Laboratory Testing for Biologicals

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GMP FORUM 2024

Agenda

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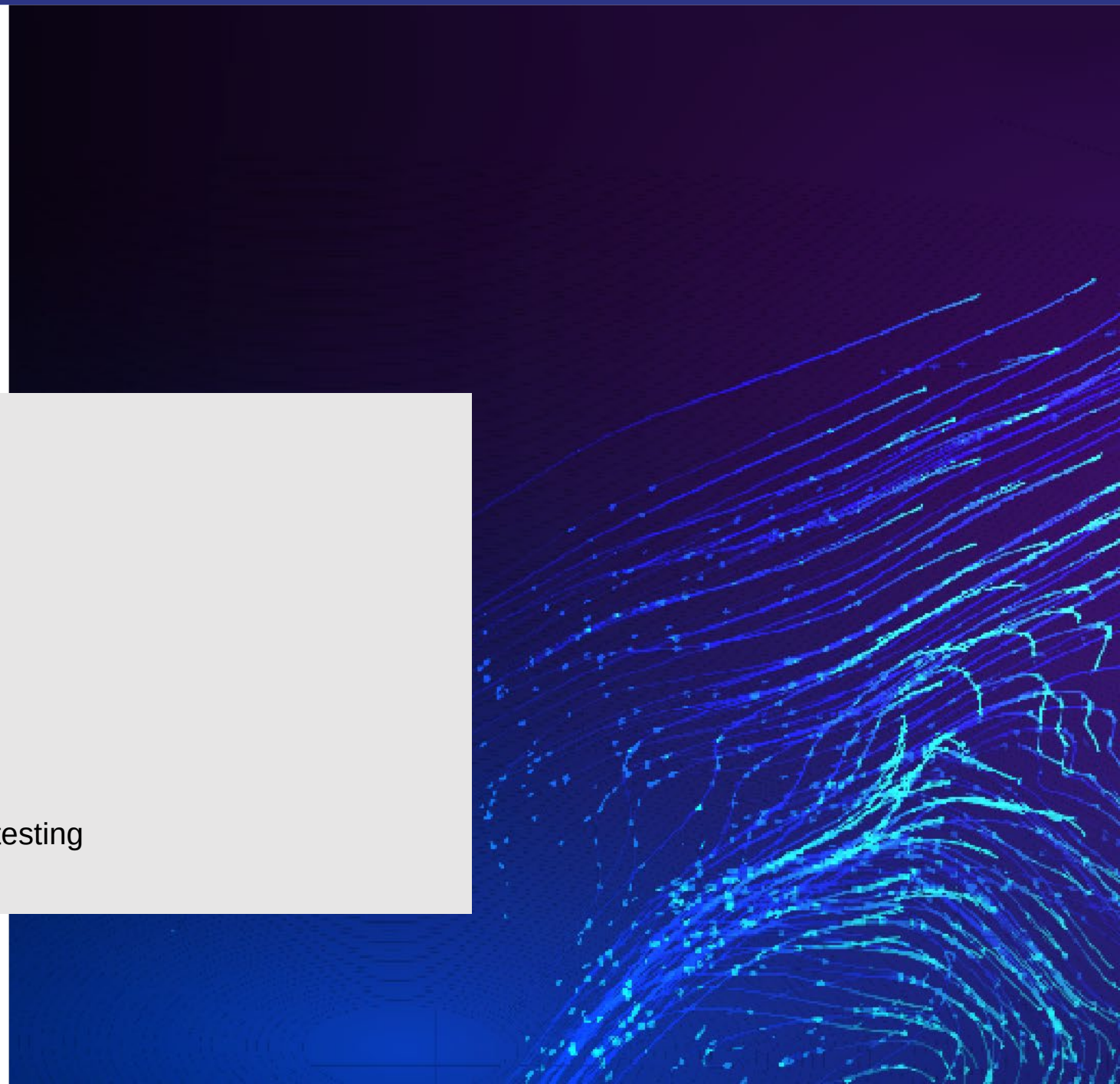
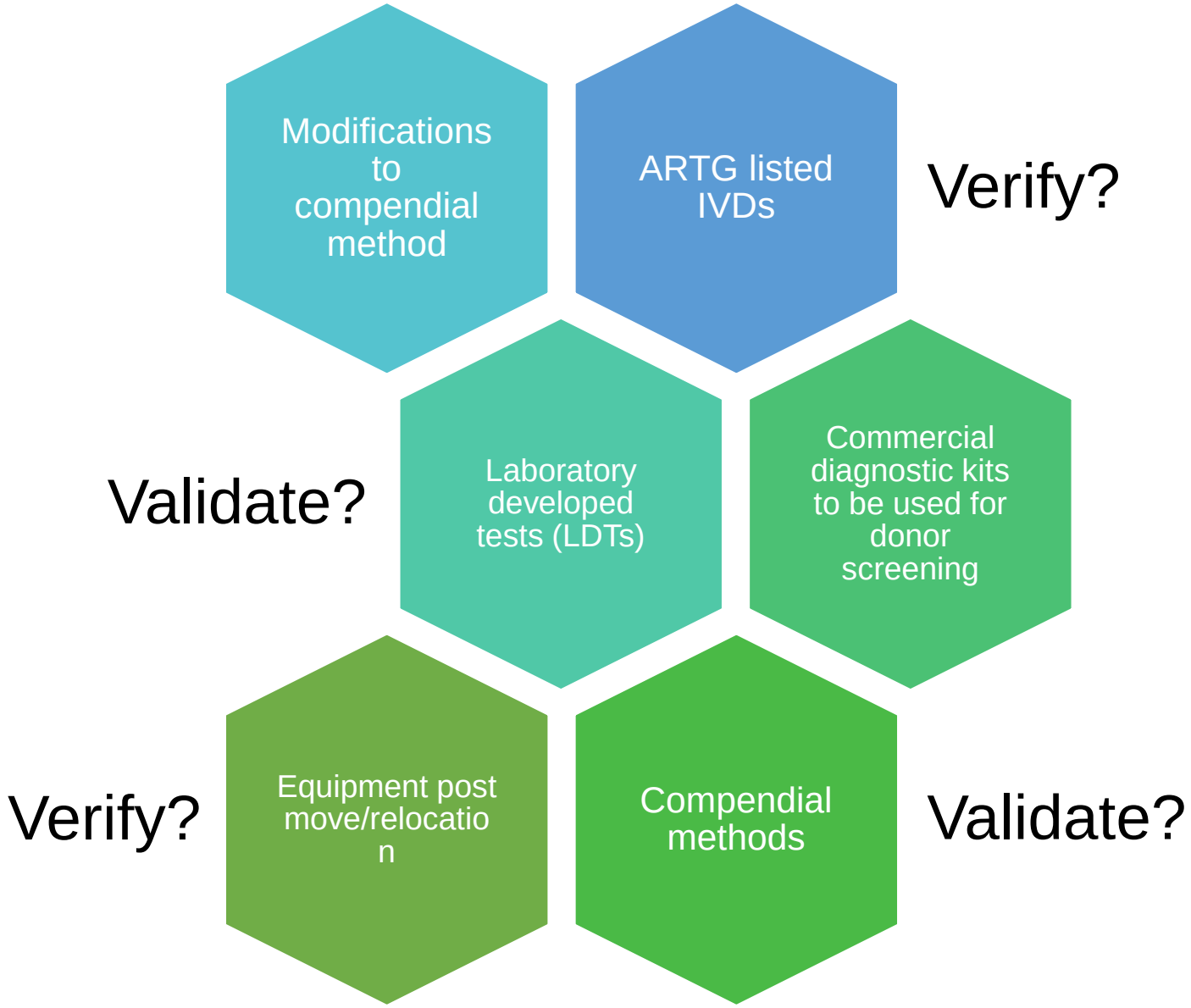
- Regulations
- Guidelines
- Definitions
- To validate or not to validate? (verify)
- Detection limits
- Strategies for method verification and validation
- Independent control – why?
- Microbiological contamination testing
- Commonly observed deficiencies for biologicals testing
- Mock Validation Protocol



