hospital

HIS.GEN.P.9d Histopathology User Guide - Paediatric Samples for Metabolic Diseases

There is also a Histopathology ICE requesting guidance

HIS.GEN.P.25 Histopathology ICE Requesting Userguide

2 METHOD / PROCEDURE

2.1 Location, Contact Details availability of clinical advice

The Cellular Pathology laboratory (Histopathology and Cytopathology) is based at the Cotman Centre, which is located at the junction of Colney Lane and the Watton Road (B1108).

- Technical Advice available from Histopathology on x2029 (01603 286029)
- Trust BEAT Pages (Departments - Cellular Pathology).
- Report enquiries available from Histopathology office x2013/14/15/16.
- Advice on interpretation of results is available from the reporting consultants, to whom you can be transferred from the Histopathology office
- Pathology porters X3456
- Porter manager X5430
- Routine specimen collection porter ext 6022 (01603 646021)
- Urgent specimen porter ext 6021 (01603 646021). Please see section 2.14 for examples of specimens requiring urgent transport. If you are unable to contact the urgent porter please try bleep 1113 or try the routine porter. (Note the urgent porter is unavailable after 16:30 Out of hours transport must be arranged by the sender/East Atrium).

2.2 Cellular Pathology Department Opening hours

Monday to Friday 0800 – 1700 hrs

Saturday and Sunday – Closed

There is a formal on-call Histopathology **technical** service provided outside of the opening hours stated above for JPUH and NNUH trusts. To speak to the on-call Biomedical Scientist, they can be contacted via ALERTAIVE or hospital

switchboard. If going through switchboard, please ensure that you specifically ask for the **Histopathology Biomedical Scientist on-call.** This will prevent going through to the wrong Biomedical Scientist within another Pathology discipline and prevents any unnecessary delays.

For any **reporting** (eg Frozen Section) service required outside hours, prior consultation and agreement between trust clinician and consultant histopathologist is necessary. If you cannot contact a consultant histopathologist during normal working hours, it may be possible to contact a pathologist by telephoning the hospital switchboard.

There is no formal on-call Cytopathology technical service outside the department opening hours.
There is no formal on-call system or laboratory service outside working hours for either Histopathology or Cytopathology in regard to SPIRE Hospital or HealthShare clinic patients or samples.

On Fridays, please make sure that as many samples as possible are sent by your last collection time, otherwise samples will remain in your department until Monday. There is no Cellular Pathology porter collection over weekends.

2.3 Specimen Types and Handling Procedures

2.3.1 Specimen types for Histopathology

- Routine tissue samples in fixative (10% Formalin)
- Fresh tissue samples for Frozen Sections
- Fresh tissue samples for pre sampling via the Biorepository
- Muscle biopsies (fresh & unfixed only)
- Sural nerve biopsies
- Skin samples for immunofluorescence IMF (In Michels medium)
- Skin Samples for Mohs
- Sentinel Lymph Nodes
- Post Mortem histology
- Fresh placentas
- Whole Genome Sequencing.

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Key Points

- **Consent for Histopathology should have been obtained (consent is inferred when specimens are received within the laboratory. Other specific specimens such as POC/PM's may have differing levels of consent and these will be stated on the accompanying request form).**
- **Before sending check the completion of the request form, always confirm the identity of the patient (See 2.7)**
- **Check that the Specimen pot is correctly labelled**
- **Ensure that the specimen is in an adequately sized specimen pot, and determine if the specimen should be sent with or without fixative (See 2.3)**
- **Minimise the risk of specimen interchange by not pre labelling pots and double checking request forms before sending.**

Please read the Health and Safety considerations (See 2.12)

For specimen packaging information please refer to section 2.4 of this document.

We request that two request forms are completed when the patient has both Histopathology and Non-Gynaecological samples, and information regarding both specimen types is completed on both forms for Clinical Governance purposes. It may be easier to photocopy the request form in this instance. Advice about specimen preparation is available on the Trust Intranet site (Cellular Pathology which includes Histology and Cytology Departments) or via the BEAT.

2.3.2 Routine tissue samples in fixative

Tissue samples for routine histology should be sent in 10% Formalin. Ideally the volume of fixative should be 10 times that of the specimen and the size of the container appropriate to the specimen (i.e. no tissue squashing). For routine specimen collection please refer to section 2.5 of this document.

2.3.3 Fresh tissue samples for Frozen Sections

All frozen sections should be **booked in advance**, if possible with the Duty Consultant Pathologist (can be contacted via the Histopathology office exts: 2014/2015/2016). The Histopathology office will pass your call onto the Duty Consultant Pathologist who will take all the necessary information.

It would be appreciated if clinicians could inform the laboratory on x2029 of any changes to the operation time, or if the operation is cancelled or running late. This enables laboratory staff to make the necessary arrangements.

All specimens for frozen section must be accompanied by a request form marked 'FROZEN SECTION', with the contact telephone number clearly written.

Samples for frozen sectioning will be collected by calling the urgent pathology porter on ext 6021 (01603 646021). Please allow at least 10 minutes for the porter to reach you, so that delays are minimised.

All specimens must reach the Histopathology laboratory by 4:30pm due to there being no urgent porter service available after 4:30pm. Therefore, please phone no later than 4pm, to guarantee that the porter can collect the specimen and deliver to Histopathology.

Important: A frozen section service cannot be guaranteed outside hours, prior consolation and agreement with a Histopathologist must be made in advance. There is also no urgent porter service after 16:30 so transport with a courier or taxi service must be arranged by the user.

2.3.4 Fresh tissue samples for Tissue Banking

These specimens should only be sent by prior arrangement with the Biorepository. 01603 289428

Samples for Tissue Banking will be collected by calling the urgent pathology porter on ext 6021 (01603 646021). Please allow at least 10 minutes for the porter to reach you, so that delays are minimised

Please note that high risk specimens are not suitable and will not be accepted for Tissue Banking.

2.3.5 Muscle biopsies (for dystrophies, myopathies and neurological diagnosis)

This type of specimen is only accepted by **prior** arrangement with the laboratory ext. 2029 or 2022. It is preferable that the laboratory receives at least **2 days notice**, to ensure that the necessary equipment/reagents are available to prevent unnecessary delay.

As the samples require lengthy preparation, they must be received in the laboratory no later than 4 p.m. It would be appreciated if clinicians could inform the laboratory on ext. 2029 of any changes to the operation time, or if operation is cancelled.

There is no need for clinicians to stretch the tissue, support it in any way or wrap in lint. All preparatory work is done in the laboratory.

Muscle biopsies for the above diagnoses **MUST** be sent fresh and arrive in the laboratory soon as possible after removal, accompanied by the request form.

Samples will be collected by calling the urgent pathology porter on ext 6021 (01603 646021). Please allow at least 10 minutes for the porter to reach you, so that delays are minimised.

These must be received by the laboratory within 30 minutes of being taken.

2.3.6 Sural nerve biopsies

This type of specimen is only accepted by prior arrangement with the laboratory ext 2029 or 2022. This will enable the laboratory to send a fixation Kit over to the clinic. The fixation kit contains the formalin and glutaraldehyde specimen pots and full instructions in the form of a form to detail use

Samples will be collected by calling the urgent pathology porter on ext 6021 (01603 646021). Please allow at least 10 minutes for the porter to reach you, so that delays are minimised

2.3.7 Skin samples for immunofluoresence 'IMF'

Specimens for IMF should be placed in 'Michels Medium'. This is a transport medium which can be order from the Path Hist fixative list on the 'Powergate' ordering system. Using this transport medium means there is not an immediate urgency to send the specimen to Histopathology! Sending the specimen via the routine collection is sufficient. If however, this transport medium is not used specimens for IMF should be wrapped in saline soaked gauze and sent to Histopathology immediately (by contacting the urgent porter on ext 6021).

Please note; the use of Michels Medium is strongly recommended, all specimens for IMF will be expected to arrive in this transport medium. If they do not arrive in this medium, tissue integrity can not be maintained.

2.3.8 Mohs Specimens

Mohs specimens are booked directly by the dermatology Mohs coordinator and are sent fresh by the Mohs Porter. Mohs specimens are placed in labelled Petri dishes with an accompanying request form and Mohs map.

2.3.9 Sentinel lymph node specimens

The specimen should be fixed in 10% Formalin and left in theatres for approximately 6 hours to ensure that the radiation level is below 10mb and therefore safe to transport. Theatres must check the levels are below 10mb before they are sent.

These specimens must be transported to the Histopathology Department one at a time, otherwise radiation levels for transport will be over 10mb and become a Health and Safety issue. These specimens should be transported to the Cotman Centre by the urgent porter. However the porter's priority will still be the really urgent samples.

