VALIDATION/VERIFICATION & CHANGE CONTROL PROFORMA

Supplemental data

This form is intended as a guide to aid laboratories in developing a suitable validation/verification procedure. A suggested methodology for using the form has been given below.

The terms 'investigating scientist' and 'senior scientist' have been used for illustration of an authorisation procedure – organisation of authorisation systems is a matter for individual laboratories.

1. Section 1 should be completed as far as possible to establish the goals and general format of the validation/verification/change control.
    - Sections 1.1 "**Intended use or application**" and 1.2 "**Requirements**" *must* be completed at the start of the procedure. The assessment of the validation/verification depends formally on confirmation, through the provision of objective evidence, that these requirements have been fulfilled[i].
    - If mentioned (1.2), the "Expected Performance" should be distinguished from the "Requirements", which must be shown to have been fulfilled.
      E.g. *The statement "should detect all known point mutations of haemophilia A" could be included as a guide in the Expected performance; if stated as a requirement, however, it would need to be proved.*

2. Section 2 covers the validation of utility which should be carried out for all validations and verifications. In the majority of cases this section can be completed on objective evidence from developmental work, design procedures (e.g. SNP checking primers) or by the use of limitations or controls in the on-going test. Where this is not the case, work plans for relevant parameters should be prepared as in 3 below.

3. Appropriate parameters for experimental investigation should be identified with the aid of the table appendix A – a checklist is also provided at the top of section 3. For each parameter required, the investigating scientist develops a work plan based on section 3 (these are referenced 3.1, 3.2 to 3.*n*) by completing copies of sections 3.*n*.1 ('Aims', 'Samples' and 'Methodology'). It is suggested that these be maintained in a single document.
     Note: several parameters may be tested in a single experiment, for example sensitivity and specificity.

4. The work plan[s] should be agreed and authorised by the investigating and the senior scientist by signing and dating in the boxes provided.

5. The experimental work is performed and analysed by the investigating scientist who should then complete the 'experimental results' and 'interpretation' sections 3.*n*.2.

6. The 'outcome and limitations' should be agreed between the investigating and senior scientists by signing and dating in the boxes provided.

7. Points 3 to 6 should be repeated for each parameter to be tested.

8. If there is any non-compliance between the experimental results and the required performance specification detailed in section 1.2 the parameter in question should be re-examined to determine if the methodology can be changed or new limitations introduced to rectify the non-compliance. Any further work should be recorded in a new section 3 work plan. Alternatively the implementation can be abandoned.

9. Once all the parameters have been satisfactorily investigated the investigating and senior scientist can agree and sign off the final conclusions in section 4.

10. Assuming the validation/verification has been completed satisfactorily an implementation plan can be drawn up. Appendix B provides a basis for an administrative checklist for the implementation.

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[i] (ISO 9000:2005 3.8.4, 3.8.5)

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1. Validation/verification[i] details

Test name	Validation of anonymiser_0_9_december_2020	**Q-Pulse Reference**	TT.VAL071

1.1 Test details

Intended use or application	Combine normalised MFI values obtained from multiple samples tested using Luminex Single Antigen Beads in an Excel spread sheet to facilitate easy viewing and decision making for listing and delisting unacceptable HLA mismatches in highly sensitised patients. Additionally intended for use in research with the NIHR data aggregator (currently version 11) to assess patient DSA development. anonymiser_0_9_december_2020 is intended to replace normalised_combiner_0_9_august_2017 which contains several bugs resulting in missing data. Anonymiser_0_9_december_2020 is also more user friendly as allows selection of a folder rather than individual files, and separates data clearly into separate sheets for Class I and Class II.
Locus / Gene / Marker	Luminex-SAB normalised MFI values.
Reference Sequence	N/A
Outline methodology	Luminex HLA class I & class II SAB MFI test results for a given patient are exported from Fusion 4.4 as csv files (or Fusion 4.2 if test files exported prior to 25/08/2020). These files are uploaded into the in-house Excel algorithm (Anonymiser) to facilitate sorting and presenting a timeline of normalised MFI values. Sample data (normalised MFI, molecular and serological specificities) generated by anonymiser_0_9_december_2020 are compared to original Fusion export files for accuracy. This was carried out for a random selection of 10 patients from Cardiac and Multi-visceral research cohorts and discrepancies noted. 4 additional patients were selected to perform targeted checks on known issues. Up to 2 random SAB I & II files were checked for each patient (if available).
SOP	TT.SOP047
References	TT.VAL015 Version 0_9 of the Normalised Combiner Random number generator: https://www.random.org

