Service Description for Lynch Syndrome (incl MSI)

1 Background

OMIM#120435

Lynch Syndrome (previously known as HNPCC - Hereditary Non-Polyposis Colon Cancer) is an autosomal dominant cancer predisposition syndrome characterised by colorectal adenocarcinoma without (Lynch Syndrome type I) or with (Lynch Syndrome type II) extracolonic cancers (including ovarian, endometrium, small bowel, stomach, ureter or renal pelvis and others). It is estimated that Lynch Syndrome accounts for 4-6% of colorectal cancer. Lynch Syndrome is caused by inactivating mutations in genes of the DNA mismatch repair system (MMR genes) of which at least five have been identified. MMR gene mutations result in failure to repair errors during DNA replication. Cancer is likely to develop when unrepaired replication errors inactivate genes including tumour suppressors. The MMR genes MLH1 and MSH2 account for approximately 80-90% of Lynch Syndrome, and it is the screening for mutations in these two genes that forms the basis of the laboratory service offered. Testing is also offered for gene screening of MSH6 and PMS2.

In conjunction, MMR gene inactivation may be revealed as extra microsatellite alleles in tumour DNA as compared to matched normal DNA, known as MSI (Microsatellite Instability). MSI tumours are characteristic of Lynch Syndrome (although not exclusively so), and patient tumour blocks (if available) are also tested to determine their MSI status.

2 Standard service

A Essential referral information

In addition to supplying standard patient identification and referral information (see Section I below), the following should be clearly indicated:

1. Full clinical details.
2. Family history: because sporadic colorectal cancer is common, some “Lynch Syndrome –like” families may occur by chance clustering. Specific selection criteria are recommended in order to target mutation screening resources to those families most likely to segregate MMR gene mutations.

   These are known as the Bethesda criteria (2000)* and are as follows:
   - Patients with cancer in families that fulfil Amsterdam criteria, which are:
     - three relatives with colorectal cancer (CRC), one of whom is a first degree relative of the other two, and
     - CRC involving at least two generations, and
     - one or more CRC cases diagnosed before the age of 50.
   - Patients with two Lynch Syndrome related cancers, including synchronous and metachronous CRCs or associated extracolonic cancers (e.g. cancer of the ovaries, endometrium, small bowel, ureter or renal pelvis).
   - Patients with CRC and a first degree relative with colorectal cancer and/or Lynch Syndrome related extracolonic cancer and/or colorectal adenoma; with

one of the cancers diagnosed at age <45 years and the adenoma diagnosed at age < 40 years.

Meeting all features listed under any of these points is sufficient to comply with the Bethesda criteria.

3. In addition, FAP should be excluded in the CRC cases and all tumours should be verified by pathological examination.
4. In cases where the Bethesda criteria are not met, but where there is CRC under the age of 45 (even without family history), Microsatellite Instability (MSI) analysis of the patient's tumour (generally supplied as paraffin embedded block) is performed before mutation screening is considered. Patients whose tumours exhibit MSI-H (MSI-high or high instability) status will normally be forwarded for MLH1 and MSH2 mutation screening (further direction as to what gene to screen is aided by immunohistochemistry (IHC) tests – performed in the Histopathology Department of OLCHC).

Syngal et al, Sensitivity and specificity of clinical criteria for hereditary non-polyposis colorectal cancer associated mutations in MSH2 and MLH1. J Med Genet 2000; **37:641-645.*

Note: It is the responsibility of the referring clinician to ensure consent has been obtained for testing and storage.

B Samples required

- Generally 5-10ml of EDTA blood (FBC bottle) is required. Sample identification policy is detailed at (see Section I below).
- Blood specimens must be appropriately packaged (see Section I), and preferably sent by courier to arrive as soon as possible. Do not freeze prior or during postage.
- For MSI analysis blood samples along with two paraffin embedded tumour blocks per patient, accompanied by a full histological report, and a diagram indicating the relative position of the tumour material in the blocks submitted (or if submitting sectioned block on slides the tumour and normal tissue can be indicated on an H & E stained slide – in this case it is recommended that 10-15 slides are sent). Please note that if the position of the tumour on the block has not been noted but the sample is to have IHC performed in OLCHC they will locate the tumour and the normal sections on the block there whilst doing the IHC assay.
- N.B. The preferred tumour type for MSI testing is colorectal, endometrial or ovarian. All other tumour types will be assessed on a case by case basis to determine whether or not they are suitable to accept for testing. Please contact the lab directly for any further queries on this matter.
- Please note that extracted DNA is stored from patient's samples at the National Centre for Medical Genetics, and kept indefinitely unless a written request for its disposal is received from the patient or their parent/guardian.

C Restrictions on testing

Requests for diagnostic Lynch Syndrome testing are usually only accepted from our own clinical team. The one exception to this rule is samples from St. Vincent's Hospital, which we also accept. Samples may be received from St. James's Hospital only when the case has been discussed previously with Prof Green (should be indicated on the referral form). Predictive testing is not considered for children under the age of 16, and is only accepted from referrals through our own clinical team in NCMG.
For further information regarding referrals to Clinical Genetics at NCMG, please see http://www.genetics.ie/clinical/ or phone 01-4096739.

