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SIMTI

Validation of diagnostic process in Immunohematology: SIMTI's guidelines proposal

Francesco Fiorin

Transfusion Department ULSS 8 Berica - Vicenza

SIMTI President

Il sottoscritto, in qualità di Relatore
dichiara che

nell'esercizio della Sua funzione e per l'evento in oggetto, NON È in alcun modo portatore di interessi commerciali propri o di terzi; e che gli eventuali rapporti avuti negli ultimi due anni con soggetti portatori di interessi commerciali non sono tali da permettere a tali soggetti di influenzare le sue funzioni al fine di trarne vantaggio.

Scope of the document

- The validation of immunohematological diagnostic process involve all the three phases of it:
  - pre-analytical (sample management),
  - analytical
  - post-analytical (final report management)
- We consider to focus our attention only on analytical phase

Main preliminar doubts

With respect to the content of the **Guide to Validation Activities of Transfusion Processes** edited by Italian National Blood Center (CNS):

- the analysis should be limited to pre-transfusion tests (group determination and irregular anti-erythrocyte antibody testing/identification) or should be extended to additional tests (e.g. antibody titration)?
- With respect to the general indications of the Guide itself, which find a specific application to analytical processes aimed at biological validation, it asks whether there are principles that can also be applied to the validation of immunohematological analytical processes (an example of "immunohematological declination" of the principles enunciated for the qualification of biological validation assays follows).

Main preliminar doubts

- With a view to process validation, it would be necessary to define standards for the verification of the integration between the analyzer and the Blood Establishment computer software (and/or the LIS of the Laboratory).
- Pre-analytical phase:
  - In this phase is important to verify at least some of the elements that may influence the tests: for example, the transport conditions of the samples and the time from collection to the analytical phase.
  - In addition, evaluate a specific control phase, in the design of the tests, of the manual labeling of the samples (aka IT failure management).
- Post-analytical phase: it's necessary to define a minimum standard of the final report and a minimun set of information for every test that can help the patient or the doctor to interpret the results (for example the minimum significant titre of anti-eritrocyte antibodies for the prevention of FNHD)

Steps necessary for validation

- Design qualification (verify that the design on the request in a tender is coherent and applicable with the organization)
- Installation qualification (verify the conformity of what done by the supplier with regulation and security requirements)
- Operational qualification (verify the conformity of instrumentation and reagents with tender specifications, tests must include worst case condition)
- Performance qualification (verify that in real world all the test are concordant with what expected in terms of sensitivity, specificity, reproducibility. It’s important in this phase to verify if TAT is what expected, especially in urgent requests)

Samples for PQ

- Positive and negative samples (commercial or archive samples of blood plasma or serum)
- Positive samples with known concentration (if applicable) expressed in IU or other measure unit recognized by WHO (international standards, reference preparations or commercial samples. For example, human anti-D sera with concentration ≤ 0.5 UI/mL
- Dilution matrix (AB plasm with negative IAT or commercial plasma matrix). Consider viscosity effect especially in microcolumn agglutination that may lead to false positive results

How many samples to test

- It’s important to reach a statistical significancy in the number of test that must consider numbers of fails permitted and interval of confidence (see tables in EDQM Guide)
- If you don’t reach a sufficient number of samples you can use a concurrent approach to validation instead of prospective validation (for example DAT in newborns)

Evaluation of results

We must keep in mind that results in immunohematological tests are for the most part qualitative and often operator dependent.

The use of readers, also non incorporated in instruments, can help to standardize the score of reactivity. (+/-, 1+, 2+ ….. in microcolumn agglutination or agglutination score in solid phase techniques).

Alternatively use two ore more operators to read the same reaction

Evaluation of results

Sample («true» value)	Diagnostic Sensibility and Specificity		Total
	Positives	Negatives	
Positive	# true positives (TP)	# false positivesv(FP)	TP+FP
Negative	# false negatives (FN)	# true negatives v(TN)	FN+TN
Total	TP+FN	FP+TN	N

a) Diagnostic sensitivity (% TP) = 100 x [TP/(TP+FN)].
b) Diagnostic specificity (% TN) = 100 x [TN/(FP+TN)].
c) % of positive concordance (positive percent agreement) =100 x TP/(TP+FP).
d) % of negative concordance (negative percent agreement)=100 x TN/(TN+FN).
e) Compare results obtained with what declared by the manufactorer

Evaluation of results

Repeatability evaluation

- It evaluates the dispersion of the results of repeated tests on the same sample in the same analytical session or in several analytical sessions due to random factors.

Cross-contamination assessment

- It evaluates the possibility of contamination of a negative sample with a positive one due to a carryover effect. You can put a positive sample every three negative known samples e.g. and evaluate if they are repeatedly negative

PARAMETER	GENERAL PRINCIPLE	VERIFICATION METHODS	GROUP	IAT	Ab identification	Anti-AB titration
Diagnostic accuracy **Testing this parameter is to determine whether the diagnostic kit generates true and valid results.**	Biological samples known to be positive or negative (commercial samples, reference samples, archive samples, ….)	Analyze a significant number of samples negative for the antigen/antibody and an equal number of positive samples.	Samples known for antigenic expression, at least for ABO, RhD, RhCE, Kk.	(at least Anti-D) **Blood donors:** positive result in 0.5 UI/mL serum. Test RBC heterozygous (R1r, R2r) **Patients:** positive result in 0.1 UI/mL serum. Test RBC heterozygous (R1r, R2r)	(at least Anti-D) **Blood donors:** positive result in 0.5 UI/mL serum. Test RBC heterozygous (R1r, R2r) **Patients:** positive result in 0.1 UI/mL serum. Test RBC heterozygous (R1r, R2r)	NIBCS standard

A heartfelt thank you to the Working Group that product the following guidelines:

Serelina Coluzzi

Antonella Matteocci

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