Assessment of HER2 Gene Amplification Status Using the Dako Omnis HER2 IQFISH pharmDx™ Assay

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Liverpool Clinical Laboratories (LCL)

- Liverpool Clinical Laboratories (LCL) is the largest pathology service provider in Cheshire and Merseyside.

- Formed from the merger of the pathology services at the Royal Liverpool & Broadgreen University Hospitals NHS Trust (RLBUHT) & Aintree University Hospital NHS Foundation Trust (AUHT).
LCL Molecular Pathology Service

- LCL Molecular Pathology service is based in Cellular Pathology Department at the RLUH.

- Offers a range of testing assays to support clinical service activities.

- Deliver cancer arm of 100k genome project - North West Coast.

- Deliver a number of regional testing services including a HER2 FISH testing service (breast tumours) for users in the Merseyside, Cheshire, Preston & Blackpool areas.

- Stain approx. 700 HER2 FISH slides per annum.
Human Epidermal Growth Factor Receptor-2 (HER2)

- HER2 protein is a 185-kD transmembrane glycoprotein with tyrosine kinase activity.

- Plays central role in growth factor signal transduction: regulation of cell growth & survival.

- 1987 clinical importance of HER2 in breast cancer was recognised.
**HER2 (ERBB2 or NEU) Gene**

- HER2 gene is located on chromosome 17 (17q11.2-q21) & encodes the HER2 protein.

- Normal diploid cells-HER2 gene is present in two copies.

- HER2 gene amplification &/or protein over-expression demonstrated in approx. 20% of breast cancers.

- HER2 gene amplification &/or protein over-expression is associated with poor prognosis, increased risk of recurrence & shortened patient survival.
HER2 Assessment

- Determination of HER2 status serves as critical predictive test for HER2 targeted therapy in breast cancer (Herceptin, Perjeta & Kadcyla).

- HER2 status assessment is currently one of the most frequently used companion diagnostic (CDx) tests used in histology.

- Number of different slide-based assays are available for assessment of treatment eligibility.
HER2 Assessment at LCL

- Initial testing phase uses an automated IHC assay to evaluate over-expression of the HER2 receptor (Ventana Benchmark Ultra staining platform & the 4B5 HER2 antibody clone, Roche Diagnostics).

- HER2 gene amplification is assessed on HER2 equivocal or 2+ cases using a manual fluorescence in situ hybridisation (FISH) assay (PathVysion HER2 DNA Probe Kit II, Abbott Diagnostics).
HER2 Assessment - challenges

- FISH testing is a time-consuming & technically challenging process (TAT of 24 hours from section de-waxing to finished FISH slide ready for interpretation).

- Current testing schedule at LCL= one FISH run per week.

- Challenges of current testing schedule:
  - service users have MDT meetings on different days/ results miss some MDTMs
  - sample transportation delays & batch delivery of samples from external users present difficulties in ensuring compliance with TATs recommended by NICE (TAT of 14 days)
  - difficulties dealing with urgent requests.

- Could automation address these issues?
Dako Omnis HER2 IQFISH PharmDx™ Assay

- Dako Omnis is a fully automated staining platform capable of staining FFPE samples for both IHC & FISH.

- Early 2016 - a newly developed assay for the Dako Omnis automated staining platform was launched: HER2 IQFISH PharmDx™ Assay.

- Fully automated method on the Dako Omnis instrument uses a direct FISH protocol to quantitatively determine HER2 gene amplification in FFPE tissue from patients with breast cancer.

- Employs a non-toxic buffer that significantly reduces FISH protocol hybridisation times (TAT of 4 hours from section de-waxing to finished FISH slide ready for interpretation).
HER2 IQFISH PharmDx™ Assay

- HER2 IQFISH PharmDx™ Assay uses an IQISH probe mix.

- IQFISH probes are designed in silico & chemically synthesized using oligonucleotide library synthesis (OLS) technology.

- HER2 IQFISH PharmDx™ probe consists of a mixture of Texas-Red labelled DNA probes covering a 218kb region including the HER2 gene on chromosome 17.

- Plus a mixture of fluorescein-labelled PNA probes targeting the centromeric region of chromosome 17 (CEN-17).

- Specific hybridisation of the two targets results in a distinct red fluorescent signal at each HER2 gene locus & a distinct green fluorescent signal at each chromosome 17 centromere.
Dako Omnis HER2 IQFISH PharmDx™ Assay

- All protocol steps are pre-programmed into the Dako Omnis software.

- Automated FISH staining on Dako Omnis includes de-paraffinization of tissue sections, target retrieval, pepsin digestion, hybridisation & stringent wash.

