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GUIDELINE ON VALIDATION OF IMMUNOASSAY FOR THE DETECTION OF ANTIBODY TO HUMAN IMMUNODEFICIENCY VIRUS (ANTI-HIV) IN PLASMA POOLS

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7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 85 45
E-mail: mail@emea.europa.eu http://www.emea.europa.eu

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Introduction

Immunoassays for the detection of antibodies to Human Immunodeficiency Virus (anti-HIV) are qualitative tests for the presence of anti-HIV in pooled plasma for fractionation. The validation requirements are laid down in the following documents:

- The test is considered to be a qualitative limit test for the control of impurities. Therefore, according to the "Note for guidance on validation of analytical procedures: definitions and terminology (CPMP/ICH/381/95)", ICH topic Q2A, the two characteristics regarded as the most important for validation of the analytical procedure are specificity and the detection limit. However, the note for guidance adds "those validation characteristics are regarded as the most important, (…) but occasional exceptions should be dealt with on a case-by-case basis" and Q2A requires that robustness needs to be considered.
- The Ph. Eur. Monograph 01/2005:0853 "Human plasma for fractionation" requires the use of anti-HIV test methods of suitable sensitivity and specificity for plasma pool testing.
- The "Note for guidance on plasma derived medicinal products" (CPMP/BWP/269/95, 3.2.2) specifies that the sensitivity of the test in relation to pool size has to be stated. The intention of the test is defined to be a safeguard against errors in testing or pooling.
- The Ph. Eur. chapter 2.7.1 "Immunochemical methods" requires the use of international reference material. Furthermore, the chapter suggests the use of commercial assay kits.
- The GMP Guide (Volume 4, Chapter 6, 6.21) as well as ISO 17025 (4.6.2) require critical reagents to be under control.

In accordance with these guidelines, the validation characteristics are described as:

- Specificity is the ability to unequivocally assess antibodies to HIV in the presence of other components, which may be expected to be present.
- The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value. In the context of plasma pool testing for anti-HIV, the detection limit should be expressed as endpoint dilution titre(s) of well-characterised positive samples.
- The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal performance.

Validation Guidelines

1. SCOPE

Anti-HIV assay kits, for single donation purposes, marketed in Europe are CE-marked devices and are classified in Annex II A of the Directive 98/79/EC on in vitro diagnostic medical devices. The kits are therefore subject to the Common Technical Specifications (CTS, 2002/364/EC)

Anti-HIV assay kits are validated for use in single donation testing either through CE-marking in the EU or through non-EU regulation. The use of such assays for the testing of pooled plasma for fractionation is a change of the intended use and this application is not covered by the validation undertaken by the test kit manufacturer. The application for testing plasma pools for fractionation therefore has to be accompanied by appropriate validation.

If non-CE marked test kits are used for plasma pool testing purposes, equivalent quality to a CE-marked test kit for individual donation testing should be proven in addition to the validation for pool testing described in this guideline.

The CTS defines minimal requirements for diagnostic sensitivity for assay kits.

Furthermore, the CTS requires that specificity should be demonstrated in a broad variety of patient samples. However, this approach to validation is not necessarily relevant for plasma pool testing purposes, because those patient samples which may give aberrant responses (e.g., those from patients with autoimmune diseases or having cross-reactive infections) will normally have been excluded by the donor selection process. In addition, non-specific interfering factors will be diluted in the plasma pool.

Plasma pool serology is not capable of detecting all contaminated single donations that may have escaped single donation screening. Anti-HIV is not a defined analyte, but can be described as the sum of reactivity of an individual humoral immune response to a virus subject to high genetic variability. In particular, samples from early infection stages contain low affinity antibodies that show poor dilution kinetics.

Plasma pool serology should therefore not be considered as a test to ensure viral safety, but as a measure to detect serious GMP failures.

This document describes methods to select and validate commercial qualitative immunoassay test kits for assessing contamination of plasma pools with antibodies to HIV 1 and 2 based on the above mentioned documents.

2. SELECTION OF THE TEST KIT(S)

Commercial kits used for the analytical procedure are validated by the manufacturer for single donation testing only. Selection of a test kit for plasma pool testing should be based on high dilutional sensitivity. As preliminary selection criterion, relative end point dilution titres of a well-characterised positive sample (e.g. a commercial working Standard) should be compared.

In most cases, the manufacturer's instructions for use of reagents are adequate for the performance of the test procedure on plasma pools.

Any modification of the manufacturer's instructions should be included in the validation of use of a kit for testing plasma pools.

Evaluation criteria of the manufacturer may be adapted to plasma pool testing according to validation data relevant to plasma pools (see 3.1 Specificity and determination of a cut-off limit for pool samples, and 4. Quality assurance).

3. VALIDATION

3.1 Specificity and determination of a cut-off limit for pool samples

For commercial kits the cut-off value established by the manufacturer is a compromise between sensitivity and specificity based on results from single donation testing. Many test kit manufacturers also define a 'grey-zone' cut-off which will identify samples which give a response above background but below the cut-off. It is recommended that such samples are re-tested as if they were reactive.

