ASSAY REVALIDATION REPORT

Validation of Beckton Dickinson (BD) CD157 PE for immediate use in the PNH assay

QR-HAE-VR0000200

Report
Prepared by:
Katy Sanchez

Date: 05/09/2017

On completion this document will constitute the Revalidation Report, and should be read in conjunction with the corresponding Revalidation Plan. For revalidations where particular sections do not apply, these should be completed with 'not applicable' as the entry

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2. REASON FOR REVALIDATION

Due to a supplier issue (see QE404), the regular order of CD157 PE (clone SY11B5) from 'The Binding Site' (manufactured by 'eBioscience') was not delivered leaving the laboratory very short of reagent. Correspondence with 'The Binding Site' revealed that they were no longer able to supply this reagent and we would need to find alternative distributor/manufacturer.

Contact was made with Beckton Dickinson (BD) who also manufactures a CD157 PE (clone SY11B5) and email correspondence was made with Robert Sutherland, field expert, who was lead author for the CD157/FLAER-based assay in use within the laboratory (Sutherland et al, 2014). Robert had also previously tested CD157 from BD and reported similar expression to the eBioscience reagent giving us confidence that a switch of manufacturer from eBioscience to BD would be acceptable following validation.

Due to the very short notice period and limited stock supply whilst maintaining a full patient PNH service, a formal validation plan was not achievable. All validation information is provided in this document (or attached to the Q-Pulse file). (Remaining stock only allowed for 14 samples to be tested in parallel. Some reagent also sent to the Royal London Hospital Immunophenotyping Laboratory for validation – to be attached separately if supplied).

3. WORK RISK ASSESSMENT / COSHH CHANGES

Not applicable, no change to work processes or COSHH. Reagent constituents remain the same, only a change in manufacturer.

4. ASSESSMENT OF EQA PERFORMANCE

EQA material received and tested as part of the validation.
## 5. ASSAY PARAMETER REVIEW

Antibody performance was assessed by running samples in parallel with the existing antibody by testing correlation and linearity.

| Lab no | % Mono clone | Median Fluorescence | SI | % Neut clone | Median Fluorescence | SI |
|--------|--------------|---------------------|----|--------------|---------------------|----|
| 17h3897| 61.86        | 61.99               | 179.17 | 147.36 | 83.96 | 84.73 | 64.23 | 64.73 | 140 | 135.16 | 102.38 | 79.69 |
| 17h3898| 62.86        | 61.99               | 179.17 | 147.36 | 83.96 | 84.73 | 64.23 | 64.73 | 140 | 135.16 | 102.38 | 79.69 |
| 17h3900| 63.86        | 61.99               | 179.17 | 147.36 | 83.96 | 84.73 | 64.23 | 64.73 | 140 | 135.16 | 102.38 | 79.69 |
| 17h3901| 52.24        | 53.43               | 360.85 | 362.96 | 5.42 | 4.99 | 65.26 | 64.56 | 190.74 | 238.62 | 14.75 | 12.9 |
| 17h3902| 60.86        | 61.29               | 110.55 | 73.97 | 51.39 | 49.96 | 68.46 | 68.14 | 142.47 | 100.73 | 29.83 | 25.68 |
| 17h3903| 97.61        | 96.98               | 185.43 | 159.03 | 69.47 | 57.3 | 98.69 | 98.71 | 110.17 | 103.8 | 61.23 | 41.16 |
| 17h3904| 89.84        | 90.32               | 339.11 | 193.26 | 68.04 | 59.26 | 90.94 | 91 | 129.33 | 91.98 | 112.59 | 74.67 |
| 17h3905| 0.07         | 0.2                 | 84.31 | 201.31 | 90.13 | 30.13 | 0.07 | 0.18 | 78.38 | 112.14 | 30.8 | 20.96 |
| 17h3906| 0.01         | 0.01                | 84.23 | 103.82 | 79.78 | 63.95 | 0.01 | 0.01 | 81.55 | 144.29 | 34.84 | 46.13 |
| 17h3907| 0.31         | 0.33                | 84.23 | 103.82 | 79.78 | 63.95 | 0.01 | 0.01 | 81.55 | 144.29 | 34.84 | 46.13 |
| 17h3910| 81.9         | 82.08               | 187.65 | 149.23 | 50.76 | 50.94 | 99.55 | 99.51 | 120.83 | 130.68 | 18.5 | 15.5 |
| 17h3913| 8.34         | 8.52                | 480.9 | 432.53 | 11.64 | 11.23 | 12.33 | 10.77 | 717.56 | 665.78 | 2.28 | 3.12 |
| 17h3914| 14.29        | 13.24               | 312.17 | 253.98 | 8.41 | 9.16 | 11.79 | 11.67 | 488.93 | 387.67 | 5.21 | 6.35 |

| Mean | 42.484545 | 42.580909 | 46.486364 | 46.3 |
| SD  | 38.711397 | 38.803999 | 41.84659 | 41.951824 |
| F-Test | 0.9941203 | 0.9938192 |

The three patients without data above had no PNH clones identified (events below LLOD were excluded). The F-Test shows no significant statistical difference between the eBioscience and BD CD157 PE reagent for either monocyte or granulocyte clones.
Regression analysis shows good correlation between clone sizes for both monocyte or granulocyte clones with $R^2$ values >0.99.
Acceptable SI’s and MFI’s were also observed. Individual patient data is supplied as an attachment to the Q-Pulse file (example below – file 1=eBio, file 2=BD) but analysis shows good distinction and separation of PNH and non-PNH clones.

