Assay validation Examples

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...This presentation centres around guidelines; but ultimately is our laboratory's approach to meeting the ISO15189 standards!

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Talk overview and aim

I will try to share what seems to work in our lab…in case it helps you

- Appropriate staffing !
- Worked out a template, a structure, for assay validation

- Look at the experiments that will be required
- Prepare a work sheet with experiment instructions and type of sample required
  - Ready to go when we have a moment and a relevant sample
- Try to focus on one assay at a time
  - We still have a long way to go

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Assay purpose

Monocytosis assay validation summary
Validation was carried out during time period of October 2019-September 2022
Staff responsible: listed by respective aspect/entry.
Validation raw data is located on SHMDS shared drive: Teams > Haematology > SHMDS > Flow Cytometry Laboratory > Validations_Audits > Monocytosis
If re-validation/verification is performed after initial assay validation: The resulting data is entered onto this form, with date and staff marked by the new entry. The document is then saved as a new edition and uploaded on Q-pulse. The previous edition may be made obsolete (archived and retrievable if required).

Assay Purpose. Key sample and result information.

Clinical need/ Benefits/Relevance of assay: *Brief statement on the diagnostic and/or prognostic relevance. Who will use the results, and for what purpose. Is there a commercial alternative? If so explain why this is not used. If replacing a previous assay – why? List improvement over previous version. For example, a better gating strategy that results in less variation between analysts*

To assist the diagnostic work-up of Chronic Myelo-Monocytic Leukaemia (CMML) versus reactive monocytosis (Khoury, 2022 #22). Identification of precursor stage CMML and Myelodysplasia patients that may develop CMML may also be made (Selimoglu-Buet, 2017 #21;Valent, 2019 #20) . How well the assay may separate definite clinical CMML from definite clinical MPN with monocytosis has not been widely published by several groups yet, and has been highlighted by others as being not straight-forward (Allen, 2022 #31); and is under evaluation in our centre (2022 onwards). Given the relatively few such cases in our centre, this may take a little while. Therefore, in cases where a monocytosis is persistent, and all other tests points towards MPN rather than CMML, a positive result from this assay (supportive of a diagnosis of CMML) may be interpreted as consistent with abnormal monocytes, and not reactive.

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What should be measured? *Describe cell types and how they are evaluated.*

The total mature monocyte population is defined by lack of T-cell, NK-cell, B-cell and neutrophil antigens, and by their CD45/light scatter/CD14/CD16 patterns. Within these mature monocytes, the relative proportion of CD14^{+}CD16^{-} (classical monocytes, cMo), CD14^{+}CD16^{+} (intermediate monocytes, iMo) and CD14$^{weaker+}$/ CD16^{+} non-classical monocytes (ncMo) are gated. The relative proportion of SLAN+ monocytes within the CD16^{+} mature monocyte proportion is also determined.

How should it be measured: *Describe the labelling method and reagents (other than the mAbs) used. Note that antibodies as well as detailed sample processing information have separate entries.*

Flow cytometry immunophenotyping, following a label-lyse-acquire processing method.
Label sample (dilute with buffer to WBC count of 10 if required) with the assay's monoclonal antibody panel (see entry below) -> Red cell lyse -> Acquire within 0-20 minutes. Manual data analysis, Diva or Kaluza.
Lyse: Ammonium chloride (BD Pharmlyse). Dilution buffer is PBS with 0.1% Albumin (BD Cellwash + Albumin)

Sezary Assay:
The assay includes two components:
1. Immunophenotypic evaluation of T-cells to identify and characterize an abnormal population, and
2. Absolute enumeration (dual platform).

In what type of samples (matrix) will it be measured? *List the sample type included in this validation. Of note, only sample types that have been validated can be run for clinical purposes.*

Peripheral blood. Bone marrow and tissue fine needle aspirates may be analysed however the diagnostic accuracy for results from those samples is unknown.

Anticoagulants/stabilisation product/s to be used: *State only those validated.*

EDTA

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Assay design and traceability

Traceability: There are no consensus guidelines for this assay. It is based on clinical correlation and peer-reviewed publications) which include two large inter-laboratory validation studies (Allen, 2022 #31;Patnaik, 2017 #23;Selimoglu-Buet, 2015 #26;Tarfi, 2020 #19;Wagner-Ballon, 2021 #32) (references in full listed at the end of the document).