2.3.10 Post Mortem histology

All tissue from post mortems is received in block sized pieces in fixative, except exceptional circumstances where the pathologist has arranged for larger pieces to be taken

2.3.11 Molecular Pathology

An array of molecular pathology tests are now performed in house, please see appendix 2 for a list of the mutations that the department can detect.

2.3.12 Placentas

Placentas for routine histology are sent to the Histopathology Department fresh. Placentas should be placed directly into a patient labelled specimen pot (**not** labelled via the lid only). They should not be wrapped in any form of clinical materials or waste bags. This provides a biological hazard to the laboratory staff. Please telephone the routine porter to arrange specimen collection. On receipt of placentas into the Histopathology Department they are fixed in 10% Formalin. For routine specimen collection please refer to section 2.2 of this document.

Over weekends and bank holidays the placentas should be sent to pathology reception were it will be stored in the fridge and then forwarded onto the Histopathology Department in working hours.

Any specimens that are not adequately labelled to the laboratory requirements or as stipulated above, could/will be returned to the sender, causing delay to patient testing and care. Repeated failure to meet these requirements, could instigate Trust error logging procedures via a DATIX.

2.3.13 Whole Genome Sequencing (WGS) specimens.

Whole Genome Sequencing (WGS) can be requested. Please follow the below embedded document on how this can be requested and organised with the laboratory. Please note, the laboratory require at least 5 working days notice for this to be undertaken, due to the complex preparation requirements.

WGS flowchart.docx

2.3.14 Specimen Packaging and Labelling

Minimum Specimen Identifiers

Specimen Pots and request forms should be labelled with the following details:-

- **Full Name**
- **Date of Birth**
- **Sex**
- **Hospital Number (If NNUH Patient)**
- **NHS Number - important for accurate data and to reduce duplication of patient records**
- **Specimen information (e.g. specimen type, location, laterality etc as appropriate – e.g. Right Breast)**
- **Pots need to be labelled with specimen information, the same as what is on the request form.**

Preferably use a PAS generated addressograph label, This should be used to label the pot and is not acceptable to place on the lid of the specimen pot.

Failure to label specimens adequately, may result in the return of the specimen to the sender and delay or prevent the report

- Specimen containers should be placed into a marsupial bag or other suitable plastic bag, which should then be sealed. Please ensure that all container lids are securely sealed to prevent leakage.

- Individual marsupial bags should then be placed into a secondary polythene bag, which should be sealed or tied.

- If the specimen container is too large to fit into a marsupial bag, or if a marsupial bag is not available, the specimen should be placed into polythene bag and sealed/tied, before placing the specimen into the secondary polythene bag. These bags should then be paced in the transport box. An absorbent sheet will also be present in the transport box to absorb any spillage within the box.

- For quality/risk management purposes, a SPECIMEN/ SENDER LOG MUST be completed by senders within the trust. Patient Addressograph labels must be placed on each copy where available. A separate 'immediate

priority/urgent 'sender log should be used for urgent samples (which are coloured pink). A plastic wallet is supplied in the specimen transport box for the storage of sender log sheets. Please do not put the logs in the same bag as the specimens. The log may be damaged if there is a leakage. Also, if the specimens are mislaid the sender log would also be lost. Replacement sender logs are available from the pathology porters on X3456.

- If specimen requirements do not meet any of criteria as stipulated within this user guide, the impact to patients will be minimised. The laboratory will contact the user as soon as they become aware of any issues, and will follow the necessary procedures in place. If further advice is requested, laboratory personnel can contact the quality team, laboratory management or Duty Doctor for further advice/clarification. The risk to patients will always be mitigated, as no specimen will ever be rejected due to the nature of the discipline, in that Histology is often unrepeatable. All efforts will be made to ensure the specimen is accepted, and the necessary caveats/explanations added to the final report as appropriate. If internal or Trust incident reporting is necessary, this will be undertaken as appropriate.

- Before the porter arrives, the box should be sealed using a plastic cable tie. Collection porters are instructed not to collect unsealed specimen transport boxes. It is good practice to carry out a final check to ensure that the entries on the sender log match the specimens placed in the transport tin. It is often easier to resolve any discrepancies at this stage before the specimens are sent to the lab. Plastic cable ties are supplied by the collection porters. If you keep your supply in a box at your collection point, the porters will top this up. Alternatively, plastic cable ties can be obtained from the Pathology porters on X3456.

- If any FRESH specimens for non-urgent reporting for Histopathology or Cytopathology CANNOT be collected by Pathology porters and delivered to the Cellular Pathology laboratory during laboratory working hours, they MUST be kept refrigerated, then collected on the first following weekday. If you do not have a fridge, then please send these samples to Pathology Reception, Level 1, East Block.

- Do **not** refrigerate Histopathology samples in fixative please.

2.4 Specimen Transport

SPIRE Hospital

SPIRE samples are despatched to the Cotman Centre via the SPIRE van delivery system.

Cromer Hospital

Samples are delivered to the Cotman Centre via EMED van delivery systems.

General Practice

Samples from most Norfolk GP practices are despatched to the Cotman Centre via EMED Facility Services van delivery system.

All samples must be transported in approved transport containers, which will be supplied to you, and will be replaced with empty containers.

All specimens should be accompanied by a completed Histopathology Request form (either ICE or 'old style' yellow edged form), see 2.14

Specimens for Cellular Pathology MUST be kept separate from specimens for Microbiology, and separate from those for Clinical Pathology (Clinical Chemistry, Haematology, Blood Transfusion and Cytogenetics and Molecular Genetics.

Norfolk and Norwich University Hospital

Designated Pathology porters will collect samples throughout the NNUH and deliver them to transport vans. We CANNOT accept specimen delivery by your porters or staff directly to the Cotman Centre.

Most user departments are issued with a routine specimen collection time, if your department does not have a routine collection please contact the routine porter. See section 2.13.

There will **not** be a routine or immediate specimen collection service outside normal working hours. Any samples requiring urgent attention outside of these hours should be transferred to the Cotman Centre (with prior arrangement with Histopathology ext 2029) via a taxi arranged by the sender. Contact the west atrium reception on x5462 to arrange a taxi.

James Paget University Hospital

User departments are responsible for the despatch of samples to Cellular Pathology Reception (located within Pathology) at JPUH. Laboratory staff in Pathology, JPUH staff log the specimens onto ICE , which is interfaced to the Labtrak system at NNUH. Specimens are placed in transport containers, with a specimen log and sealed, then collected by the JPUH van driver and transported to the Cotman Centre (2 runs per day).

2.5 Specimen Priorities

Please check the information below before deciding which category your specimens are allocated to.

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Turnaround Times

The department aims to meet ensure that 90% of "Two week wait" cases are reported within 10 days in order allow for clinical decisions to be made early enough to meet the national 62 day target for the initiate to cancer treatment.
Actual individual case turnaround times vary depending on the complexity of the case and the need for subsequent investigations such as special stains or immuno. The table below gives an approximation of routine turnaround times from collection to report, based upon average TATs for cases requiring the following additional techniques.

	H&E only	Special Stains	Immunohistochemistry	ISH (Kappa / Lambda or EBER)	FISH Her-2
Expected TAT	**10 days**	**11 days**	**11 days**	**17 days**	**21 days**

Routine Specimens– If you do not have designated collection times for routine specimens, contact the routine porter to arrange collection on ext. 6022. Currently, routine specimens are prioritised in terms of RTT weeks wait and clinical escalation, NOT received date. Accordingly, TATs are not available at the moment."

Two Week Wait Specimens- Only use this if the patient is on the **62 day Cancer Pathway**, departmental targets are measured against this category as this has been deemed the most clinically important. If two week wait specimens have not been reported within 7 days, the clinician may ask for the case to escalated following the framework outlined in appendix 1.

Urgent priority Samples- Are samples in fixative (10% Formalin) where the report is required within a specified time frame, usually within 48 hours, but not the same day. Specimens should be marked urgent and the date the report is required should be stated, together with contact details. **In most cases urgent specimens can be collected by the routine porter, providing the sample will reach the laboratory before 17.00, if it is required for next-day reporting.**

Immediate Priority Samples- Are samples that require immediate transfer to the laboratory, see list below.

- Frozen sections
- Tissue Bank samples
- Muscle biopsies
- Nerve biopsies
- Skin specimens for IMF not in 'Michels Medium'.

- Specimens that need to be transferred for urgent opening to ensure adequate fixation, including but not exclusively mastectomies and colorectal resections (Friday pm when samples are ready for collection after usual collection time).
- Very urgent fixed specimens for same day processing that have been discussed and **agreed** with the Duty Pathologist.