On the basis of previous experience in testing plasma pools, the use of a lower cut-off for pool samples should be considered as non-specific factors present in single donations are diluted in a fractionation pool. The use of such a cut-off will increase the analytical sensitivity of assays and facilitate the detection of a single positive donation in a plasma pool. The grey-zone value recommended by the kit manufacturer may be suitable. Alternatively, a limit could be established by considering the signal distribution of negative pools, e.g. as mean response to cut-off ratios of negative pool samples + 3 standard deviations and routinely expressed as % of single donation cut-off. In no case should the pool cut-off be higher than the single donation cut-off.

For all practical applications in the context of this Guideline, if a grey-zone limit is used as the cut-off value for pool samples, this limit is used to identify initially and repeatedly reactive plasma pools (see 5., confirmation strategies).

3.2 Robustness

Robustness of the analytical procedure has to be evaluated, as all methods using biological and biochemical reagents may be subject of considerable batch-to-batch variation of the reagents used and may be influenced by changes in ambient conditions.

3.2.1 Inter-assay and intra-assay

Qualitative immunoassays primarily produce a quantitative signal that is compared to the calculated cut-off in an independent step. Reactive plasma pool samples are likely to give low signals due to high dilution in the pool. Batch-to-batch variability of the test kit reagents (including controls) may have a significant influence on results and should be under control, as is foreseen by the GMP guide (chapter 6, 6.21) and ISO 17025 (4.6.2).

Robustness of the method should be demonstrated for a panel of representative negative pool samples (e.g. routine pool samples which have tested negative by both the manufacturer and an OMCL), and a low positive sample (e.g. the low positive control, see 4.3).

The study should cover:

- Inter-assay variability in 6 independent assays (including variable ambient conditions, equipment if available, preferably using more than one test kit lot if available)
- Intra-assay variability with at least 6 determinations of a low positive control in 1 run

  Intra-assay variability can be expressed as %CV (Relative Standard Deviation, RSD) of the signal of the low positive sample in relation to the cut-off of the individual assay (S/CO, sample to cut-off ratio).

3.3 Detection limit

There is no International Standard for antibodies to HIV 1 or 2. As anti-HIV is not a defined analyte, the detection limit (dilutional sensitivity) has to be determined with a representative panel of positive samples reflecting different subtypes and groups taking into consideration the epidemiological situation in the respective regions where the plasma is sourced. Confirmed reactive samples from routine donor screening may be regarded as representative without further characterization, so long as the number of samples in the panel covers the main genotypes.

To facilitate comparability of detection limit data, independent reference material for HIV-1 antibodies should be included in the panel as soon as it becomes available.

The panel is serially diluted in anti-HIV negative pool plasma. The minimum and maximum number of donations in a typical pool should be taken into consideration in the dilution series used to simulate the “best” and “worst”case scenarios. Results are expressed as end point dilution titres.

4. QUALITY ASSURANCE

4.1 Standard Operating Procedures for plasma pool testing

The test procedures must be described in detail in the form of standard operating procedures (SOPs). These should cover at least the following operations:

- Storage conditions for samples
- Preparation of samples (e.g. freezing/thawing steps, mixing)
- Description of the equipment and the test kit used
- Incubation procedures (including tolerance limits for time and temperature, e.g. according to the test kit manufacturer’s specifications/instrument settings)
- Detailed formulae for calculation and interpretation of results
- Validity criteria for the individual assay

- Retesting procedures
- Reference to confirmation procedures, if applicable

4.2 Test kit controls

The test kit manufacturer's controls should always be included in every assay to ensure correct performance of reagents according to the manufacturer's specifications. Validity criteria for modified testing conditions should be defined and documented.

4.3 Test kit independent controls

The positive controls in many commercial test kits are highly reactive and therefore do not reflect the low level of reactivity likely to be found in contaminated pool samples. In addition, as for all biological reagents, these controls are subject of batch-to-batch variation. Therefore it is strongly advised to include an independent low positive control (in the dynamic range of the assay, e.g. 2-3 times the single donation cut-off) in every test used for on-going data monitoring.

4.4 Proficiency testing

Regular participation in an appropriate proficiency testing scheme which include diluted samples with low reactivity to assess the analytical sensitivity of kits is encouraged.

5. CONFIRMATION STRATEGIES

A validated confirmation strategy for initially reactive results should be in place. A pool is considered negative if the initially reactive sample gives a negative result when retested in duplicate. Repeat reactive samples have to be considered positive unless proven otherwise with an adequately validated serological method using different antigens.

A repeat reactive on repeat testing should be confirmed through the use of alternative assays. If immunoblots are used as HIV confirmation tests, great care in the formulation of interpretation criteria is advised, as the highly specific ENV bands are hardly detectable in high dilutions, and some pool samples show unspecific bands at 24 and 40 kDa. Therefore, a positive immunoblot may be used to confirm an initially reactive result. However, a negative immunoblot result should be treated with caution.

As anti-HIV may be present in donors with low or undetectable nucleic acid plasma levels, NAT should not be considered as a confirmation assay as a negative NAT results does not invalidate a positive serological result. On the other hand, positive NAT results do confirm the serological detection of contaminations.

6. IMPLEMENTATION OF THIS GUIDELINE

This guideline has been developed to respond to inadequacies in the validation of plasma pool testing for anti-HIV observed during evaluation of dossiers. Marketing Authorisation Holders and Plasma Master File Holders should review the validation of their pool testing methods in the light of this guidance. If the key aspects described in the guideline have already been covered by existing validation, no further validation is needed. If this is not the case, pool testing should be validated in accordance with this guideline and reported in the next annual update of the documentation on the plasma starting material.