Quick Check


Modifications to compendial method
ARTG listed IVDs
Verify?
Validate?
Laboratory developed tests (LDTs)
Commercial diagnostic kits to be used for donor screening
Verify?
Equipment post move/relocation
Compendial methods
Validate?

Legislation

Therapeutic Goods Act 1989 (the Act).

Therapeutic Goods Regulations 1990 Schedule 16

Therapeutic Goods (Standard for Human Cell and Tissue Products – Donor Screening Requirements) (TGO 108) Order 2021, which came into effect 30 September 2021.

Additional or higher requirements may be included at a sponsor's discretion or may be required by a product-specific order or default standard

TGO 108

Specifies the minimum criteria and screening requirements regarding donor:

- medical and social history requirements
- testing requirements for blood and other samples
- physical assessment requirements
- deferral criteria.

[Understanding donor screening rules for human cell or tissue products | Therapeutic Goods Administration (TGA)]

Guidelines

- Guideline presents information on the characteristics to be considered.
- Manufacturer to demonstrate – analytical procedure is suitable for its intended purpose.
- Validate / Verify analytical methods – whether they indicate stability or not.
- Validated by supplier of kits or by R&D before being transferred to the quality control unit when appropriate.

Definitions

- There are typical analytical characteristics used for any given analytical method validation in USP General Chapter <1225> as well as ICH Q2(R2).
- May be used slightly differently for clinical vs analytical assays and quantitative vs qualitative assays.
- Accuracy: an evaluation of how the result is related to the true value.

Repeatability

- Repeatability expresses the precision under the same operating conditions over a short interval of time.

Precision

- An assessment of repeatability on the multiple measurements.
- Intermediate precision: Intermediate precision expresses intra-laboratory variations – for example, different days, different environment conditions, different analysts and different equipment.
- Reproducibility: Reproducibility expresses the precision between laboratories (e.g. inter-laboratory studies, usually applied to standardisation of methodology) (ICH Q2).

Definitions

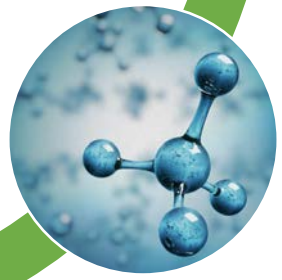
- Specificity: method is capable of identifying the desired component from the matrix components.
- Linearity: The linearity of method is a relationship, which reflects how the test result is proportional to the concentration of analyte in sample.
- Range: The range of method is an interval of different analyte concentration between lower and upper levels.
- Robustness: How a method is capable of remaining stable under normal operation despite the existence of variation from procedures.

To validate or not to validate? (verify)

Verify

Compendial methods would have already been successfully validated prior to their inclusion on the monographs.

IVD test kits on the Register (ARTG) have been assessed as fit for purpose and should be used according to the IFU.



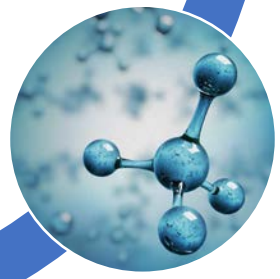
Undertake verification to ensure method is suitable under actual conditions of use in the laboratory. The goal of verification is to confirm a subset of the method’s validation performance outcomes.

To validate or not to validate? (verify)