The urgent porter can be contacted on ext 6021 (01603 646021). Please ensure that the specimen is ready for collection before calling. Please note the urgent porter service is only available between 8.30 and 17.00 hrs Mon to Fri. Please note that for late specimens the emergency specimen porter should be called by 16.30 to ensure the specimen can be collected within the 8.30–17.00 time frame.

ICE reports are available immediately after authorisation, authorised Histopathology paper reports are always printed on gold edged paper and are mailed on the following day. Please note that official authorised histology reports are always on gold edged paper, and these contain the required elements of the ISO 15189 standard, Other unofficial copies (ie printed from ICE/Lab trak may) not contain pagination or patient locations on all pages.

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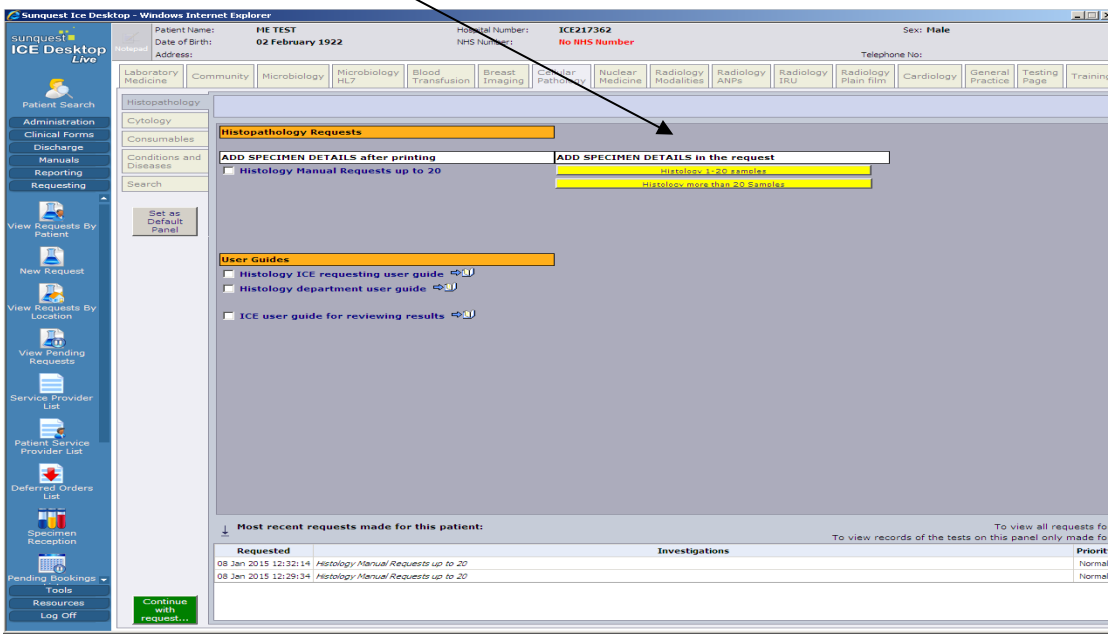
2.6 Request Forms

NNUH Histopathology requests should be made on the ICE System, a paper request is available for occasions when the ICE system is down or a computer terminal is not accessible. A paper request should only be used in the above circumstance and not as routine practice. This could lead to patient reports not being sent to the correct location if misinformation is given.

To request on ICE

- Log into ICE desktop
- Select patient.
- Choose 'New request' from the Requesting Tab on the left hand side.
- Select the Cellular Pathology tab at the top and the Histopathology tab on the left hand side.
- Click on Histology 1-20 samples or Histology 21-40 samples depending on how many samples you have taken.



- Click on the number which corresponds to the amount of samples you have taken.
- Choose which test you require.
- Enter the specimen type and site, one specimen per box.
- Using the reporting guide at the top of the page, please state if the report is needed urgently.
- Choose the patient pathway if appropriate.

- State if a Cytopathology specimen has also been taken. (A separate Cytopathology request will need to be made).
- If you have chosen urgent then a separate screen will pop up and you will be asked what date the report is needed by (format dd/mm/yy). If you have chosen routine then you will go straight to the next step
- If the patient is female, then you will be asked if this is a gynaecological sample.
- If yes then you will be asked for the patients LMP and recent hormone treatment.
- Click on the green button at bottom left to continue with request.
- Enter the following details:
    - the requesting GP/Consultant
    - patient location
    - clinical history in Global Clinical Details box
      (Failure to provide sufficient clinical data may lead to misdiagnosis and delay)
    - category of patient
    - whether the request is high risk
- If you require the report to go to another clinician or GP (i.e. not yourself), please enter those details into the Global Clinical Details box, e.g. Radiologists performing a Biopsy on behalf of a GP
- Click accept request.
- Print out request form (NB. if there is more than 20 samples then 2 request forms will be printed.)
- Please use the labels printed on the top left of the request form to label the specimen pot, or alternatively use the patients PAS label.

For more information on this please refer to the Histopathology ICE requesting user guide available on Trust Docs.

Alternately if ICE is unavailable the 'Histopathology or Non-cervical Cytopathology Request Form' form number NNU36 can be used.

These are obtained from:

- Procurement Department NNUH, for NNUH users (via Powergate)
- Stationary Department, St Andrews House – General Practitioners.
- Printing Department at Queen Elizabeth Hospital, Kings Lynn - JPUH clinicians/users

Please complete **ALL** sections on the request form except the grey shaded area (for laboratory use only). Failure to provide sufficient clinical data may lead to misdiagnosis and delay .

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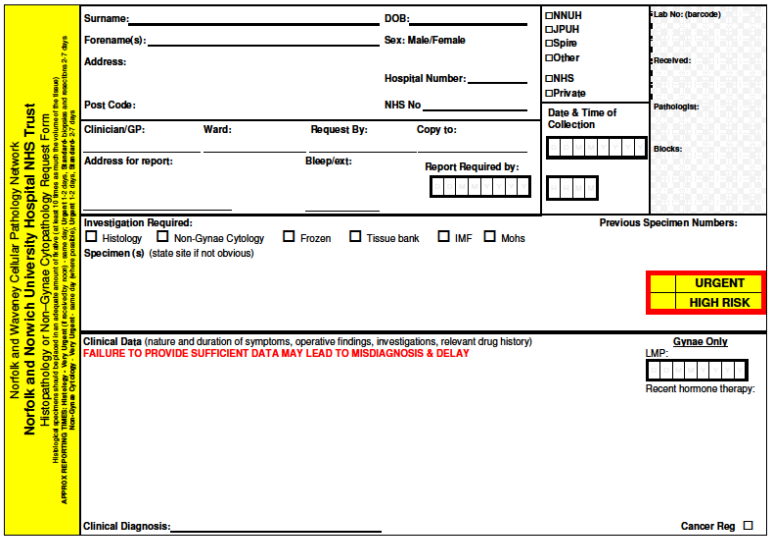
Please use an 'Addressograph' label if possible. It is essential to complete all these details, including the date and time of sample removed, and tick the source box in the yellow edge to show the patient category i.e. NNUH, JPUH, SPIRE or OTHER.

Please ensure either type of request form is placed in the pocket of the marsupial bag and the corresponding specimen in the bag itself, and ensure the form is not contaminated in any way.

Please label the request form 'HIGH RISK' if appropriate.

If the specimen is urgent please ensure a 'Report Required by:' date is provided.


Norfolk and Waveney Cellular Pathology Network
Norfolk and Norwich University Hospital NHS Trust
Histopathology or Non-Gynae Cytopathology Request Form

Surname: DOB:
Forename(s): Sex: Male/Female
Address:
Hospital Number:
Post Code: NHS No
Clinician/GP: Ward: Request By: Copy to:
Address for report: Bleep/ext: Report Required by:

☐NNUH ☐JPUH ☐Spire ☐Other ☐NHS ☐Private
Date & Time of Collection
Lab No: (barcode)
Received:
Pathologist:
Blocks:

Investigation Required:
☐ Histology ☐ Non-Gynae Cytology ☐ Frozen ☐ Tissue bank ☐ IMF ☐ Mohs
Specimen (s) (state site if not obvious)
Previous Specimen Numbers:
URGENT
HIGH RISK

Clinical Data (nature and duration of symptoms, operative findings, investigations, relevant drug history)
FAILURE TO PROVIDE SUFFICIENT DATA MAY LEAD TO MISDIAGNOSIS & DELAY
Gynae Only
LMP:
Recent hormone therapy:
Clinical Diagnosis:
Cancer Reg ☐

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Flagging of High Risk Specimens

HSE Safety Notice HID 5-2011

In clinical laboratories, specimens are sorted and processed on the basis of the information provided. If clinical details are inaccurate or incomplete or there is delay in disclosing new information to the laboratory then this can result in specimens being processed under insufficient laboratory containment conditions. Clinicians must include any recent history of relevant foreign travel that may increase the likelihood of exotic agents being present.

