UKGTN GenU Workshop 18/2/14

Revision of system applicable to cytogenetic techniques

The meeting was attended by a good cross section of laboratory scientists from combined (molecular/cytogenetic) and specialist genetic laboratories. This included several head of departments.

Following discussions of the existing system the consensus was that the current system needed a complete revamp in relation to the GenUs applied to cytogenetic techniques. Many previous queries around version 4 were clarified and more details of the way the molecular system functions were given.

Everyone agreed that the current system was not fit for purpose particularly in the current financial climate and the move to array based testing instead of karyotyping for both pre and postnatal cases.

The molecular system already incorporates add on tests in some categories e.g. large number of controls require for some predictive tests; MLPA testing required to cover all exons. The cytogenetic system has evolved as a cumulative system and the returns for the NEQAS scheme illustrated this very well. The key areas of contention have been the issue of all karyotyping in one band which several participants disagreed with; and the way FISH tests have been recorded, panels vs. single FISH.

Discussion included the way other tariffs are applied in the NHS e.g. HRG that cover a broad range of patient attendances in pathway. It was suggested that we should take a pathway based approach to setting out testing 'buckets'. This was suggested as all laboratories should be able to model how many times we do FISH follow up for a case and this could be incorporated into the weight in a similar way the system is used for molecular techniques.

The group defined the tests that we do into 4 broad categories

The system will also have a band A that is applied to every sample that is booked into the LIMS and will included extraction or cell culture to the point that material either DNA or cell suspension is stored or available for analysis. This is consistent with the molecular system and therefore the number or samples received will equal the number of band As.

1. Rapid aneuploidy testing
    a. This is the pivot point of the two system and was considered a common denominator. Discussions on other weights stemmed from this; leaving this at a band C (4)
2. Whole genome screen
    a. This can be undertaken by karyotype or array and this concept was agreed. This would give a flexible approach to the way the activity is recorded.
    b. It was felt that there was a difference between postnatal and prenatal activity whether array or karyotyping. This was focussed around the additional culturing and processing steps that were not covered in band A. This removed the need for a band B. The weighting also includes any follow up tests required for the proband required for confirmation or probe validation as part of follow up testing of other members, again preventing the cumulative

approach to bands. A postnatal whole genome screen was assigned band D (7) with a prenatal whole genome screen a band E (10)

3. Follow up/targeted testing
    a. All family follow up was included in this category. This can be achieved using as variety of techniques karyotype, FISH q-PCR, MLPA this is the choice of the lab and will depend on the question being asked. There was a lot of discussion in this point. E.g. A parental follow up sample following an abnormal karyotype in a child; most labs currently do a full karyotype even if the only question being asked was does this patient have a certain chromosome rearrangement. We have historically done more work because we can. This thinking was challenged including the idea that a parent has only consented to be tested to look for a particular abnormality. E.g. One lab stated they would report this as no evidence of t(7;11) rather than list a normal parental karyotype. Band C (4) was applied to this group. This was also deemed suitable for oncology follow up and any referral where a specific diagnosis was queried and was extended to e.g. CLL FISH panels
4. Diagnostic leukaemia samples. The example of a diagnostic ALL was considered. The current system has a value of D and E (17). Utilising band F (15) seemed too high therefore band E (10) was suggested. This would cover a very large range of referral from a MDS to a full FISH panel ALL.
5. Follow up leukaemia samples would also fit into the follow up category C (4).

There was general acceptance that this new recommendation will result in fewer bands and GenUs therefore will expect the unit cost to be higher when labs apply this system to their budgets.

Testing not discussed at the workshop includes:

- Embryo prep for PDG analysis (currently left in band B)
- Breakage syndrome testing (currently left in band E (10)

Response to comments relating to cytogenetics GenU changes

The draft GenUs for Cytogenetics was sent out to workshop delegates on 1st May 2014.

5 comments (3 delegates and 2 ACGS members) were received and discussed during a teleconference on May 29th.

The comments received about the revised cytogenetics GenUs were addressed as follows:

As mentioned at the workshop all samples booked into the LIMS will have band A assigned. The work that is undertaken on the sample will then fall into ONE band only.

1. Tissues

These were not discussed specifically at the workshop.

Proposal: These are included in the postnatal section of the table i.e. band D and propose that this band will cover tissue analysis by karyotype, array, FISH/QF-PCR **and** MLPA approach such that all chromosomes are screened in some way. This would not include tissues that just have a rapid aneuploidy screen; these should just be band C.