Design: Antigens, reagents, sample processing strategy, instrument set-up, analysis and reporting strategy were selected according to the French multicentre study that forms a key reference for why this assay was endorsed by the WHO Edition 5 to be used in context of CMML diagnostic work-up (Khoury, 2022 #22;Selimoglu-Buet, 2015 #26) and after personal communication with Dr Orianne Wagner-Ballon (member of the French multicentre group, and project lead and author of the international working groups' validation study of this assay [Wagner-Ballon, 2021 #32] . Choice of sample acquisition criteria (time to acquisition) was based on the advice of the same guidelines and to provide consistency with our own laboratory working patterns.

Flow cytometers

Flow Cytometers used: *State all cytometers that the assay was validated for*
BD LSR Fortessa
Voltages were defined and optimised *Describe how instrument setting were determined. For example, manufactures' recommendations, CLSI H62 guidelines. State any requirements for ongoing assay validation and maintenance work. This should include whether beads may be used for routine compensation*
As per standard for LSR Fortessa ("General") PMT settings are used, with light scatter and FSC threshold amended to fit the lysed-not-washed processed cells, placing the monocyte could in the centre of the FSC-A versus SSC-A plot, not being overly concerned of 'loosing' any neutrophils due to stacking on the upper frame. This is best shown by the plots in the gating strategy section below. When re-compensation is required, unlabelled peripheral blood processed as per this assay is used for negative, and beads may be used for positive signals.

Software

Flow cytometry acquisition and analysis software used: *Ensure all software used for assay is included, for example, acquisition and analysis may use different software*
BD Facs Diva and Coulter Kaluza
Statistical software used: *This is normally related to the validation process. If statistical software is required for routine assay use – this should also be stated here.*

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Traceability

- No metrological traceability
- 15189 does give suggestions

☺ You can find one or several peer-reviewed publications from individual laboratories that have used an approach/validated flow cytometry method for measuring what you want to measure
And clinical correlation!

☺☺ You can find a peer-reviewed inter-laboratory validation study of an assay that measures what you want to measure *more or less*

☺☺☺ You can find a peer-reviewed inter-laboratory validation study of an assay that measures what you want to measure *–exactly!*

☺☺☺☺ You can find above, as well as an international consensus guideline for the assay, *that spells out which mAbs, clones, gating strategy and more, to use….*
And you can carry out 'clinical correlation':
Integrated sign-out and MDT (and/or lab MDT) outcomes
5 negative , 5 positive (diagnostic accuracy/trueness)

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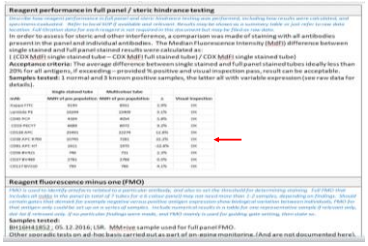
Reagent, panel defined.
Is steric hindrance check required?

- It is quick
- May show unexpected results
- If in-house design, we tend to do this.


Reagent performance in full panel / steric hindrance testing

Reagent fluorescence minus one (FMO)

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Risks?

- Are there risks involved?
  - Lab H&S
  - Regulatory requirements (Risk categories)
  - Assay-related non-conformities, incidents: High risk assay?
  - Interferences – are listed in separate entry – but could pose a risk – how is that mitigated


Risk assessment: regulatory classification, laboratory health and safety

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3.1.1.3 Quasiquantitative/Semiquantitative Data

Quasiquantitative/semiquantitative data are derived from assays that lack a calibration curve and reference standards. The data are continuous numeric and expressed in terms that are characteristic of the test samples without external normalization, calibration, or traceability. Quasiquantitative/semiquantitative data can be used to estimate the values of the measurand in the samples and to track temporal changes but not to determine absolute quantitative values. Without reference standards, accuracy and linearity for quasiquantitative/semiquantitative assays cannot be directly assessed. Flow cytometric methods in which the readout is the relative percentage of a given population of cells or the cellular concentration (number of cells/unit volume), that are not assessed against a calibration curve, would be considered quasiquantitative or semiquantitative