Ensure that clinical details supplied on specimen request forms contain clear information regarding the nature of test being requested and sufficient detail to inform laboratory staff upon the safety precautions they need to take in order to process the specimen without risk of infection.

Specimens labelled as 'High Risk' must be sent in 10% formalin. The specimen pot, form and marsupial bag must all be labelled as 'High Risk'. Please include on the request form if the patient has known or suspected TB, HIV, Hepatitis A, B, C etc. It is the responsibility of the clinical staff to identify and label the specimen.

Please note Frozen Sections, Tissue Banking and Immunofluorescence will not normally be carried out on known 'High Risk' specimens.

CJD/vCJD

The Histopathology Department **does not** receive or handle tissue samples from;

- Patients with known Creutzfeldt - Jakob disease (including vCJD)
- Symptomatic patients with definite, probable or possible forms of sporadic, variant, familial or acquired CJD
- Patients with a history of a neurodegenerative disorder, including dementia and ataxia, where the diagnosis is uncertain.

All such samples must be sent for testing to The National Creutzfeldt-Jakob Disease Surveillance Unit, address as below:

Western General Hospital
Crewe Road
Edinburgh
EH4 2XU
UK
Clinical Office Telephone: 0131 537 2128
Pathology Telephone: 0131 537 1980
Fax: 0131 343 1404

The handling of tissue samples from patients who are at risk of CJD or vCJD but are asymptomatic will be considered (by Histopathology) if the tissue is classed as either medium or low risk. This is based on the 'Guidelines for pathologists and pathology laboratories for the handling of tissues from patients with, or at risk of, CJD or vCJD' (Transmissible Spongiform Encephalopathy Agents: Safe Working and the Prevention of Infection: Annex K. Published: March 2009)

Please contact the Histopathology Department on ext 2022 or 2029 for further advice.

2.7 Specimen pots and Fixative supplies

- Service users are required to purchase their own supplies of specimen containers and fixative. 10 Litre containers (10% Formalin) and pre-filled specimen pots containing 10% Formalin (60 ml and 120 ml) must be obtained by ordering from the trust Powergate ordering system, If you do not have access, please contact the purchasing department. A shopping list for these items has been set up under the name of Path Hist fixatives. Contact the Pathology porters on x3456 (01603 287456) to arrange a collection times. The porters will arrange to meet you at the rear service corridor entrance to Pathology, level 1, East Block.
- White specimen buckets (1, 2.5, 5 and 10 litres) are also available for collection from the Pathology porters, follow the collection arrangements stated above.

2.8 Factors affecting specimen quality and reasons for specimen rejection

- Inappropriate amount of fixative or none at all (if required)
- Large specimen crammed into a small pot
- Fresh tissue not sent to Histopathology immediately
- Unlabelled or miss-labelled request form or specimen
- Poorly packed specimens resulting in spillage

All of the above can result in poor quality histology or delays in the processing of the specimen.

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2.9 Work referred to other laboratories

If any tissue samples removed require further tests at either another department within the trust or a different hospital please contact the 'Consultant Duty Pathologist' (ext 6014/16) as far in advance as possible with all the relevant details. In order to maintain turnaround times the department is sometimes having to refer some cases to a trust approved primary reporting service (SHS), where this is the case it will be mentioned in the report.

The department, on occasion will refer work to consultant colleagues outside of the trust for second opinions, where this is the case the second opinion will be attached to the original report.

Muscle Biopsies and Sural Nerve Biopsies are routinely referred to Addenbrookes Hospital Cambridge.

Details of the referral procedures and the centres used are available from the Histopathology department.

Currently routine outsource molecular tests are MDM-2 and ROS-1

2.10 Health and safety considerations

Please ensure

- All areas that use formalin have appropriate spillage kits
- All specimens and request forms are packaged correctly
- All specimen lids fit securely
- Specimen pots and request forms are clean externally (i.e., no blood stains)
- All request forms are labelled adequately including any 'High Risk' or 'Urgent' information
- All environmental and storage conditions are fulfilled.
- That correct PPE is used when handling chemical fixatives and that COSHH regulations are adhered to.
- That all materials involved in specimen collection are disposed of in a safe and correct manner
- Formalin should be stored at room temperature (5-30 °C)

More information is available from the Histopathology Department

2.11 Time limits for repeat Investigations

Histopathology paraffin blocks are kept for a minimum of thirty years, in line with the Royal College of Pathologists Guidelines. Therefore any repeat investigations on sampled tissue must be requested within this timeframe.

2.12Requests for Patients to be Discussed at Multi Disciplinary Team Meetings (MDTs)

Any requests must be made via the appropriate MDT Coordinator. The MDT Coordinator should send a list of patients to be discussed via secure email to the relevant member of the histopathology office team (MDT Coordinators will have contact information for this purpose). Any requests must be received by midday, the day prior to the meeting.

For any queries please contact the Histopathology office on 01603 286958 (Ext 2958)

2.13Verification and Measurement Uncertainty In Histopathology

All stains performed in Histopathology are verified for use by checking against control material prior to reporting, in some circumstances in house reagents or bought reagents that are not CE marked are used in diagnostic testing. Such reagents are verified for use with control tissue prior to a specimen being reported. If you would like further information on these procedures please contact Laura Wright (laura.wright2@nnuh.nhs.uk)

Any direct measurement in histopathology may be affected degree of tissue shrinkage caused by fixation or processing. We therefore are unable to assess the degree of accuracy of a measurement however where we measure or count the relevant elements that are required by the current Royal College of Pathologists minimum datasets. The reported values are checked by the pathologists, who review the cases for the MDTMs

2.14Protection of Personnel Information

Any information concerning your health is strictly confidential and all staff who deal with your medical records must keep them confidential at all times.

We have a legal duty to protect any information we collect from you and will only use your information for the purposes of providing your healthcare and for training and monitoring. In the course of your care we may need to share your information within the NHS and with partner organisations.

The laboratory adheres to the Trust Confidentiality Protocol which can be found on the NNUH website under ‘Patient Information’

2.15Complaints Procedure

The Trust is committed to looking at ways to improve the service we provide you and you can help us by telling us what you think of our service, good or bad.

Modern healthcare is a complex process and things may not always go to plan despite our best intentions, NNUH clinicians may direct complaints to the Quality Manager in Histopathology or log incidents via Datix. If patients are unhappy about their care, we would recommend they speak first to their consultant, the nurse in charge, or our Patient Advice and Liaison Service (PALS).

The Complaints Procedure, followed by the Trust, provides the method for dealing with formal complaints where there is dissatisfaction with our services. For further details of the procedure and the way that complaints are managed in the Trust, please follow the link on the Trust internet site under the heading 'Useful Documents'.

2.16Digital Pathology

The Histopathology department are moving towards all requests being reported via Digital Pathology from scanned whole slide images produced from traditionally reported light microscopy produced slides. This will not impact the way patient reports are received. Whilst we undergo this transition process, any patient reports that have been reported via Digital Pathology will have the following statement added to the report – "*"This case has been reported digitally using a validated scanning system. This is not currently part of our ISO 15189:2022 schedule of accreditation".*

When we have moved to a fully digital scanning workflow, this statement will appear on all reports and will be adapted once accreditation to ISO15189:2022 has been achieved.