As seen in all previous NEQAS sample (using eBioscience CD157 PE), separation (SI) is reduced, likely due to the way the NEQAS samples are stabilised before distribution. This observation was also see with the BD CD157 PE however the SI values were slightly higher (see last two patients of the table).
# 6. Review of Results against Acceptance Criteria

| No | Revalidation Criteria | Acceptance Criteria | Acceptable/Not Acceptable | Sign and date |
|----|-----------------------|---------------------|---------------------------|---------------|
| RV1 | EQA review            | • No significant difference with reported value | Acceptable | KSanchez 5/9/2017 |
|     |                       | • No significant difference with consensus results | Acceptable | KSanchez 5/9/2017 |
| RV2 | F-Test                | • No significant difference between results (>0.98) | Acceptable | KSanchez 5/9/2017 |
| RV3 | Regression analysis   | • Good correlation between data point ($R^2 >0.95$) | Acceptable | KSanchez 5/9/2017 |
| RV4 | Linearity             | • Dilution assay not appropriate, linearity observed in regression analysis | Acceptable | KSanchez 5/9/2017 |

Revalidation completed satisfactorily: YES

Revalidation Study Manager Signature: ...KSanchez ..................................  
Date: 05/09/2017
7. PROBLEM REPORT

Details of Problem(s)

N/A

Documentation Attached YES / NO No. of Pages

Person(s) Responsible

Actions Taken:

N/A

Performed By:

The problem(s) detailed above has / has not been fully resolved and further action is / is not required.

Revalidation Study Manager Signature: Date:

(Documentation Retained for future reference by operational department)
8. DOCUMENTATION REVIEW

The following documentation has been reviewed and amended appropriately, as a result of revalidation of the assay/process. All additional documentation required has been prepared & issued and is listed below.

| Document Type & Identifiers | Comment (e.g. reviewed/ amended/ withdrawn/ implemented) |
|-----------------------------|----------------------------------------------------------|
| N/A                         |                                                          |
|                             |                                                          |
|                             |                                                          |
|                             |                                                          |
|                             |                                                          |
|                             |                                                          |
|                             |                                                          |

The above documentation has been reviewed / amended / introduced as appropriate

Revalidation Manager Signature:  Date:
9. REVALIDATION REPORT SUMMARY & RECOMMENDATIONS

Report Recommendations

This validation report confirms the correlation of BD CD157 PE (clone SY11B5) with the existing (validated) eBioscience BD CD157 PE (clone SY11B5) (distributed by The Binding Site). No significant differences in clone sizes were seen, all clinical interpretation remained constant. This antibody can be put into immediate use following the withdrawal of the distribution by the Binding Site and use of any remaining stock. Stability data to confirm use in a cocktail to follow (see appendix 1).

Validation Manager Signature:  
KSanchez  
Date: 05/09/2017

10. REVALIDATION REPORT AUTHORISATION

The staff below, as designated “Responsible Persons” approve and authorise the Revalidation Report.

| Name          | Position                    | Signature     | Date    |
|---------------|-----------------------------|---------------|---------|
| Alan Dunlop   | Head of Immunophenotyping   | Alan Dunlop   | 6/9/17  |

Comments where applicable:
11. BIBLIOGRAPHY

1. SOPs
   1.1 LP-HAE-IM1035 Flow Cytometric Testing for PNH

2. Literature References
   2.1 Sutherland et al (2014). ‘Use of CD157 in FLAER-Based Assays for High-Sensitivity PNH Granulocyte and PNH Monocyte Detection.’ Cytometry Part B 86B:44-55

3. Policy References
   3.1 N/A

4. Other Documents
   4.1 N/A
12. APPENDIX 1

Cocktail stability containing PE CD157 PE

Antibody cocktails containing the new BD CD157 PE were made and tested over a period of 2 working weeks to ensure no changes in antigen expression were detected over time. In the population histogram above and the parameter histogram, below-right, the median fluorescence remained constant for both the negative control (lymphocyte) population (blue) and the CD157 positive monocyte (red) and granulocyte (green) populations. The data was collected on day 1 (file 1 on y axis), day 2 (file 2), day 4 (file 3), day 7 (file 4), day 9 (file 5) and day 11 (file 6) to cover 2 working weeks usage. When viewed overlaid in a dot plot (below-left), the median fluorescence of each time point (seen as a coloured dot) remains within the 2SD perimeter marker for each population type. It is therefore concluded that the WBC cocktail stability containing the new BD CD157 PE is stable for up to 11 days (2 working weeks).

Katy Sanchez 18/9/17