The enumeration of CD19⁺ B cells, for which there are currently no reference standards, provides a clear example of quasiquantitative or semiquantitative data. CD19⁺ B cells are typically evaluated by staining whole blood with at least CD19 and CD45. The events satisfying the definition of CD19⁺ lymphocytes (low side scatter [SSC], low forward scatter [FSC], high levels of CD45 expression, CD19⁺) are reported as the relative percentage of events satisfying the definition of lymphocytes (low SSC, low FSC, high levels of CD45 expression), through a hierarchy of logical operations known as "gating." During data analysis, each decision point in the process that identifies the CD19⁺ B cells is based on properties of the test sample. Properties such as the expression level of each antigen and the light scatter are numeric; however, calibrators for antigen expression are not used, and the final reported result is not derived from a standard curve. This is not to say that the measurement of CD19⁺ B cells by flow cytometry cannot be validated. As with all assays reporting quasiquantitative or semiquantitative data, selectivity, detection capability, precision, stability, and reference intervals for CD19⁺ B cells can be validated, but the validation of accuracy and linearity would not be conducted in the same manner typically used for biochemical or ligand-binding assays (see Subchapter 3.2). Approaches for demonstrating accuracy and linearity as appropriate to a given COU and relative risk are provided in Subchapter 6.1.

3.1.1.4 Qualitative Data

As opposed to numeric data, qualitative data are descriptive and are used to determine characteristics of the test sample. Data are reported in nominal (eg, yes/no) terms[11]. Examples of qualitative data include leukemia/lymphoma immunophenotyping by flow cytometry and genetic markers.

H62

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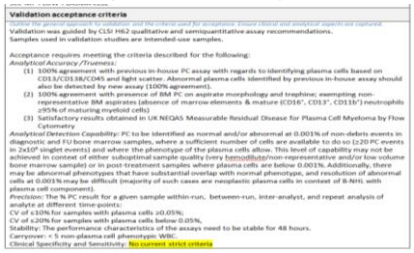
Purpose, general design & traceability & risk

- Has now been stated.
- What are our acceptance criteria for our 'dream assay' ?


Validation acceptance criteria

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Sample volume, detailed processing protocol, assay controls

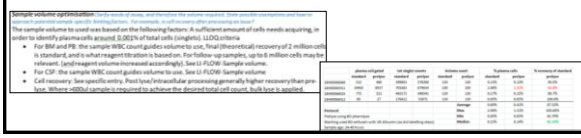
Volume used:

As directed by the guideline/validation paper you trace your assay to, or other,

Such as based on cell recovery

If more than one matrix used – is volume always the same. Is processing always the same.


Development and Optimisation


Sample volume optimisation

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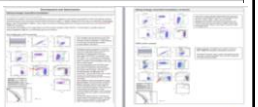
Assay internal controls

- List, also highlight in gating figure

Internal positive and negative controls		
Antigen	*Negative*	*Positive*
CD45	Un-lysed red cells/debris, CD45^{weak} neutrophils	Mature lymphocytes
CD14	Lymphocytes	Monocytes
CD16	Lymphocytes (bar some NK-cells)	Neutrophils and some NK-cells
CD7	Neutrophils, CD24⁺ lymphocytes (B-cells)	T- and NK-cells
CD24	CD7⁺ Lymphocytes (T-cells, NK-cells)	B-cells and Neutrophils
SLAN	Lymphocytes	CD16⁺ monocytes (where available)
CD56	Most lymphocytes	NK-cells



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Here, also reagent (mAb) information and optimisation data


Development and Optimisation

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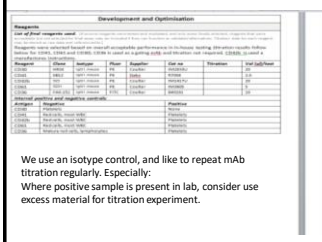
Tricky assay example: Platelet glycoprotein:

(Almost) no internal negative controls,
few diagnoses (rare to find a positive control)


Development and Optimisation

We use an isotype control, and like to repeat mAb titration regularly. Especially:
Where positive sample is present in lab, consider use excess material for titration experiment.