2.17Accreditation

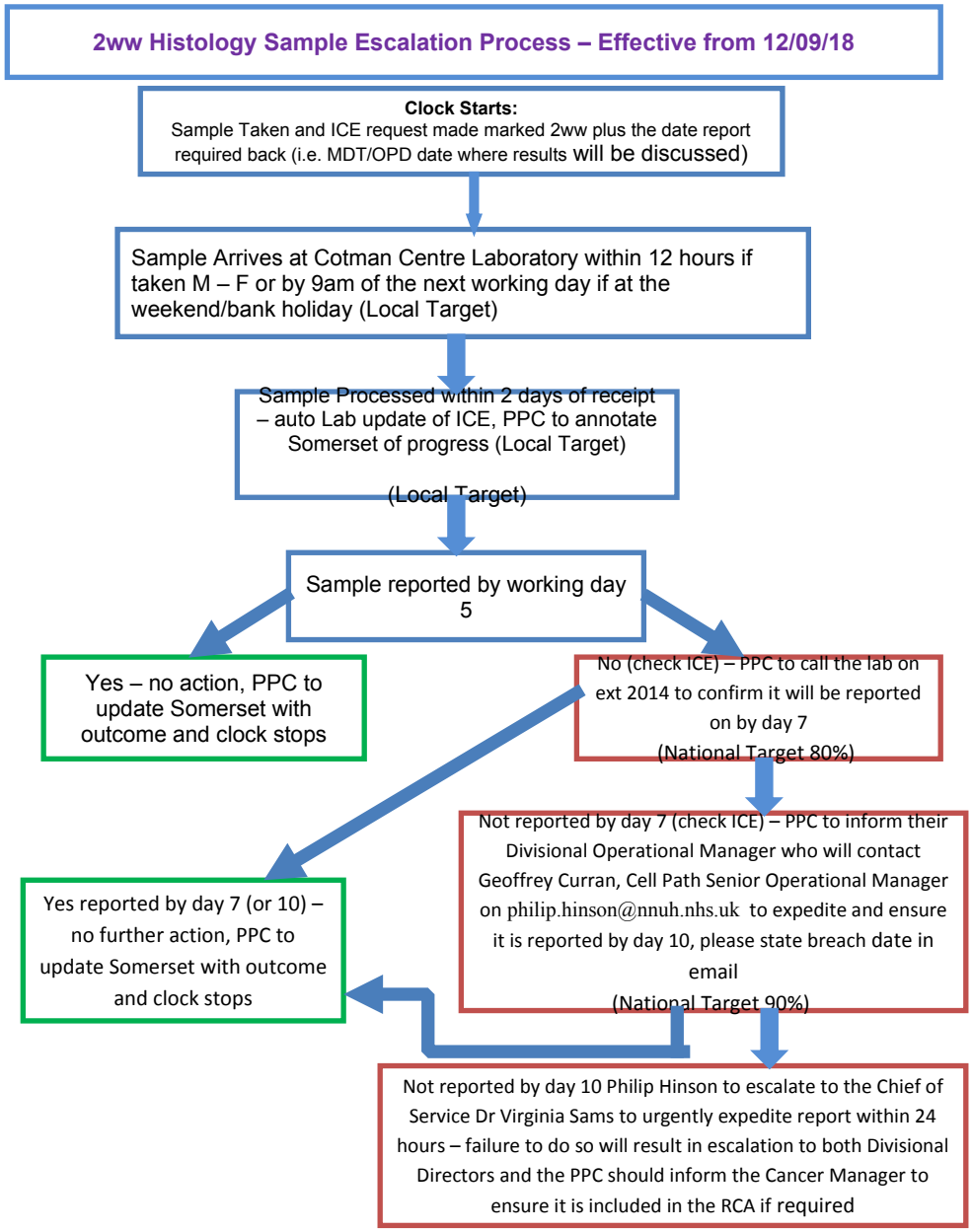
The department of Cellular Pathology is accredited to ISO 15189 (2012), our current up to date accredited repertoire is available on the UKAS website (www.ukas.com). The department is in the process of transitioning to ISO15189:2022, which when accredited, will replace the 2012 accreditation.

The department has recently added: Molecular tests - BRAF, KRAS and EGFR and the following antibodies - Arginase-1, Carbonic Anhydrase IX, Cathepsin K, Glutamine Synthetase, HNF1β, NKX3.1, PAX2, PD-L1, PTEN, TFE3 and TLE1to its accredited scope

An extension to scope for Digital Pathology is in the process of being submitted, with the aim that this will be assessed in 2025. The department aims for this to be added to its accredited scope.

New tests may be added to the department's repertoire throughout the year however these do not form part of the 'accredited scope' of the lab, until they have been formally assessed by UKAS. Please be assured that all tests are internally verified by the laboratory prior to introduction.

Appendix 1 Two Week Wait Escalation



NB *If immunocytochemistry is required or extra sections are needed in order to provide an accurate a diagnosis as possible this may add up to 2 extra working days to the flow*

Please note these clock start and stop times are only for the purpose of monitoring turnaround times of histo samples and do not relate to the overall cancer or RTT pathways, for which the access policy should be used

Histopathology User Guide

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AUTHOR: Senior Staff/ Laura Wright	DATE OF ISSUE:24/10/2024

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Reporting time frames and KPIs

2WW samples

90% in 10 days from the sample date

Appendix 2 Molecular Test Mutations and Analytcal Sensitivity/Specificity

BRAF Mutations Detectable by the Biocartis Idylla Mutation Test

Mutation Group:	V600E/V600E2/V600D
Protein HGVS:	p.Val600Glu/p.Val600Glu/p.Val600Asp
Base Change:	c.1799T>A/c.1799_1800TG>AA/c.1799_1800TG>AT; c.1799_1800TG>AC
Mutation Group:	V600K/V600R/V600M
Protein HGVS:	p.Val600Lys/p.Val600Arg/p.Val600Met
Base Change:	c.1798_1799GT>AA/c.1798_1799GT>AG/c.1798G>A

BRAF Idylla Mutation Test Analytical Specificity

Analytical specificity of the Idylla™ BRAF Mutation Test was evaluated by testing the following specimen types using high input amounts:

- Twenty artificial FFPE reference samples
- Wild Type (6.6*105 copies per Cartridge),
- 100% V600E mutant (6.1*105 copies per Cartridge)
- 100% V600K mutant (5.9*105 copies per Cartridge).
- Synthetic target oligos (106 copies per Cartridge) combined with human Wild Type gDNA (106 copies per
- Cartridge) for V600A and V600G
- Plasmids representing the BRAF homologues BRAF P1, ARAF and CRAF (106 copies per Cartridge)

Results obtained show that the genotypes Wild Type, V600E, V600K, V600A, BRAF P1, ARAF and CRAF were correctly identified and that no cross-reactivity was observed.

When using high input amounts of V600G (106 copies per Cartridge) the Idylla™ BRAF Mutation Test reports V600E/E2/D, indicating V600G cross-reactivity.

BRAF Idylla Mutation Test Analytical Sensitivity

The LoD is defined as the lowest input level (copies/PCR) where the estimated mean probability of detection is, with 95% confidence, at least 95%.

Analytical sensitivity was determined using 1% V600E and 1% V600K artificial FFPE samples with known DNA content. Based on the DNA content, copy numbers for V600E and V600K mutations were calculated and serial dilutions were prepared.

Per liquefied slice, 10 serial dilutions were tested in replicates of 12:

- V600E dilution range: 20 - 0.15 mutation copies per PCR

- V600K dilution range: 40 – 0.15 mutation copies per PCR

Logistic regression modelling was used to determine the LoDs for both V600E and V600K. This resulted in LoD claims for V600E and V600K of 4 copies/PCR and 10 copies/PCR, respectively.

To verify the LoD for each mutant, 1% V600 mutation standards at the determined LoD were prepared and twenty replicates were tested. For both mutants, all replicates tested positive. The LoDs were used to deduce the corresponding tissue areas for both 1% V600E and V600K mutants.

Limit of Detection for V600E and V600K

Sample	Number of mutant copies/PCR	Tissue area per Cartridge for 5 µm tissue section	Mutation positive/tested
1% V600E	4 copies/PCR	10.4 mm^2	20/20
1% V600K	10 copies/PCR	24.9 mm^2	20/20

EGFR Mutations Detectable by the Biocartis Idylla EGFR Mutation Test

Whilst the Idylla test detects an array of Exon 19 deletions and Exon 20 insertions (below) it does not distinguish in it's test result output which specific deletion or insertion was present in the sample.

GENOTYPE	EXON	MUTATION	PROTEIN CHANGE	NUCLEOTIDE CHANGE
G719A/C/S	18	G719A	p.Gly719Ala	c.2156G>C
		G719C	p.Gly719Cys	c.2155G>T
		G719C2	p.Gly719Cys(2)	c.2154_2155delinsTT
		G719S	p.Gly719Ser	c.2155G>A
Exon 19 deletion	19	Deletion 9	p.Leu747_Ala750delinsPro	c.2238_2248delinsGC
				c.2239_2248delinsC
			p.Leu747_Ala750delinsSer	c.2240_2248del
			p.Leu747_Glu749del	c.2239_2247del
		Deletion 12	p.Leu747_Thr751delinsPro	c.2239_2251delinsC
			p.Leu747_Thr751delinsSer	c.2240_2251del
		Deletion 15	p.Glu746_Ala750del	c.2235_2249del
				c.2236_2250del
			p.Leu747_Thr751del	c.2239_2253del
				c.2240_2254del
				c.2238_2252del
			p.Glu746_Thr751delinsAla	c.2237_2251del
			p.Glu746_Thr751delinsIle	c.2235_2252delinsAAT
			p.Glu746_Thr751delinsVal	c.2237_2252delinsT
			p.Lys745_Ala750delinsThr	c.2234_2248del
			p.Glu746_Thr751delinsLeu	c.2236_2253delinsCTA
			p.Glu746_Thr751delinsVal	c.2237_2253delinsTA
			p.Glu746_Thr751delinsAla	c.2235_2251delinsAG