2. Prenatal whole genome screen where a rapid aneuploidy is done before the karyotype/array.

Proposal: The aneuploidy screen is one of the additional tests done as part of this investigation

e.g. If a Down screen positive case that was only eligible for QF-PCR is abnormal and the karyotype is done to confirm the finding this would be a band E test not a band C

3. Breakage testing

Proposal: We didn't discuss this at the workshop but comments received back indicate that people think that band E is right for a chromosome breakage test.

4. Cell freezing and storage currently attracts and additional band A and will continue to do so ONLY if this is the only thing that is being done to the sample. If any other testing is done then the storage element will automatically be included that band.

5. Diagnostic haematology

There have been several comments received on this proposal.

One relates to the idea of all follow up testing being band C regardless of whether this is a 'score' for diagnostic abnormalities or a complete reanalysis in the case of a transformation or relapsed sample.

Proposal: the wording be changed in band E to diagnostic, transformed or relapsed haematology samples (similar to the categories we had in the version 1 units) thus covering all occasions where a full analysis would be needed for a haematology sample.

The second relates to putting all FISH only testing for acquired samples into band C, the wording in the table is ambiguous and needs to be clearer.

Proposal: wording added to relate number of FISH hybridisations with amplicons. This will enable FISH only testing to be assigned to Band B, C or E depending on number of hybridisations carried out. NB. A single hybridisation in band B could include two informative probes e.g. TP53/ATM combination probe.

There have been some comments regarding PETS FISH taking longer to prepare and score. The **proposal** is not to split FISH in this way at the moment. The data collection will ask for the FISH activity to be broken down to traditional and PETS FISH so that we can have a look at the activity and see if a distinction is warranted. By treating the first sample as a band E this will cover the sample where most FISH probes on PETS are used.

6. The issue on embryo preparation for PGD has not been discussed. Comments are welcome from laboratories (Liverpool, Birmingham, Glasgow and Guy's) routinely doing this work on where this should fit. Please also provide comments if your laboratory is routinely doing PGD.

Proposed GENUs for NGS - June 2014

based on February 2014 round table discussions and comments received

1. Sample prep will not be split from analysis

4/5 tables favoured **NOT splitting the two components**. It will all be included in the banding. MLPA/CNV and any Sanger confirmations will also be included in the banding allocated.

2. Genes analysed will be the unit analagous to the amplicon for Sanger

3/5 tables wanted genes. The unit to be trialled will be **genes analysed** i.e. not genes prepped.

3. No addition of bands at the top of the existing system

Opinion on this was evenly spread. Objections to adding new bands was the implication that capacity is open ended which may not be the case and that as NGS technology and pipelines become more streamlined in laboratories there is evidence that processes are becoming more efficient.

The initial proposal circulated to workshop participants suggested:-

Band G weight 25, 50-100 amplicons or 1-10 genes analysed

Band H weight 40 >100 amplicons or 11-100 genes analysed

Introduce a new band for > 100 genes but note that well characterised genes e.g mitochondrial genome, should perhaps be in a Band lower than the number of genes analysed would indicate.

However, to reflect the fact that NGS technology is becoming more established it may be simpler at this stage to use a logarithmic scale for the number of genes analysed with Band G (1 to 50 genes) and Band H (50 - 500 genes) and review the banding again for 501 -5000 genes (eg exome OMIM morbid genes) and whole genome analysis when they are in routine diagnostic use.

4. Summary of proposed bandings for 2014:-

A, weight 1, extractions and send aways

B, weight 2, single amplicon tests

C, weight 4, 2-4 amplicons

D, weight 7, not used for molecular

E, weight 10, 5-19 amplicons

F, weight 15, 20-49 amplicons

G, weight 25, 50-100 amplicons or 1-50 genes analysed

H, weight 40, >100 amplicons or 51-500 genes analysed.

This report was circulated to NGS laboratories on the 5th June 2014 and 4 comments were received.

- For clarification , where NGS is used as a replacement technology to sequence less than the equivalent of 50 amplicons the band assigned should be the same as the Sanger sequencing band
- It seems likely that there will be instances where labs may only undertake part of the NGS sequencing and reporting pipeline and we will ask labs to suggest GenUs for sample preparation; sequencing, alignment and filtering; analysis, interpretation and reporting.
- One laboratory felt strongly that the unit of measurement for NGS work should be kbp rather than genes but this round of audit will use the system defined above.

We will collate the data to inform further discussion and refinement of the use of GenUs for NGS.

Next steps:

UKGTN will coordinate 2 sets of data collection to check that the GenU system is being applied consistently across laboratories.

1. Data collection: Retrospective 2013/2014 data co-ordinated through the UKGTN annual return process.
2. Data collection: UKGTN request for GenU as part of NEQAS. Molecular GenUs will be requested after the second round of samples/questions has been distributed.