An audit of the quality of lymphocyte preparations produced using the Hanabi PIII robotic harvester and a technique to ensure good quality metaphases from newborns

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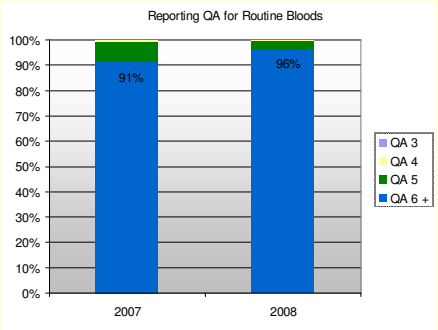
Hanabi PIII Robotic harvester

- Previously (ACC Spring Conference 2008), we presented our validation data for the Hanabi-PIII automated suspension harvester (ADStec – ADScience Technologies, Japan) showing that the peripheral blood lymphocyte chromosome preparations obtained are suitable for diagnostic use and that the harvester is user friendly, reliable and efficient.
- At Guy's, since April 2008, all blood sample cultures have been harvested using the PIII.
- As part of laboratory quality assurance programme, a retrospective audit of the reporting quality has been carried out.

Audit of the quality of preparations

- Data was collected for the first six months (15/04 – 15/10/2008) since full automation and compared to samples processed in the same period the previous year.
- Reporting quality (QA) was used to evaluate the preparations from samples harvested with the PIII Robotic Harvester against samples harvested manually. Data for routine samples (adults and children) and samples from neonatal patients were analysed separately.

Routine samples



- In the first six months routine blood samples were good quality, with 96% reported at QA 6 or higher. This is an increase of 5% compared to the previous year when samples were harvested manually.

Neonatal samples

- Initial observations indicated that the quality and consistency of chromosome preparations from neonates were not as good as those for routine samples, and sometimes produced yellow & sticky pellets, resulting in poor preparations.
- Experiments were carried out to optimise the quality of preparations from neonatal bloods, using different programmes on the harvester and testing with different volumes of blood.

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Technique for Neonatal bloods

Neonatal bloods

- Neonatal blood samples can be challenging. Due to the increased levels of protein, a layer of gelatinous material can form within the fixed cell suspension, which can adversely affect the spreading of metaphase cells.
- Cytogenetics laboratories use various methods to prevent poor preparations from these samples. They include:

  -- Setting up cultures with less blood than cultures for adults and children. *At Guy's 0.2ml of blood (rather than 0.25ml used in cultures for adults and children) was used in neonatal blood cultures.*

  -- Neonatal bloods can be pre-fixed during the harvest – *this was used at Guy's for neonatal cultures harvested manually.*

  -- Water fixation can also be used to clean up poor preparations.

Harvesting neonatal bloods using the PIII

- The option of adding a pre-fixation step is available on the PIII; however the optimum programme for routine samples does not use the pre-fixation step, as trials have shown that this step results in more condensed/shorter chromosome preparations.
- The pre-fix option would have to be applied to the whole run, it is not possible to apply it to the neonatal bloods only; it would also be inefficient to harvest neonatal bloods separately.
- Experiments were set up using smaller volumes of blood to initiate cultures for neonates:

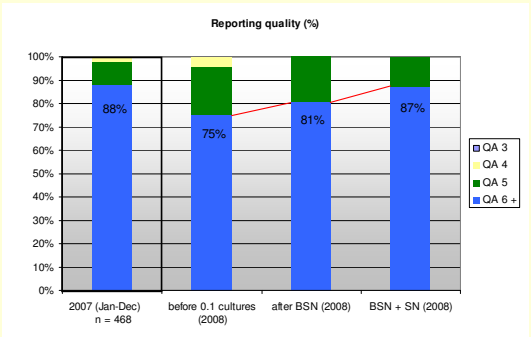
  -- 0.15 ml } preparations were suitable but occasionally dirty & sticky.

  -- 0.05 ml } had no metaphases

  -- **0.1 ml } long, clean preparations with good yields**

Introducing 0.1ml cultures and audit

- Initially an additional culture with 0.1ml blood (BSN) was set up and monitored for two months. These were very successful and so a second 0.1ml culture (SN) was introduced for our alternative culture medium. We continued with the original 0.2ml cultures for a few months but these were discontinued once prospective audit confirmed that the 0.1ml cultures were consistently better.
- A retrospective audit was carried out to evaluate the effectiveness of the 0.1ml cultures for neonatal bloods. Using data from the previous year for comparison, there was a clear reduction in quality when the harvester was first introduced. However the change to 0.1ml cultures resulted in a significant increase (12%) in samples reported at QA 6 or higher.



Conclusions

- The quality chromosome preparations from neonatal bloods was initially a concern and required further work - introducing the 0.1ml cultures has significantly improved reporting quality.
- The quality of preparations from adult and child blood samples has improved since the harvest was automated.
- Automation of blood harvesting has changed that dynamics within the laboratory; harvesting on the PIII is more efficient and has significantly reduced manual work, leaving experienced staff available to concentrate on other tasks.

Reference:

Rooney D.E. (2001) Human Cytogenetics – constitutional analysis. Third Edition. Oxford University Press.