			p.Glu746_Thr751delinsGln	c.2236_2253delinsCAA
			p.Ile744_Ala750delinsValLys	c.2230_2249delinsGTCAA
		Deletion 18	p.Leu747_Pro753delinsSer	c.2240_2257del
			p.Glu746_Ser752delinsVal	c.2237_2255delinsT
			p.Leu747_Ser752del	c.2239_2256del
			p.Glu746_Thr751del	c.2236_2253del
			p.Leu747_Pro753delinsGln	c.2239_2258delinsCA
			p.Glu746_Ser752delinsAla	c.2237_2254del
			p.Glu746_Ser752delinsAsp	c.2238_2255del
			p.Glu746_Pro753delinsValSer	c.2237_2257delinsTCT
			p.Glu746_Ser752delinsIle	c.2236_2255delinsAT
				c.2236_2256delinsATC
			p.Glu746_Ser752delinsVal	c.2237_2256delinsTT
				c.2237_2256delinsTC
				c.2235_2255delinsGGT
		Deletion 21	p.Leu747_Pro753del	c.2238_2258del
			p.Glu746_Ser752del	c.2236_2256del
		Deletion 24	p.Ser752_Ile759del	c.2253_2276del
T790M	20	T790M	p.Thr790Met	c.2369C>T
S768I		S768I	p.Ser768Ile	c.2303G>T
Exon 20 insertion		InsG	p.Asp770_Asn771insGly	c.2310_2311insGGT
		InsASV(9)	p.Val769_Asp770insAlaSerVal	c.2307_2308insGCCAGCGTG
		InsASV(11)	p.Val769_Asp770insAlaSerVal	c.2309_2310delinsCCAGCGTGGAT
		InsSVD	p.Asp770_Asn771insSerValAsp	c.2311_2312insGCGTGGACA
		InsH	p.His773_Val774insHis	c.2319_2320insCAC
L858R	21	L858R	p.Leu858Arg	c.2573T>G
				c.2573_2574delinsGT
				c.2573_2574delinsGA
L861Q		L861Q	p.Leu861Gln	c.2582T>A

EGFR Idylla Mutation Test Analytical Sensitivity

The Limit Of Detection (LOD) is defined as the lowest allelic frequency at which the mutant alleles can consistently be detected in ≥ 95% of the test cases at a given input. An input of 2500 copies of wild-type (WT) FFPE background in each of the five multiplex PCR reactions of one Cartridge is representative for a standard NSCLC FFPE tissue sample.

The LOD was estimated for 20 mutations targeted by the Idylla™ EGFR Mutation Test. Therefore, serial dilutions covering a range of allelic frequencies (from 1% to 15%) for each mutant were tested at different input levels of WT FFPE background in the PCR reaction (from 200 copies to 5000 copies). The serial dilutions were prepared from synthetic target DNA oligonucleotides harbouring the mutation and spiked in liquefied EGFR WT FFPE Reference Standard.

Testing was performed in a two-step approach: (1) a dose range study with six repeats for each dilution (3 dilutions at 3 input levels), and (2) a refined study with twelve repeats for a narrowed range of dilutions near the estimated LOD (2 dilutions at 2 input levels). Cartridges originating from three different Idylla™ EGFR Mutation Test lots were used.

Mutation positivity rate of the serial dilutions was used to calculate the LOD by logistic regression. In the table below the LOD for each mutant at an input level of 1000 and 2500 copies of WT background is given, with the corresponding Cq of the EGFR control (average EGFR control Cq value of all LOD tests done at the corresponding input level).

Estimated LOD levels for each mutant at three input levels as measured on three lots.

Gene	Exon	Mutation	Nucleotide change	Cosmic ID	Lowest allelic frequency with ≥95% Positivity Rate (LOD)	
					In 1000 copies WT FFPE Cq of EGFR control = 21.3	In 2500 copies WT FFPE Cq of EGFR control = 19.8
EGFR	18	G719A	c.2156G>C	COSM6239	3.0%	1.7%
		G719C	c.2155G>T	COSM6253	2.1%	1.0%
		G719S	c.2155G>A	COSM6252	4.9%	1.7%
	19	Deletion 9	c.2239_2248delinsC	COSM12382	2.1%	1.0%
		Deletion 12	c.2239_2251delinsC	COSM12383	2.2%	1.1%
		Deletion 15	c.2235_2249del	COSM6223	1.0%	1.0%
			c.2236_2250del	COSM6225	2.0%	1.4%
		Deletion 18	c.2240_2257del	COSM12370	3.5%	1.7%
		Deletion 21	c.2238_2258del	COSM255211	1.0%	1.0%
			c.2236_2256del	COSM133189	1.4%	1.1%
		Deletion 24	c.2253_2276del	COSM13556	7.5%	3.0%
	20	T790M	c.2369C>T	COSM6240	2.4%	1.5%
		S768I	c.2303G>T	COSM6421	2.5%	1.4%
		InsG	c.2310_2311insGGT	COSM12378	1.0%	1.0%
		InsASV(9)	c.2307_2308insGCCAGCGTG	COSM12376	1.3%	1.0%
		InsASV(11)	c.2309_2310delinsCCAGCGTGGAT	COSM13558	1.8%	1.1%
		InsSVD	c.2311_2312insGCGTGGACA	COSM13428	2.0%	1.3%
		InsH	c.2319_2320insCAC	COSM12377	2.3%	1.6%
	21	L858R	c.2573T>G	COSM6224	1.0%	1.0%
		L861Q	c.2582T>A	COSM6213	2.5%	1.3%

The most prevalent mutations within each of the seven possible genotype calls were included to confirm the estimated LOD level.

This was performed by testing 50 replicates near 3 times the corresponding upper limit of the 95% confidence interval at an input level representative for a standard NSCLC FFPE tissue sample (i.e. 2500 copies of WT FFPE background in the PCR reaction), equally divided over two Idylla™ EGFR Mutation Test lots. In the table below the lowest allelic frequency (LOD) with a positivity rate of at least 95% with 95% confidence is given.

					Lowest Allelic Frequency with ≥95% Positivity Rate (LOD)	
Gene	Exon	Mutation	Nucleotide change	Cosmic ID	In 2500 copies WT FFPE	Positivity Rate
EGFR	18	G719A G719S	c.2156G>C c.2156G>T	COSM6239 COSM6252	10.0% 5.0%	50/50 50/50
	19	Del15	c.2235_2249del	COSM6252	5.0%	50/50
	20	T790M S768I insASV9	c.2369C>T c.2303G>T c.2308_2309insGCCAGCGTG	COSM6240 COSM6241 COSM12376	5.0% 5.0% 5.0%	50/50 50/50 50/50
	21	L858R L861Q	c.2573T>G c.2582T>A	COSM6224 COSM6213	5.0% 5.0%	50/50 50/50

EGFR Idylla Mutation Test Analytical Specificity

In silico analysis of the human genome sequence did not identify reactivity of any of the oligonucleotide primers outside the EGFR gene that could possibly result in non-specific detection.

Twenty EGFR mutations targeted by the Idylla™ EGFR Mutation Test were confirmed in the in silico analysis to be detected by the Test. Screening of mutations reported for the human EGFR gene revealed other variants than the targeted mutations that could be detected by the Test. Inclusivity for 31 tested mutations was confirmed since at least 1 of 2 repeats generated the correct genotype call (table below).

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Inclusivity of 31 additional EGFR mutations (besides 20 targeted mutations) detected by the Idylla™ EGFR Mutation Test.