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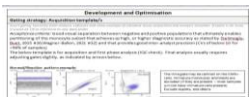
Gating strategy.

Work out template. Our experience: gating template is a main influence on inter-analyst precision. So, sometimes we have refined the template after carying out analyst precision testing.


Development and Optimisation

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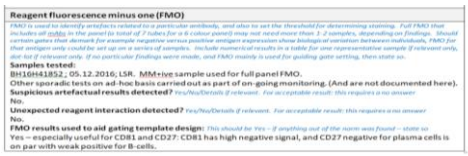
FMO?

- Our approach: a must for any in-house design
- If adapting from validated published assay: not necessarily required
  - PNH
- Always useful for gating template


Reagent fluorescence minus one (FMO)

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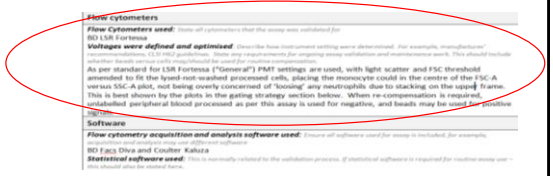
Cytometer settings also captured

Most assays follow a standard approach , so where this is not the case, we aim to state the specifics for the assay. Useful to have bead/harmonemia like strategy for voltage settings for the assay.
Compensaiton strategy also included, for example whether cells or beads are used as compensation controls.


Flow cytometers

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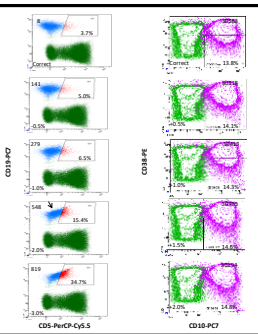
Robustness

Suboptimal compensation:
How does it affect my assay?

Yes – we would like to have perfect compensation at all times

Familiarity of how incorrect compensation (tandem lot-to-lot variation for exammple) influences our interpretation of the data is useful.



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Assay locked down - validation

- That said: If precision data shows high (too high) inter-analyst CVs for example – the gating protocol may need changing perhaps.
- Or if any other issues are found, that are not acceptable.

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Analytical accuracy

PCS example old vs new assay

If no old/previous assay:
We use diagnostic accuracy (Sens/spec calculated).
Sample share/EQA not always available.


Validation

Analytical Accuracy / Trueness and Specificity

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Limit of Blank (LOB)

- Examples of assays with established LOB:
  - B-ALL
    - 'Empty space' (LAIP)
  - PNH
    - There is only one composite phenotype to monitor
  - Sezary (specific phenotype)
    - There are several composite phenoytpes
  - MM (specific phenotype)
  - CAR-T19
    - We considered two ways of measuring this
- Examples of assays for which we do not intend to establish LOB:
  - Monocytosis
  - Acute leukaemia lineage determination
  - Diagnostic L&L assay

For these assays, 'blank' samples are easy to find in our labs.
H62 advices 5 samples (that each may be acquired 5 times)

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LOB for assays with several different disease phenotypes?

- Grateful for your thoughts!
- For Sezary, we elected to use a defined phenotype: CD3+ CD4+ CD7- CD26-/wk+.
- This does not cover all Sezary phenotypes encountered
  - PD-1/CD28/5/TRBC-1/and others
- However a check of the 'core/spine' gate was helpful for us to understand the limitations of the assay.

  'What are we up against' in this phenotypic area,
  in pathological controls?

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LOB: Sezary example

Analytical Sensitivity: Limit of Blank (LOB)

[Minimum 10 normal samples and, if possible, 10 pathological controls. Include sample description: Normal or pathological/reactive control]. 5 negative samples in final version

Samples: 10 normal/reactive samples were tested. A cohort of pathological controls is being built up.