1.2 Validation details

Overall Aims	To ensure completeness and accuracy of sample data generated by anonymiser_0_9_december_2020 by comparison with data exported from Fusion. If data matches, anonymiser_0_9_december_2020 can be put into routine use, replacing normalised_combiner_0_9_august_2017.

[i] Delete as appropriate

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Requirements	Normalised MFI values for specificities imported into the combiner are identical to those in the original Fusion export files. Serological specificities have imported in the format A, B with Bw4/6, Cw, DR, DQ, DP. Molecular specificities have imported as standardised second field nomenclature. Eg. A*01:01.
Validation/verification	Validation (new in-house software).
Type	Validation performed prior to implementation.
Scope / limitations	None
Turnaround time	N/A
Other considerations	Accuracy of HLA Fusion 4.2/4.4 SAB export programme. Updates to Excel may affect software function.

2. Validation of Utility

Test name	Validation of anonymiser_0_9_december_2020	**Q-Pulse Reference**	TT.VAL071

Applicability of measurements	Accuracy of combined data compared to exported data is appropriate and sufficient to achieve the desired results.
Selectivity	There are no selectivity issues, limitations or control measures required to ensure test utility.
Interferences	None

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Cross-reactivity	N/A

Authorisation	Name	Signature	Date
Investigating scientist	Rebecca Cope & Rhea Langeveld		06/07/2021
Senior Scientist (Authorisation)	Sarah Peacock		07/07/2021

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3. Validation of anonymiser_0_9_december_2020

A copy of this section should be filled in for all parameters to be tested.

Test name	Validation of anonymiser_0_9_december_2020	**Q-Pulse Reference**	TT.VAL071

	Sensitivity		Trueness		Reproducibility		Limit of quantification
	Specificity		Repeatability		Robustness		Linearity
X	Accuracy		Intermediate precision		Limit of detection		Measurement uncertainty

3.1 Work plan

Section aims	To compare SAB data generated by the anonymiser_0_9_december_2020 vs. data files exported from Fusion 4.2/4.4 to ensure accuracy of data combination.
Samples	Random number generator used to select 10 patients from Multi-visceral and Cardiac research cohorts. Random number generator used to select up to 2 each of SAB I & II files per patient if available. 4 additional patients selected for targeted checks on previously identified files with combiner issues. Record of all patients,samples tested and validation notes/workflow is available in Z:\Tissue Typing\Quality Management\Change Control & Validation Forms (SOP 133)\TT.VAL071 Validation of anonymiser.
Methodology	Upload patient csv files from Fusion into anonymiser_0_9_december_2020. Run combiner programme. Copy MFI, serological and molecular specificity data from the combiner into the original Fusion export file, below their corresponding rows. Use Excel column differences function to compare the original and combiner data. Where discrepancies are highlighted, manually check data.