D Tests offered

Four types of tests can be performed:

1. Pre-screen: Microsatellite Instability (MSI) analysis of patient tumour blocks and normal (blood) DNA. It is recommended that all affected patients referred for Lynch Syndrome testing undergo MSI analysis.
2. Pre-screen: Immunohistochemistry (IHC) of the tumour block tissue for the MLH1/MSH2/MSH6/PMS2 genes of patients who are affected with Lynch Syndrome
3. Diagnostic: Mutation screening of the MLH1/MSH2/MSH6/PMS2 genes of patients who are affected with Lynch Syndrome, in an effort to confirm a diagnosis, and provide a direct mutation test for "at risk" relatives (predictive test). Mutation screening is usually only performed after a patient has shown either a MSI-high result and/or a loss of MMR genes on IHC. Given the obvious anxiety and stress of the patient and their families undergoing (lengthy) mutation screening, we would recommend a referral to the genetic counselling team present at the National Centre for Medical Genetics, to discuss their concerns.
4. Predictive: testing for asymptomatic individuals who have a confirmed family history of Lynch Syndrome (i.e. gene mutation known and characterised). These tests are only performed in conjunction with the genetic counselling team present at the National Centre for Medical Genetics. Patients should be referred to the Director, Professor Andrew Green.

Tests are performed:

1. MSI: In house at NCMG
2. IHC: In the Histopathology Department of OLCHC. Contact person is John O'Brien (ext 6436)
3. MLH1/MSH2/PMS2 screening: Birmingham Women's Hospital.
4. MSH6: Kennedy-Galton Centre.
    - MLH1 mutation screening is €655 (Birmingham)
    - MSH2 mutation screening is €655 (Birmingham)
    - MSH6 mutation screening is €750 (Kennedy-Galton centre)
    - PMS2 mutation screening is approx €700 (Birmingham)

Although the mutation screening services are now sent-out, they were originally done in-house and therefore we incur the costs for all referring clinicians.

E Diagnostic Sensitivity of tests

- Microsatellite Instability (MSI): greater than 90% of Lynch Syndrome tumours display MSI. However, approximately 15% of sporadic CRCs also show MSI.
- Mutation screening: new methods are currently being introduced, and the sensitivity of the actual screening process (i.e. detection of mutations present) is estimated to be >95%.
- Predictive testing sensitivity is ~100%.

F Interpretation:

- Results are given in the form of a written interpretative report to the referring clinician.
- Following MSI analysis, a report stating the MSI status of the patient's tumour will be issued. The IHC screen is usually performed by the Histopathology Department in OLCHC and therefore the results of this analysis are included and sent to the referring clinician along with the MSI results. Patients with MSI-H and/or loss of MMR proteins will be forwarded for MLH1/MSH2/MSH6 mutation screening.
- Following mutation screening, if a mutation is found, a report stating the exact nature and likely pathogenicity of the mutation will be issued.
- Following predictive testing a report stating whether the patient has inherited the family mutation i.e. is at high risk of developing the disease, or has not inherited the mutation, i.e. risk reduced to general population risk of developing CRC, will be issued.

G Target reporting times:

As reporting times are constantly evolving, please refer to www.genetics.ie/molecular, or contact the molecular genetics laboratory, to receive up-to-date information on anticipated reporting times for your referral.

- The current target reporting times for each category of test offered (information correct as of 13/01/10):
- For MSI/IHC – 3 to 6 months from receipt of both the tumour and blood samples (samples are batched, hence the range of time given).
- MLH1/MSH2 testing – approx 4 to 5 months from receipt of sample
- PMS2 testing – approx 3 months from receipt of sample
- MSH6 testing – 3 to 4 months from receipt of sample.

- Please contact the laboratory if you have not received a report within a week of your patient being due back in clinic.
- Please note it is our policy not to issue verbal results.
- Request for copies of reports on the day that your patient is in clinic cannot normally be accommodated. We usually require 24 hours notice in which to fax a copy of a report.

H Further tests

As the pathway for testing of Lynch Syndrome samples can be quite complex it is advisable to contact the Division of Molecular Genetics, or the clinical genetics team, for further information required on this.

I Web Links to Related Documents

Standard referral information/NCMG request form	http://www.genetics.ie/pir/2006_NCMG_Referral_Form.pdf
Sample/Patient identification policy	http://www.genetics.ie/pir/SampleIdentificationPolicyWeb.pdf
Packaging of specimens for transport	http://www.genetics.ie/pir/sending_samples.pdf

Please note that hard copies of the above documents may be requested from:
Division of Molecular Genetics, National Centre for Medical Genetics, Our Lady's Children's Hospital, Crumlin, Dublin 12. Tel: 01 4096733; Fax: 01 4096971

The NCMG Molecular Genetics laboratory participates in external QA schemes run by the UK NEQAS for Molecular Genetics, the European Molecular Genetics Quality Network (EMQN), and the Cystic Fibrosis European Network. Results of assessments are available for inspection upon request.