		1	2	3	4	5	6	7	8	9	10
events	WBC	321948	254974	241550	356251	373297	340300	293547	398043	378430	218231
	45 Lymphs	146058	73381	124246	146963	79611	124655	97494	117575	144748	65393
	Ly	145735	75977	123682	145740	78901	123027	93237	115206	141894	63542
	3+	113174	44926	74190	102130	65733	99452	65097	102832	102997	39152
	3+8+	34749	14862	20544	28408	8874	7036	14081	45498	33474	10841
	3+4+	68347	27684	38818	64560	54393	90607	49143	51707	52351	25660
	3+4+7-26-	1421	984	2905	1330	1172	4901	1889	2467	1851	850
% of Ly	3+	78%	59%	60%	70%	83%	81%	70%	89%	73%	62%
	3+8+	24%	20%	17%	19%	11%	6%	15%	39%	24%	17%
	3+4+	47%	36%	31%	44%	69%	74%	53%	45%	37%	40%
	3+4+7-26-	1.0%	1.3%	2.3%	0.9%	1.5%	3.9%	1.9%	2.1%	1.3%	1.3%
% of WBC	3+4+7-26-	0.4%	0.4%	1.2%	0.4%	0.3%	1.4%	0.6%	0.6%	0.5%	0.4%

Results: 80% of tested samples had less than 1.0% CD3+CD4+CD7-CD26- T cells of total WBC. The composite phenotype of the cases with >1.0% values were deemed within norm.

Samples run as part of routine clinical work – integrated report: Not CTLC (or other neoplasm)

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LOD and LLOQ

Limit of Detection LoD
Determined by utilising both the measured LoB and X test replicates of a sample known to contain a low concentration of analyte.

$$LoD = LoB + 1.645 \times SD$$

Limit of Quantitation LoQ
The lowest concentration at which the analyte can not only be reliably detected but at which ***some predefined goals for bias and imprecision are met***.

"Functional sensitivity" is defined as the concentration that results in a CV=20% (or some other predetermined % CV), and is thus
a measure of an assay's precision at low analyte levels (without addressing bias).

Armbruster, David A., and Terry Pry. "Limit of blank, limit of detection and limit of quantitation." *Clin Biochem Rev* 29 Suppl 1 (2008): S49-52.

Clinical and Laboratory Standards Institute. Protocols for Determination of Limits of Detection and Limits of Quantitation, Approved Guideline. CLSI document EP17. Wayne, PA USA: CLSI; 2004.

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LOD, LLOQ and linearity

- We often need to work from a mock sample
  - Plasma cell assay: If we have very low level, 0.0001% sample, insufficient sample to set up x6 for example
  - PNH red cell assay: There can use a native sample
- Creating a mock sample
  - Dilute a positive sample in relevant negative sample
    - A little inexact, even if you correct for the different WBC counts of the two samples (the 'spike' and the 'dilutant' samples, resulting in a spiked sample.
    - The dilutant (native sample) of course need to be a blood for PNH, but a BM for plasma cell assay, for example. The correct matrix.
  - How to calculate the expected #events of cell of interest?
    - Correct for the spiking and dilutant samples WBC count
  - Our experience is that a dilution series is useful: we want to make sure we create a mock sample with the (low) level of disease that we want to achieve detecting (what acceptance criteria did we set?)
  - This then works also as your linearity test.

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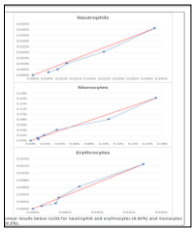
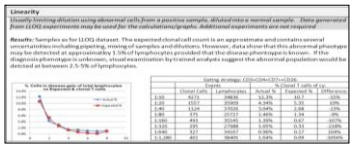
Linearity



Provides a pretty good idea of what our detection level might be.

And we can select the relevant dilution to be used for our repeat testing, to have x5 replicated to create our LOD.



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We now have a mock sample with low level disease…

Repeat analysis.

H62 suggests x5 replicates for medical laboratories
(for clinical quantitative assays, sugges 5 samples…may need interpretaton)

And we can calculate our Limit of Detection :
Determined by utilising both the measured LoB and 6 test replicates of a sample known to contain a low concentration of analyte.