Authorisation	Name	Signature	Date
Investigating scientist	Rebecca Cope & Rhea Langeveld		06/07/2021
Senior Scientist (Authorisation)	Sarah Peacock		07/07/2021

3.2 Partial results and conclusions

Experimental results	All data is present and accurate – see 'Notes validation of anonymiser_0_9_december_2020' saved Z:\Tissue Typing\Quality Management\Change Control & Validation Forms (SOP 133)\TT.VAL071 Validation of anonymiser software The anonymiser_0_9_december_2020 has the following notes/ limitations: - A maximum of 200 SAB files can be processed at a time - Beads, and their corresponding HLA antibody specificities, are not consistent across all samples tested over a wide period of time. When the lot number changes, this may result in different beads being included in the SAB tests. Therefore a patient may have had

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	HLA antibodies to particular specificity on one lot but then this specificity is no longer tested on a future lot so the MFIs will no longer be listed. In theory the positivity of this HLA antibody should be picked up by another bead but it is possible this coverage is not full. - The specificity of bead 41 on lot LS2A01_006_00 has two DQAs: DQA1*03:03,DQA1*05:05,DQB1*03:01. The combiner strips off the DQB1*03:01 so just calls the bead DQA1*03:03,DQA1*05:05. DQA1*05:05,DQB1*03:01 combination is covered by bead 69. DQA1*03:03,DQB1*03:01 is not covered by any other bead so data will not exist for this specificity, however other lots do not cover DQA1*03:03,DQB1*03:01 either. The bead is still recognised correctly as DQ7. - anonymiser_0_9_december_2020 still works when there is just one SAB file to process or when there are zero files to process (a blank version of the programme might be needed to work with the aggregator for example, i.e. for negative patients). - If a SAB bead lot has two beads with the same specificity (rare – reason unknown why beads are duplicated), the combiner will just select one MFI value from the original data from one of the beads. As the specificity is the same, the MFI values shouldn't vary but this is just something to be aware of. - Beads in Fusion files must be in numerical order, otherwise the combiner is unable to properly identify the correct MFIs and will populate a proportion of the columns with 'x'. SOP047 update required.
Interpretation	anonymiser_0_9_december_2020 reached its objective in generating accurate MFI, molecular and serological data into single sheets for class I and II from multiple SAB files.
Outcome / limitations	anonymiser_0_9_december_2020 can be used clinically once SOP047 has been updated to reflect the new instructions for use. It may also be used for research.

Authorisation	Name	Signature	Date
Investigating scientist	Rebecca Cope & Rhea Langeveld		06/07/2021
Senior Scientist (Authorisation)	Sarah Peacock		07/07/2021

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4. Validation Final Conclusions

Test name	Validation of anonymiser_0_9_december_2020	**Q-Pulse Reference**	TT.VAL071

Overall Conclusion	anonymiser_0_9_december_2020 generates accurate normalised MFI values from Fusion export files, with serological and molecular specificities in the correct standardised format. anonymiser_0_9_december_2020 is safe to use clinically and in research.
Estimates of accuracy and measures of uncertainty	Sample MFI and specificity data is 100% correct. Measurement uncertainty – N/A.
Limitations and/or predictable interferences	Limitations are as follows: - A maximum of 200 SAB files can be processed at a time - Beads, and their corresponding HLA antibody specificities, are not consistent across all samples tested over a wide period of time. When the lot number changes, this may result in different beads being included in the SAB tests. Therefore a patient may have had HLA antibodies to particular specificity on one lot but then this specificity is no longer tested on a future lot so the MFIs will no longer be listed. In theory the positivity of this HLA antibody should be picked up by another bead but it is possible this coverage is not full. - The specificity of bead 41 on lot LS2A01_006_00 has two DQAs: DQA1*03:03,DQA1*05:05,DQB1*03:01. The combiner strips off the DQB1*03:01 so just calls the bead DQA1*03:03,DQA1*05:05. DQA1*05:05,DQB1*03:01 combination is covered by bead 69. DQA1*03:03,DQB1*03:01 is not covered by any other bead so data will not exist for this specificity, however other lots do not cover DQA1*03:03,DQB1*03:01 either. The bead is still recognised correctly as DQ7. - anonymiser_0_9_december_2020 still works when there is just one SAB file to process or when there are zero files to process (a blank version of the programme might be needed to work with the aggregator for example, i.e. for negative patients). - If a SAB bead lot has a two beads with the same specificity (rare – reason unknown why beads are duplicated), the combiner will just select one MFI value from the original data from one of the beads. As the specificity is the same, the MFI values shouldn't vary but this is just something to be aware of. - Beads in Fusion files must be in numerical order, otherwise the combiner is unable to properly identify the correct MFIs and will populate a proportion of the columns with 'x'.