Validate

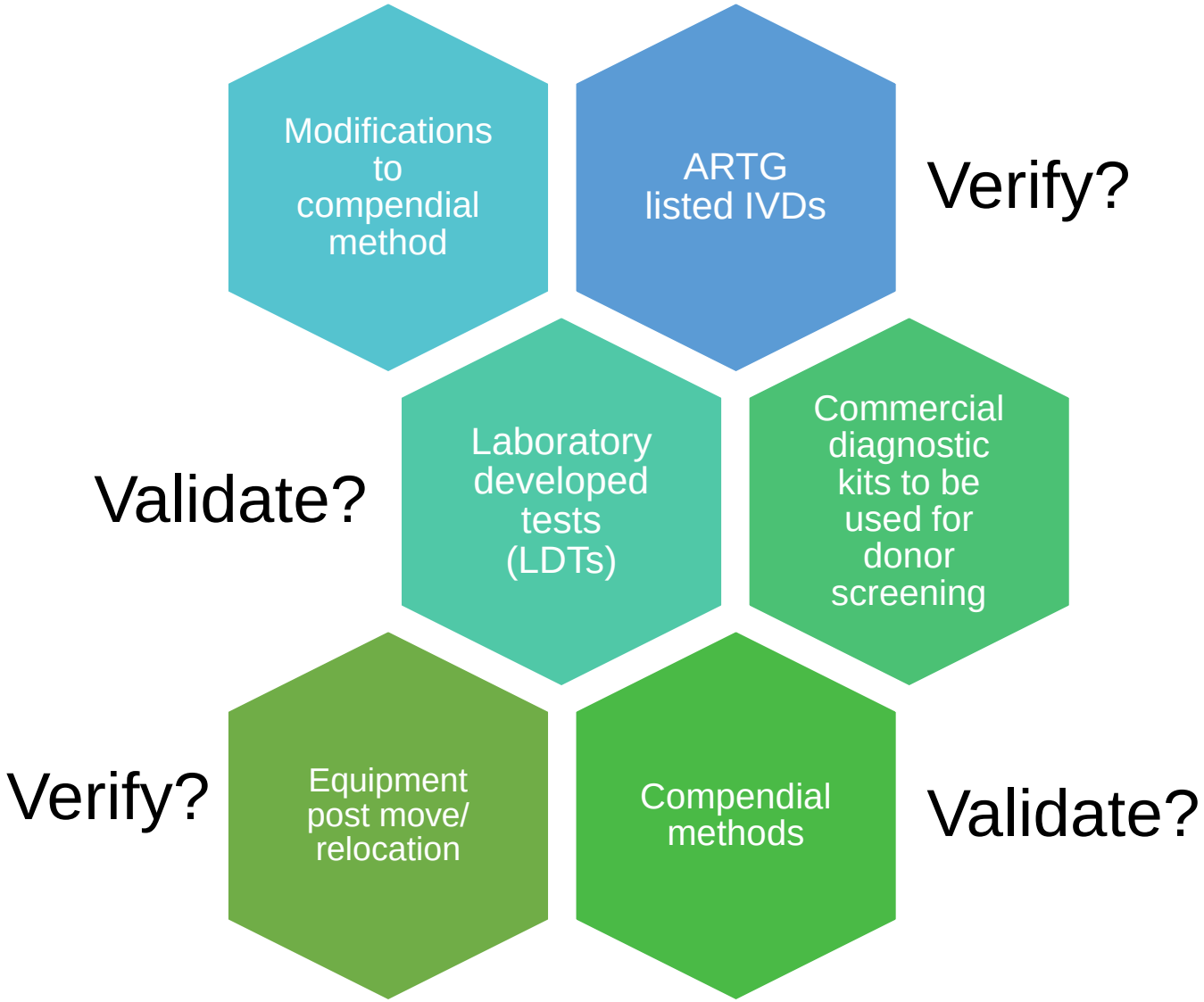
Using diagnostic kit for donor screening.

Using kits validated for living donor to test cadaveric specimens.



Significant changes to a standard method requires that the method be revalidated. This includes non-standard, modifications to standard methods (if applicable) and laboratory-developed methods.