LoD = LoB + 1.645×SD

Raw event data	Neutrophils	Type 2 N	Type 3 N	Monocytes	Type 3 monos	RBC	Type III RBC
Dilutant	422561	0	0	13367	0	562830	6
PNH + sample	148273	90	416	17132	11	542103	72
1	886366	195	558	51749	37	1053021	70
2	1149267	82	268	55169	30	895408	58
3	1077752	89	269	50504	26	900120	58
4	1103381	93	386	51104	22	914270	62
5	1227949	101	217	53906	21	920915	71
6	1051648	90	196	46990	19	898940	55

% GPI neg		Type 2 N	Type 3 N		Type 3 monos		Type III RBC
Dilutant		0.000%	0.000%		0.000%		0.001%
PNH + sample		0.061%	0.292%		0.064%		0.013%
1		0.012%	0.018%		0.071%		0.007%
2		0.007%	0.023%		0.054%		0.007%
3		0.008%	0.025%		0.071%		0.006%
4		0.008%	0.018%		0.043%		0.007%
5		0.008%	0.018%		0.039%		0.008%
6		0.009%	0.019%		0.040%		0.006%
Average		0.009%	0.020%		0.053%		0.007%
SD		0.00002	0.00003		0.00015		0.00001
CV		18%	16%		28%		8%

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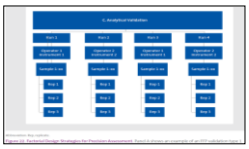
Precision

- Approach is pragmatic: Maximise sample and data point use
- Check through requirements (number of samples for each type of precision we need to measure)
- For example

  "LOD: When the assay is an LDT to be used in a clinical lab it is recommended to use five negative and five low-positive samples".



Let all analyst's analyse these = inter-analyst precision (help with gating template maybe)

Part of Accuracy/Truenes: The +ive sample we used to create the mock, is part of our 10 cases to show accuracy and trueness

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Example:

One sample was set up by 2 staff, each staff set up the sample in triplicate . At two different time points.
The resulting six tubes were acquired on two cytometers.

		EVENT COUNT						PERCENTAGE			
Sample no	Total	FSC/SSC singlets		PCS	clonal PCS	19+ PCS	56+PCS	56+ of 56	PCS of tot	cPCS of PCS	cPCS of tot	
1	750000	670981		459	171	196	55	13.8	0.07	37.3	0.025	Staff A set-up am
2	896365	660507		441	166	183	54	13.8	0.07	37.6	0.025	
3	750000	669471		488	184	199	64	14	0.07	37.7	0.027	
4	750000	676938		478	213	164	57	14	0.07	44.6	0.031	Staff B set-up pm
5	750000	673826		434	183	151	60	16.9	0.06	42.2	0.027	
6	750000	668726		431	171	159	54	17.6	0.06	39.7	0.026	
1	750000	676000		452	159	162	95	46.7	0.07	35.2	0.024	Staff B set-up am
2	721099	633689		447	142	179	41	29.3	0.07	31.8	0.022	
3	750000	688365		495	154	188	97	51.2	0.07	31.1	0.022	
4	750000	674881		489	164	187	82	45	0.07	33.5	0.024	Staff A set-up pm
5	750000	683981		504	158	223	71	47	0.07	31.3	0.023	
6	750000	688650		434	116	186	78	43.8	0.06	26.7	0.017	
Average		671075	Average	455	181	175	57	15	0.07	39.8	0.027	
SD		6271	SD	24	17	20	4	2	0.003	3.0	0.002	
CV		1	CV	5	9	12	7	11	5.0	7.4	8.9	
Average		670928	Average	470	149	188	75	44	0.07	31.6	0.022	
SD		19533	SD	29	18	20	19	8	0.003	2.9	0.002	
CV		3	CV	6	12	11	25	17	4.9	9.0	11.2	
Paired t-test:		0.982		0.241	0.005	0.457	0.064	0.000	0.218	0.004	0.005	

Repeatability (within run) for each operator/sample processing staff;
Between cytometer, And: Once all tubes are also analysed by all analysts : inter-analyst precision.
analyte stability. The sample is a 'low presence of disease' type sample – can be used for comparison with gold standards/limit of detection data,

If precision exceeds expected/acceptance criteria: Any obvious cause? (dead/dying cells/gating template may be improved, something went wrong during processing, aso

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Analyte stability

Native and processed

- In our experience, one of the most important tests.
- Realistic approach that fits with our labs routine
  - What age is sample usually when we receive it?
  - How long time does it normally take until we can process it?
  - What is the maximum time we would like to/require to process it?
  - How would we store it until then?
    - Do we need to check processed sample stability?
    - Fixed/unfixed, stored in fridge after processing?