GENE	EXON	MUTATION	NUCLEOTIDE CHANGE	COSMIC ID
EGFR	18	G719C	c.2154_2155delinsTT	COSM18441
	19	Deletion 9	c.2238_2248delinsGC	COSM12422
			c.2239_2247del	COSM6218
			c.2240_2248del	COSM4170221
		Deletion 12	c.2240_2251del	COSM6210
		Deletion 15	c.2237_2252delinsT	COSM12386
			c.2238_2252del	COSM23571
			c.2239_2253del	COSM6254
			c.2240_2254del	COSM12369
			c.2234_2248del	COSM1190791
			c.2236_2253delinsCTA	COSM133187
			c.2237_2253delinsTA	COSM133192
			c.2235_2251delinsAG	COSM13549
			c.2236_2253delinsCAA	COSM133187
			c.2230_2249delinsGTCAA	COSM85798
			c.2235_2252delinsAAT	COSM13551
			c.2237_2251del	COSM12678
		Deletion 18	c.2238_2255del	COSM6220
			c.2237_2254del	COSM12367
			c.2237_2257delinsTCT	COSM18427
			c.2236_2255delinsAT	COSM133188
			c.2236_2256delinsATC	COSM133190
			c.2237_2256delinsTT	COSM133194
			c.2237_2256delinsTC	COSM18426
			c.2235_2255delinsGGT	COSM85797
			c.2239_2256del	COSM12403
			c.2237_2255delinsT	COSM12384
			c.2236_2253del	COSM12728
			c.2239_2258delinsCA	COSM12387
	21	L858R	c.2573_2574delinsGT	COSM12429
			c.2573_2574delinsGA	COSM133630

KRAS Mutations Detectable by the Biocartis Idylla KRAS Mutation Test

MUTATIONS IN KRAS CODON 12	
Mutation	G12A
Protein Change	p.Gly12Ala
Nucleotide Change	c.35G>C
Mutation	G12C
Protein Change	p.Gly12Cys
Nucleotide Change	c.34G>T
Mutation	G12D
Protein Change	p.Gly12Asp
Nucleotide Change	c.35G>A
Mutation	G12R
Protein Change	p.Gly12Arg
Nucleotide Change	c.34G>C
Mutation	G12S
Protein Change	p.Gly12Ser
Nucleotide Change	c.34G>A
Mutation	G12V
Protein Change	p.Gly12Val
Nucleotide Change	c.35G>T

MUTATIONS IN KRAS CODON 13	
Mutation	G13D
Protein Change	p.Gly13Asp
Nucleotide Change	c.38G>A
MUTATIONS IN KRAS CODON 59	
Mutation	A59T/E/G
Protein Change	p.Ala59Thr / p.Ala59Glu / p.Ala59Gly
Nucleotide Change	c.175G>A / c.176C>A / c.176C>G
MUTATIONS IN KRAS CODON 61	
Mutation	Q61H
Protein Change	p.Gln61His
Nucleotide Change	c.183A>C ; c.183A>T
Mutation	Q61K
Protein Change	p.Gln61Lys
Nucleotide Change	c.181C>A ; c.180_181delinsAA
Mutation	Q61R/L
Protein Change	p.Gln61Arg / p.Gln61Leu
Nucleotide Change	c.182A>G / c.182A>T
MUTATIONS IN KRAS CODON 117	
Mutation	K117N
Protein Change	p.Lys117Asn
Nucleotide Change	c.351A>C ; c.351A>T

MUTATIONS IN KRAS CODON 146	
Mutation	A146P/T/V
Protein Change	p.Ala146Pro / p.Ala146Thr / p.Ala146Val
Nucleotide Change	c.436G>C / c.436G>A / c.437C>T

KRAS Idylla Mutation Test Analytical Sensitivity

The Limit Of Detection (LOD) is defined as the lowest KRAS mutation copy number that, with 95% confidence, can consistently be detected in ≥ 95% of the test cases. This LOD is depending both on the allelic frequency as well as the tested amount of tissue.

The LOD values of the KRAS mutations that are detected by the Idylla™ KRAS Mutation Test were established by testing dilution series of the individual KRAS mutations at a certain allelic frequency and known copy number input. The dilution series were prepared from the following materials:

- Liquefied KRAS mutant artificial FFPE specimens with known KRAS gene copy number,
- Liquefied KRAS Wild-Type artificial FFPE specimens with known KRAS gene copy number,
- Synthetic Target DNA oligonucleotides harboring the KRAS mutation.

Liquefied KRAS Wild-Type and KRAS mutant materials were produced from artificial FFPE specimens corresponding to 5 µm sections with an area of 58 mm². For KRAS mutations G12A, G12C, G12D, G12R, G12S, G12V, G13D, Q61H, Q61L, and A146T, specimens with a 5% allelic frequency (or 1% for G12R) were prepared by blending liquefied KRAS mutant FFPE material with liquefied KRAS Wild-Type FFPE material to the desired allelic frequency. Subsequently, these specimens were used to generate two-fold dilution series.

For mutations Q61K (variant c.180_181delinsAA), K117N (variant c.351A>T), and A59E, Synthetic Target DNA was spiked to the desired allelic frequency into liquefied KRAS Wild-Type FFPE material for the preparation of the dilution series.

The two-fold dilution series were prepared in 12-fold and tested with cartridges from different Idylla™ KRAS Mutation Test lots. Tested amounts were equivalent to 58 mm² down to 3.6 mm² of section, i.e. the equivalent of 1.0 to 0.063 FFPE section with a thickness of 5 µm and a total area of 58 mm². Copy numbers for the KRAS mutations in these 5% allelic

frequency dilution series ranged from 900 to 56 per PCR reaction. Mutation call rates for these dilution series were used to determine the LOD by logistic regression.

Determined LOD values were confirmed by testing 27 replicates at the corresponding upper limit of the 95% confidence interval with cartridges from three Idylla™ KRAS Mutation Test lots (9 replicates per lot). This resulted in the claimed KRAS mutant copy numbers with a positivity rate of at least 95% shown in the table below.

Confirmed LOD values and corresponding tissue area equivalents

KRAS Mutation	KRAS Mutant Material	Allelic Freq.	KRAS mutant copy number with ≥ 95% positivity rate		
			Copy N°	Tissue area Equivalent (mm² of 5µm tissue section)	Positivy rate
G12A	FFPE specimen	5%	450	29.0	27/27
G12C	FFPE specimen	5%	56	3.6	27/27
G12D	FFPE specimen	5%	113	7.3	27/27
G12R	FFPE specimen	1%	90	5.8	27/27
G12S	FFPE specimen	5%	225	14.5	27/27
G12V	FFPE specimen	5%	225	14.5	27/27
G13D	FFPE specimen	5%	900	58.0	27/27
A59E	Synthetic Target	5%	56	3.6	26/27
Q61H	FFPE specimen	5%	113	7.3	27/27
Q61K	Synthetic Target	5%	225	14.5	27/27
Q61L	FFPE specimen	5%	113	7.3	27/27
K117N	Synthetic Target	5%	56	3.6	27/27
A146T	FFPE specimen	5%	900	58.0	27/27

KRAS Idylla Mutation Test Analytical Specificity

In silico analysis of the human genome sequence did not identify reactivity for any of the oligonucleotide primers outside the KRAS gene that could possibly result in non-specific amplicon formation and/or detection, thereby excluding cross-reactivity of the Idylla™ KRAS Mutation Test primers with sequence homologues in the NRAS gene or the KRASP1 pseudogene.

Screening of mutations reported for the human KRAS gene by in silico analysis, identified three mutations outside the codons targeted by the Idylla™ KRAS Mutation Test that could potentially lead to false-positive results for the Idylla™ KRAS Mutation Test, i.e. p.G10_A11insG (c.30_31insGGA), p.C118S (c.353G>C) and p.G60D (c.179G>A), respectively. However, their prevalence is low and the likelihood for a false-positive result is small if not negligible.

Known variants for the KRAS mutations targeted by the Idylla™ KRAS Mutation Test were identified in the in silico analysis to be detected and correctly reported by the Idylla™ KRAS Mutation Test. The only exceptions that will not be detected, are three rarely occurring variants for G12D (c.35_36delinsAC), G12V (c.35_36delinsTC) and Q61R (c.182_183delinsGT), respectively.

Of the known mutations within the codons covered by the Idylla™ KRAS Mutation Test but for which the primers were not primarily designed, mutation G12W (c.34_36delinsTGG) will be detected and reported as G12C, and -24 KRAS mutations G13N (c.37_38delinsAA) and G13E (c.38_39delinsAA or c.38_39delinsAG) will result in a G13D call.

All other variants will result in ‘No mutation detected’.