Recheck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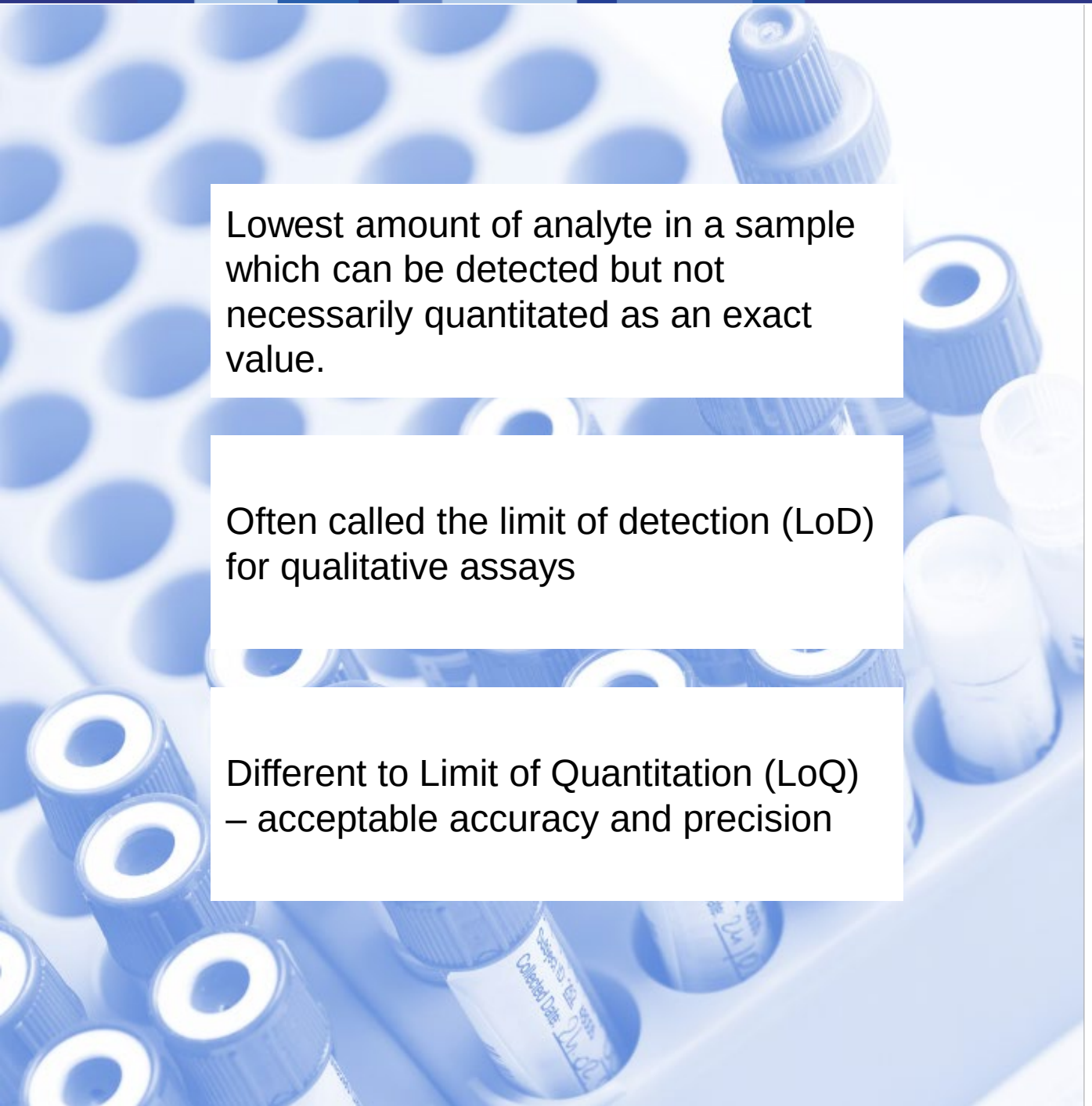


Detection Limits

Lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Often called the limit of detection (LoD) for qualitative assays

Different to Limit of Quantitation (LoQ) – acceptable accuracy and precision

Strategies for method verification and validation

	Confidence Level			
FN or FP rate	80%	90%	95%	99%
< 1%	161	230	299	459
< 2%	0	114	149	228
< 5%	32	45	59	90
<10%	16	22	29	44

Samples required to determine false positive/negative rates (qualitative test)[1]. It demonstrates the increased statistical validity of a qualitative test as the number of samples increases.

- Consider spiking (contrived samples)
- Sample size matters!