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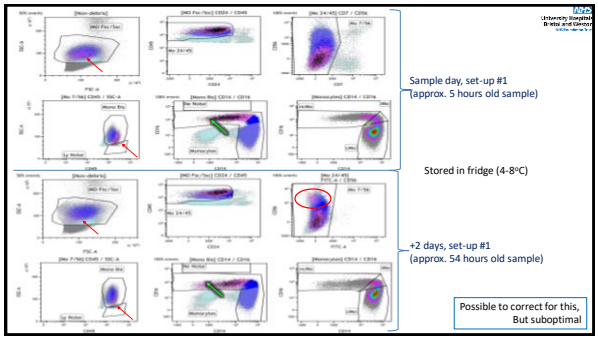

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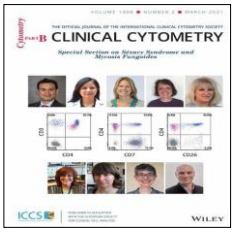
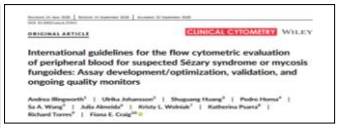
Assay validation template available in this paper





Illingworth, A et al . *Cytometry Part B: Clinical Cytometry, 100*(2), 156-182.

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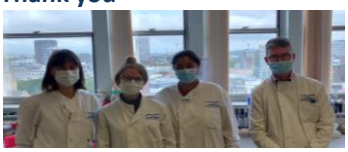
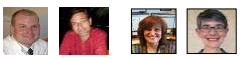
Thank you





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Spare slides

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Precision:
creating the 'mega experiment' layout

- H62 may ask for (check the table and sections relevant for your assay)…
  - Analyte stability= 3 samples
  - Repeatability = 6 samples, each in triplicate
  - Reproducibility (same sample, at different time points) = 2-3 samples, 3 replicates
- And we could fit nearly all that into a large experiment on a quiet day…

- There may not be sufficient material to carry out a 'mega experiment'
- Our experience also – things can go wrong (a Mab was not added, cytometer played up)
  - So 2 slightly smaller 'mega experiments' rather than one massive can work better.
- For certain sample types (matrix)/assays: just difficult;
  - CSF, some FNAs, MM MRD precision on native samples, and so on.

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What are our results used for?

- Monocytosis assay: report is based on a % cut-off. Supportive
  - Monocytes are identified. They are divided into 3 subsets (MO1, MO2, MO3). If MO1 is at or above 94% of total monocytes: This supports a diagnosis of CMML over reactive monocytosis.
- Sezary assay: reports clonal T-cells x 10^9/L. Staging, monitoring
  - Dual platform @UHBW
- Acute Leukaemia Linage Determination: reports description of an abnormal population, as well as its relative size in the sample (% of total nucleated cells)
  - The clone size is less likely to influence management, if there is an integrated laboratory report (aspirate, trephine, genetics, imaging)
- MRD: reports description of an abnormal population, as well as its relative size in the sample (% of total nucleated cells)
  - The clone size will or may well influence management

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Interferences?

- Monocytosis –
  - Therapy affecting read-out?
- Various assays
  - Anti-CD38
  - Anti-CD20 and many others

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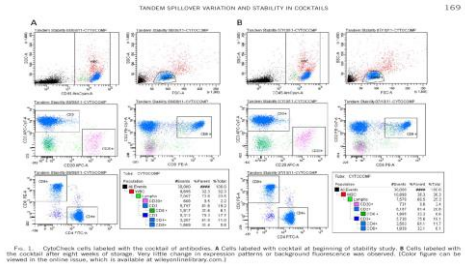
Reagent stability



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Traceability – example 1

- B-ALL MRD– CEIVD labelled assay , purchased
  - Still include the papers that the company refers to
  - Verification process (not included in this setting, but along similar vein)

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Traceability – example 1

PNH

- Consensus guidelines (ICCS/ESCCA 2018, 4 papers)
  - Comments and the authors individual laboratory experience of
    - Analyte stability
    - Anti-coagulant
    - Which antigens to study
- Inter-laboratory validation studies (several)
- Can choose to use exactly the same reagents…
- Or slightly different (cytometer used, finance..)