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Internal QC	N/A
External QA	N/A

Authorisation	Name	Signature	Date
Investigating scientist	Rebecca Cope & Rhea Langeveld		06/07/2021
Senior Scientist (Authorisation)	Sarah Peacock		07/07/2021

Reference: Mattocks CJ, Morris MA, Matthijs G, et al. A standardized framework for the validation and verification of clinical molecular genetic tests. European Journal of Human Genetics. 2010;18(12):1276-1288. doi:10.1038/ejhg.2010.101 (supplementary material) available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3002854/

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Appendix A: Types of test. (Refer to main paper for full descriptions of test types)
*NB. In addition to the parameters detailed below appropriate **robustness** testing should be carried out for all types of test.*

	Description	Examples	Sensitivity[a]	Specificity[b]	Accuracy[c]	Trueness	Precision[d]	Limits of detection	Probability[e]
A	Quantitative tests. The result can have any value between two limits (including decimals).	Determination of methylation load (%); characterization of a mosaic mutation; heteroplasmy of mitochondrial variants.				++	++	++	
B	Categorical tests where the quantitative signal is placed into an ordinal series to give the final result.	Sizing a PCR product; determination of triplet repeat size (FRAXA, Huntington disease, etc.)			+	++	++	++	+
C	Categorical tests where the quantitative signal is placed into one of a limited series of predefined categories to give the final result.	Determination of copy number using PCR or MLPA.: exon deletion / duplication in *BRCA1*; *PMP22* gene dosage in CMT and HNPP;			+	To establish correction factors and/or cut-offs			++
D	Qualitative tests where the true quantitative signal can have one of many possible values, but the required result can only have one of two possible values.	Mutation scanning for unknown mutations e.g. by sequencing or high resolution melt.	++	++	+	To establish correction factors and/or cut-offs		++[f]	
E	Qualitative [binary] tests where the true quantitative signal can only have one of two possible values	Genotyping for a specific mutation e.g. *CFTR* Phe508del in cystic fibrosis or *HFE* Cys282Tyr in hemochromatosis.	++	++	+	To establish correction factors and/or cut-offs		++[f]	+

Legend

				Notes
▮ (red)	Metric used for implementation validation	a.	Sensitivity = True Positive / (True Positive + False Negative)	
▮ (purple)	Metric used for implementation or ongoing validation	b.	Specificity = True Negative / (True Negative + False Positive)	
▮ (blue)	Metric used for ongoing validation	c.	Accuracy = True Result / (True Result + False Result)	
++	Recommended parameter	d.	Precision should be measured in terms of repeatability and intermediate precision (as well as reproducibility for inter-laboratory validations)	
+	Applicable parameter (less used)	e.	The term 'probability' is used to describe situations where a probability that the result is correct can be assigned – primarily in ongoing validation (e.g. competitive hypothesis testing)	
		f.	Should be used in tests where genotyping of low level variations is required for example mitochondrial DNA	

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Appendix B: Administrative checklist

☐ Validation completed and approved

☐ Complete SOP

☐ Order reagents

☐ Health and safety aspects (personal, reagents)

☐ Equipment (electrical testing, maintenance)

☐ Subscribe to EQA

☐ Update request forms

☐ Update website and any directory listings

☐ Billing procedure

☐ Training

☐ LIMS functionality

☐ Worksheets

☐ Inform clients

☐ Report template

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