*[1] FDA Guidelines for the validation of Chemical Methods in Foods, Feeds, Cosmetics and Veterinary Products (3rd ed. 2019)

Strategies for method verification and validation

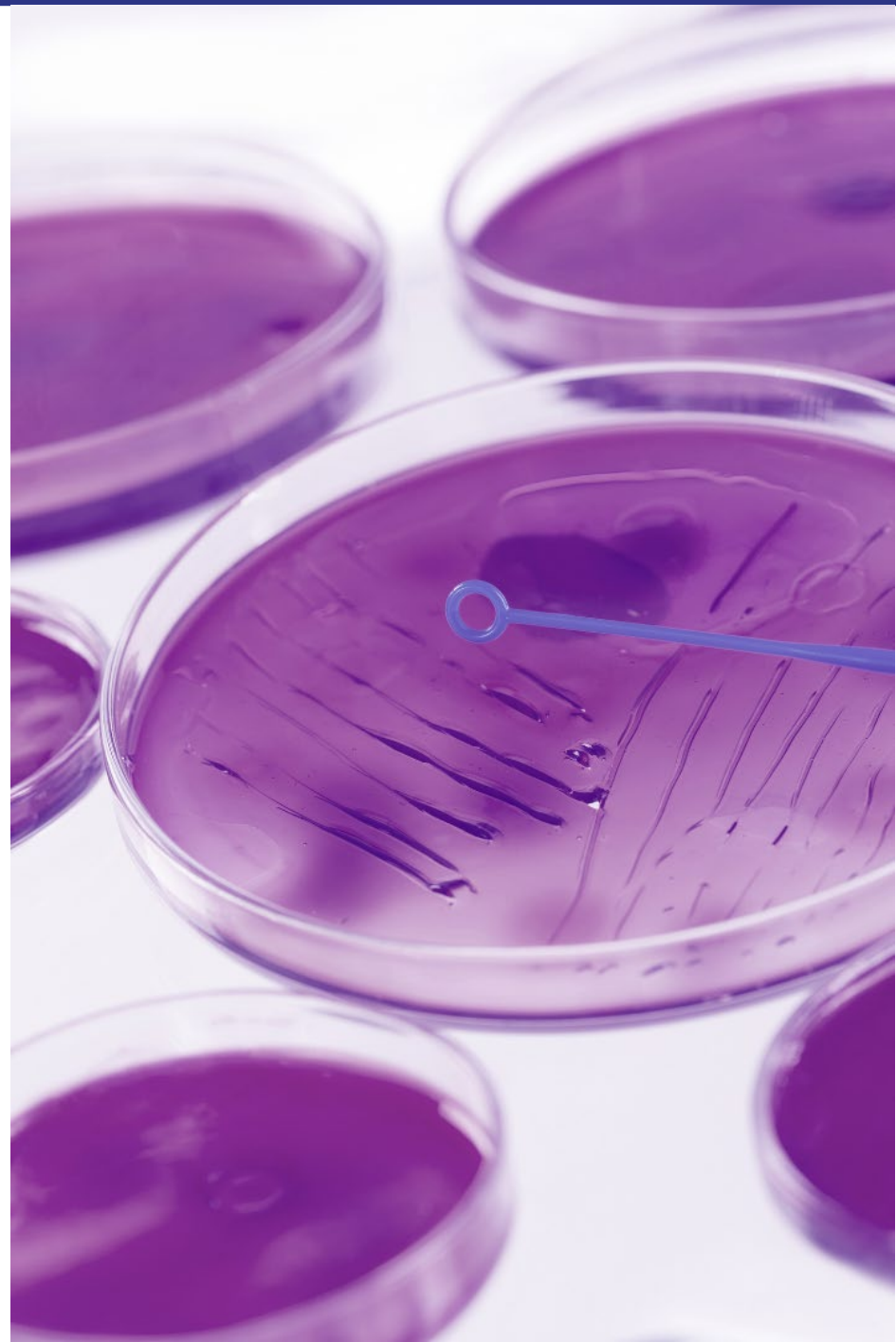
- USP General Chapter <1225> as well as ICH Q2(R2).
- CLSI EP5-A2, CLSI EP10-A3, CLSI EP12-A2, CLSI EP15-A2 etc.
- Accuracy: Minimum of 20 specimens with known results within the measuring interval at various concentrations (low and high reactive) and non-reactive samples.
- Precision: Minimum of 20 data, from low-reactive and non-reactive specimens from three precision runs. 95% agreement with expected results.
- Samples can be clinical or contrived.

Independent Control – Why?

- Viral screening tests can be broadly categorised as either “indirect” or “direct”.
- “Indirect” serology markers.
- Direct tests, used to complement serology test. NAT also shortens the detection window.
- Use of suitable external controls to track assay performance.
- Ongoing monitoring and trending of the control.
- Participation in a QAP (external) is required, If not available, the laboratory can design internal QAP.