Similar for CLL, also much available guidelines and relevant publications for MDS

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Traceability – example 2

- Platelet glycoprotein analysis (for bleeding disorders)
- There are consensus guidelines (et al)
  - Comments and the authors individual laboratory experience of
    - Analyte stability
    - Anti-coagulant
    - Which antigens to study
  - Some backed up by references
  - No validation data, as far as I can see, the comments are backed up by the authors experience (did they validate their assays? Probably yes – but there are no data here)
  - No specific mAb clones recommended

So here, need to search literature, find papers that show clinically documented cases, which antibodies (specificity, clones) did they use?

Lots of different bleeding disorder related gene variants:
A given mAb may work for one case, but not another. (integrated reporting)

Similar for many L&L assays
Often companies will list publications on mAb Product Inserts
but check up to date literature.

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Traceability – example 4

- CAR-T-cell measurement assay
  - Uses reagent not previously published
  - Reagent itself needs validation
  - Unusual for clinical flow assay, albeit
    - Monoblast identification
      - Cell sorting, correlating with morphology/IHC
    - Epitope specificity of any Ab, for example CD30 in NHL
    - The reagents we use – have traceability?

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H62 strategies Qualitative / Quantitative

Table 22. Validation Scenarios Based on Intended Use

Regulatory Setting	Intended Use of Data	Assay Type	Recommended Validation Strategy
Nonregulated	Basic research	Novel assay	FFP validation type 1
Nonregulated	Drug discovery	Novel assay	FFP validation type 1
Nonregulated	Exploratory end points in clinical trials	Novel assay	FFP validation type 1
Nonregulated (GCLP recommended)	Secondary end points in clinical trials	Novel assay	FFP validation type 2
Medical laboratory (CAP, CLIA, or ISO[15189])	Patient care and/or treatment	IVD	Verification[a]
Medical laboratory (CAP, CLIA, or ISO[15189])	Patient care and/or treatment	Qualitative LDT assay	CLIA/IMDRF qualitative validation
Medical laboratory (CAP, CLIA, or ISO[15189])	Patient care and/or treatment	Quantitative LDT assay	CLIA/IMDRF quantitative validation
Medical laboratory (CAP, CLIA, or ISO[15189])	Patient care and/or treatment	Laboratory-initiated assay revision	Laboratory-initiated assay revision validation
GLP, GCLP[b]	Primary end point in clinical development	Novel assay	Analytical validation type 1
Manufacturing (GMP, ISO[13485])	Regulatory submission for new diagnostic test	Novel assay	Analytical validation type 2
Manufacturing (GMP, ISO[13485])	CDx	Novel assay	Analytical validation type 2

Abbreviations: CAP, College of American Pathologists; CDx, companion diagnostic; CLIA, Clinical Laboratory Improvement Amendments of 1988; GCLP, Good Clinical Laboratory Practice; GLP, good laboratory practice; GMP, good manufacturing practice; IMDRF, International Medical Device Regulators Forum; ISO, International Organization for Standardization; IVD, in vitro diagnostic; LDT, laboratory-developed test.
[a]Verifications are performed when an IVD assay, which the manufacturers have already validated, is set up. Verifications require that the laboratory follow all documented procedures that accompany the validated assay. The same documentation outline should be followed for verifications.
[b]The regulatory requirements for primary end point data are not clear. Many laboratories will follow GLP.

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A little outside the concept of validation perhaps – but still;
Other areas within ISO standard where new assay is captured,

and that the 'super template' perhaps could capture:

- Training (competencies)
- Risk mitigation
- Quality indicator – assay TAT
- Standards are a lot about minimising risk, also to **identify opportunities** for improvement.
- **4.11 preventative action:**
  - ISO says to determine action to **eliminate the causes of *potential* nonconformities** in order ***to prevent their occurrence***.
  - "By employing an additional anti-CD30mAb, I reduced risk of not detecting CD30+ lymphoma"

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