- Slides are unloaded into the dry unloading station ready for coverslipping.
Overview of HER2 IQFISH PharmDx™ staining protocol steps

<table>
<thead>
<tr>
<th>Step</th>
<th>Reagent</th>
<th>Time &amp; temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>De-wax</td>
<td>*Clearify™ clearing agent</td>
<td>10 mins, 38°C</td>
</tr>
<tr>
<td>Target retrieval</td>
<td>ISH Pre-Treatment Solution (Dako Omnis)</td>
<td>15 mins, 97°C</td>
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<tr>
<td>Wash</td>
<td>ISH Ethanol Solution, 96% (Dako Omnis)</td>
<td>2 x 3 mins, 32°C</td>
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<tr>
<td>Digestion</td>
<td>ISH Pepsin (Dako Omnis)</td>
<td>*10, 15 or 20 mins</td>
</tr>
<tr>
<td>Drying</td>
<td></td>
<td>15 mins, 45°C</td>
</tr>
<tr>
<td>Denaturation</td>
<td></td>
<td>10 mins, 66°C</td>
</tr>
<tr>
<td>Hybridization</td>
<td>HER2 IQFISH PharmDx™ (Dako Omnis)</td>
<td>75 mins, 45°C</td>
</tr>
<tr>
<td>Stringent wash</td>
<td>ISH Stringent Wash Buffer (Dako Omnis)</td>
<td>10 mins, 61°C</td>
</tr>
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*Three pepsin digestion times can be chosen using the HER2 IQFISH protocol
Method Comparison of Dako Omnis HER2 IQFISH PharmDx™ Assay with PathVysion HER2 DNA Probe Kit II

- In Summer 2016 a comparative assessment was initiated to compare performance of the Dako Omnis HER2 IQFISH PharmDx™ automated FISH assay with the manual PathVysion HER2 DNA Probe Kit II (Abbott Diagnostics).

- Critical role is played by HER2 assays in HER2 targeted treatment decisions.

- Thus it is a mandatory that a thorough analytical validation is undertaken before a companion diagnostic assay is implemented for routine use.

- Guidance on CDx assay validation is provided in the ASCO/CAP guidelines for HER2 Testing in Breast Cancer.

- These guidelines were used as an indicative reference guide for the assessment study design.
Method Comparison of Dako Omnis HER2 IQFISH PharmDx™ Assay with PathVysion HER2 DNA Probe Kit II

- ASCO/CAP guidelines recommend that validation of a CE-marked or FDA approved HER2 CDx assay must include a minimum of 40 test cases.

- Validation samples should include a range of HER2 gene amplified, borderline & non-amplified cases.
Method Comparison of Dako Omnis HER2 IQFISH PharmDx™ Assay with PathVysion HER2 DNA Probe Kit II

- A total of 46 cases were included in the method comparison study.

- Study included samples from all current HERFISH service users: RLUH, UHA, APH/COCH & Royal Preston Hospital/Blackpool Victoria Hospital.

- Tumour samples included in study represented broad range of HER2/CEP17 ratios; amplified & non-amplified cases.

- Samples stained using the PathVysion HER2 DNA Probe Kit II & the Dako Omnis HER2 IQFISH PharmDx™ Assay.
Method Comparison of Dako Omnis HER2 IQFISH PharmDx™ Assay with PathVysion HER2 DNA Probe Kit II

- Study compared concordance between the two assays with regards to HER2 gene status (amplified/ non-amplified) as well as HER2/CEP17 ratio & copy number.

- In accordance with ASCO/CAP guidelines a method results concordance rate of 95% was set.

- Slides were stained in accordance with the working procedures for both assays.

- Evaluation & enumeration of the manual & automated stained slides was performed by two trained/competent observers.
Method Comparison of Dako Omnis HER2 IQFISH PharmDx™ Assay with PathVysion HER2 DNA Probe Kit II- results

- In 44 of the 46 cases (95.7%) a concordance rate of 100% achieved with regards to HER2 gene status results.

- In the 2 of the 46 cases (4.3%) no matched HER2 gene status scores were available possibly due to pre-analytical factors.
Method Comparison of Dako Omnis HER2 IQFISH PharmDx™ Assay with PathVysion HER2 DNA Probe Kit II - results

- Staining Quality of both assays compared:
  
  - Dako Omnis HER2 IQFISH PharmDx™ Assay showed comparatively little background staining when compared with the PathVysion assay.

  - HER2 signal was bright in the Dako Omnis HER2 IQFISH PharmDx™ Assay however the CEP17 signal was not as bright as with the PathVysion assay.
HER2 amplified case stained with Dako Omnis HER2 IQFISH PharmDx™ x40 magnification
HER2 amplified case stained with Dako Omnis HER2 IQFISH PharmDx™ x100 magnification
HER2 non-amplified case stained with Dako Omnis HER2 IQFISH PharmDx™ x100 magnification
Method Comparison of Dako Omnis HER2 IQFISH PharmDx™ Assay with PathVysion HER2 DNA Probe Kit II - results

- This validation study has shown that the performance of the automated Dako Omnis HER2 IQFISH PharmDx™ Assay is comparable with PathVysion HER2 DNA Probe Kit II.

- Results indicate that the Omnis assay is precise & the staining quality is as good as the PathVysion assay.

- Omnis HER2 IQFISH PharmDx™ Assay offers added benefits of more consistent & standardised HER2 FISH staining of FFPE breast cancer specimens, full traceability of reagents & each individual slide assay & the potential to significantly improve FISH assay TATs.
Thank you

• Questions?