Microbiological contamination testing

- Critical materials require pre-acceptance testing – e.g. sterility and growth promotion. A defined sampling plan based on ASTM E2234-09, ANSI/ASQ Z1.4 for example.
- Micro testing laboratory to work collaboratively with manufacturers and provide expert advice .
- Micro validation – ability to recover low CFU (<100 CFU) organisms including stasis testing.
- Appendix XVI A. Test for Sterility (Ph. Eur. 2.6.1) and Appendix XVI E. Microbiological Examination of Cell-based Preparations (Ph. Eur. 2.6.27) – method suitability and growth promotion of aerobes, anaerobes and fungi.



Commonly observed deficiencies – Biologicals testing

- Swabs for diagnostic testing with organism load in the x 10^{6-7} not suitably validated to pick up low CFU, representative of typical bioburden of tissue samples.
- Cross contamination (wipe test) and carry over study have not been considered.
- Exception report not clearly documented and explained. Example, if not meeting 95% agreement with expected results.
- Inadequacy with monitoring and tracking of external control. Example, once every monthly and breaches outside 3SD are not investigated.
- Inadequate sampling or pre-acceptance testing (PAT) of critical materials. Example, growth promotion of plates or bottles does not include pharmacopeial organisms and EM organisms.
- Recovery of EM organisms not performed.
- Poor verification/validation protocol and execution.
- LoD not suitably demonstrated.

Mock Validation Protocol

2.1.2.1. Bacterial Organisms- To create the mock bacterial specimen, each organism will be grown overnight in CO_2 atmospheric conditions. A 0.5 McFarland was made in saline creating a 10^8 colony forming unit (CFU)/mL concentration of each bacterium. For a straight mock bacterial sample, 100µL of this 0.5 McFarland was combined with 400µL of confirmed negative stool. For the limit of detection (LOD)

What is the temperature for incubation? The 0.5 McFarland corresponding to 10^8 CFU/mL may vary depending on the bacterial strains. Suggestion: To perform a plate count at low CFU/mL to assess actual concentration.

Mock Validation Protocol

4.1. Analytical Sensitivity

4.1.1. Limit of Detection (LOD) - Since the BIOFIRE® FILMARRAY® Gastrointestinal (GI) Panel package insert was explicit in determining the LOD for each organism, we propose that we perform the LOD studies outlined in **Table 2** with the representative organisms listed above in **Table 1**. We will also set up one negative stool sample for control comparison. Once the LOD is determined from an initial dilution series, we will run the mock positive pooled sample above, at and below the LOD three times to confirm the LOD.

The recommendation (CLSI EP17A) is 20 times to allow the test to be negative 1 time (i.e. 95%). There is a risk when tested 3 times if there is no growth in one plate the LOD might not be what you expect.

Mock Validation Protocol

Table 2. Limit of Detection Studies

Bacterial Organisms	
Initial Concentration	**Estimated Final Concentration (1:5 Pooled)**
1.0×10^7	2.0×10^6
1.0×10^6	2.0×10^5
1.0×10^5	2.0×10^4
1.0×10^4	2.0×10^3
1.0×10^3	2.0×10^2
1.0×10^2	2.0

Suggestion to use this initial 10 log dilution series to determine where the LoD is. Then maybe a 3x or 4x serial dilution studies above and below ~ LoD to be used for validation.

Mock Validation Protocol

4.4.1. Stability

4.4.1.1. Below is a table (**Table 3**) that outlines the proposed experiments for the stability of specimens. We will create three mock positive pooled specimen for each of the organisms listed in **Table 1** at the determined LOD. Each sample will then be placed into each temperature condition listed in **Table 3**. At the designated time point, one of the mock positive pooled specimens will be removed and then set up for culture three times. We will also set up one negative stool sample for control comparison.

As per ISO 23640, 3 types of specimens need to be tested, low positive: near the LOD, moderate positive: 2-4x LOD and high positive: >6xLoD

Questions?

Scan this QR code with your device to submit a question

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