NRAS Mutations Detectable by the Biocartis Idylla NRAS-BRAF Mutation Test

MUTATIONS DETECTED IN NRAS CODON 12	
Mutation	G12D
Protein Change	p.Gly12Asp
Nucleotide Change	c.35G>A
Mutation	G12C
Protein Change	p.Gly12Cys

Nucleotide Change	c.34G>T
Mutation	G12S
Protein Change	p.Gly12Ser
Nucleotide Change	c.34G>A
Mutation	G12A/V
Protein Change	p.Gly12Ala/ p.Gly12Val
Nucleotide Change	c.35G>C/ c.35G>T

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MUTATIONS DETECTED IN NRAS CODON 13	
Mutation	G13D
Protein Change	p.Gly13Asp
Nucleotide Change	c.38G>A
Mutation	G13R/V
Protein Change	p.Gly13Arg/p.Gly13Val
Nucleotide Change	c.37G>C/c.38G>T
MUTATION DETECTED IN NRAS CODON 59	
Mutation	A59T
Protein Change	p.Ala59Thr
Nucleotide Change	c.175G>A
MUTATIONS DETECTED IN NRAS CODON 61	
Mutation	Q61K
Protein Change	p.Gln61Lys
Nucleotide Change	c.181C>A
Mutation	Q61R
Protein Change	p.Gln61Arg
Nucleotide Change	c.182A>G
Mutation	Q61L
Protein Change	p.Gln61Leu
Nucleotide Change	c.182A>T

Mutation	Q61H
Protein Change	p.Gln61His
Nucleotide Change	c.183A>C ; c.183A>T
MUTATION DETECTED IN NRAS CODON 117	
Mutation	K117N
Protein Change	p.Lys117Asn
Nucleotide Change	c.351G>C ; c.351G>T
MUTATION DETECTED IN NRAS CODON 146	
Mutation	A146T/V
Protein Change	p.Ala146Thr / p.Ala146Val
Nucleotide Change	c.436G>A / c.437C>T

BRAF Mutations Detectable by the Biocartis Idylla NRAS-BRAF Mutation Test

MUTATIONS DETECTED IN BRAF CODON 600	
Mutation:	V600E/V600D
Protein Change:	p.Val600Glu/p.Val600Asp
Base Change:	c.1799T>A;c.1799_1800delinsAA/ c.1799_1800delinsAC
Mutation:	V600K/V600R
Protein Change:	p.Val600Lys/p.Val600Arg
Base Change:	c.1798_1799delinsAA/c.1798_1799delinsAG

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NRAS-BRAF Idylla Mutation Test Analytical Sensitivity

The Limit Of Detection (LOD) is defined as the lowest allelic frequency at which the mutant alleles can consistently be detected in ≥ 95% of the test cases. An input of 16000 copies of wild-type (WT) FFPE background in each of the five multiplex PCR reactions of one Cartridge is representative for one FFPE Reference Standard specimen.

To estimate the LOD for each of the 23 mutations detected by the Idylla™ NRAS-BRAF Mutation Test, serial dilutions containing a range of allelic frequencies for each mutant were tested at three input levels of WT FFPE background in the PCR reaction. The input levels ranged from 25% to 1% in 1000 copies (4 dilutions), 20% to 1% in 4000 copies (4 dilutions), and 10% to 1% in 16000 copies (3 dilutions). The serial dilutions were prepared from liquefied mutant FFPE Reference Standard (for the commercially available mutations) or synthetic targetDNA oligonucleotides harboring the mutation and spiked in liquefied NRAS-BRAF WT FFPE Reference Standard.

Testing was performed in a two-steps approach: (1) a dose range study with six repeats for each dilution; and, (2) a refined study with twelve repeats for a narrowed range of dilutions near the estimated LOD. Cartridges originating from three different Idylla™ NRAS-BRAF Mutation Test lots were used.

Mutation positivity rate of the serial dilutions was used to calculate the LOD by logistic regression. In Table 7 the LOD for each mutant at the three input levels is given, with the corresponding Cq of the NRAS control (average NRAS control Cq value of all LOD tests done at this input level).

Estimated LOD levels for each mutant at three input levels as measured on three lots.

					Lowest Allelic Frequency With ≥95% Positivity Rate (LOD)		
Oncogene	Exon	Codon	Mutation	Nucleotide Change	In 1000 copies WT FFPE Cq of NRAS Control = 35.0	In 4000 copies WT FFPE Cq of NRAS Control = 32.7	In 16000 copies WT FFPE Cq of NRAS Control = 30.9
NRAS	2	12	G12D	c.35G>A	5.5%	1.4%	1.0%
			G12C	c.34G>T*	1.0%	1.0%	1.0%
			G12S	c.34G>A*	>30%	21.0%	5.5%
			G12V	c.35G>C	5.0%	2.2%	1.0%
			G12A	c.35G>T*	1.3%	1.0%	1.0%
		13	G13D	c.38G>A	23.9%	6.0%	1.5%
			G13R	c.37G>C*	4.6%	1.0%	1.0%
			G13V	c.38G>T*	1.0%	1.0%	1.0%
	3	59	A59T	c.175G>A	25.0%	5.0%	3.0%
		61	Q61K	c.181C>A	20.4%	5.0%	1.3%
			Q61R	c.182A>G	15.0%	5.0%	1.6%
			Q61L	c.182A>T	19.9%	5.0%	1.3%
			Q61H	c.183A>C*	1.4%	1.0%	1.0%
			Q61H	c.183A>T	14.5%	4.0%	1.0%
	4	117	K117N	c.351G>C	5.0%	3.3%	1.0%
			K117N	c.351G>T*	1.0%	1.0%	1.0%
		146	A146T	c.436G>A°	>30%	>30%	8.5%
			A146V	c.437C>T*	6.0%	1.5%	1.0%
BRAF	15	600	V600E	c.1799T>A	5.7%	1.5%	1.0%
			V600E	c.1799_1800delinsAA*	1.7%	1.0%	1.0%
			V600D	c.1799_1800delinsAC*	1.6%	1.0%	1.0%
			V600K	c.1798_1799delinsAA	9.0%	2.5%	1.0%
			V600R	c.1789_1799delinsAG	4.0%	1.0%	1.0%

* Serial dilutions were prepared from synthetic target DNA harboring the mutation since mutant FFPE Reference Standard wascommercially not available.

° For NRAS A146T, the dose range study was performed using synthetic target DNA whilst for the refinement study FFPE ReferenceStandard was available.

The most prevalent mutations (i.e. ≥3% among NRAS- or BRAF-mutated colorectal cancer (CRC) patients were included to confirm the estimated LOD level. At least one mutation for each codon was evaluated. This was performed by testing 25 replicates near the corresponding upper limit of the 95% confidence interval with an input level representative for a FFPE Reference Standard specimen (i.e. 16000 copies of WT FFPE background in the PCR reaction) on one Idylla™ NRAS-BRAF Mutation Test lot. In the next table, the lowest allelic frequency (LOD) with a positivity rate of at least 95% with 95% confidence is given.

Confirmed LOD levels for 10 NRAS-BRAF mutants with a positivity rate of at least 95% with 95% confidence at an input level representative for a FFPE Reference Standard.

					Lowest Allelic Frequency with ≥95% Positivity Rate (LOD)	
Oncogene	Exon	Codon	Mutation	Nucleotide Change	in 16000 copies WT FFPE	Positivity Rate
NRAS	2	12	G12D G12C G12V	c.35G>A c.34G>T c.35G>C	1.7% 1.0% 5.0%	25/25 25/25 25/25
		13	G13R	c.37G>C*	10.0%	25/25
	3	59	A59T	c.175G>A	9.0%	25/25
		61	Q61K Q61R	c.181C>A c.182A>G	2.0% 3.3%	25/25 25/25
	4	117	K117N	c.351G>C	1.0%	25/25
		146	A146T	c.436G>A	15.0%	25/25
BRAF	15	600	V600E	c.1799T>A	5.0%	25/25

* Serial dilutions were prepared from synthetic target DNA harboring the mutation since mutant FFPE Reference Standard was commercially not available.

The Idylla™ NRAS-BRAF Mutation Test is able to detect allelic frequencies:

- ≤ 5% in the NRAS gene in codons 12 and 61, and in the BRAF gene in codon 600 (most prevalent mutations i.e. ≥3% among NRAS- or BRAF-mutated CRC patients)
- ≤ 10% for mutations in the NRAS gene in codons 13, 59, 117 and ≤ 15% in the NRAS gene in codon 146 (rare mutations i.e. <3% among NRAS- or BRAF-mutated CRC patients)

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NRAS-BRAF Idylla Mutation Test Analytical Specificity

In silico analysis of the human genome sequence did not identify reactivity for any of the oligonucleotide primers outside the NRAS or BRAF gene that could possibly result in non-specific detection, thereby excluding cross-reactivity of the Idylla™ NRAS-BRAF Mutation Test primers with sequence homologues in the KRAS gene. Moreover, evaluation of 45 KRAS positive samples from metastatic CRC patients by the Idylla™ NRAS-BRAF Mutation Test resulted in 'No mutation detected'.

The NRAS and BRAF mutations targeted by the Idylla™ NRAS-BRAF Mutation Test were identified in the in silico analysis to be detected by the Test. Screening of mutations reported for the human NRAS and BRAF gene revealed that there were no other variants than the targeted mutations reported in colorectal tissue samples.

Of the known mutations within the codons covered by the Idylla™ NRAS-BRAF Mutation Test but for which the primers were not primarily designed, mutations V600fs*11 (c.1799_1800delTG) and V600Q (c.1798_1799delinsCA) can be detected and reported as V600E/D or V600K/R. All other variants will result in 'No mutation detected'.